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PROCEEDINGS
OF THE
INTERNATIONAL CONFERENCE ON THE
PEACEFUL USES OF ATOMIC ENERGY

Volume 10
RADIOACTIVE ISOTOPES AND
NUCLEAR RADIATIONS IN MEDICINE

**Proceedings of the International Conference
on the
Peaceful Uses of Atomic Energy**

**Held in Geneva
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**Volume 10
Radioactive Isotopes and
Nuclear Radiations in Medicine**



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PREFACE

The Proceedings of the International Conference on the Peaceful Uses of Atomic Energy are published in a series of 16 volumes, as follows:

<i>Volume Number</i>	<i>Title</i>	<i>Sessions Included</i>
1	The World's Requirements for Energy; The Role of Nuclear Power.....	2, 3.2, 4.1, 4.2, 5, 24.2.
2	Physics; Research Reactors	6A, 7A, 8A, 9A, 10A.1.
3	Power Reactors	10A.2, 3.1, 11A, 12A, 13A, 14A.
4	Cross Sections Important to Reactor Design	15A, 16A, 17A, 18A.
5	Physics of Reactor Design	19A, 20A, 21A, 22A, 23A.
6	Geology of Uranium and Thorium	6B, 7B.
7	Nuclear Chemistry and the Effects of Irradiation	8B, 9B, 10B, 11B, 12B, 13B.
8	Production Technology of the Materials Used for Nuclear Energy.....	14B, 15B, 16B, 17B.
9	Reactor Technology and Chemical Processing	7.3, 18B, 19B, 20B, 21B, 22B, 23B.
10	Radioactive Isotopes and Nuclear Radiations in Medicine	7.2 (Med.), 8C, 9C, 10C.
11	Biological Effects of Radiation	6.1, 11C, 12C, 13C.1.
12	Radioactive Isotopes and Ionizing Radiations in Agriculture, Physiology and Biochemistry	7.2 (Agric.), 13C.2, 14C, 15C, 16C.
13	Legal, Administrative, Health and Safety Aspects of Large-Scale Use of Nuclear Energy	4.3, 6.2, 17C, 18C.
14	General Aspects of the Use of Radioactive Isotopes; Dosimetry	7.1, 19C, 20C.
15	Applications of Radioactive Isotopes and Fission Products in Research and Industry	21C, 22C, 23C.
16	Record of the Conference	1, 24.1, 24.3.

These volumes include all the papers submitted to the Geneva Conference, as edited by the Scientific Secretaries. The efforts of the Scientific Secretaries have been directed primarily towards scientific accuracy. Editing for style has been minimal in the interests of early publication. This may be noted especially in the English translations of certain papers submitted in French, Russian and Spanish. In a few instances, the titles of papers have been edited to reflect more accurately the content of those papers.

The editors principally responsible for the preparation of these volumes were: Robert A. Charpie, Donald J. Dewar, André Finkelstein, John Gaunt, Jacob A. Goedkoop, Elwyn O. Hughes, Leonard F. Lamerton, Aleksandar Milojević, Clifford Moshbacher, César A. Sastre, and Brian E. Urquhart.

The verbatim records of the Conference are included in the pertinent volumes. These verbatim records contain the author's corrections and, where necessary for scientific accuracy, the editing changes of the Scientific Secretaries, who have also been responsible for inserting slides, diagrams and sketches at appropriate points. In the record of each session, slides are numbered in numerical order through all presentations. Where the slide duplicates an illustration in the submitted paper, appropriate reference is made and the illustration does not appear in the record of the session.

Volume 16, "The Record of the Conference," includes the complete programme of the Conference, a numerical index of papers and an author's index, the list of delegates, the records of the opening and closing sessions and the complete texts of the evening lectures.

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Session 7.2

ISOTOPES IN MEDICINE, BIOLOGY AND AGRICULTURE

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Radioisotopes in Medicine

By S. Warren,* USA

Medicine tends to progress by quantal strides rather than gradually. The discoveries and initial work of Malpighi, Leeuwenhoek, Virchow, Pasteur, Shiga, Pavlov, Freud, and Florey, for example, have each resulted in a stimulus to the field of medicine as a whole that resulted in great progress. Similarly, the discovery and wide availability of radioactive tracers have already led to many significant advances in medicine^{1,2,3} and will lead to many more. The field of endeavor is so great, the number of workers so large, that it will not be feasible for me to mention by name the many, in a wide variety of countries, who have already laid the firm foundations upon which active building is now being done by hundreds of investigators. Isotopes were known and used before the advent of atomic energy, but radioisotopes were in limited supply and often of low specific activity, and stable isotopes costly and cumbersome to detect.

Two major fields have already been found for these new substances. One is their usefulness in the field of investigation, the other their usefulness in the field of radiation therapy. Related to the latter is the use of large sources of radiation in food preservation or sterilization.

RESEARCH

In research the outstanding advance of radioactive tracers is the fact that amounts so minute as to have no toxic effect nor any significant alteration of normal physiologic or biochemical processes can be used to follow vital processes in detail, both in space and in time. These tracers follow the same metabolic pathways as do their normal counterparts among the elements. Their specific radioactivity may be made so high that they can be readily identified in all stages of metabolism and can be added without influencing the biologic processes, the course of which one desires to trace.

Fortunately, most radioisotopes in tracer quantities may be expected to behave chemically as do the corresponding elements. For example, there is no evidence that $C^{14}O_2$ differs in its chemical reactions from $C^{12}O_2$. However, isotopes are not truly chemically identical as is best demonstrated by the isotopes of hydrogen. In the case of most elements, however, the difference is so slight as not to be significant and even in the case of the isotopes of hydrogen much

valuable information is being found by the use of tracer isotopes. For practical purposes, it can be assumed in most biologic reactions that the substitution of radioactive atoms at a level below the threshold of response to the radioactivity itself will not influence the reaction significantly.

Radiation is so readily and accurately detected that even in very small amounts a radioisotope makes an ideal labelling substance.

Radioautography is a convenient technique. It was first used by Becquerel in detecting the radioactivity of uranium samples. Relatively crude radioautographs were made a quarter of a century ago with the aid of radium and its relatives. Tremendous refinement is now possible, as photographic emulsions have been specifically developed for this and related purposes and as artificially radioactive substances ranging from simple elements to complex organic compounds have become available.

The high efficiency of the scintillation counter which depends upon the detection of photons emitted from certain organic or inorganic substances, as anthracene or zinc sulfide, when hit by ionizing radiations, has led to its widespread use, particularly when measurement of very minute amounts of radiation or of poorly penetrating radiation is required. However, the Geiger-Muller counter is still the workhorse of most laboratories and carries the great bulk of the research load. The electroscope is used primarily in determinations of β - γ radiation, particularly in dose levels planned for therapy.

It must be remembered that a few of the elements have so significant an effect even in very small amount that no more should be administered in the radioactive form than is required for detection purposes. Thus, iodine in even microgram quantities may affect thyroid function.

Radioactive isotopes may be used experimentally in a great variety of ways but these may be approximately grouped in six classes.

1. The administration of a radioactive isotope itself or combined in very simple chemical form to determine the metabolism of the element or to influence specifically a physiologic, biochemical, or pathologic process.

2. The use of the radioisotope in chemical combination as, for example, the use of an organic compound with specific atoms labelled, such as dextrose with the 6-carbon atoms labelled, to determine the metabolism of that compound.

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3. The use of isotopes loosely combined with complex organic substances, for example, I^{131} combined with antigenic proteins or zinc-65 combined with insulin to determine time or locus of reaction.

4. The use of a combination of several isotopes with distinctive types of radiation and different half-lives within a single molecule, as, for example, sucrose labelled with C^{14} and H^3 .

5. The administration of one or more isotopes in simple form to a plant or animal for biosynthesis of more complex tagged substances to be used in turn, as, for example, providing $C^{14}O_2$ to medicinal plants and extracting C^{14} -labelled drugs.

6. The experimental activation *in situ* of elements in the experimental animal by neutron radiation in order to determine the function and amount of some of the trace elements, for example, cobalt, zinc or barium. This technique of activation by external neutron irradiation has been of advantage in learning of the distribution of minute amounts of trace elements in the body and interpretation of their probable metabolic roles, for example, cobalt, zinc or copper. Although this technique, because of the potential harm to the organism, is applicable only to plants and animals rather than to humans, much can be learned by it and some work has been done with the study of post-mortem human tissue.

The use of radioactive isotopes permits the identification of any substance so tagged at any stage of its metabolism. The information already gained has indicated a much greater lability of the constituents of the body than had previously been dreamt of, and has profoundly altered some concepts.

For example, the rapidity of exchange of mineral salts to bone and in teeth has seriously modified our views as to the metabolic activity of these tissues and should lead to much clearer understanding of their normal state and metabolic faults. Our knowledge of the pathways of intermediary metabolism in the body has been greatly advanced and significant opportunities still lie ahead.

In the field of biochemistry, the ready availability of easily measured isotopically-labelled compounds often permits simple physical methods to replace laborious chemical estimations. By this use of radioactivity to detect compounds the sensitivity of our measurements has been increased by several orders of magnitude. At the same time, the very nature of the method of isotopic labelling permits these measurements to be made without undue disturbance of the normal cellular processes. No longer has it been necessary to work only in highly abnormal conditions where some product accumulated in amounts that could be measured chemically.

The innocuousness of the tracer method of living cells and its great sensitivity have been exploited in both intact living tissues and animals and in isolated systems. By charting the interchange of atoms between different compounds and with the living substance itself the dynamic equilibrium of the body constituents as proposed by Schoenheimer⁴ has been

amply confirmed. Closely related to this tracing function of the isotopic method is its ability to measure the formation of a substance even when it is being degraded more rapidly than formed. Experimenters were therefore freed from the need to measure only net synthesis. The relative lifetime of many of the body substances in the normal cell could then be measured. These were found to vary from the very short life of rapidly turning over compounds like the energy source, ATP, to some very long-lived compounds in nerve cells and cell nuclei, which are necessarily much more stable in order to perform their usual functions. The general pathways of synthesis and of breakdown of most of the body constituents have been surveyed, and a number of simple molecules which act as building blocks for all kinds of materials, whether fat, protein or carbohydrate, have been identified. In this "pool" of acetate, CO_2 , glycine, and related compounds, the qualitative distinctions between the major types of body substance have disappeared, since all are interconvertible through these simpler compounds.

When the broad outlines of an unfamiliar metabolic pathway had been roughly outlined by *in vivo* isotope experiments, the elucidation of many of the discrete enzyme reactions which make up such pathways became possible. In such studies the isotope method combined with classical enzyme isolation techniques has proved to be an immensely powerful tool. Systems which were previously unrecognized because only small, though possibly very important amounts of material reacted, or because the material formed was simultaneously degraded, have become amenable to the usual kinds of enzymatic experimentation when followed with isotopes. The synthesis of porphyrine, of glutathione, or of cholesterol, which can now all be done in cell-free enzyme preparations, became possible only after initial detection of these systems in isotopic studies. Even after manifold concentration of the enzymes, analysis of the now palpable amounts of product formed is still best made isotopically. This combination of isotopic analysis with enzyme isolation promises to extend greatly the knowable number of the individual reactions of cells, although our knowledge for a time appeared to be limited to the degradation reactions which occurred in sufficient amount to be detected chemically.

Especially in research in man, it is essential that no harmful concentration of the isotope should occur. This is particularly a problem in the case of the bone-seeking isotopes because of their long biologic half-life and the heavily ionizing character of the radiations given off by some of them. A sufficient amount of work has been done to establish safe standards for the tracer use of most of the radioactive isotopes.

DIAGNOSIS

A number of diagnostic uses of radioisotopes have already become well-standardized procedures. The uptake of I^{131} in the thyroid determines its metabolic

activity with a high degree of accuracy. The presence of functioning metastases of tumors of the thyroid may be readily demonstrated by concentrations of I^{131} in them. In a more complex combination, as, for example, tetraiodophenolphthalein, radioactive iodine has been of value in the localization of brain tumors. Protein-bound radioiodine has been helpful in blood-volume studies.

P^{32} has proved useful to determine the margins of certain intracranial tumors, and for other types of tumor localization.

Isotopic methods of determination of the blood volume, circulation time and other aspects of cardiac function are being tested but as yet an insufficient body of data has been established to permit their routine utilization.

THERAPY

There are two main therapeutic uses of radioisotopes: (1) as external sources of radiation, and (2) as internal sources. In addition, neutron activation of certain substances as, for example, boron, within the tissue has been used to intensify a radiation effect there.

The therapeutic use of radioisotopes depends on the radiation that they emit, their preferential concentration in regions where radiation is desired and the radiosensitivity of the tissues or tumors it is desired to control or destroy. The ability of the internal radioactive emitters to localize and concentrate in certain tissues offers a very real advantage over the use of any source of external radiation since in this way the irradiation of normal tissue is minimized.

Therapeutic use is advantageous in those instances where selective concentration of the radioisotope may be brought about and where the biologic half-life is short, either due to rapid loss of radioactivity, rapid elimination of the substance, or both. In general, therapeutic applications have been limited by: (1) the need for sharp localization of the material administered in the tissue to be treated, (2) the sensitivity of the tissue to radiation, and (3) the level of radiation given to the remainder of the body.

Concentration or localization of internal emitters can be achieved in two ways: (1) by the natural uptake of a given element by a given organ or tissue, as, for example, the uptake of iodine by the thyroid and (2) by the addition of the substance to some particular compound absorbed by that organ or tissue, as, for example, the combination of a radioactive element with an antigen or antibody that might be concentrated at specific sites.

A third method of localization of radiation effect is physical, either by the direct injection of radioisotopic material in insoluble form into a tumor for example, or its instillation into a body cavity.

Radioactive phosphorus was the first isotope to be applied to therapy of human disease. This was based upon its convenient half-life of 14.3 days, its concentration in the nuclei of rapidly growing cells, and

the great radiosensitivity of leukemic cells, which all combine to make it a very satisfactory substance to use in the control of this disease. Subsequent study has shown that it has no broad advantages over other types of radiation nor over some of the anti-leukemic chemical agents. It is now used chiefly when X-ray irradiation has failed or when the patient suffers from radiation sickness when treated by conventional means.

In polycythemia vera it is the treatment of choice. In plasma cell myeloma it has proved to be a significant palliative aid.

Radioactive colloidal gold has been utilized in two ways: (1) direct injection into a tumor to bring about local regression, and (2) for intracavitary injection to retard the growth of tumor metastases and to retard the formation of excess fluid by the mesothelial surfaces.

A variety of colloidal preparations of radioisotopes particularly P^{32} have been used to treat various malignant conditions. Among these may be mentioned suspensions of smaller colloidal particles to be phagocytized by the reticulo-endothelial system and affect neoplastic processes involving it, and of larger particles which may be localized by embolic impaction.

Finally, external sources must be considered. The chief of these is cobalt-60, utilized as a convenient substitute for radium in multicurie quantities and used to a lesser extent in the form of interstitial therapy as needles or beads. The chief advantages of radiocobalt are its availability in large quantities at low dose and its very high specific activity. The characteristics of its radiations, however, are not such as to give significant qualitative advantages over radium or supervoltage X-ray radiation.

Cesium-137 and tantalum-182 have considerable interest as external sources of radiation at lower voltages producing softer gamma rays than those of cobalt. Their competitive position in relation to X-rays has yet to be established.

Strontium-90 provides a useful external source of fairly intense beta emission for superficial therapy, penetrating up to 3 mm of tissue.

Radioactive thulium with the equivalent of 85-kv radiation and a 129-day half-life has been utilized as the equivalent of an X-ray generator and has potentialities for portable diagnostic use.

While some isotopes of short half-life can be obtained as daughter products from other isotopes, such as I^{132} with a 2.4-hour half-life obtained from tellurium-132, only in special instances are these available and for the bulk of short-lived isotopes one must still depend upon a reactor.

Thus far, as may be seen from the foregoing summary the major uses of radioisotopes have been in the field of research. Their utilization in laboratory diagnostic procedures has been as yet inadequately explored but possesses great potential value. For purposes of therapy thus far the radioisotopes have proved somewhat disappointing with fairly nar-

row fields of application. However, much work remains to be done before their evaluation is complete.

In any consideration of medical applications of atomic energy, the advantages of an atomic reactor of simple type with a relatively low power level become apparent. While its cost would prevent its being made available in every medical center, its potential value is such that a considerable number widely distributed would find useful employment. Isotopes having half-lives of a few days or longer can readily be provided from the major centers of preparation and separation as Chalk River, Harwell or Oak Ridge. However, a local source must be at hand for investigation of isotopes with half-lives measured in minutes or hours. The usefulness of many of these has not yet been explored. Their potential interest is high as their known

physical and chemical characteristics suggest a variety of possible uses. Any medical center would have a very real advantage with both the source of neutrons and a source of radioisotopes, both short- and long-lived, as many yet to be explored have characteristics that would make them of great interest in medical research, diagnosis and treatment.

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Importance of Isotopes in Biology and Medicine

By A. S. McFarlane,* UK

In any appraisal of the current value of isotopes in medical and biological research it is important to distinguish between their indispensability and the superior convenience which they offer. Since the unique character of the kind of information which their use provides is being more fully realised with each new development the former is the more difficult of the two to assess. It will be attempted in the second part of this review while the first will deal with isotope applications of which the least that can be said is that they make the life of the research biologist a great deal easier.

In many applications the use of radioactive isotopes depends on the fact that they can be measured with a higher order of accuracy than is possible with most chemical or biochemical methods, or that they can be detected at a distance. For these two main reasons they are often preferred to alternative procedures but, of course, the choice is only made possible in the first instance by the fact that isotopic compounds in general can take part in living processes without altering the normal course of events. Thus, a few hours after feeding C^{14} -amino acids to an immunised animal a significant proportion of the carbon atoms in the blood plasma antibody are radioactive ones. This labelled protein still protects an unimmunised animal against the disease. Although it is slowly metabolised the small proportion which can be recovered from the serum several weeks later by specific precipitation has precisely the same specific radioactivity as the injected material.^{1,2} This shows that no exchange of amino acids has occurred between the injected protein and the free amino acids of the lymph and plasma of the host. Having once established in this way that the label is firmly attached, the technique may be used in reverse to measure the amount of antibody or other specifically precipitable protein in the ground-up tissues of an animal in circumstances where these cannot be quantitatively extracted.³ The procedure indeed is one well known to chemists as "isotope dilution" but with specific immunological precipitation in place of crystallisation as the criterion of purity in the sample recovered.³ Since proteins are notoriously difficult to extract quantitatively from tissues and a more precise knowledge of their distribution in the body is a matter of great importance, this technique has great potentialities. It has been

used to study the composition of interstitial fluid in muscle,⁴ and protein synthesis in tissue slices.⁵

Another facet of the same general problem is the estimation of body spaces. Traditionally these spaces or "milieux", such as the blood plasma, lymph, and cerebrospinal fluid spaces, have been measured by the introduction of non-toxic substances normally foreign to the space and believed to be confined to it. With greater accuracy and immensely greater convenience isotopic modifications of compounds which occur naturally in these spaces are now used. Thus $Na^{24}Cl$ or $Na^{22}Cl$ are routinely injected with adequate safeguards into humans and a sample of salt recovered by crystallisation from the appropriate fluid and its radioactivity measured. Many kinds of labelled substance have been used, e.g., salts of potassium, calcium, strontium; phosphates and chromates as well as chlorides, bromides and iodides; red cells, proteins and colloidal substances such as gold,⁶ chromic phosphate⁷ or caesium-labelled glass microspheres.⁸ Even body water is conveniently measured by injecting the heavy⁹ or tritiated¹⁰ varieties. There is a qualification, however, in all these measurements in that the test substance is seldom confined exclusively to one compartment, and therefore a knowledge of equilibrium times is important. Thus sodium chloride shows a transient equilibrium at 24 hours and full equilibrium after 7 days with a 10% larger pool.¹¹ In patients it is necessary to measure body sodium over several days to be certain of including sodium associated with intracellular, ascitic, amniotic, or pleural fluids and for this purpose Na^{22} , the 2.6-year isotope of sodium, is ideal. The physical definition of the spaces being measured is in many cases not fully apparent and may vary from a compact fluid or gas volume to an infinitely convoluted surface layer which can be measured with the aid of labelled surface-active agents. How important some of these "spaces" may prove to be in the clinical field has yet to be shown.

Similar general considerations apply to measurements of rates of movement of solids, gases and fluids in the body. In particular, greater confidence in the results arises from labelling and using substances normally found in the body. Examples which may be mentioned are measurements of blood flow in a limb with labelled sodium chloride,¹² of efficiency of cardiac mixing with I^{131} -labelled albumin,¹³ of bladder and intestinal absorption with Na^{22} , Cl^{38}

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and $K^{42,14}$ of placental and intestinal transfer with heavy^{15,16} and tritiated¹⁷ water and of the spread of labelled proteins along nerves.¹⁸

The second broad category of isotope advantages depends principally on the measurement of gamma-radiation. The facility of measuring at a distance is of course most appreciated in the clinical field, the most familiar example being the assessment of thyroid function by measuring the uptake of iodide-131 directly over the gland. In recent years, however, it has been recognized that this procedure should be supported by measurements of thyroid hormone radioactivity in the blood.¹⁹ In general directional measurements of gamma-rays are not as accurate as the biologist would like them to be, although notable advances have been made with the introduction of scintillation detectors and scanning methods.²⁰ They are used among other things to locate brain tumours after injecting I^{131} di-iodofluorescein or I^{131} -albumin, to measure the adequacy of new circulation in pedicle skin grafts (Na^{22}), to diagnose placenta praevia (I^{131} -albumin), to estimate the vigour of blood regeneration in bone marrow (Fe^{59}), and to locate retrosternal thyroid tissue and functional thyroid metastases. It is not unlikely in the near future that purely objective radiographic methods of outlining the viscera will be available which will far exceed in accuracy the time-honoured percussion techniques. We may also see an extension of the "telephone box" technique of measuring body sodium in which the patient is surrounded by gamma-sensitive counters.¹¹ Such methods of measuring the retention of injected substances would be altogether more convenient than conventional ones requiring the collection and measurement of excreta.

The use of labelled-colloidal materials is increasing and may well prove to be of fundamental importance, since control over the distribution of particles of a wide range of size is crucial to the well-being of higher animals. Injected bacteria, viruses, and larger particles, e.g., of carbon and gold, are rapidly deposited in the lungs, while smaller particles which escape the pulmonary trap are removed by phagocytosis in reticulo-endothelial cells, especially of the liver.²¹ The half-period of the latter process in man is 3–4 minutes and is prolonged in disease conditions which reduce the circulation in the liver.²² For removal of particles as small as plasma protein or dextran molecules a different mechanism comes into play and at much slower rates. Thus the physical basis of humoral immunity appears to be the aggregation of foreign proteins into a larger particle class with correspondingly quicker removal by phagocytic cells.²³ The intense metabolic and reproductive activities of these cells appears to make them more vulnerable to radiation damage.²⁴ Once damaged their function is rapidly taken over by neighbouring cells so that it is extremely difficult if not impossible to achieve experimental suppression of phagocytosis by massive blockade with inert or radioactive particles. This kind of accumulation of gamma-emitting

particles in the liver can be followed by external measurements, and the use of colloidal radioactive particles opens a new field in the treatment of primary and secondary malignant disease. Accessible tumour-bearing areas are infiltrated with destructive doses of the colloid and inaccessible ones can sometimes be reached by parenteral injection of suspensions of suitable particle size.²⁵ There appears to be scope here for development, especially in exploiting the local destructive power of soft beta-emitters prepared in suitable colloidal form.

Turning now to more metabolic aspects of the isotope field we are faced with a more difficult assessment. At first it seemed that the use of isotopes would solve most of the more difficult problems of animal nutrition. Many answers have indeed been supplied by the use of isotopes, but in general not the simple ones which were expected. A most complex state of affairs has been uncovered and for every problem which has been definitely settled probably several new ones have come to light. Indeed, it is clear that a new outlook is necessary to visualise the rapidly changing events and the complex interrelationships of isotopic biochemistry and some time must elapse before a common viewpoint is established.

It is clear, especially from Hevesy's pioneer work, that practically every tissue, even the apatite structure of the skeleton, is in continuous exchange, and in spite of the enormous numbers of atoms and molecules in the animal body relatively few that are there in youth are likely still to be present in old age. The speeds of the individual exchanges vary over a wide range and in some cases the true two-way nature of the exchange has been obscured by an obvious unbalance. Thus the marked uni-directional shift of chloride ions between red cells and plasma which is associated with respiratory exchanges has been shown to be part of much more rapid exchanges in both directions. Indeed, after adding Cl^{38} ions to blood, equilibrium between plasma and red cells is achieved inside 10 minutes.²⁶

Such fast exchanges have usually a purely physical basis but many others are clearly dependent on a supply of energy from concomitant biological reactions. Thus the passage of various ions through the stomach,²⁷ bladder or intestinal mucosa¹⁴ cannot be explained solely on kinetic grounds nor can the absorption of phosphate ions into cells.²⁸ The precise cycle of chemical events taking place within living membranes which are responsible for a particular ion being taken in on one side and ejected on the other against normal diffusional tendencies have in most cases not been worked out, but no doubt are mediated by salt linkages with proteins and their solution may very well have to await an extension of our knowledge of protein metabolism.

The synthesis of amino acids into proteins has been the subject of numerous investigations specially directed towards the discovery of polypeptide intermediates, but with results which have been singu-

larly negative. This is the case even with systems which lend themselves to precise investigation, such as the production of egg proteins in the oviduct of the hen²⁹ or of plasma proteins in isolated perfused rat liver.³⁰ Most if not all cells seem to use only amino acids for protein synthesis, and possibly mainly amino acids from the surrounding medium. The phosphate required for the desoxy-ribonucleic acid of the developing bacteriophage is derived incidentally from the culture medium and not from intracellular phosphate of the host, a phenomenon which could not have been demonstrated without the use of isotopically-labelled phosphate.³¹ Whatever the precise mechanism for assembling amino acids into proteins it can be accomplished quickly. Thus 25 minutes is estimated³² as the minimum time required in the rabbit for synthesis of plasma proteins. Plasma albumins and globulins reach peak labelling in the blood 4–6 hours after injecting C¹⁴-amino acids but this time is increased to 50–100 hours if the protein originates in tissue sites not in close contact with the blood stream. This observation may be used to diagnose the intra- or extra-vascular origin of a protein.³³

In spite of the skill of the organic chemist, progress in the field of protein metabolic investigation has been retarded by lack of adequate supplies of C¹⁴-labelled amino acids, especially the L-forms. Recently this difficulty has been overcome by the development of biosynthetic procedures. The alga, *Chlorella*³⁴ and the yeast, *S. cerevisiae*³⁵ have both been grown on synthetic media with C¹⁴ as the source of carbon. Hydrolysis of the new protein followed by chromatographic separation of the amino acids provides commercial sources of all the commoner amino acids at more than adequate specific radioactivities,³⁶ yeast also providing C¹⁴-nucleotides, purines and pyrimidines.

By feeding C¹⁴-amino acids to animals, labelled plasma proteins have been obtained of such high activity that only negligible volumes of plasma have to be transferred to an unlabelled animal to enable plasma protein metabolism in the latter to be studied for periods of a month or more.³⁷ From such investigations it is evident that the rapid synthesis of these proteins stands in marked contrast to their gradual elimination. In which tissue of the body and by what mechanism they are degraded is a controversial matter. Some maintain that plasma proteins enter cells in a wide variety of tissues, constituting there an intra-cellular pool several times larger than the lymph and plasma pool and one which can be drawn on in emergencies associated with blood loss.³⁸ An alternative view has been argued, viz., that plasma proteins cannot penetrate cells as such but are rapidly broken down to amino acids at the cell surface and that these are re-used in different parts of the body for intra-cellular protein synthesis.⁸ The matter is of fundamental importance since it is becoming increasingly realised that the role of plasma proteins in nutrition is far removed

from the passive one formerly accepted and may indeed concern the activities of nearly every cell in the body. Agreement on these divergent views cannot be far off. Meantime, the fact that such views are tenable we owe to the use of isotopes. Without them the fact that plasma proteins enter into dynamic exchanges at all must have remained unrecognised.

The same can be said of numerous compounds to be found in the body. Sugars, fats, phospholipids, nucleic acids, are all in process of continuous exchange even in the fasting animal. The speed of these exchanges depends on numerous reactants and enzymes being brought together in correct proportions in favourable physical conditions, and abnormal speeds may be expected to result from genetic abnormalities or acquired disease. A major difficulty, however, in tracing cause and effect with the help of isotopes arises from the immense adaptability of living matter. Just as cells without specific function lie dormant in the animal until called upon to differentiate and fulfil a new requirement, such as phagocytosis or erythropoiesis, it is evident that the organism has impressive resources of a metabolic nature. Thus, while the normal trend is towards the breakdown of carbohydrate and expiry of CO₂, the use of C¹⁴O₂ shows that the reverse process can occur in animals as in plants. Similarly, dietary protein carbon which does not normally find its way to any great extent into tissue fat can do so in special circumstances. The existence of such alternative metabolic pathways presents a serious technical difficulty in tracer research which is directed towards finding the immediate precursor of a particular substance. In general the fact that a label which is attached to substance A subsequently appears in B is inadequate and a more quantitative relationship must be established.³⁹ By establishing such relationships exact metabolic pathways by which many important compounds are made have been worked out, one of the most complete examples being the biosynthesis of the blood pigment haematin due mainly to Shemin. Many simple substances have achieved a new importance in biological economy as a result of this, e.g., glycine and D-amino-laevulinic acid in the synthesis of blood pigments,^{40,41} acetate and squalene in the synthesis of cholesterol,⁴² formate in the synthesis of uric acid.⁴³

The metabolic story is, however, not exclusively one of alternative pathways, an example of specificity being found in the precise transference of methyl groups among a limited number of compounds in the body. The revelation of the body's remarkable economy in this particular group we owe largely to the use of isotopes by Du Vigneaud and his associates.⁴⁴ At the moment the whole field of metabolic investigation with isotopes is in a state of flux and generalisations are both difficult and dangerous. It is, however, safe to say that the present rate of progress in the biological field will be maintained and that the future holds promise of further discoveries of practical benefit to mankind.

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Importance of Isotopes in Medicine

By Masanori Nakaidzumi,* Japan

Radioisotopes were first imported into Japan from the United States in 1950. But now we are importing them not only from the United States but also from Canada, the United Kingdom, Holland, etc. and the amounts as well as the cases of isotope applications have considerably increased. Besides, the kinds of isotope have become diversified.

As to the national production of radioisotopes, we must rely solely on the cyclotron. In fact, since 1953 certain particular kinds of isotopes such as Na^{24} , whose half-life is very short and whose importation is therefore difficult, have been produced on a small scale and used for experimental purposes.

Radioisotopes are used in medicine in Japan not only in basic research but also in diagnosis and therapy.

Among various isotopes used for diagnostic purposes, I^{131} is most used, mainly for the functional test of the thyroid gland. For the external measurement of γ -rays, scintillation counters as well as Geiger-Müller counters are used. The Thyroid-Scintigram recording apparatus by scintillation counter is not yet on the market in Japan, but good results have been already gained in many cases by a simple trial apparatus in a laboratory stage.

As a therapeutic application, telecurie irradiation with Co^{60} has been used. Two kinds of irradiation apparatus for 50 curies and 100 curies are being manufactured and are now used by university hospitals and national hospitals for deep therapy. Although at present the amount of Co^{60} actually used is limited to less than 100 curies, plans are pushed on to increase them over a few hundred curies.

Co^{60} needles have already been imported, but plans are being made to make in Japan a very inexpensive Co^{60} wire and sell it in the market, putting

it in a needle-like case. Next to Co^{60} in terms of the amounts used is I^{131} , which is used very extensively throughout the country for the therapy of thyroid gland diseases, especially for hyperthyroidism.

P^{32} , Sr^{89} , and Sr^{90} , which are permeated evenly into a filter-paper of good quality, are used for skin diseases as the external irradiation sources of beta rays, especially for the therapy of angioma.

Instances of application of isotopes as a tracer are too many to enumerate. Among others, I^{131} , Ca^{45} , Fe^{59} , Fe^{55} , P^{32} , S^{35} , etc. are most frequently used for the study of absorption, excretion and utilization, etc. in the living body, and Na^{24} domestically produced is used for the study of Na sodium-space and also for the radiocardiography. For the purpose of autoradiography home-made films are used.

Formal plans are undertaken for the synthesis of organic compounds and compounds labeled with C^{14} are already being produced. In addition, studies are being conducted on sterilization.

Research committees on radioisotopes have been organized at various universities and now they are dealing with problems of acquisition of materials, furnishing of research facilities, purchasing of instruments of measurement and health management of researchers, etc. The Central Coordinating Research Center for Isotopes has also been set up. These laboratories have rooms for ordinary adjustments, storage, tracer experiments, measurements, radioautography, etc. In planning the construction of these laboratories, full attention is being paid to matters of ventilation and decontamination. Among the instruments of measurement we are using Japanese-made Geiger-Müller counters. But the scintillation counter we are now using is imported. As to the monitoring apparatus such as survey meters, pocket-chambers, film-badges, etc., we are using both domestic and imported ones.

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Use of Radioisotopes in France, in the Field of Medicine and Biology

By M. J. Coursaget,* France

At the very end of the second world war, the responsible organizations gave thought to the introduction or development of new research methods in France. Among these, the use of isotopes, both stable and radioactive, as labelling or "tagging" agents, opened broad vistas to the biologists and the physicians by providing means of investigating all the reactions or movements of various chemical compounds within living matter itself. The method was of such interest as to warrant a special effort for the training of specialized investigators and the equipping of laboratories.

As early as 1947, thanks to the efforts of Professor L. Bugnard, Director of the Institut National d'Hygiène, some ten medical researchers a year were enabled to attend specialized laboratories or hospitals in foreign countries, particularly in the United States, there to be trained in these new methods the development of which had been delayed, in France, due to the war. More than one hundred scholarship holders now have profited by such useful stays abroad. A score or so of them concentrated more specifically on the use of nuclear tracers, which they applied, on their return, in various fields of medicine and biology.

Other public entities, as well as private foundations, also provided for courses of study abroad, thus contributing to the training of this hard core of investigators so necessary to give research a new impetus in our country.

The Commissariat à l'Energie Atomique, created in 1946, also found itself in a position, as early as 1947, to produce some of the radioelements required by the biologists or the physicians.

Shortly thereafter, in 1950, the Centre National de la Recherche Scientifique, jointly with the Commissariat à l'Energie Atomique and the Institut National d'Hygiène, was to set up courses of study meant for the technical training of the users of radioelements. These courses are designed for qualified investigators of diverse backgrounds, trained in biology and medicine in particular, who wish to use radioactive substances either in basic research, or in the development of possible practical applications for these substances.

Training lasts six weeks, and two sessions have been organized, each year, since 1950. More than 300 students, of whom some 200 are biologists or physicians, have thus been initiated into the uses of the isotopes and the precautions they require, as well as faced with the difficulties encountered when endeavoring to interpret findings.

At the same time, thanks to subsidies granted mostly by the Institut National d'Hygiène, the Centre National de la Recherche Scientifique and the Caisse Nationale de Sécurité Sociale, various laboratories and hospital departments were supplied with the equipment required for the use of radioactive elements or radioactive compounds. A mass-spectrometry laboratory was created at Necker Hospital for the utilization of N^{15} and C^{13} . At this writing, some 20 biological research laboratories, and approximately 30 hospital departments, are provided with the required equipment.

Finally, the Commissariat à l'Energie Atomique, whose production of radioelements currently meets most of the demand, created a special department for the synthesis of tagged molecules, along, in 1953, with a Department of Biology.

CLINICAL APPLICATIONS

Some 30 hospitals, of which about one half are located in the Paris region, routinely use artificial radioelements, either for diagnostic tests, or for radiation therapy.

The table below shows, for each radioelement used in medicine, the number of patients investigated or treated during the year 1954.

Radioelements	Total number of patients	Number of diagnostic tests	Number of treatments
I ¹³¹	6500	5900	600
P ³²	800	275	525
Au ¹⁹⁸	250	275	250
Br ⁸²	80	275	80
Miscellaneous	150		

The large number of diagnostic tests carried out with I¹³¹ enabled us to get a better idea of its value for the evaluation of thyroid function, and the detection of an aberrant gland, or thyroid cancer metastases. Various iodine-containing radioactive compounds, iodine-containing proteins, di-iodo-fluoresceine, etc., have been used for the detection and localization of brain tumors.

Original language: French.

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In the area of therapeutic applications, we have endeavored to determine the optimal therapeutic regimen for hyperthyroidism and cancer of the thyroid.

As against what was done with I^{131} , P^{32} was used mostly for therapeutic purposes. Out of 525 patients treated in 1954, there were 210 cases of myeloid or lymphoid leukemia, 150 of polycythemia and 165 of cancerous affections of various types (Hodgkin's disease of the lymph node and bone-marrow types, osteosarcomata, reticulum cell sarcomata, etc.). The colloidal chromium radiophosphate first prepared and studied at the Strasbourg Cancer Center has been in use in several hospitals for months.

Au^{198} has been used as a colloidal preparation for the treatment of pleurisy and neoplastic ascites, and, as a wire, for the treatment of some brain tumors.

Br^{82} has been used only for the treatment of cancer of the urinary bladder.

As regards the isotopes having more limited uses let us mention radioarsenic, applied to the treatment of superficial lesions such as those of fungoid mycosis or Hodgkin's disease; radiobismuth, tried in the treatment of some syphilitic lesions, and radiogallium, to which resort was had for the study and treatment of osteosarcomata and metastases in bones.

FUNDAMENTAL AND APPLIED RESEARCH

Some twenty laboratories have the equipment needed for the use of the radioelements used as tracers, and numerous research projects are in progress. These are mostly in the field of physiology and biochemistry. They bear mostly on the following.

1. Absorption, diffusion, distribution or movements of various substances, particularly of water and mineral substances. Aside from basic research on the permeability of the biologic membranes, we also undertook research work applicable to medicine (circulation time, attempts at localizing tumors, etc.) or agronomy (movement of the ions in the soil and plant tissues, fertilizers uptake).

2. Incorporation of tagged materials into various organic components through exchange or synthesis. Thus, P^{32} is in very wide use; it is possible to investigate the renewal of this element in bone tissue, as well as in substances of great biological importance, such as the nucleic acids. In medical research, use is made of these potentialities, more particularly for the study of fracture repair and bone grafting. Similarly, N^{14} or C^{15} labelled amino acids are being used extensively for the study of the protein turnover, radioactive acetate for that of the lipids and cholesterol.

3. The mechanism of some biochemical reactions. C^{14} , N^{15} or F^{18} are most widely used. In the Biological Division of the Commissariat à l'Energie Atomique, studies are being made on the mechanism of utilization of inorganic sulfur by higher organisms and on that of photosynthesis.

4. Endocrinology. Many research projects are under way on thyroid function, both in man and mammals, birds, refrigerated warm-blooded animals, and fish. Interesting studies on congenital myxedema have been carried out at the Lyons Faculty of Medicine. Thyroid hormones are being investigated biochemically, particularly at the Collège de France.

Other studies also are being carried out on various hormone substances, particularly those of the adrenal cortex.

5. Hematology. Radiophosphorus, radioiron, radio-carbon, radiochromium and N^{15} are being used to follow the fate of the various blood cells or their constituents, such as hemoglobin, in normal and pathological conditions. Studies of that nature are actively being conducted at Beaujon, at the Enfants Malades, and at the Centre Anticancéreux G. Roussy, of Villejuif.

Aside from these main research fields, use has been made of tagged substances in a great variety of studies in pharmacology, bacteriology, immunology, zootechnics, agronomy, etc. Taken jointly, these projects account for the yearly consumption of radioelements the total radioactivity of which is less than 5 curies, while several tens of curies are being used yearly in clinical applications. This low consumption mostly reflects the fact that each therapeutic procedure brings a radioactivity of tens of millicuries into play while, very often, a few microcuries will be sufficient for research work. It is also a consequence of the inevitable delay in preparing the experiments and interpreting the results obtained, which necessarily reduce the rate of the demand for radioelements. The fact should also properly be emphasized that preparation of tagged molecules necessarily raises new difficulties of a financial and technical nature, which often bring about long delays.

The Commissariat à l'Energie Atomique has cooperated with the research laboratories in this field. The Organic Chemistry and Biology Divisions prepared and supplied to users, at a reasonable price, a fairly large number of labelled molecules: sodium acetate (C^{14} -methyl); sodium acetate (C^{14} -carboxyl); indolacetic acid (C^{14}); DL-glutamic acid (1-2 C^{14}); aminosalicic acid (C^{14} -carboxyl); nicotinic acid hydrazide (C^{14}); DL valine (4-5 C^{14}); glycylvaline (C^{14} -methyl); glycine (C^{14} -carboxyl); proline (C^{14} -carboxyl); pentanidine (C^{14})-(trypanocide); pyruvic acid (C^{14} -carboxyl); cystine (S^{35}); arginine (N^{15}); deuterium containing cholesterol; sodium methylarsinate (As); Diiodo-fluoresceine (I^{131}); iodized albumins (I^{131}); iodized globulins (I^{131}); *p*-iodophenylhydroxylamine (I^{131}); and cupric complexes of amino acids (Cu^{60}).

Preparation, unfortunately, often requires several months, which considerably delays the implementation of the research programs on the one hand, while, on the other hand, due to appropriations which are, at times, much-too small, many laboratories are not in a position to purchase the tagged

substances. Despite these difficulties, the use of the nuclear tagging method is under continuous development. An ever growing number of biological or medical research laboratories resort to radioelements.

Thus, French physicians and investigators are now in a position to make use of the radioelements and tagged molecules for diagnostics, therapeutics or basic and applied research. The techniques making use of nuclear tracers in biological studies are acquiring so much importance that they should receive ever growing use in order to be accessible to the laboratories which find themselves in an unfavorable position. To this end, one should take a certain number of measures such as:

1. Development of specialized training designed for researchers, and intensification of exchanges with foreign countries. Along such lines, one could mention, in particular, the opening, in October 1955, at the Nuclear Research Center at Saclay, of a yearly course for the training of investigators in the field of the applications of Atomic Energy to Biology and Medicine.

2. An increase in the means available for the performance of the work, measured in men and equipment, to the laboratories now in existence, and the setting up of new laboratories. However, an undue dispersal of efforts should be avoided and, while broad generalization of the techniques making use of isotopes is unquestionably desirable, it must also be remembered that the results given by well-equipped

specialized centers generally are better than those of multipurpose laboratories which have no such adequate means available to them. Thus, for instance, four specialized hospital departments treated more patients, in 1954, than all other departments together, although the latter were more or less well-equipped.

It would also be wise to resort more broadly to five year programs: in most cases, the appropriations and technical personnel made available to the laboratories are subject to a more or less haphazard yearly renewal system. The completion of research work calling for the use of complex techniques such as those of radioactivity and nuclear tracers often require several years. A guarantee of adequate financing for a five-year period, in order that a given work program may be carried out, would doubtless make it possible to engage in more complete and extensive studies.

3. The organization of a European "pool" of tagged molecules. It would be advisable for a European organization to make up, and keep up to date, a list of the labelled molecules which may be prepared by the various centers. Such an organization could maintain liaison between those who call for such tagged molecules and the organic chemists who can rapidly synthesize them. By this means, it would be possible to increase the number of available tagged molecules while reducing both the delays in their preparation and their cost.

Record of Proceedings of Session 7.2 (Med.)

THURSDAY MORNING, 11 AUGUST 1955

Chairman: General B. C. de Mattos (Brazil)

Scientific Secretaries: Messrs. L. F. Lamerton, A. Finkelstein and I. D. Rojanski

PROGRAMME

P/309 Radioisotopes in medicine..... S. Warren
DISCUSSION

Mr. S. WARREN (USA) presented paper P/309 as follows:

Mr. Chairman, fellow delegates and guests, medicine tends to progress by quantal strides rather than gradually. The discoveries and initial work of Malpighi, Leeuwenhoek, Virchow, Pasteur, Shiga, Pavlov, Freud, and Florey, for example, have each resulted in a stimulus to the field of medicine as a whole that resulted in great progress. Similarly, the discovery and wide availability of radioactive tracers have already led to many significant advances in medicine and will lead to many more. The field of endeavour is so great, the number of workers so large, that it will not be feasible for me to mention by name the many in many countries who have already laid the firm foundations upon which active building is now being done by hundreds of investigators. Isotopes were known and used before the advent of atomic energy, but radioisotopes were in limited supply and often of low specific activity, and stable isotopes costly and cumbersome to detect.

Two major fields have already been found for these new substances in biology and medicine. One is their usefulness in investigation, the other in radiation therapy. Related to the latter is the use of large sources of radiation in food preservation or sterilization.

In research the outstanding advantage of radioactive tracers is the fact that amounts so minute as to have no toxic effect nor any significant alteration of normal physiologic or biochemical processes can be used to follow vital processes in detail, both in space and in time. These tracers follow the same metabolic pathways as do their normal counterparts among the elements. For example, there is no evidence that $C^{14}O_2$ differs in its chemical reactions from $C^{12}O_2$.

Radiation is so readily and accurately detected that even in very small amounts a radioisotope makes an ideal labelling substance.

Radioautography is one convenient technique of investigation. It was first used by Becquerel in detecting the radioactivity of uranium samples. Tremendous refinement is now possible, as photographic emulsions have been specifically developed for this and related purposes, and as artificially radioactive substances ranging from simple elements to complex organic compounds have become available.

The high efficiency of the scintillation counter has led to its widespread use, particularly when measurement of very minute amounts of radiation or of poorly penetrating radiation is required. However, the Geiger-Müller counter is still the workhorse of most laboratories and carries the great bulk of the research load. The electroscope is used primarily in determinations of beta gamma radiation, particularly in dose levels planned for therapy.

It must be remembered that a few of the elements have so significant an effect even in very small amount that no more should be administered in the radioactive form than is required for detection purposes. Thus, iodine or astatine in even microgram quantities may affect thyroid function.

Radioactive isotopes may be used experimentally in a great variety of ways but these may be approximately grouped in six classes:

1. The administration of a radioactive isotope itself or combined in very simple chemical form to determine the metabolism of the element or to influence specifically a physiological, biochemical or pathological process.

2. The use of the isotope in more complex chemical combinations as, for example, the use of an organic compound with specific atoms labelled, such as dextrose with the 6-carbon atoms labelled, to determine the metabolism of that compound.

3. The use of isotopes loosely combined with complex organic substances, for example, iodine-131 combined with antigenic proteins or zinc-65 combined with insulin to determine time or locus of reaction.

4. The use of a combination of several isotopes with distinctive types of radiation and different half-lives within a single molecule, as, for example, sucrose labelled with both carbon-14 and tritium.

5. The administration of one or more isotopes in simple form to a plant or animal for synthesis by the organism of more complex tagged substances to be used in turn as, for example, providing $C^{14}O_2$ to medicinal plants and extracting from them C^{14} -labelled drugs.

6. The experimental activation *in situ* of elements in the experimental animal by neutron radiation in order to determine the function and amount of some of the trace elements, for example, cobalt, zinc or barium. Although this technique, because of the

potential harm to the organism, is applicable only to plants and animals rather than to humans, much can be learned by it, and some work has been done in the study of post-mortem human tissue.

The use of radioactive isotopes permits the identification of any substance so tagged at any stage of its metabolism. The information already gained has indicated a much greater lability of the constituents of the body than had previously been dreamt of, and has profoundly altered some concepts.

For example, the rapidity of exchange of mineral salts to bone and in teeth has seriously modified our views as to the metabolic activity of these tissues and should lead to much clearer understanding of their normal state and metabolic faults.

In the field of biochemistry, the ready availability of easily measured isotopically-labelled compounds often permits simply physical methods to replace laborious chemical estimations. By this use of radioactivity to detect compounds the sensitivity of our measurements has been increased by several orders of magnitude. At the same time, the very nature of the method of isotopic labelling permits these measurements to be made without undue disturbance of the normal cellular processes.

By charting the interchange of atoms between different compounds and within the living substance itself the dynamic equilibrium of the body constituents as proposed by Schoenheimer has been amply confirmed. Closely related to this tracing function of the isotopic method is its ability to measure the formation of a substance even when that substance is being degraded more rapidly than formed. Experimenters are therefore freed from the need to measure only net synthesis. The general pathways of synthesis and of breakdown of most of the body constituents have been surveyed, and a number of simple molecules which act as building blocks for all kinds of materials, whether fat, protein or carbohydrate, have been identified. In this "pool" of acetate, CO₂-glycine, and related compounds, the qualitative distinctions between the major types of body substance have disappeared, since all are interconvertible through these simpler compounds.

When the broad outlines of an unfamiliar metabolic pathway had been roughly outlined by *in vivo* isotope experiments, the elucidation of many of the discrete enzyme reactions which make up such pathways became possible. Systems which were previously unrecognized because only small though possibly very important amounts of material reacted, or because the material formed was simultaneously degraded, have become amenable to the usual kinds of enzymatic experimentation when followed with isotopes. The synthesis of porphyrine, of glutathione, or of cholesterol, which can now all be done in cell-free enzyme preparations, became possible only after initial detection of these systems in isotopic studies. Especially in research in man, it is essential that no harmful

concentration of the isotope should occur. This is particularly a problem in the case of the bone-seeking isotopes because of their long biological half-life and the heavily ionizing character of the radiations given off by some of them. A sufficient amount of work has been done to establish safe standards for the tracer use of most isotopes.

Many diagnostic uses of radioisotopes have already become well-standardized procedures. The uptake of I¹³¹ in the thyroid determines its metabolic activity with a high degree of accuracy. The presence of functioning metastases of tumors of the thyroid may be readily demonstrated.

Isotopic methods of determination of blood volume, circulation time and other aspects of cardiac function are being tested but as yet an insufficient body of data has been established to permit their routine utilization.

There are two main classes of therapeutic uses of radioisotopes: (1) as external sources of radiation, and (2) as internal sources. In addition, neutron activation of certain substances as, for example, boron, within the tissues has been used to intensify local radiation effect.

The therapeutic use of radioisotopes depends on the radiations that they emit, their preferential concentration in regions where radiation is desired and the radiosensitivity of the tissues or tumors it is desired to control or destroy. The ability of the internal radioactive emitters to localize and concentrate in certain tissues offers a very real advantage over the use of any source of external radiation since in this way the irradiation of much normal tissue is minimized.

In general, therapeutic applications have been limited by: the need for sharp localization of the material administered in the tissues to be treated, the sensitivity of the tissue to radiation, and the level of radiation given to the remainder of the body.

Concentration or localization of internal emitters can be achieved in two ways: (1) by the normal uptake of a given element by a given organ or tissue as, for example, the uptake of iodine by the thyroid, or (2) the addition of the substance to some particular compound absorbed by that organ or tissue, as, for example, the combination of a radioactive element with an antigen or antibody that might be concentrated at specific sites.

A third method of localization of radiation effect is physical, either by the direct injection of radioisotopic material in insoluble form into a tumor, for example, or its instillation into a body cavity.

Radioactive phosphorus was the first isotope to be applied to therapy of human disease. This was based upon its convenient half-life of fourteen days, its concentration in the nuclei of rapidly growing cells, and the great radiosensitivity of leukemic cells, which all combine to make it a satisfactory substance to use in the control of leukemia. Subsequent study has shown that it has no startling advantages over

other types of radiation in this field, however, and is one of a number of useful tools.

Radioactive colloidal gold has been utilized in two ways: (1) direct injection into a tumor to bring about local regression, and (2) for intracavitary injection to retard the growth of tumor metastases and to retard the formation of excess fluid by the mesothelial surfaces.

A variety of colloidal preparations of radioisotopes, particularly P^{32} , has been used to treat various malignant conditions.

Finally, external sources must be considered. The chief of these is cobalt-60, utilized as a convenient substitute for radium in multi-curie quantities and used to a lesser extent in the form of interstitial therapy as needles or beads. The chief advantages of radiocobalt are its availability in large quantities at low cost and its very high specific activity. The characteristics of its radiations, however, are not such as to give significant qualitative advantages over radium or super-voltage X-ray therapy.

The competitive position of cesium-137 and tantalum-182 in relation to X-rays has yet to be established.

Radioactive thulium with the equivalent of 85-kv radiation and a 129-day half-life has been utilized as the equivalent of an X-ray generator and has potentialities for portable diagnostic use.

While some isotopes of short half-life can be obtained as daughter products from other isotopes, such as I^{132} with a 2.4-hour half-life obtained from tellurium-132, only in special instances are these available and for the bulk of short-lived isotopes one must still depend upon a reactor.

In any consideration of medical applications of atomic energy, the advantages of an atomic reactor of simple type with a relatively low power level become apparent. While its cost would prevent its being

made available in every medical center, its potential value is such that a considerable number of them widely distributed would find useful employment.

DISCUSSION OF P/309

Mrs. P. CAMBEL (Turkey): I should like to ask Mr. Warren what is the prospect of using cesium-teletherapy instead of cobalt-teletherapy. Will the cesium source be cheaper and superior to the cobalt source in cancer treatment?

Mr. WARREN (USA): It is not easy to answer this question at the present time. The chief saving in cost with regard to the cesium source as contrasted with the cobalt source is that the radiation from the cesium is rather less penetrating, and consequently less shielding will be required in the construction of the apparatus and in the region used for therapy. However, I do not believe that the less penetrating radiations of cesium can replace the more penetrating radiations of cobalt. I would feel that, rather than these two types of applicators being in competition with one another, one could be utilized where more penetrating radiation is required and the other where less penetrating radiation will serve.

Mr. S. MILCOU (Romania): Has it been possible to synthesize radioactive hormones, such as thyroxine, the corticosteroids, etc., and have these substances any therapeutic applications?

Mr. WARREN (USA): It is quite possible to synthesize the various hormones either by chemical synthesis or by biosynthesis in the organism. However, the radioactivity that they carry and the concentration at any specific site is so slight that there will not be a therapeutically helpful radiation. The value in utilizing them will come from a clearer understanding of the metabolic and physiologic processes involved rather than from using them as a therapeutic aid.

Session 8C

RADIOACTIVE ISOTOPES AND NUCLEAR RADIATIONS IN MEDICINE—THERAPY

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Medical Application of Some Radioactive Isotopes

By A. V. Kozlova, USSR

Radioactive isotopes are used in the medical establishments of the Soviet Union for treating various diseases. Their greatest importance, however, has been proved in treating malignant and benign tumours, including some systematic diseases of blood-forming organs. In the last few years isotopes have been used in dermatology as well. Among the various radioactive isotopes employed for medical purposes, radioactive cobalt, phosphorus, iodine and gold have won the greatest recognition.

The centralized supply of a large number of medical establishments with radioactive isotopes, and free medical service easily available to the public have made it possible in a comparatively short time to carry out clinical observations of large groups of patients. The observations as well as numerous experimental investigations permit the appraisal of the various methods of treatment with radioactive isotopes.

Before the introduction of radioactive isotopes into medical practice, irradiation therapy was effected by radium, radio-mesothorium and various methods of roentgenotherapy. When radioactive cobalt was discovered, the study of its physical properties and the peculiarities of its biological action, as compared with natural radioactive substances became the most important task of scientific research institutions. Work on dosimetry has been carried out simultaneously. Thus K. K. Aglintsev gave an analysis and theoretical original computation of the photographic effect of ionizing radiations.¹ Computations have been made of protection from gamma irradiation and, notably, from radioactive cobalt.²

K. K. Aglintsev has designed a special device to reproduce the roentgen unit in the region of gamma rays with energy up to 1.5 Mev. The possibility of using germanium phototransistors in dosimetry has been investigated.³ Special-type plastics have been recommended for scintillation dosimeters instead of crystals.

A method of detecting and measuring alpha and beta radiation of low activity by means of diffusion chambers has been suggested. Computations of dose fields of gamma radiators for big areas were made.⁴

Calculations have been made of the gamma constants of various isotopes and the methods of absolute computations of beta and gamma radiators have been specified.⁶

Considering the differences in the spectra of gamma-radiation of radium and cobalt, experiments have been performed to study the specific features of the biological effect of cobalt in normal as well as pathological tissues and, notably in tumour tissues.

Both experimental investigations and clinical observations conducted on nearly 2000 patients affected with various kinds of malignant tumours have revealed some advantages of cobalt as compared with radium and radio-mesothorium: the use of cobalt resulted in slighter injury to surrounding tissues. This was particularly manifest during intra-tissue, intra-cavity and application methods of treatment. Irradiation with radioactive cobalt, with total and single doses, radiation power, screens and skin to focus distance being equal, caused a reaction on the skin and mucosa, which ended 5–10 days earlier than during radium irradiation. Under the usual conditions of radium treatment, the reaction on the skin mostly assumed the form of a moist epidermitis. During cobalt irradiation the reaction quite often was confined to a dry epidermitis. For instance, a dose of 6000 r of radium possessing a dose rate of 30 r/hour, as a rule, caused a moist epidermitis. With radioactive cobalt irradiation the same degree of reaction was caused by a dose of 7000 r with the same dose rate.

The discovery of the specific features of the biological action of Co^{60} allowed the extension of its use in medicine. Needles with radioactive cobalt could be used with less risk than those with radium, for the intra-tissue method of treating cancer complicated with an infection, or the involvement into the process of the cartilaginous or bone tissue.

In view of the lesser degree of injury caused to the normal tissue, it was possible to administer repeated irradiation therapy to patients with changes of skin as a result of the previous irradiation effect. The frequency of complications such as perichondritis, osteonecroses and necroses of the soft tissue has been reduced. Complications did not exceed three per cent in patients suffering from recurrences or late stages of the disease.

One hundred and sixty oncological establishments in the Soviet Union have almost simultaneously been supplied with sets of radioactive cobalt preparations in the shape of small tubes and needles of various sizes containing from 0.5 mg to 35 mg radium equivalents. In addition, gamma installations have been manufactured and set up, with a charge of 400 curies

and skin to focus distance of 35 and 60 cm, and a charge of 20 curies with a skin to focus distance of 6 cm.

In the Soviet Union radioactive cobalt treatment has been administered to a large number of patients with malignant tumours of the skin, the oral cavity mucosa, the tonsils, the upper jaw, the nasal cavity, the larynx, the oesophagus, the lungs, the mammary gland and with metastasis in lymphatic nodes and bones, as well as patients with tumours of genitalia. It has been found that the results of treatment, both immediate and distant, are not inferior to those when using natural radioactive substances. For example, treatment of 450 patients with eyelid skin tumour in the Kharkov Institute of Roentgenology and Radiology resulted in recovery of 94 per cent of cases, no complications being caused by the treatment.

The results of treatment of 1552 patients with various malignant tumours in the first, second and third stages, achieved by means of Co^{60} at the Herzen Oncological Institute, far from being inferior to the results of treatment with natural radioactive substances, in some cases of localization showed an even higher percentage of favourable results. Thus, when treating gullet ring tumours with radio-mesothorium, immediate recovery was obtained in 61 per cent of cases, while radioactive cobalt produced favourable results in 70 per cent of cases; in larynx cancer cases of 1st and 2nd stages prolonged recovery was obtained in 73 per cent, while with cobalt treatment (140 patients) the percentage was as high as 77. Treatment of cancer metastases of the mammary gland in the supraclavicular area has produced 22 and 36 per cent of recoveries respectively.^{8,9,10}

When treating patients with malignant tumours of small radium-sensitivity, such as oral-cavity cancer, some forms of gullet ring cancer, oesophagus cancer, etc., a combination of various methods of irradiation therapy is widely used. Thus, in cases of tongue cancer of all stages, treatment begins with telecobalt therapy and terminates with the intra-tissue method of treatment. When treating 558 such patients, the percentage of recovery has reached 40. In cases of oesophagus cancer, telecobalt therapy is used in combination with the intra-cavity method of treatment, which makes it possible to increase the local dose without increasing the intensity of action on the normal tissues surrounding the tumour.

When treating 1295 patients with cancer of the lower lip and skin in all four stages, the percentage of recovery amounted to 76.

The application of combined methods of irradiation therapy permits one, in the majority of cases, to bring the necessary dose of radiation to the area of lesion with the least injury to the surrounding normal tissues. The preservation of the regenerative capacity of the normal tissue, as is known, is of great importance for the patient's recovery. In view of the importance of this factor, a number of methods have been elaborated in the Soviet Union aimed at pre-

venting the injury of normal tissues, directly subjected to irradiation, as well as at the improving of the general condition of the patient during irradiation therapy.

The application of a bilateral one-stage ligation of the external carotids proved most effective for reducing the local manifestations of reaction to irradiation in cases of tumours (II and III stages) of the upper and lower jaws, the oral cavity, the crane and the lower lip (III and IV stages). Observations made on 340 patients showed that no complications resulted from the operation. Skin resistance to irradiation increases on the average by 50 per cent. The disorders of blood supply of the tumours causes its degeneration which makes it more susceptible to injury during irradiation. The method proves effective, provided irradiation begins shortly after the operation, not later than 1-3 days.¹¹

In cases of tumours located on the trunk, the decrease of local reaction can be attained by a repeated (4-5 days) circular vagosympathetic of paranephral novocaine block.¹²

In order to prevent irradiation injury which frequently follows irradiation therapy, the patients are given blood transfusions to be performed premedically, weekly during treatment and during 1-2 weeks following the completion of the irradiation therapy course. Observations have shown that blood transfusion is most effective when done before the development of irradiation disease. Blood transfusion in developed leukopenia proves to be less effective. Apart from blood transfusion, the patients are given a number of pharmacological aids and vitamins (vitamin B₁₂, K, C, antianaemin, etc.)

The guiding principle of irradiation therapy—to act only on the tumour, without injuring the surrounding normal tissues—was until recently difficult to fulfill.

Artificial radioactive isotopes with a short half-life, which can be introduced directly into the tumour, are manufactured in the Soviet Union. The radioactive substance is produced as a suspension of insoluble salt or colloid solutions.

A method of determination of the activity in the tissues by measuring the inhibitive roentgen radiation has been worked out.

Observations have shown that the action of radioactive isotopes is mostly confined to the site of its injection. While the isotope is in the tumour, its dose caused by its decay reaches a value sufficient to destroy the most radioresistant tumours.

This is not accompanied by an injury to the normal tissues surrounding the tumour (absence of any clinical symptoms of general or local reaction). Among the isotopes produced for the treatment of malignant tumours, radioactive colloidal gold and, to a lesser degree, radioactive chromium phosphate are the most effective.

Observations of 47 patients with forms of tumour most difficult for treatment (cancer recurrence after irradiation therapy, metastases not requiring sur-

gical intervention or repeated irradiation therapy, and metastases of malignant melanoma) have shown that the introduction of isotopes into the tumour resulted in its degeneration. Thus, of 11 patients with metastases of malignant melanoma thus treated, no further spread of the process has been found in 8 cases. The period of observation lasted up to two years. About half the cases of recurrent tumours recovered (Institute of Roentgenology and Radiology).^{13,14}

When treating cancer of the urinary bladder, we apply a combined method: external irradiation at a distance of 35 or 60 cm with a local dose of some 7000 r followed by *sectio alta* two or three weeks after the end of irradiation; the remaining tumour is then infiltrated by a solution of colloidal gold or a chromium phosphate suspension; the wound is sutured blindly.

Treatment with telecobalt of 450 children with cavernous angioma was effective in 92 per cent of cases. Treatment was administered in fractions with daily doses of 400–500 r and a total dose of 2000 to 3000 r, depending on the age of the child.

When treating malignant tumours of the eyelids, sclera, and conjunctiva and the cornea with radioactive phosphorus (external irradiation) it is possible to attain favourable results without injuring the organ of vision. In these localized tumours only surgical intervention, such as enucleation of the eye is known to have been applied so far. To determine the dose from a flat applicator with radioactive phosphorus, the average beta-irradiation dose rate has been computed and dosimeters for measuring beta-irradiation elaborated.¹⁵ The findings are very promising for the method in question, which will make it possible to achieve recovery in such localizations of malignant tumours and states of the body for which the methods existing hitherto proved to be either unrealizable or ineffective.

One of the leading trends in Soviet oncology is prophylaxis of malignant tumours.

Out-patients with pre-tumour state of skin or mucous tissue, recognized at medical examinations in large groups of the population, or those applying for consultation on their own initiative, are subject to special examinations and observations at oncological dispensaries.

Examination of the motor and secretory function of the gastro-intestinal tract in 170 patients with leukoplakia of the oral-cavity revealed the presence of anacide or hypoacide gastritis in 75 per cent of the cases. The patients were given treatment for the gastro-intestinal tract disorders and local treatment with radioactive phosphorus for leukoplakia. In most of the cases leukoplakia disappeared after treatment. In those cases, however, where no marked improvement of the gastro-intestinal tract function set in, recurrent leukoplakia was recorded.

Nearly 100 per cent of recoveries were recorded as a result of the application of radioactive phos-

phorus in pre-tumour states of the skin—hyperkeratosis and Bowen's disease.

Treatment with radioactive isotopes was based on the study of the reactivity of the skin, the state of peripheral blood circulation, permeability of skin capillaries and the morphological changes caused by local irradiation with beta rays.

Soviet dermatologists have used radioactive isotopes as applicators in various skin diseases. For this purpose radioactive phosphorus was most widely used to treat capillary angiomes, pre-tumour conditions, neurodermatoses and other skin diseases.

The total dose for the treatment should not exceed 600–900 r when treating eczema, psoriasis and neurodermites. In treating capillary angiomes, the dosage is determined by the age of the patient. The treatment of pre-tumour cases, basalioma and keratoderma called for doses causing moist epidermitis.

The findings in 1396 cases of capillary angioma showed good therapeutic results in 1124, i.e., 88 per cent of patients. Of these, 16 per cent exhibited a complete disappearance of angioma without atrophic cicatrices, while in 72 per cent an improvement was recorded expressed in a change in the colour of the foci from bluish-red to light pink.¹⁶ Better results were observed in children of an early age.

In a group of 733 cases of erythemo-squamous dermatosis (eczema, psoriasis, red lichen), 60 per cent of the patients showed satisfactory results: itching rapidly disappeared followed by resorption of the inflammatory infiltration. In 50 per cent of cases of limited forms of eczema the result was stable (observations lasted for three years).^{17,18} In foci with an intense growth of the corneous layer (warty neurodermites and red lichen) daily applications of phosphorus in most cases brought about a moist epidermitis with a change-over to a cicatricial atrophy. In cases like keratoderma, the daily application of phosphorus, following the removal of thick corneous growths by keratolytic means, while having a depressing effect on rapid horn-formation, resulted in recovery.

Modern clinics are making wide use of the various methods of irradiation therapy in treating diseases of the blood system.

Specialized institutions (blood transfusion institutes and centres in all the republican and regional centres) have been set up by the Ministry of Health of the USSR, dealing with problems of aetiology, pathogenesis, clinical investigations and treatment of the blood system diseases.

The institutions are working at scientific and practical problems of experimental and clinical haematology. They carry on systematic work of dispanserization and treatment of patients with diseases of the blood system. It is these institutions that investigate problems dealing with the practical application of radioactive isotopes in diseases of the blood system, notably for treating erythremia and leukoses.

Radioactive phosphorus is not only the most effective means of treating erythremia; it is also most

convenient in application. This has been confirmed by data published in the USSR and abroad.¹⁹

It has been considered of paramount importance to search for ways of achieving stable remission when treating erythremia with small doses of radioactive phosphorus. This method is very important since the administration of comparatively small doses of radioactive phosphorus throughout the course of treatment (8-9 mc) may sometimes bring about a state of anaemia, leukopenia or thrombocytopenia.

The works of N. V. Nikolayeva²⁰ prove this advisability, prior to injections of radioactive phosphorus, of 1-3 phlebotomies, 400-500 ml each, to be followed by injections of 300-400 ml of fresh plasma with intervals of 5-6 days. In such cases the author recommends the use of radioactive phosphorus in doses not exceeding 4 mc for the whole course. This method led to an improvement in 122 patients within the first few weeks, while in 120 cases prolonged remissions were recorded lasting from 1 to 3 years. Pronounced disposition for thromboangiitis is a contraindication for such a complex method of treatment of erythremia.

Of great interest was the analysis not only of haematological, but also haemodynamic data in patients affected with erythremia and treated with radioactive phosphorus. Prior to and during treatment with radioactive phosphorus, a study was made of the rate of the blood flow, the electrocardiograms, the arterial and venous pressure, capillaroscopy, the basal metabolism, the amount of circulating blood, the antitoxic liver function, blood coagulations, etc.

Modern clinics do not possess any effective methods of treating acute leukoses. In some isolated cases a temporary weakening of symptoms of the disease and a certain prolongation of the patient's life are brought about by complex therapy comprising the use of antibiotics, frequent transfusions of erythrocytal mass, hormones of hypophysis and adrenal cortex, and ascorbic acid.

The experience of applying radioactive phosphorus in a great number of patients with chronic leukaemia has led Soviet scientists to believe that the medical effect of this preparation is higher in chronic lymphadenosis than in chronic myelosis.

On the basis of their experiments, the investigators consider it advisable to prescribe radioactive phosphorus in combination with roentgenotherapy, particularly in myeloid forms of leukaemia; the spleen or individual large lymphatic nodes are subjected to irradiation in doses of 100-150 r 2-3 times a week.

We make use of methods of haemotherapy, notably transfusion of erythrocytal mass (which the patients bear easier than transfusion of whole blood) in treating leukaemia, complicated by anaemia, as well as in preventing the development of anaemia.

Along with haemotherapeutic methods, antibiotics and hormonal therapy are employed to treat leukaemia.

The problems of indications and contra-indications for this method are being investigated at present as well as that of dosage. The scientific research insti-

tutes are engaged in searching for the most effective methods of using radioactive phosphorus in combination with other remedies for the treatment of blood disorders. Special observation of the patients who had completed their course of radioactive phosphorus treatment has been instituted and the remote results are being studied. A systematization and recording of the results of treatment in all the health establishments of the USSR is effected by the institutes of blood transfusion and institutes of roentgenology and radiology; instructions on the application of radioactive phosphorus in haematology are worked out on the basis of the findings.

We use radioactive iodine in treating some diseases of the thyroid gland. Thus, the injection of radioactive iodine causes a considerable decline in or a complete disappearance of the symptoms of the disease in various forms of hyperthyreosis (toxic diffuse, toxic nodulous, etc.). Soviet experimenters use I¹³¹ in doses not exceeding 6-8 mc. Such doses did not lead to hypothyreosis or any other complications. According to the clinics of the Institute for Medical Advanced Training good results were obtained in 107 out of 118 cases of hyperthyreosis; the observations lasted up to 18 months.

Hence our experience in the field of medical radiology leads us to the conclusion that curative application of radioactive isotopes is of considerable value and holds out great possibilities.

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Some Problems of Radiotherapeutics

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So much has been published about the many valuable applications of nuclear physics to medical treatment that it seems opportune to consider some of the difficulties associated with these developments. This paper deals with problems in connection with present methods of radioactive isotope therapy and of high energy roentgen therapy and with the mechanism of the therapeutic actions of ionising radiations. It is based essentially on clinical experience and investigations at Cambridge.

In general in the practical uses of these newer methods in radiotherapy, as distinct from research and investigations including tracer studies, insufficient time has elapsed for the long-term follow-up of adequately large numbers of patients to provide a satisfactory assessment of the results of treatment. For example, there are relatively few cases of carcinoma of the thyroid after treatment with radioiodine who have been observed even for 5 years and, taking into account the natural history of this disease, it is suggested that substantially longer follow-up, for at least 10 years, is essential to evaluate this type of treatment. Also, in the case of 20 to 30 Mev roentgen therapy, follow-up of all patients for at least 5 years is essential not only to estimate the survival rate in different types of tumour after treatment but also to determine the relative biological efficiency of the new radiation with reference to conventional radiations for permanent healing of the tumours and for late effects on the normal tissues. Such information is essential for correct dosage and cannot be assumed to have been provided by clinical studies of immediate reactions or by most animal experiments which are essentially short-term studies.

In all these new developments, perhaps especially with the high energy radiations on account of the high costs of equipment and treatment, the need for properly designed clinical trials must be emphasised. Although there are many difficulties, the basic principles involved are well understood as a result of the classical statistical studies of Fisher^{1,2} on the design of experiments and on the work of Bradford Hill³ on the design of clinical trials. The most important single feature of the design is random allocation of the patients to two or more alternative forms of treatment, the value of which is being compared. It is absolutely essential to provide for each individual patient treatment which is at least the best

according to present knowledge before the trial begins. It may be, and probably is, necessary to plan to depart from what may be termed the theoretically ideal form of experiment with deliberate sacrifice of some information. For assessment of the results of treatment, it is necessary to select relevant criteria capable of objective measurement such as the survival time suitably specified. In addition to the statistical estimation of survival times from the first symptom and the first treatment, it is important to assess the detailed progress of the course of the disease in the individual patients and in particular to try to obtain an estimate of the effect of the different types of treatment; for this purpose a useful method of approach appears to be by means of the Performance Status defined by Karnofsky *et al.*⁴ Details of a clinical therapeutic trial of a radiosensitiser have been published, with special reference to the evaluation of tetra-sodium 2-methyl-1:4-naphthohydroquinone diphosphate as a radiosensitiser in the radiotherapy of inoperable cases of carcinoma of the bronchus.^{5,6,7} For types of malignant tumour with substantially longer survival, it would seem to be essential to devise methods of estimation of the results based on the mortality during the first few years after treatment.⁸

RADIOACTIVE ISOTOPES IN THERAPY

At present the largest use of radioactive isotopes is as γ -ray sources, and β -ray emitting isotopes have an accepted place in the treatment of a number of very superficial lesions.

Of more fundamental interest is the therapeutic use of radioactive isotopes administered internally but at present this application is limited to the use of a few elements in inorganic form. Usually only one isotope of the element is easily available and this has been used even though its radiations may not be ideal. The possibility of alternative isotopes of the same element with more suitable radiations must be considered despite difficulties of preparation, e.g., I^{124} instead of I^{131} for treatment of carcinoma of the thyroid to obtain substantially greater uniformity of dose,^{9,10} and probably P^{33} instead of P^{32} .

At present, for internally administered radioactive isotopes, dosage is largely empirical. It is essential to try to estimate in relation to the amount, usually millicuries of isotope administered to the patient, the relevant doses of radiation specified in rads, rep, or ergs per gram received by both the abnormal

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cells to be treated and the normal cells of other tissues inevitably irradiated, especially bone marrow, lymphoid tissue, reproductive organs and kidneys and also the reticulo-endothelial system, generally including the liver, spleen and bone marrow, in the case of intravenous administration of radioactive colloids. At present, it is rarely possible to make more than a crudely approximate evaluation of the mean tissue dose, at the best within $\pm 20\%$ and often with much greater error. Serious difficulty arises from inhomogeneous distribution. It is well known that the required information can be obtained in some cases by quantitative autoradiographic studies which are useful but time consuming. For example, in operation specimens of carcinoma of the thyroid, Phillips^{9,10} has shown that in some regions of tumours which concentrate radioiodine, the β -ray dose falls practically to zero, so that the minimum tumour dose depends upon the γ -rays, but the γ -ray dose can rarely, if ever, be large enough to be effective therapeutically with I^{131} which is the isotope commonly used. Progress is closely linked with the development of more sensitive methods of measurement. However, the decisions as to the treatment and management of the patient must be made on the basis of the clinical picture as a whole, taking into account the physical measurements and other laboratory findings in proper perspective.

An important unsolved problem which must be considered but not over-emphasised, is whether there is any real risk of late carcinogenesis or of leukaemogenesis. The apparently small risks associated with isotope therapy must be compared with those of other forms of treatment for the individual patient. In the radioiodine treatment of thyrotoxicosis, if it is found that the risk of carcinogenesis is less than 0.1% after 25 years, this method of treatment is safer than either surgery or anti-thyroid drugs for patients over a minimum age of 45. However, if the risk of late carcinogenesis should prove to be in the region of 2% or more after 25 years, then the use of radioiodine therapy is justified only in the relatively few patients over 45 in whom the risks of surgery and of the use of anti-thyroid drugs, including those associated with failure to respond, are large. Particular care is necessary in assessing the possible risks in those patients under the age of 45 who have to be treated with radioiodine when all other methods have failed.

No cases of carcinoma of the thyroid following radioiodine treatment of thyrotoxicosis appear to have been reported to date. This form of therapy has been used since 1942.^{11,12} However, although this finding is very satisfactory, it is suggested that a period of observation of 13 years is not sufficiently long in relation to the probable latent period of radiation carcinogenesis to be able to reach a reliable quantitative conclusion of the type required.

It is relevant to refer to the considerable number, probably several thousand, cases of thyrotoxicosis treated by means of X-ray therapy between 1920

and 1939. The dose received by the thyroid was often in the region of 2500 r but rarely more than 6000 r delivered in over-all times often of several months and sometimes of several years. Several cases of carcinoma of the pharynx and post-cricoid carcinoma have been encountered in these patients and some of them are probably attributable to the radiation, with a long latent period in the region of 25 to 30 years.^{13,14} The rather numerous malignant tumours of the skin observed in these patients are not so relevant because of the high skin doses often given with the soft radiations used some years ago. Although the doses of X-radiation delivered to the thyroid were considerably smaller than the mean doses delivered by radioiodine, hypothyroidism and myxoedema considered to be attributable to the radiotherapy occurred, e.g., in 5 out of 42 cases of thyrotoxicosis treated with X-rays in one series (see Martin¹⁵). Nevertheless, no cases of carcinoma of the thyroid attributable to X-ray therapy of thyrotoxicosis have been reported so far. Mention must be made of Kindler's case of carcinoma of the thyroid observed 23 years after X-ray treatment of bilateral tuberculous cervical glands, though it seems that there was no histological examination of the original enlarged lymph nodes. The normal thyroid gland has been included in the radiation fields in the radical radiotherapy of large numbers of cases of carcinoma of the larynx and pharynx and no attributable cases of carcinoma of the thyroid have been reported. From this evidence it is clear that the thyroid gland, both normal and in thyrotoxicosis is substantially less radiosensitive from the point of view of late carcinogenesis than the skin or the mucous membrane of the pharynx and upper oesophagus. However, the mean doses of radiation delivered to the thyroid gland in the radioiodine therapy of thyrotoxicosis are usually at least 3 to 4 times those delivered in the X-ray therapy of this condition, in very much longer over-all times, and at least 2 to 3 times those delivered to the normal thyroid gland in radical radiotherapy in comparable over-all times. Also there is the inhomogeneous distribution of the radioiodine. It is relevant to mention here the experiments of Goldberg and Chaikoff¹⁶ in which 7 out of 25 rats that had received a single intra-peritoneal injection of 400 microcuries of I^{131} developed carcinoma of the thyroid between 1½ and 2 years afterwards; in 5 of these 7 animals with tumours, metastases were found. Assuming an uptake of one third in the rat thyroid of weight about 24 mg, the mean dose of radiation was about 600,000 rep. This mean dose is, of course, much higher than that used in the treatment of thyrotoxicosis but only by a factor of between about 30 and 100. These considerations confirm the continued need for a cautious approach to radioiodine therapy of thyrotoxicosis.

In connection with the long latent period of radiation carcinogenesis, it is of interest to refer to an extreme example. A case of squamous-celled carcinoma of the skin of the upper part of anterior ab-

dominal wall and adjacent area over the costal margin, arising 49 years after a single diagnostic X-ray exposure in 1898, has been described.¹⁷

In the radiophosphorus therapy of polycythaemia vera the possible small risk of inducing acute leukaemia (Stroebel, Hall and Pease¹⁸; but see Lawrence¹⁹ and Lawrence, Berlin and Huff²⁰) appears to be offset amply by the reduced incidence of haemorrhage and thrombosis and the associated substantial increase in period of survival.

It is suggested that the risk of genetic injury is not always negligible in radioactive isotope therapy as will be evident from the consideration that intravenous administration of 6 millicuries of P³² delivers a dose in the region of 100 rep to the testis or ovary.

The value, and limitations, of many of the uses of radioactive isotopes by internal administration are becoming established. There are, however, many practical problems especially in connection with the selection of patients and techniques of treatment which are best illustrated for the present purpose by one's own clinical experience (c.f., Mitchell,²¹ and reference 7 for the more physical aspects).

RADIOIODINE (¹³¹I) TREATMENT OF THYROTOXICOSIS

On account of concern about the possibility of late malignant changes, radio-iodine therapy has been limited to those patients with thyrotoxicosis in whom (a) symptoms have recurred after partial thyroidectomy and also have often failed to respond to anti-thyroid drugs, or occasionally to X-ray therapy, (b) there has been failure of response or development of toxic symptoms or intolerance following the use of anti-thyroid drugs, mainly methyl thiouracil, or (c) surgery was contra-indicated by, e.g., a previously treated malignant tumour, especially carcinoma of the breast, previous coronary thrombosis, anxiety states, and threatened mental breakdown, or by exophthalmic ophthalmoplegia. The more gradual endocrine disturbance produced by radioiodine therapy may be associated with less risk of progressive exophthalmos. Radioiodine therapy has been employed in a few cases with a very small thyroid which the surgeon felt unable to deal with.

An attempt has been made in each patient to estimate the mean dose of radiation delivered by the radioiodine, mainly in order to collect information. The disease is so variable in its natural history and still so little understood that the approach has been largely empirical, though influenced by previous experience of X-ray therapy. Fractionated doses have been used especially when there is no urgency and also when it is particularly important to avoid myxoedema. However, it is often difficult to reach a satisfactory end result by the fractionated method. Single doses have been used more and more. The single-dose method is particularly valuable in cases of thyrotoxicosis with congestive cardiac failure where urgent treatment is required and where any risk of myxoedema is not a serious matter. One

problem is that after administration of radioiodine the level of thyroid function as it is settling down appears in some cases to oscillate slowly over periods of months. Sometimes there is a temporary state of hypothyroidism and then the patient spontaneously recovers in the course of perhaps 18 months to an almost normal state of thyroid function no longer requiring administration of thyroid extract. There is a real need for an early test of thyroid function during the period of radioiodine treatment; it is suggested that I¹³² may be used for this purpose. Cases in which symptoms have recurred after the use of anti-thyroid drugs often seem particularly difficult to treat and for such cases the fractionated method is usually preferable. In this connection, it is worthwhile mentioning the difficulty of persistent increase in thyroid uptake of radioiodine in some patients treated with anti-thyroid drugs. In such cases the routine measurements of protein-bound iodine in the plasma were usually helpful. In deciding when to stop in fractionated radioiodine treatment, one has to be guided by the clinical findings primarily and sometimes it seems that the repeated radioiodine tracer studies can be misleading. In patients with troublesome exophthalmos, it is important to obtain residual hyperthyroidism after treatment or to keep the patient hyperthyroid by means of thyroid medication if there is hypothyroidism. However, a number of patients with residual mild hypothyroidism after treatment keep very well if given a small amount of thyroid, e.g., 1 grain (65 mg) daily by mouth for the rest of their lives. Residual hypothyroidism must be treated adequately with thyroid. A very small proportion of cases seem never to respond to radioiodine therapy.

In considering the dosage of I¹³¹, in most cases the maximum uptake into the thyroid gland and the rate of decay in the thyroid gland, usually expressed in terms of the total half-life have been estimated both for the tracer and the therapeutic doses. In 38 such pairs of tests, the values of the maximum uptake agreed within 10% in 31 cases and within 20% in 37 cases but the values of the total half-life agreed only within 10% in 16 cases, within 20% in 19 cases and within 33⅓% of the smaller value in 29 cases. Since in half the cases the values of the total half-life differ by more than 20%, it is important to make measurements for the therapeutic dose in each case and not rely on the tracer dose, although this is necessary to determine the approximate amount of radioiodine in millicuries to be given. In 3 of these cases the decay curves could not be represented by a single exponential; the sum of two exponentials has been tried but for dose determination the irregular curves obtained were integrated graphically.

Measurement of the thyroid weight is one of the most difficult problems in this form of therapy. In practice, one can estimate the thyroid weight approximately by means of palpation but this method is very crude and liable to large errors. Among

others, we have made studies to try to measure the thyroid weight objectively by deduction from iso-count contours projected on to two planes at right angles, each at 45 degrees to the medium plane (L. Burkinshaw, unpublished). Even when measured accurately, the significance of the thyroid weight is at least sometimes difficult to interpret.

In 44 cases of thyrotoxicosis treated by radio-iodine, satisfactory results were obtained in 11 of 13 cases in which the mean thyroid dose was in the region of 8000 to 9300 rep and in 12 of 31 cases in which the mean dose was either below 8000 rep (3500 to 7700 rep), or 10,000 rep and higher, mainly 10,000 to 14,000 rep but in one case as high as 33,000 rep. This result is numerically statistically significant ($\chi^2 = 6.00$ for 1 d f, so that $P = 0.014$). However, it must be pointed out that the cases in the different dose groups are not strictly comparable. The smaller radiation doses have been used in some of the younger patients and the larger doses in severe and resistant cases, in patients with congestive cardiac failure, and in a few patients with a large toxic nodular goitre where surgery was contra-indicated or pre-operatively as in one case. With single-dose treatment good results were obtained in 9 of 11 cases with mean doses of 8000 to 9300 rep and in 12 of 16 cases with doses of 8000 to 14,000 rep. With total mean doses lower than 8000 rep, the probability of cure seems to be rather lower, e.g., satisfactory results were observed in 6 out of 17 cases treated with mean doses of 3500 to 7700 rep. Myxoedema is uncommon after doses below 9000 rep but has been observed after 6000 rep. In the 44 cases reported, moderate residual hypothyroidism occurred in 7 and myxoedema in 2. This limited experience suggests that in many but not all cases a good result can be obtained by a single treatment with radioiodine I^{131} which delivers a mean radiation dose of 8000 to 9000 rep to the thyroid. However, an attempt has been made to plan the method of radio-iodine treatment for each patient with thyrotoxicosis, mainly on the basis of the clinical picture.

On the whole the best results appear to have been obtained with patients unsuitable for surgery, who have received no previous treatment and in whom the thyroid gland shows a moderate degree of diffuse enlargement without nodularity or tracheal compression. In general, any clinically detectable nodularity is an indication for surgery, but it should be noted that some patients develop a transient nodularity about 3 weeks after the administration of the radio-iodine sometimes lasting for a further six weeks but ultimately clearing up completely. Contra-indications for radioiodine therapy include primary thyrotoxicosis in children and adolescents, pregnancy, non-toxic goitre and uncertain diagnosis of thyrotoxicosis.

In connection with the problem of extra-thyroidal concentration of radioiodine, the pituitary of a woman patient with severe thyrotoxicosis recurrent after partial thyroidectomy and difficultly controlled

diabetes, who died of a pulmonary embolus 23 hours after administration of 2 millicuries of I^{131} was examined by counting and by autoradiography. The mean differential absorption ratio for radio-iodine in the pituitary was 8, and high local concentrations of organically bound radioiodine were found with a differential absorption ratio in the region of 300. The greater part of the radioactivity was concentrated in small groups of cells in close relation to the capsule of the posterior lobe and in the central cleft. In another case, autoradiographs of sections of the wall of the carotid artery showed foci of I^{131} activity in groups of cells in relation to the adventitial coat (Phillips,¹⁰ and unpublished).

CARCINOMA OF THE THYROID

There are many problems in connection with the use of radio-iodine in the treatment of carcinoma of the thyroid. These difficulties are largely associated with the wide variations in extent of the growth, general condition of the patient, and radiosensitivity of the tumour and especially with the present lack of knowledge about many aspects of the concentration behaviour and inhomogeneity of distribution of the radio-iodine in both the primary tumours and the metastases in different patients. Methods of induction of uptake of radio-iodine in tumours showing no initial absorption have been widely studied. The most important of these is ablation of normally functioning thyroid tissue by means of surgery or radio-iodine. The use of anti-thyroid drugs is well recognised. Testosterone has been used successfully. Thyroid stimulating hormone (TSH) does not appear to be as valuable as seemed earlier because although it increases radioiodine uptake into some lesions of carcinoma of the thyroid, it appears usually to increase the turnover rate so that there is then no substantial increase in radiation dose delivered. Histology, while essential, does not appear to be as valuable a guide to treatment as has been generally considered. For example, I have observed two cases of carcinoma of the thyroid with anaplastic structure in good specimens examined histologically which responded usefully to radioiodine treatment; in one of these patients with recurrences after thyroidectomy despite no detectable uptake, retrogression of pulmonary metastases was observed following radio-iodine treatment.

Although surgery and conventional radiotherapy especially γ -ray teletherapy are still the most important lines of treatment, at present almost every case of carcinoma of the thyroid is investigated from the point of view of possible radioiodine therapy, which appears to be useful in about one-third of the cases seen. Radical surgery should be carried out wherever possible and almost invariably followed by radical post-operative conventional radiotherapy. For this purpose γ -ray teletherapy, which at Cambridge is carried out with the Ir^{192} unit, has been found to be useful especially because of the reduction of un-

wanted radiation absorption in bone. Even if radical surgery cannot be carried out, palliative surgery is particularly valuable to remove as much normal and malignant thyroid tissue as possible, including any technically operable metastases. If possible a satisfactory biopsy specimen should always be obtained. Conventional radiotherapy is essential if after surgery there is localised residual tumour with no evidence of radioiodine uptake and especially if the histology shows an undifferentiated carcinoma or an unusual tumour of the thyroid such as squamous carcinoma. When the general condition of the patient has been poor, palliative X-ray therapy has often been started without delay and may be of great value especially in producing relief of pressure symptoms and relief of pain from metastases in bones. However, in some of these advanced cases, empirical palliative radioiodine therapy is useful and does not seem to be contra-indicated by tracheal compression.

In one case of a well-differentiated acinar large cubical celled carcinoma of the thyroid with extensive regional lymph node metastases in a man of 26, the condition appears to have been finally rendered operable by radioiodine therapy. In cases with mixed differentiated and anaplastic histology, an attempt has been made to combine therapy with external radiation with radioiodine.

Mapping of the whole body for localisation of areas of radioactivity must be carried out repeatedly in the course of radioiodine treatment. In addition, systematic studies of autoradiography of surgical specimens have been made especially from the point of view of trying to get some information about dosimetry in radio-iodine therapy of carcinoma of the thyroid.^{9,10} It is emphasised that radioiodine therapy is not necessarily excluded in any particular patient by the finding of absence of uptake before thyroid ablation. As a rough empirical rule, an amount of I^{131} has been given 2 to 3 days before operation such that the radioactivity at operation in any one mass of tumour of radius r cm should be $10\ r^2$ in microcuries for reasonable ease of measurement by means of autoradiography; usually there has been a considerable margin of safety in the radiation received by the surgeon's fingers. Autoradiography with photometry and standardisation makes it possible to measure approximately both the β - and γ -ray doses at different points throughout the surgical specimen. Counting measurements on the operation specimens give values of the average specific activity and mean β -ray dose. Comparison of the γ -ray dose measured by means of autoradiography and that calculated for uniformly radioactive spheres shows that it is satisfactory to rely on the calculated values of the γ -ray dose. The distribution of radio-iodine almost invariably is inhomogeneous, resulting in negligible or zero β -ray dose with I^{131} in some regions in iodine-concentrating tumours. It never seems to have been possible to obtain a sufficiently high γ -ray dose to be therapeutically effective with I^{131} . The reality of areas of inadequate

dosage in carcinoma of the thyroid treated with I^{131} has been confirmed by the finding of groups of apparently viable tumour cells embedded in fibrous tissue in an operation specimen at 20 months after the last radio-iodine treatment; these surviving cells did not show radioiodine uptake in the microscopical autoradiographs. This type of evidence raises the question as to whether radioiodine therapy with I^{131} can ever be genuinely curative, although it may produce very long-term freedom from disease, and it is not unlikely that many of the groups of surviving carcinoma cells imprisoned in fibrous tissue will remain dormant for the rest of the patient's life.

The ablation of thyroid tissue by radio-iodine in cases of carcinoma of the thyroid has been carried out in general by 50 to 75 millicuries of I^{131} , usually given in 25 millicurie fractions on alternate days or weekly if there is any possible risk of increased thyrotoxicosis.

These amounts have been rather larger than those used in cardiac cases where 35 to 40 millicuries have been given, usually in fractions. In some cases of carcinoma of the thyroid recurrent after partial thyroidectomy, destruction of the thyroid remnant is accomplished by smaller amounts such as 25 millicuries.

The treatment with radioiodine is essentially empirical. After removal or destruction of normal thyroid tissue, radioiodine treatment has been carried out mainly by one of two techniques, either (a) the repeated massive dose method by administration of unit amounts of 100 to 150 millicuries of I^{131} every 6 to 8 weeks with increasing intervals of up to 6 months as the tumour uptake is reduced, or (b) courses of 25 millicuries weekly for 4 to 8 weeks with intervals of 8 to 12 weeks, and longer. Probably the massive dose method is the better but the advantage does not appear to be as clear-cut as was thought previously. Whichever method is used, the radioiodine treatment is continued until there is no detectable uptake into the region of tumour under consideration and then an attempt has been made to continue further by an additional total amount of I^{131} , if possible of 50%, in order to destroy the residual carcinoma. The limiting factor is the tolerance of the bone marrow and lymphoid tissue. Although measurements of the total and protein-bound radioiodine of the plasma are made, treatment has been controlled by serial sternal-marrow examinations in addition to blood counts, including platelet counts and especially taking note of the absolute lymphocyte-count.

It is often considered that the risk of renal damage is negligible but it is to be noted that albuminuria has been observed after single doses of 100 millicuries of I^{131} (my attention was drawn to this by Dr. J. Efskind of Oslo). With multiple metastases, the property of concentration of radioiodine may appear in only one lesion after ablation of the thyroid and then

later in a second metastatic lesion after treatment of the first, and so on.

RADIOPHOSPHORUS, P^{32}

It is now general experience that the one disease in which P^{32} treatment has given better results than any other form of treatment, and is the treatment of choice in most cases, is polycythaemia vera. In this disease, P^{32} treatment has reduced substantially the frequency of deaths from hæmorrhage and thrombosis, with associated prolongation of life.²⁰ Remissions as long as 5 years have been observed following single doses of P^{32} . A clinical observation is that in cases of polycythaemia vera, usually it becomes rapidly obvious when further P^{32} treatment is required. Most cases respond extremely satisfactorily to P^{32} treatment and the development of radioresistance after repeated doses of P^{32} does not appear to be a serious problem. There are occasional cases in which the diagnosis seems to be established but which do not respond to P^{32} ; some of these rare radioresistant cases respond usefully to nitrogen-mustard therapy. Some cases of Gaisböck's syndrome do not respond well to P^{32} treatment. In addition to the usual laboratory studies, paraffin sections of the sternal marrow appear to be of value in the diagnosis of a few cases of polycythaemia vera and in following the relation between the hæmopoietic marrow and the fatty marrow throughout P^{32} treatment.²² Although in the majority of cases there is no difficulty, the need for certainty of diagnosis before treatment with P^{32} must be emphasised.

The scope of radiophosphorus in the treatment of other diseases is limited. In chronic myeloid leukaemia useful control can be obtained but in general there has been no evidence that more satisfactory results are obtained than with X-ray therapy. In a few selected cases of chronic lymphatic leukaemia with moderate enlargement of a number of groups of lymphatic nodes where it is essential to treat to relieve symptoms, radiophosphorus has been found to have a useful place. In a few cases of myelomatosis an unexpectedly good response appears to have been obtained with P^{32} .

In patients with lymphosarcoma, reticulum cell sarcoma, mycosis fungoides, and various types of generalised malignant disease, an attempt has been made to measure the differential absorption ratio in biopsy and operation specimens taken usually at 24 hours after administration of P^{32} as a tracer dose of $\frac{1}{4}$ –1 millicurie or as a therapeutic dose in the usual range of 2 to 10 millicuries. Autoradiography has been attempted but did not often add substantially to information otherwise available.

A value of the differential absorption ratio of about 5 is sometimes associated with a clinical response to therapeutic doses of P^{32} in highly radiosensitive lesions, e.g., some cases of mycosis fungoides and of reticulum cell sarcoma. Further work is required on the quantitative relation between the biological effects

of internal radiation from radioactive isotopes and external radiation. It is clear that there is no great difference between the biological effects of equal absorbed doses in the two cases but there is often a clinical impression of slightly greater effect with P^{32} than expected. However, no substantial error is introduced by the assumption of a value of unity for the relative biological efficiency and it must be emphasized that this assumption has been of great value in the development of radioactive isotope therapy.

In most cases of lymphosarcoma, reticulum cell sarcoma, Hodgkin's disease and mycosis fungoides, and of advanced malignant disease, it is not possible to give a therapeutically useful dose of radiation by internal administration of P^{32} . In general, radiophosphorus has no place in the palliative treatment of patients with advanced and generalised malignant disease.

Mention may be made of the occasional palliative value of the injection of 10 to 15 millicuries of P^{32} into cysts in inoperable cerebral tumours where aspiration is necessary and no other form of treatment has been successful.

RADIOACTIVE COLLOIDAL GOLD, Au^{198}

The application of radioactive colloidal gold by introduction into the peritoneal and pleural cavities to inhibit the accumulation of effusions due to secondary carcinomatosis is now generally accepted as a palliative radiotherapeutic measure. My experience of this method has not given as good results as some reported in the literature, especially for intra-pleural injection but in some cases there has been great improvement. The dosimetry has been discussed by Mitchell.⁷ Twenty-eight patients with peritoneal carcinomatosis were treated with unit amounts mainly of 100 to 125 millicuries but ranging from 25 millicuries to 150 millicuries, with repetition up to 3 times. Six showed real improvement. Two of these were out of 19 cases of carcinoma of the ovary and survived 13 months and more than 14 months after the first injection; both had an unusually severe reaction with abdominal pain for several days after the radiogold injection. One case of carcinoma of the colon with peritoneal carcinomatosis improved dramatically and lived, though invalided for 12 months after the first injection; she had three injections of 105, 83 and 35 millicuries. Of cases of carcinoma of the breast with peritoneal carcinomatosis, 2 out of 4 improved usefully. There was also dramatic improvement in an unusual case of generalised hæmangiomatosis with survival of more than 2 years and 7 months after the first intra-peritoneal injection of radioactive colloidal gold. For intra-pleural injection, mainly of 50 millicuries, there was moderate improvement only in 2 out of 8 cases; those were both from carcinoma of the breast in a very advanced stage of pleural carcinomatosis.

A special study has been made of interstitial infiltration of radioactive colloidal gold²³ in and around

inoperable and usually otherwise untreatable malignant tumours to effect what may be termed as "injection implant" (see Mitchell^{21,7}). It is useful to remember that one millicurie of Au^{198} destroyed per gram of tissue delivers a dose of 70,600 rep in *mean* time 3.9 days. It is valuable to measure the biological decay in the area of the injection and to obtain information about redistribution and leakage from the injection sites and accumulation of radioactivity in the regional lymph nodes and in the reticulo-endothelial system, especially in the liver, spleen and bone marrow. In 27 of these extremely advanced cases, useful palliation has been obtained in 11 cases. One of the most striking cases was a lady of 56 with a profusely bleeding fungating fibrosarcoma of the upper part of the right shoulder recurrent with bone necrosis after partial excision and much X-ray therapy. She refused a fore-quarter amputation because she had a useful hand and forearm. Infiltration of various areas of recurrent tumour with radioactive colloidal gold on 8 occasions between 16.8.51 and 11.3.54 to give calculated β -ray doses between 26,000 and 70,000 rep has rendered the tumour quiescent with almost complete healing. In recurrent carcinoma of the mouth and tongue when no further surgery or radiotherapy was possible, palliation was obtained in 3 out of 10 cases. In very advanced carcinoma of the breast improvement was obtained in 2 out of 7 cases. Scar recurrences, e.g., of carcinoma of the bladder after surgery and radiotherapy can sometimes be treated usefully.

With "injection implants" of this type it is usually necessary to deliver a β -ray dose of 30,000 to 60,000 rep in a tumour of volume of up to 50 ml. For small volumes such as those encountered in the case of otherwise untreatable recurrent secondary carcinoma in lymph nodes, e.g., after block dissection and radical radiotherapy, doses of 70,000 to 100,000 rep can be given. With higher doses there may be cystic degeneration. In two cases of inoperable squamous carcinoma of the bronchus, while the chest was open at thoracotomy, the primary tumour of diameter 6–7 cm was infiltrated to a dose of about 18,000 rep and the inoperable lymph nodes and tumour adherent to the great vessels to a dose of about 100,000 rep. Models were constructed and measurements were made externally and with a Sievert chamber in a bronchoscope. The estimated dose to the liver was 300 rep and 1100 rep in the two cases which are tolerable levels. The corresponding total amounts injected were about 85 and 107 millicuries. The patients survived respectively 6 months and 13 months so that no certain benefit was obtained.

The "injection implant" raises anew the questions of optimal dose and tissue tolerance in radiotherapy. In some cases a useful therapeutic response can be obtained by means of an injection implant in tumours which in the ordinary way are regarded as radio-incurable or radioresistant. Another example of this was a case of adenocarcinoma probably arising in the

sebaceous glands of the scalp which had recurred after surgery and shown no response to radical X-ray therapy but showed a good palliative response with almost complete retrogression after an injection implant to a *mean* β -ray dose of 100,000 rep. Autoradiography has demonstrated the gross inhomogeneity of distribution of the Au^{198} in most specimens and in some cases, including a case of carcinoma of the bronchus injected before pneumonectomy, it is clear that the radiogold has not penetrated into parts of the tumour, even though there are considerable areas of fairly uniform activity. In many cases, the γ -ray dose contribution is sufficient to produce some palliative effect, e.g., 8.5 millicuries of Au^{198} destroyed in 10 ml of infiltrated tumour delivers a *mean* β -ray dose of approximately 60,000 rep and the associated central γ -ray dose is about 2250 r. However, in some cases it seems that a useful therapeutic response has been obtained by means of Au^{198} in a tumour usually regarded as radioresistant by the delivery to a small volume of a β -ray dose in the region of 50,000 to 100,000 rep.

SOME EXPERIMENTAL STUDIES IN PROGRESS

Attempts have been made to develop organic compounds which are selectively concentrated in malignant tumours and carry incorporated radioactive atoms into the tumour cells. It is suggested that reduction of the required differential absorption ratio might be effected by the use of compounds which act as radiosensitisers. Laboratory studies and clinical trials of some chemical radiosensitisers including tetra-sodium 2-methyl-1:4-naphthohydroquinone diphosphate (Synkavit) have been summarised.⁵ Some radioactive derivatives of this and related compounds have been prepared. Experiments with rats with the Walker carcinoma have been described.^{24,25} In the case of the tetra-sodium salt of 2-methyl-3-bromo-1:4-naphthohydroquinone diphosphate incorporating Br^{82} , values of differential absorption ratio in the Walker carcinoma of 2–3 were found but surprisingly a large proportion of the bromine in this compound when administered by intravenous injection appears within one hour as bromide ion, perhaps as a result of interaction with —SH compounds. Studies including clinical investigations with Synkavit labelled with C^{14} in the methyl group are in progress. Of interest is 6-iodo-2-methyl-1:4-naphthohydroquinone diphosphate but so far it has not been possible to prepare the compound incorporating I^{131} in sufficiently high specific activity.

EXPERIENCE WITH A RADIOIRIDIUM γ -RAY THERAPY UNIT

Because of the difficulties in obtaining Co^{60} , a γ -ray therapy apparatus with radioiridium Ir^{192} was constructed. The first unit, containing about 20 curies and with lead shielding, was described by Freundlich, Haybittle and Quick²⁶ and Mitchell²¹ and started in routine use at the Radiotherapeutic Centre, Adden-

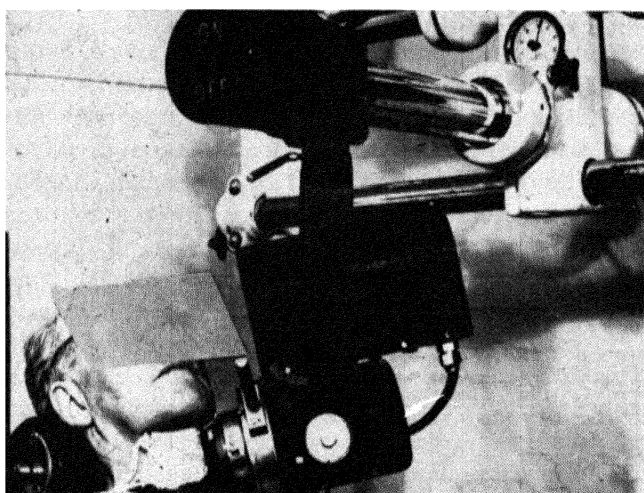


Figure 1. Radioiodium γ -ray therapy unit treating a case of carcinoma of the larynx

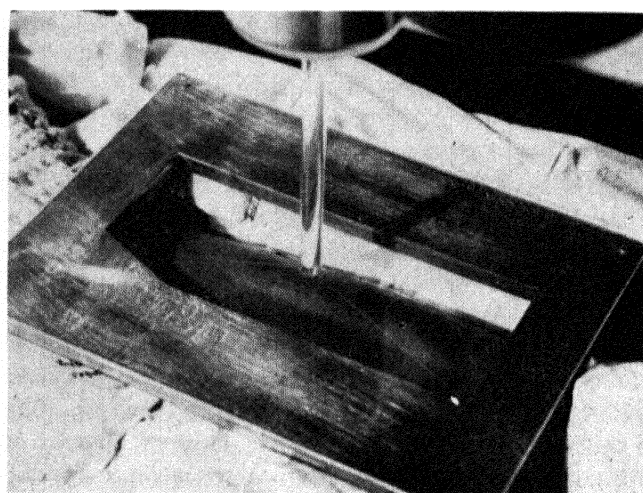


Figure 2. The use of the radioiodium γ -ray therapy unit in the treatment of a case of Ewing's tumour of the tibia

brooke's Hospital, Cambridge, in May 1950. This apparatus was succeeded by a unit of improved design containing about 75 to 80 curies of Ir^{192} with uranium metal shielding enabling the size to be reduced.^{27,7}

Figure 1 shows an example of the routine use of the radioiodium teletherapy unit in the treatment of a case of intrinsic carcinoma of the larynx. In all such cases individual applicators are made. A perspex former with a moulded black tray support is shown for the positioning of the two fields used for this particular patient. An example of a three-field arrangement for the treatment of intrinsic carcinoma of the larynx has been illustrated.²¹ The uranium metal head carries the applicator. The motor in the larger case above the head rotates the uranium cylinder containing the source and is controlled by the operator who watches through the water window seen at the left-hand edge of the photograph. In the Off position, the shielding is 4.5 cm of uranium in all directions, except toward the nozzle where it is 3.5 cm. When switched On, a motor driving through a Geneva mechanism rotates the uranium cylinder carrying the source to bring the source in line with the nozzle; the cylinder is locked firmly in the On or Off position. This apparatus is of the type now termed a short distance unit. The maximum dose rate is about 28 r per minute at source skin distance (SSD) 9 cm and 14 r per minute at 13 cm. Longer SSDs are often used at low dose rates. The *mean* energy of the primary radiation is approximately 0.38 Mev on the basis of half-value layer measurements. It is thus high enough to minimise excess energy absorption in bone. An important use for the unit has been the treatment of tumours overlying and involving bone as, for example, the radical radiotherapy of a Ewing's tumour of tibia (Fig. 2).

The disadvantage of the short half-life (74 days) is overcome by replacement of half the source for re-activation in the pile at Harwell every 4 weeks. The **exchange of sources** is carried out with safety by means of remote control from behind a mobile lead bench (Fig. 3).

The over-all dimensions of the source are 3-cm diameter by 3 cm long. The present apparatus has been investigated with special reference to the effect of source size upon the integral dose²⁸ and the physical requirements of beam-defining systems for medium distance teletherapy units.²⁹ Radiochemical measurements of the integral dose have been made by means of the ferrous sulphate dosimeter and suggest that the problem of penumbra has been over-

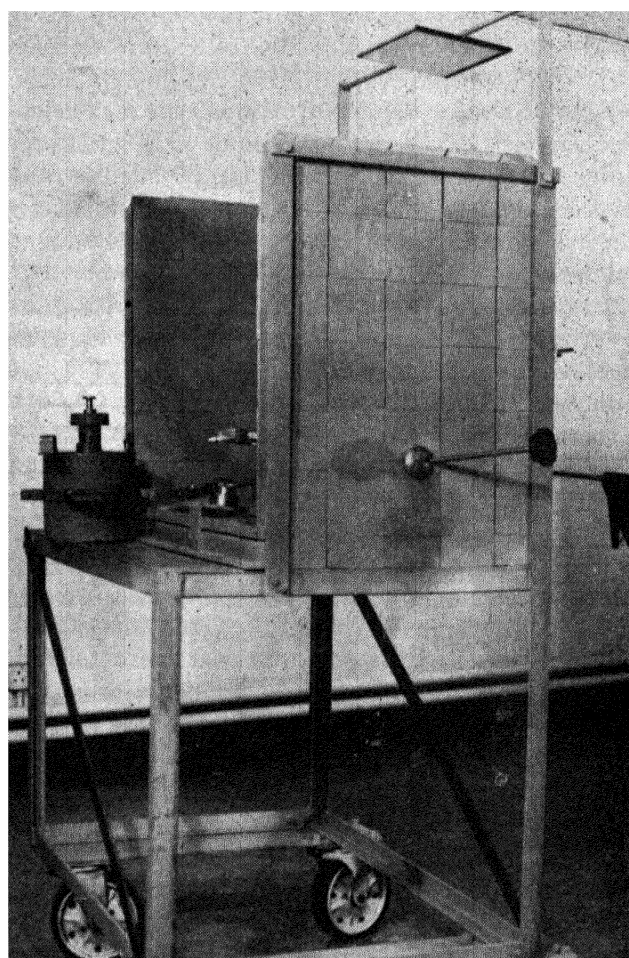


Figure 3. Mobile lead bench used in changing the sources in the radioiodium γ -ray therapy unit

emphasised. To reduce the integral dose the most effective method is to reduce the distance from the defining aperture to the skin. The source size in large γ -ray therapy units need not increase the integral dose delivered to the patient above the levels that have been tolerated in the past.

These quantitative physical studies made with the radio-iridium units and the clinical experience gained are of interest in relation to the proposed use of Cs^{137} . It is suggested, on the basis of these investigations and of 5 years' routine clinical use of our radio-iridium units, it seems reasonable to suggest that Cs^{137} is eminently suitable as a γ -ray source in therapy units with intermediate SSD of 30 to 40 cm and that there is every reason to expect that such units will come into routine use as standard apparatus in radiotherapy.

Between May 1950 and June 1955, 282 patients with various types of malignant tumours have been treated with the radioiridium X-ray therapy units at Cambridge. The numbers of patients with the different types of tumours may be summarised as follows:

Carcinoma of pharynx and extrinsic larynx	26
Carcinoma of larynx—intrinsic	17
Carcinoma of lower lip, usually extensive	15
Carcinoma of tongue	18
Carcinoma of other sites in the mouth, mainly floor	19
Malignant tumours of tonsil	6
Post cricoid carcinoma	5
Carcinoma of breast, mainly lesions at edge of breast in lower inner quadrant and recurrences over bone	14
Carcinoma of thyroid	6
Malignant tumours of bone—one Ewing's tumour of tibia and one chondrosarcoma of tibia	2
Carcinoma of penis	7
Carcinoma of skin basal and squamous cell types—in sites immediately over or involving bone or cartilage:	
Nose	40
Pinna of ear	26
Hand, mainly dorsum	15
Fingers	3
Inner canthus	3
Post-auricular region	6
Other areas	44
Miscellaneous tumours	10

It is concluded that from the point of view of practical radiotherapy, the radioiridium γ -ray therapy unit is proving to be equivalent to a standard radium beam unit. Long-term follow-up is essential before a final assessment can be made. In all this work, the biologically equivalent dose of radioiridium γ -rays has been 1.2 times the dose of the standard filtered 220 kvp X-radiation used under comparable conditions.

A point of considerable practical importance is that the use of the radio-iridium unit has replaced the use of radium moulds in the treatment of most cases of carcinoma of the pinna, nose, dorsum of hand and fingers, skin overlying bone, e.g., on the forehead and of carcinoma of the penis. Among the 17 cases of intrinsic carcinoma of the larynx treated, 2 have shown persistent oedema probably due to

localised cartilage necrosis and not associated with persistent or recurrent growth. This proportion is considerably lower than the frequency of this complication usually observed with conventional deep X-ray therapy, but long-term follow-up on larger numbers of patients is essential.

Ce^{144} BETA-RAY APPLICATOR

Artificial β -emitting isotopes have an accepted place in the treatment of superficial lesions especially benign conditions including selected cases of naevi in infants and various diseases of the eye.

A group of isotopes has been studied in which there is a long-lived parent which emits low energy β -particles but has a short-lived daughter product which emits the required high energy β -particles, usually together with weak γ -radiation which must be of negligible intensity. The use of Ce^{144} - Pr^{144} , proposed by Freundlich³⁰ has been developed into a practical clinical β -ray applicator by Haybittle.³¹ The use of the source in the treatment of a patient with mycosis fungoides is shown in Fig. 4. The source con-



Figure 4. The Ce^{144} beta-ray applicator in use in the treatment of a case of mycosis fungoides

tains 40 millicuries of Ce^{144} spread over a 3-cm diameter circle and will treat areas of up to 5-cm diameter at source-skin distance 3.5 cm, with surface dose rate about 37 r per minute. If necessary, larger areas may be treated; in particular we use a 7-cm diameter circular field at SSD 5 cm with dose rate about 18 r per minute. The half-value depth for SSD 3.4 cm is 3.2 mm of unit density tissue.

HIGH-ENERGY ROENTGEN-RAY THERAPY

The 30 Mev synchrotron provided by the Medical Research Council was installed in the Radiotherapeutic Centre, Addenbrooke's Hospital, Cambridge, in November 1949. Considerable effort has been needed to make the machine work with the degree of reliability necessary for its use in the treatment of patients. The intensity of roentgen radiation in the filtered

beam at FSD 1 metre has been as high as 12 to 15 "r" per minute and has much more usually been in the region of 3 to 5 "r" per minute which is inconveniently low. We have only been able to treat 25 patients between May 1951 and May 1955. Realising the limitations of the machine, we have concentrated on the radical treatment of a few patients with histologically verified malignant tumours occurring at sites for which radiotherapy by the usually available methods is unsatisfactory. It seemed reasonable to select patients with lesions in which the probability of permanent cure is low and difficulty of accessibility with conventional radiotherapy appears to be the most important factor limiting radio-curability. All the patients treated have been inoperable and almost invariably the lesion was advanced.

In the development of the 30 Mev synchrotron as a radiotherapeutic instrument it has been necessary to carry out detailed investigations of dosimetry and relevant physical measurements, together with biological experiments to obtain information about the relative biological efficiency of the filtered roentgen ray beam. A detailed account of our experience up to February 1953 has been published.³² This paper deals almost entirely with more recent studies.

As yet no reliable method of dosimetry for megavoltage roentgen radiation has been developed. Following Laughlin³³ experimental studies of calorimetric dosimetry have been started. Until it is possible to express the absorbed dose in terms of the rad, it is generally agreed that it is necessary as a provisional basis for dosimetry to refer to an experimental sub-standard which is easily reproducible and carefully defined. We have used a 100 Victoreen chamber placed with its axis transversely to the axis of the roentgen ray beam at the centre of a "cubical" block of perspex of measured density 1.19 (1) grams per cm³ and with edges of lengths 10.0, 10.0, and 10.5 cm, the last dimension being parallel to the long axis of the Victoreen chamber. The volume of the chamber determined radiographically was 0.405 cm³. The unit based on this experimental arrangement is called "r" in this paper. The isodose curves were measured with a small thin-walled perspex ionisation chamber of diameter 9 mm and effective collecting volume of 0.572 cm³. Readings were taken of the ionisation chamber current in a 30 cm cubic water phantom. Measurements near the surface were made by means of extrapolation chambers with front walls of polythene films and of aluminium.

The roentgen radiation from the synchrotron is produced in main pulses at the rate of 50 per second. Each main pulse is of duration about 100 microseconds and consists of 50,000 short pulses each of duration about 1×10^{-9} seconds due to bunching of the circulating electrons. At a mean dose rate of 15 "r" per minute the intensity in the main pulse is 50 r per second and in the constituent short pulses is about 100 r per second. Using the theory of Boag^{34,35} to calculate the collection efficiency, it was found that

for the 100 r Victoreen chamber and the probe and monitor chambers, the ionisation current readings were respectively 100, 99 and 98% of the theoretical values. These conclusions were supported by measurements of the saturation curves using radium sources and the synchrotron.

The depth dose distributions in the water phantom for the filtered roentgen ray beam from the synchrotron have been published.³² An example of the depth dose distribution for the filtered beam with the "5 cm diameter" circular field at FSD 100 cm for peak energy 30.0 ± 0.4 Mev is given in Fig. 5. The cen-

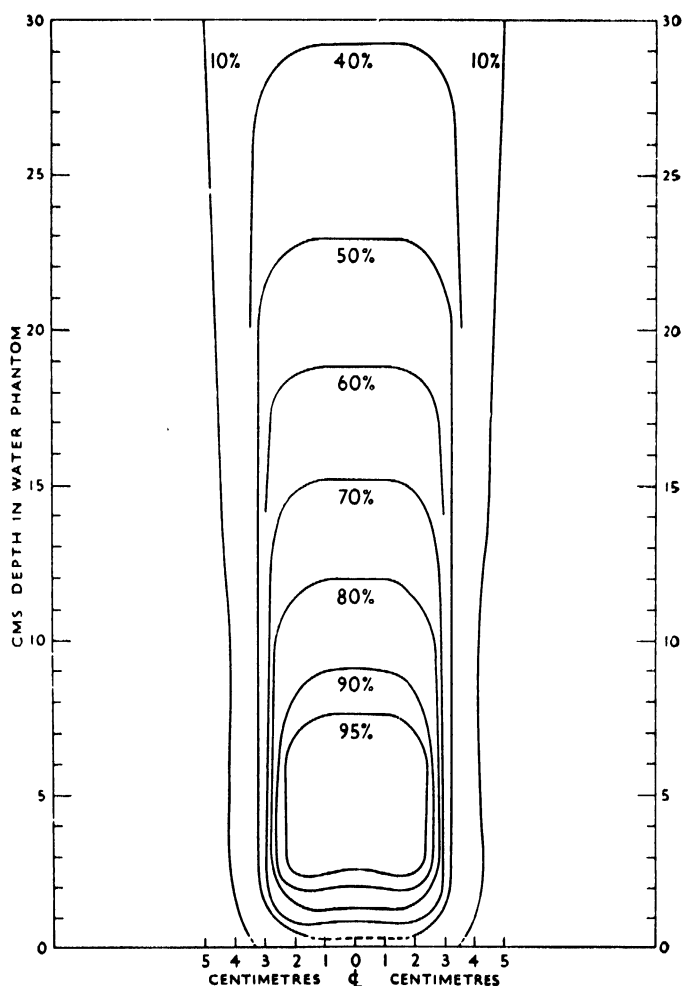


Figure 5. A typical depth dose distribution with filtered 30 Mev roentgen rays produced by the Cambridge synchrotron. "5 cm diameter" circle. Peak energy = 30 ± 0.4 Mev; "conical" copper filter in situ; FSD = 100 centimetres (readings 8.4.52; redrawn, 15.11.52)

tral axis depth dose curve for peak energy 33 Mev is shown in Fig. 6. The mass absorption coefficient in water corresponds to mono-energetic radiation of energy 7.6 Mev for maximum energy 30 Mev. It is of particular interest that for the filtered beam, the mass absorption coefficient has the same value within the accuracy of the measurements for water and for bone. Thus, taking into account the density of bone, this result makes it possible to correct the depth dose distribution for transmission through intervening bones as in the treatment of intra-cranial tumours.

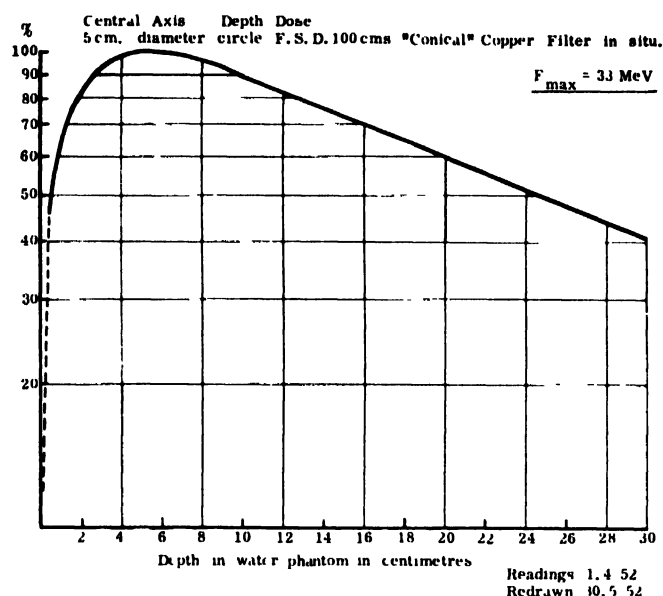


Figure 6 Example of central axis depth dose curve for filtered 33 MeV roentgen radiation produced by the synchrotron. Central axis 5 cm diameter circle Depth dose FSD 100 cm "Conical" copper filter in situ. $E_{max} = 33$ MeV

For the treatment of the patients the routine methods for accurate localisation of small volume irradiation with beam direction have been used. Plaster shells have been frequently made to fit on the head in the treatment of intra-cranial tumours or tumours in the region of the mouth and neck as in the treatment of the case of recurrent carcinoma of the tongue shown in Fig. 7, where, it is to be noted, the beam-directing device is removed before treatment. Paraffin wax blocks are often required to adjust the depth dose contours to the lesion. In most cases radiographic checking of the field positions is carried out at lower voltages and entrance and exit points are marked on the applicators.

THE PROBLEM OF RELATIVE BIOLOGICAL EFFICIENCY

The best value of the relative biological efficiency for the filtered 30 MeV roentgen radiation in relation to the usual filtered 220 kV roentgen radiation has been found to be 0.85 on the basis of the immediate mucosal and tumour reactions. This value corre-

sponds to a reciprocal value for the practical dose ratio of 1.2. Thus, if the minimum tumour dose taken as prescribed at the 95% isodose contour is used, this must be 1.2 times the dose used under comparable conditions of fractionation with 200–250 kV filtered roentgen radiation. The difference in dose distribution with the 30 MeV roentgen radiation of the synchrotron and the usual filtered 200–250 kV roentgen radiation makes it particularly important to specify carefully the minimum and maximum tumour doses.

All our clinical experience agrees with the value of approximately 1.2 for the practical dose ratio. This factor is certainly not as high as the value of 1.4 which was originally thought to be likely.

The intensity of the roentgen ray beam from the Cambridge synchrotron has hitherto been too low for experiments on animal tumours. Meanwhile, chromosome cytological studies have been made by Dr. K. C. Bora using microspores of *Tradescantia bracteata* to obtain information concerning the relative biological efficiencies of filtered 220 kV roentgen rays of IIVL 2.55 and 3.66 mm copper, Ir^{192} gamma rays of mean energy 0.38 MeV and of IIVL 8.1 mm copper and the filtered 30 MeV roentgen rays produced by the synchrotron. The shoots of *Tradescantia bracteata* were irradiated in water in a perspex tank or chamber at $24 \pm 1^\circ\text{C}$. The measured mean dose rate at the position of the shoots was the same for each of the three radiations in each experiment. Chromosome aberrations have been studied at several dosage levels, 150–300, 100–600 and 500–1000 r and "r". At lower dosage levels equal doses of 30 MeV roentgen rays and of the 0.38 MeV gamma rays produce equal frequencies of chromosome aberrations but at high dose levels, 600–1000 r, the same dose of the former radiation produces consistently lower frequencies of all types of aberrations. For producing equal biological effects of the types examined, the required doses of these two radiations are greater than that of 220 kV roentgen rays by a factor of about 1.4–1.45 at lower dose levels, but at high dose levels this factor is about 1.15–1.20 for 0.38 MeV gamma rays and 1.2–1.3 for filtered 30 MeV roentgen rays³⁶

APPLICATIONS IN TREATMENT

The main advantages of the use of filtered 30 MeV roentgen rays in radiotherapy appear to be:

1. The possibility of radical radiotherapy of inoperable malignant tumours which are otherwise untreatable or difficult to treat by other radiotherapeutic techniques. It is useful to comment that with the 30 MeV the 90% isodose surface intersects the axis at depths 2.0 and 9.5 cm and the 95% surface at 2.8 and 8.2 cm. The reduction of energy absorption in bone is useful from the point of view of increasing the accessibility to the radiation of some tumours, e.g., of the middle ear. The low surface dose makes it possible to carry out radical treatment of recurrent tumours through previously heavily irradiated skin, as in recurrent carcinoma of the parotid gland.

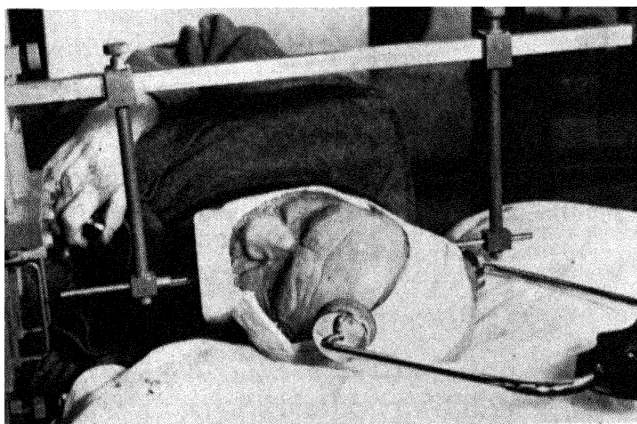


Figure 7. Beam direction method for treatment of a patient with recurrent carcinoma of the tongue by means of filtered 30 MeV roentgen-rays produced by the synchrotron

2. Substantial reduction of the integral dose per unit tumour dose and hence reduction of radiation sickness and general effects. For example, in a case of an inoperable cerebral tumour (glioblastoma multiforme) of the right temporo-frontal region, with radical 30 Mevp roentgen ray therapy with a single 7.5-cm diameter field at FSD 100 cm, the integral dose was 970 gm "r" per minimum tumour, "r" taken as 90% (Fig. 8). For treatment of this patient with 220 kvp roentgen rays of HVL 1.5 mm copper and FSD 50 cm, three fields would be required, right and left lateral fields 10×10 cm and an anterior field 7×10 cm. With this arrangement, assuming all fields receive 100% applied dose, the integral dose per minimum tumour r at 120% would be 2720 gm r.

3. The possibility of simplification of techniques of treatment including the use of single field methods.

A real disadvantage of 30 Mevp roentgen rays is that clinical control of the treatment is in general very difficult when the maximum tissue reactions occur in the depths and bear no obvious relation to the skin or mucosal reactions. It is necessary to know the dose contribution at the sites of the visible reactions and try to extrapolate from these. Moreover, even though the immediate reactions are visible there is as yet no substantial body of clinical evidence to relate the late reactions and behaviour of the normal tissues and tumours to these.

Single field techniques are useful. With single fields, it appears to be undesirable to use peak energy

of the roentgen rays of less than 25 Mev, and 30–35 Mev is slightly preferable. For techniques with two opposing coaxial fields, there is little advantage in using energies higher than 25 Mev and only slight improvement above about 15 Mev. The advantages of the high energy radiation are reduced with increasing numbers of fields. There would appear to be no advantage of 30 Mev rotation therapy over rotation therapy with 2 to 4 Mev roentgen rays or with Co^{60} γ -rays.

CLINICAL INDICATIONS FOR 30 MEVP ROENTGEN RAY THERAPY

It is now becoming possible to form a picture of the conditions for which radiotherapy with 30 Mevp roentgen rays is of value. The indications for radical treatment include the following inoperable tumours:

1. Cerebral tumours, including glioblastoma multiforme (Fig. 8).
2. Other intra-cranial tumours, including chordoma arising from the body of the sphenoid. The distribution of radiation in a case of this condition treated in May 1951 is shown in Fig. 9.
3. Tumours of the middle ear, e.g., glomus jugulare tumour.
4. Carcinoma of the bladder.
5. Tumours of the parotid gland, especially recurrent after previous radiotherapy.

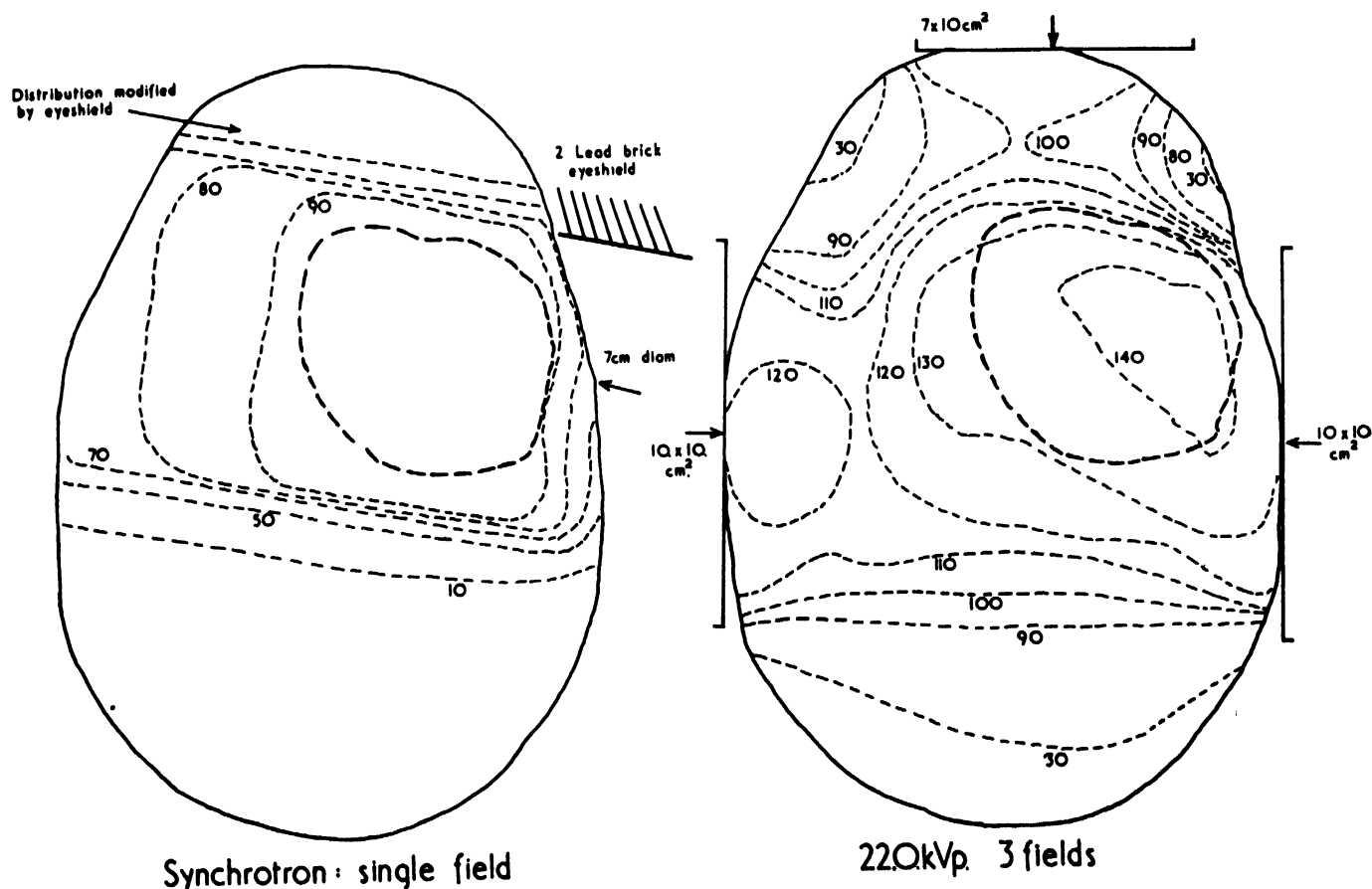


Figure 8. Comparison of radiation distribution in treatment of inoperable glioblastoma multiforme by 30 Mevp roentgen-rays with distribution which would be used with filtered 220 kvp roentgen-rays

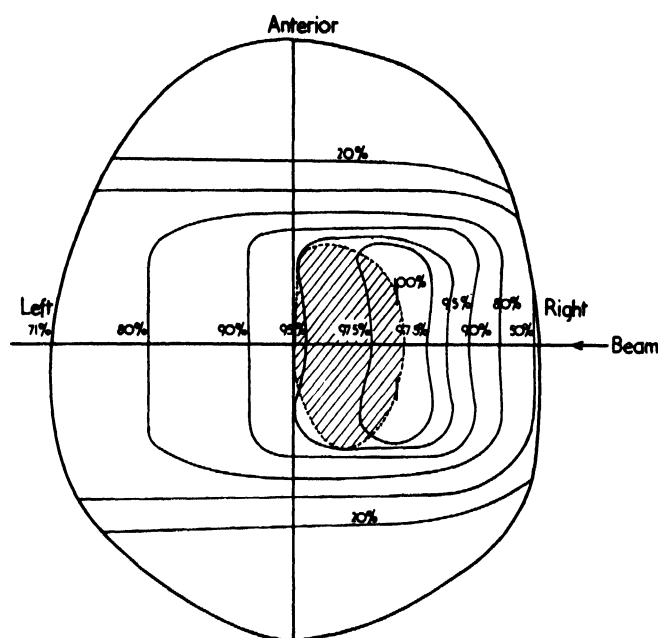


Figure 9. Distribution of 30 Mevp roentgen radiation used in the treatment of a case of chordoma of the sphenoid

6. Carcinoma of the mouth, especially tongue and floor of mouth, and palate.

7. Tumours of the nasopharynx, especially with involvement of the base of the skull.

8. Carcinoma of the ethmoid and of the nasal fossa.

9. Carcinoma of the posterior pharyngeal wall.

10. Post cricoid carcinoma.

11. Carcinoma of the trachea; an unexpectedly good result has been obtained in one case.

12. Carcinoma of the bronchus—cases which are inoperable on the basis of proximity to the carina but are otherwise still localised. This form of treatment does not overcome the problem of formation of metastases but may minimise local vascular and pulmonary fibrosis.

13. Some cases of carcinoma of the uterine cervix, especially in Stage III with isolated metastases on the pelvic wall in conjunction with radium treatment.

There has been an indication for palliative treatment with 30 Mevp roentgen rays in a case of inoperable carcinoma of the rectum. Post-operative radiotherapy with this high energy radiation has been considered in some cases of carcinoma of the bronchus after pneumonectomy to irradiate the bronchial stump and in a few cases of carcinoma of the breast to irradiate the region of the apex of the axilla in unusually large patients.

Much further work is required. The present investigation must be regarded as the preliminary stage of a much more extensive clinical trial.

SOME OBSERVATIONS ON MECHANISMS OF ACTION

Far too little is known about the mechanism of the therapeutic action of ionising radiations to help constructively in improvement of the results of treatment.

A general aspect to which often insufficient attention has been paid is the importance of the stroma

response and its relation to direct effects of radiation on the tumour cells.

However, at the present time it is essential to ascertain whether or not inhibition of synthesis of desoxyribonucleic acid (DNA) is a fundamental process.

Although the methods of classical histology show no obvious changes shortly after irradiation, studies which I have carried out over a number of years with the use of ultra-violet photomicrography have shown changes in cellular nucleic acid metabolism in several types of cells within relatively short times after irradiation. In my early work on the accumulation of pentose nucleotides in cytoplasm after irradiation and inhibition of synthesis of desoxyribonucleic acid by irradiation,^{37,38,39,40} a substantial increase in cytoplasmic absorption at 2537 Å was found in a number of human malignant tumours at 80 minutes after irradiation. The magnitude of the change was consistent with the presence in the irradiated cytoplasm of ribonucleotides in local concentration often of the order of 3%. In further work^{41,42} experiments using ribonuclease in conjunction with quantitative ultra-violet photomicrography showed that the cytoplasmic nucleotides accumulating after irradiation were mainly ribopolynucleotides closely resembling and probably identical with ribonucleic acid. Extraction methods showed characteristic nucleotide absorption with a maximum at the wavelength of 2620 Å. It is important to note that the increase in ribonucleotide absorption occurs almost entirely after irradiation with doses less than the region of 750–1000 r; at higher doses usually there is no accumulation of ribonucleotide absorption and often a definite decrease, presumably due to interference with less radiosensitive processes in the disturbance of cellular nucleic acid metabolism (see L. D. Hamilton⁴³ and unpublished work). Subsequent experiments have detected increased cytoplasmic absorption in a biopsy of a human basal-celled carcinoma of the skin taken immediately after delivery of 40 r of gamma radiation in 43 minutes.

Since 1940 I have been carrying out experiments on the irradiation of tissue cultures mainly of chick fibroblasts *in situ* on the stage of the ultra-violet microscope. Very little of this work has yet been published because of the difficulties of precise quantitative interpretation. The result of an experiment in which chick fibroblasts in culture (provided by Mrs. I. Simon-Reuss) received a dose of 435 r of X-radiation delivered in 6 minutes has been published (p. 313 of ref. 42). There appeared to be an increase in cytoplasmic absorption at 2537 Å and also an increase in diffusible absorbing material in the photograph taken at between 2–6 minutes after the end of the X-ray exposure. At about 87 minutes after the irradiation the characteristic absorption was lost, the cells were breaking down and there appeared to be gross damage to the chromosome. A great many control experiments were carried out which showed that the ultra-violet radiation used for these exposures did not

produce comparable effects. There was evidence of a hyperchromatic effect after the first photographic exposure but then no change in ultra-violet absorption for about 20 photographic exposures.

More recently, Dr. G. H. Hjort, who worked with me in Cambridge, has used the methods of ultra-violet photomicrography to study the effects of total body irradiation with doses between 75 and 1200 r on the lymphocytes of lymphoid tissue in adult rats. The sections were prepared from frozen dehydrated tissue. It has been found that an increase in nucleotide type of absorption mainly in the cytoplasm can be detected as early as 15 minutes after irradiation and this biochemical change appears before any morphological changes can be detected. There is, however, wide variability in the behaviour of different cells in respect of these changes and much further quantitative examination of the experimental material is necessary.

It is useful to express the effects of irradiation on inhibition of synthesis of desoxyribonucleotides by means of a crude ionic efficiency.⁴¹ In a small human tumour, e.g., a typical squamous carcinoma of the skin, cure may be effected by a single dose of 2000 r of X-radiation. It can be assumed as an approximation that 2000 r stops the formation of an amount of DNA equal to that already present in the nucleus. It is reasonable to assume that the mean diameter of the nuclei is 7 microns and that the mean DNA content per nucleus is 6×10^{-12} grams. Hence, there are approximately 1.10×10^{10} monomeric desoxyribonucleotides of assumed molecular weight 330 per nucleus, and 2000 r corresponds to the formation within each nucleus of 6.5×10^5 ion pairs. Hence the ionic efficiency which is the number of monomeric desoxyribonucleotides whose formation (or duplication) is inhibited per ion pair is roughly 1.7×10^4 . Such a value would correspond to inhibition of formation of a polymeric molecule of DNA of molecular weight 5.6×10^6 . All the figures used are rough approximations. However, it is not unreasonable to consider that a DNA may have a molecular weight of about 6×10^6 , so that these calculations suggest that one ion pair stops the formation of one molecule of DNA. The possibility of a direct action mechanism on DNA or a DNA protein must be raised. It may perhaps be suggested that damage anywhere in the DNA molecule stops self-duplication.

It would seem to be very important to find out the details of the mechanism by which X- and γ -rays interfere with the synthesis of DNA and nucleoproteins in proliferating cells in order to try to provide a more rational starting point for the chemotherapy of cancer.

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Statistics of the Therapeutical Use of Radioisotopes in Brazil

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Radioisotopes in experimental work related to problems of human physiopathology have been used at the Institute of Biophysics of the University of Brazil since 1944. Several papers^{1,2,3,4} have been published on the subject, in spite of the difficulty in receiving the isotopes, prepared in the cyclotron of the Massachusetts Institute of Technology, through the courtesy of Professor Robley D. Evans. In 1947 the Pan-American Sanitary Bureau was officially designated to represent Brazil at the American Atomic Energy Commission. Through that Bureau it was a little easier to receive shipments of isotopes from the USA. Consequently a few experimental programs using radioactive isotope techniques could be developed; at the same time a system of cooperation between the Institute of Biophysics of the University of Brazil and several hospitals or medical groups could be established.

Thus many patients with bloody dyscrasias, malignancy, hyperthyroidism, and cardiac decompensation, have been treated with radioactive phosphorus and iodine in Rio de Janeiro. The largest group of patients so treated was studied at the Hospital dos Servidores do Estado. In São Paulo, through a close cooperation between the University of São Paulo and the Rockefeller Foundation, a Laboratory of Isotopes was created. This has been a most important achievement, as this Laboratory has been an invaluable aid to many clinical and research groups in São Paulo and other centers. It acts as a very well organized center, receiving and redistributing isotopes regularly (these are shipped mainly from the USA). Thus it has worked with or redistributed many types of isotopes (P^{32} , I^{131} , Zn^{65} , C^{14} , Ce^{134} , Co^{60} etc.) to several other institutions connected with the University of São Paulo (University Hospital, Department of Histology, School of Engineering, School of Philosophy, Science and Literature; School of Medicine of Ribeirão Preto, São Paulo; School of Agriculture, Piracicaba, São Paulo), or not (School of Medicine, Pôrto Alegre, Rio Grande do Sul); Serviço Nacional do Câncer, Rio de Janeiro; several private clinics. We assume that the various medical groups from São Paulo altogether should have a larger number of patients treated with radioactive isotopes. However, we could not have their data collected in time to be presented in this paper. So we had to analyse

only the cases treated by us or with our assistance, up to June 5, 1955, in Rio de Janeiro, mostly at the Hospital dos Servidores do Estado. We are now receiving the complete equipment for an Isotope Unit to be installed at the latter Hospital and directed by a committee consisting of members of the Hospital and the Institute of Biophysics of the University of Brazil. So we hope that the work initiated in 1950, of which the first reports^{5,6} were published in 1951-52, will continue and greatly develop very soon.

THERAPEUTICAL USE OF RADIOACTIVE PHOSPHORUS IN RIO DE JANEIRO

Radioactive phosphorus has been used for the treatment of patients with blood dyscrasias in Rio de Janeiro since 1944. The first cases have been observed with no accuracy regarding the proper hematological control, in the sense that no complete follow-up could be obtained. Since 1949 at the Hospital dos Servidores do Estado, in cooperation with the Institute of Biophysics of the University of Brazil, we have been able to administer P^{32} to a series of patients with chronic granulocytic leukemia and polycythemia vera. It is the purpose of this communication to (1) describe our own experience during the past seven years and based on the treatment of fifteen patients, and (2) evaluate the effectiveness of this therapy and compare it with other methods of treatment. We admit that our series is too small. However, we think that, in presenting it and following the patterns of study suggested by Reinhard *et al.*⁷ we are able to sum up our data to the others similarly and conveniently collected elsewhere. The circumstances of having followed up our patients from 1949 to the present, and the fact that most cases had been previously or consecutively submitted to other forms of therapy allowed us to reach some tentative conclusions which are worthy of presentation. This International Conference seems to be an excellent opportunity of analysing data collected throughout the world in order to establish common points of view on the subject.

MATERIAL AND METHODS

Our series consists of: 9 patients with chronic granulocytic leukemia; 5 with polycythemia vera; and 1 with chronic lymphocytic leukemia. The diagnosis was established in every case by peripheral blood examinations, bone marrow aspiration and/or lymph node biopsy. Of these 15 patients, 5 have died

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and the remaining 10 are still alive. Post-mortem examination was performed on 4 of the 5 who died.

The radioactive phosphorus used was prepared by Oak Ridge National Laboratory, Tennessee, USA, or Harwell Laboratory, England. We received it as a dibasic phosphate solution. The doses used were calculated in millicuries (mc) and were administered intravenously; oral administration was not used. Partial single doses varied between 0.10 mc and 5.2 mc. The total amount of P^{32} varied from 2 mc to 19 mc for each patient. The intervals of administration varied from 10 to 296 days, depending upon the availability of the isotope and the clinical condition of the patient. Until we had acquired some experience on the use of P^{32} , we decided to keep our cases hospitalized during intervals of time varying between 10 and 104 days. The clinical course was carefully observed and all the data recorded in the patients' charts. As a better acquaintance with P^{32} therapy was achieved, we allowed our patients to be followed as out-patients.

Hematological controls included frequent blood counts according to the usual methods. All our patients at the Hospital dos Servidores do Estado were submitted to, at least, one sternal aspiration before starting the therapy. They have been under our control during intervals varying between 1 and 67 months.

One patient with polycythemia (Case No. 5), one patient with chronic granulocytic leukemia (Case No. 9), and the only one with chronic lymphocytic leukemia, were followed by other doctors in Rio de Janeiro. We have tried to follow as strictly as possible the patterns of observation and presentation of cases published by Reinhard *et al.*⁷ in their excellent paper, where an extensive review of literature up to 1946 was made.

POLYCYTHEMIA VERA

Analysis of Results Obtained in Treating Five Patients with Polycythemia Vera

During the last five years, five patients with polycythemia vera have been adequately treated with P^{32} in Rio de Janeiro; four of them under our direct supervision (Cases 1-4) and one followed by a local physician, who has kindly collaborated with us. Cases 1, 2, 3 and 4, in addition to the routine blood and bone-marrow studies, have also had their blood volume determined. (Evans blue-dye method—photoelectric colorimeter technic), chest X-ray and circulation time taken. It is believed the diagnosis of polycythemia vera was definitely established in this series.

Effect of Therapy on the Cellular Elements of the Blood

Hematologic data on these 5 patients are summarized in Table I. The figures in column 3 reveal that our patients were given doses varying between 2 and 9.75 mc for each course (usually in one single injection, except Case No. 5, in whom two injections of 3.6 and 6.15 mc were administered at a 10 days'

interval of time). The above doses are somewhat smaller than the usual in other clinics. However, the RBC counts fell below 4.0 million/mm³ in three of the five patients. Patients No. 3 and 5 were slightly overtreated, as the fall in RBC levels and thrombocytopenia with transient purpura seem to indicate (unfortunately we have no WBC counts to follow at the same time). It is worth mentioning she was given only 5.2 mc in one single injection and her remission has lasted 12 months up to the present time.

The figures in column 5 reveal that the erythrocyte level began to fall from 4 (Case No. 4) to 17 weeks (Case No. 2), after the P^{32} injection was given. This more or less latent period between the isotope administration and the fall in RBC has to be taken into consideration when treating such patients. If symptoms of plethora are too severe and persist for some time after the injection, phlebotomies should be performed in order to prevent complications such as cerebral or myocardial thrombosis. This was the case in patients Nos. 1, 2 and 5. We believe that it would be even more advisable to bleed patients before administering the P^{32} whenever they have hematocrit values equal/or higher than 65 vol.% or the symptoms suggesting impending thrombosis are too evident (constant headache or dizziness too severe, precordial discomfort or pain, thrombotic foci in retinal venules, etc).

The depressing effect of P^{32} on white corpuscles and platelets is evident in Table I. However, we should emphasize this is not a long-lasting effect and usually it lasts for 2 to 5 weeks. Cases No. 3 and 5 developed purpura of the skin, especially in the lower extremities, which lasted for two weeks approximately.

Here again the variability in the degree of sensitivity of patients to the P^{32} radiation effect is evident. It has been definitely established that "therapy must be individualized to a high degree, and it is impossible to predict in advance how much therapy any given patient will require."⁷

Effect of Therapy on the Clinical Manifestations of Polycythemia Vera

Table II shows the symptoms which were present in our five patients and what response they presented to therapy. Contrary to Reinhard's experience,⁷ all our patients who had more or less severe headache and dizziness experienced a maximum benefit from the P^{32} injection, to the point of complete disappearance of the symptom. The itching and the paresthesias also disappeared completely after therapy in 2 cases. However, the other symptoms (fatigability (aching pains in extremities, blurring of vision, burning of eyes, and weight loss) did not subside completely in all cases. Notwithstanding we must emphasize all patients stressed the fact that they felt much better after the treatment reached its maximum effect and were able to get along without much trouble.

Table I. Hematologic Data on Five Patients with Polycythemia Vera

Patient	Age and sex	Total P ³² for each course of therapy (mc)	Duration of course of therapy (days)	Time from first treatment to initial drop in R.B.C. level (days)	R.B.C. count at time therapy was begun (millions/mm ³)	Lowest R.B.C. count (millions/mm ³)	Platelet count at time therapy was begun (per mm ³)	Lowest platelet count after therapy (per mm ³)	W.B.C. count at time therapy was begun (per mm ³)	Lowest W.B.C. count after therapy (per mm ³)	Duration of hematologic remission (from time R.B.C. count fell below 5.5 M., until it again rose above 6.0 M., or interval between courses of treatment) (month)
1-J.G.C.D.	52, F	5	1	85	8.79	5.2*	300,000	140,000	18,600	3,400	4 +
		2	1	43	6.43	4.1	358,000	210,000	9,000	5,400	51
2-E.C.	50, F	3	1	120	8.46	3.72	380,000		7,075		43
		4.5	1	112	7.21	3.6	454,000		12,200	4,750	8
3-B.L.	54, F	5.2	1	40	6.8†	2.84	480,000	40,000†			12
4-S.P.	59, M	4.6	1	32	8.1	5.1					3
5-A.T.	59, M	9.75	10	46	10.1	5.0	922,000	± 30,000†	14,300		1

* Six phlebotomies were done in addition, because of delay in R.B.C., depression.

† Transient episode of purpura of the skin (lasting 2 weeks).

+ Patient treated by "Chloronaphthina" until 8 months prior to P³² therapy.

In Table III are listed the abnormal physical signs present in our series. All such signs completely or partially subsided after therapy except in two cases in whom retinal venules, distension and hypertension respectively persisted unaltered.

Duration of Remissions Produced

Three of our five patients have been followed for a year or more. According with data expressed in Table I, the longest remission so far observed is 51 months and is still continuing. One patient has been followed for just one month after RBC fell below 5.5 millions/mm³ and for two and a half months since the first injection of P³². No deaths have occurred so far in our series.

Comparison of Radioactive Phosphorus With Other Forms of Therapy Used in the Treatment of Polycythemia Vera

There is no doubt that P³² induces both clinical and hematological remissions, in many instances of a quite long duration, in patients with polycythemia vera. In spite of similar results obtained with roentgen therapy and/or phenylhydrazine, the absolute majority of hematologists and clinicians thinks that radioactive phosphorus represents the best form of treatment that we have for this condition at the present moment. Phlebotomies are still advocated by a few workers. However, (1) the absence of radiation sickness, (2) the fact that there is no obligation of regulating the doses of the medicine at frequent intervals, (3) the ease and rapidity with which the therapy is completed without any need for complicated equipment or highly specialized personnel, make P³² therapy the most convenient form of treating polycythemia vera. If the physician knows well how to administer the isotope, there is a very little, if any, risk of doing any harm to the patient. The possibility of developing anemia, leucopenia and thrombocytopenia of some clinical significance is quite rare and due to overdosage. The only drawbacks for this therapy which are really important

Table II. Five Patients with Polycythemia Vera
Effect of P³² Therapy on Symptoms

	Number of patients			
	With symptom	Completely relieved	Partially relieved	Un-improved
Fatiguability	2	1		1
Headaches	4	4		
Dizziness	4	4		
Aching pains in extremities	1			1
Itching	2	2		
Blurring of vision	4	2		2
Burning of eyes and/or lacrimation	2	1		1
Weight loss	2	1		1
Paresthesias	1	1		

Table III. Five Patients with Polycythemia Vera Changes in Physical Signs Following P^{32} Therapy

	<i>Number of patients</i>			
	<i>Who had abnormal physical sign at onset of therapy</i>	<i>In whom abnormal finding completely disappeared after therapy</i>	<i>In whom abnormal finding partially subsided</i>	<i>In whom there was no improvement</i>
Skin and/or mucous membranes bright red	3	2	1	
Cyanosis	5	3	2	
Conjunctivae red	3	2	1	
Retinal veins distended	4	2	1	1
Splenomegaly	5	3	2	
Hepatomegaly	4	3	1	
Systolic B.P. 150 mm Hg or higher	3	1	1	1

are: (1) the relative scarcity and moderately high cost of the isotope, which have been decreased year after year, and (2) the latent period which occurs between injection of P^{32} and fall in RBC levels.

CHRONIC GRANULOCYTIC LEUKEMIA

Analysis of Results Obtained in Treating Nine Patients with Chronic Granulocytic Leukemia

Of this series of nine patients with chronic granulocytic leukemia, eight have been followed at the Hospital dos Servidores do Estado directly by us, and the remaining one was observed by a local physician (Case No. 9). Five of these patients have died and four are still alive. Cases No. 1 to 5 (the first to be treated with P^{32}) were admitted to the Hospital dos Servidores do Estado and were seen daily by one of us, during a period of 10 to 104 days. After such a period, the patients were followed in the clinic every one or two weeks according to the particular situation. Cases No. 6, 7 and 8 have been followed in the out-patient department since the first consultation; however they have been examined at intervals of approximately one month.

The hematologic control included complete blood counts by the usual methods. Hemoglobin was measured by Sahli's method until 1952, and by the photolorimetric method thereafter. Platelets were measured by Fonio's method. All patients had a sternal bone-marrow aspiration done before starting the P^{32} and it was repeated at variable intervals according to the course of the disease. A total of one to 4 sternal punctures were done on our patients, who have been under our observation for variable periods of time (one to five and a half years). Of our nine patients, five are male and four are female. The age at the beginning of the clinical symptoms varied between 24 and 41 years, for the males; female between 30 and 50 years. Of our nine patients, four are colored and five are white.

Effect of Therapy on the Cellular Elements of the Blood

Table IV summarizes the hematological course of our nine patients. Seven of these had some other form of therapy prior to P^{32} administration. When treated with the isotope, however, every case had elevated WBC count with many immature forms in the peripheral blood; none had been submitted to any form of therapy in the period of five months preceding the first injection of P^{32} . No anemia or thrombocytopenia were observed at the time when radioactive phosphorus was started.

The dosage of P^{32} necessary to bring the WBC to approximately normal levels (15,000–4000) has varied between 5 and 18 mc in the first course. Time required to show such response varied between 4 and 14 weeks. In our series there apparently was a close relationship between the height of the initial count and the total amount of P^{32} required to show a satisfactory remission. On the other hand, the time required to have the first remission has also been in close relationship with the height of the initial WBC level.

At the same time as the leucocyte count decreased, the percentage of polymorphonuclear neutrophils increased and the percentage of immature cells (myeloblasts to myelocytes included) went down to zero in all but two cases. We think that the probable dose of P^{32} which will induce a good remission in a determined case is in a close relation with the percentage of immature cells present in the peripheral blood. On the other hand, the prognosis of a case depends much more on the disappearance or not of these immature cells than on just the decreasing of the WBC level.

The few patients who had a slight anemia have shown a perfect correction of it to normal levels without any other therapeutic measure. Platelets did not fall appreciably and we had no purpuric manifestation after P^{32} , depending on a thrombocytopenia

Table IV. Hematological Data on Nine Patients with Chronic Granulocytic Leukemia Treated with Radioactive Phosphorus

Patient	Age and sex	Previous treatment*	Blood counts at time P ³² therapy was started					P ³² therapy required to restore W. B. C. count to approx. normal (4000-15,000)					Most normal blood counts after P ³² therapy					Duration from restoration of normal W. B. C. count until death or 6/5/55 (mo)	Treatment and blood counts during first year or fraction thereof following restoration of leucocyte count to approx. normal (4000-15,000)					
			W. B. C. (per mm ³)	R. B. C. (millions per mm ³)	Hg (% of normal)	Platelets (per mm ³)	% polymorpho-nuclear neu-trophils	% myeloblasts	mc	days	W. B. C.	R. B. C.	Hg (% of normal)	Platelets	% polymorpho-nuclear neu-trophils	% myeloblasts	mc or P ³²		Other	W. B. C. range	R. B. C. range†			
1-O.L.S.	26,M	RX	152,200	5.04	94	280,000	94.4	22.4	18	102	7700	5.0	100	400,000	62	4	3	+	None	FO	4800-117,000	2.39-4.75		
2-A.C.F.	32,F	RX	180,800	4.0	70	441,000	97.1	19.6	13.3	85	6000	5.08	90	400,000	66	0	44	None	RX	10,500-185,000	0.96-3.12			
3-W.R.A.	41,M	RX	52,800	4.76	100	243,000	77.5	15.5	15.4	85	6100	5.01	100	344,800	64	0	12	None				T.26		
4-H.T.M.	50,F	RX	82,200	5.17	97	300,000	90	9.5	8	60	7200	5.02	97	380,000	69	0	34	4			14,000-84,000	3.64-4.65		
5-E.R.A.	28,M	RX	62,000	5.06	91	380,000	82	12	6.8	54	8800	5.09	97	410,000	66	0	14	2.3			9800-80,000	3.3-5.4		
6-R.S.M.	24,M	RX	67,000	3.9	75	150,000	79	9	5	29	8500	5.4	102	300,000	75	0	7	None			9400-33,300			
7-E.F.N.	30,F	None	54,000	4.1	79	390,000	63	10	5.1	35	12,500	4.3	92	300,000	74	0	11	+	None		20,400-83,500			
8-V.B.C.P.	46,F	None	280,000	4.05	84	280,000	61.5	18.5	12.8†	29	40,200	4.1	86	320,000	63	6		None			7000-8000			
9-J.Z.§	35,M	FO	53,000		86		84	12	9.5	29	4000		85	300,000	70	0	63		None					
Treatment and blood counts during second year or fraction thereof following initial restoration of leucocyte count to approx. normal																								
Patient	mc of P ³²	Other	W. B. C. range		R. B. C. range		mc of P ³²		Other		W. B. C. range		R. B. C. range		mc of P ³²		Other		W. B. C. range		R. B. C. range		Duration of disease (mo.) from first symptom	
			to approx. normal		to normal		to normal		to normal		to normal		to normal		to normal		to normal		to normal					
1-O.L.S.																								
2-A.C.F.	None	FO	6600-55,000	3.0-4.13	T.3	27.5	FO	4350-97,500	3.3-4.9	None	6000-97,000		-2.25	T.3										
3-W.R.A.																								
4-H.T.M.	None	URE	50,800-33,700	3.5-4.0		None	RX	42,000-585,000	3.8-1.5															
5-E.R.A.																								
6-R.S.M.																								
7-E.F.N.																								
8-V.B.C.P.																								
9-J.Z.§	9.5	No	8000-15,000			8	No	7000-12,000	Normal	8	No	7000-27,000	4.2-5.5	18	Tem	3000-67,000	3.4-4.9	3	66					

* XR = X-ray therapy; FO = Fowler's solution; URE = Urethane.

† T = Transfusion. The figure indicates the number of transfusions given.

§ Patient followed by other local M.D.; less uniform data.

† Figure represents the total number of millicuries given, but leucocyte has not reached approx. normal levels yet. Patient will soon re-start P³² which has been impossible to obtain since last shipment received in October 1954.

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Headaches	6	5	1	
Spontaneous bruising	1		1	
Bloating, dyspepsia	2	2		
Anorexia	4	4		
Abdominal pain	1	1		
Cough	2	2		
Pain in extremities	5	4	1	
Night sweats	7	5	1	1
Chills and feverishness	4	3	1	
Nervousness	8	6	1	1
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Symptoms	Number of patients			
	Who had abnormal physical sign at onset of therapy	In whom abnormal finding completely disappeared after therapy	In whom abnormal finding partially subsided	In whom there was no improvement
Fever	7	7		
Tachycardia	5	4	1	
Petechiae and/or ecchymoses	1		1	
Splenomegaly	9	7	2	
Hepatomegaly	7	5	2	
Systolic cardiac murmur	2	1	1	
Lymph node enlargement	2	1		1

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Table IV. Hematological Data on Nine Patients with Chronic Granulocytic Leukemia Treated with Radioactive Phosphorus

Patient	Age and sex	Previous treatment*	Blood counts at time P ₃₂ therapy was started						P ₃₂ therapy required to restore W. B. C. count to approx. normal (4000-15,000)				Most normal blood counts after P ₃₂ therapy						Duration from restoration or normal W. B. C. count until death or 6/5/55 (mo.)	Treatment and blood counts during first year or fraction thereof following restoration of leucocyte count to approx. normal (4000-15,000)	
			W. B. C. (per mm.)	R. B. C. (millions per mm.)	Hg (% of normal)	Platelets (per mm.)	% polymorpho-nuclear neutrophils	% myeloblasts	mc	days	W. B. C.	R. B. C.	Hg (% of normal)	Platelets	% polymorpho-nuclear neutrophils	% myeloblasts	mc or P ₃₂	Other		W. B. C. range	R. B. C. range
1-O.L.S.	26,M	RX	152,200	5.04	94	280,000	94.4	22.4	18	102	7700	5.0	100	400,000	62	4	3	None	FO	4800-117,000	2.39-4.75
2-A.C.F.	32,F	RX	180,800	4.0	70	441,000	97.1	19.6	13.3	85	6000	5.08	90	400,000	66	0	44	None	RX	10,500-185,000	0.96-3.12
3-W.R.A.	41,M	RX	52,800	4.76	100	243,000	77.5	15.5	15.4	85	6100	5.01	100	344,800	64	0	12	None		14,000-84,000	3.64-4.65
4-H.T.M.	50,F	RX	82,200	5.17	97	300,000	90	9.5	8	60	7200	5.02	97	380,000	69	0	34	4		9800-80,000	3.3-5.4
5-E.R.A.	28,M	RX	62,000	5.06	91	380,000	82	12	6.8	54	8800	5.09	97	410,000	66	0	14	2.3		9400-33,300	T.2
6-R.S.M.	24,M	RX	67,000	3.9	75	150,000	79	9	5	29	8500	5.4	102	300,000	75	0	7	None		20,400-83,500	
7-E.F.N.	30,F	None	54,000	4.1	79	390,000	63	10	5.1	35	12,500	4.3	92	300,000	74	0	11	+			
8-V.B.C.P.	46,F	None	280,000	4.05	84	280,000	61.5	18.5	12.8†		40,200	4.1	86	320,000	63	6		None			
9-J.Z.§	35,M	FO	63,000		86		84	12	9.5	29	4000		85	300,000	70	0	63	None		7000-8000	

Patient	Age and sex	Previous treatment*	Treatment and blood counts during second year or fraction thereof following initial restoration of leucocyte count to approx. normal						Treatment and blood counts during fourth year or fraction thereof following initial restoration of leucocyte count to normal						Treatment and blood counts during fifth and sixth year following initial restoration of leucocyte count to normal						Duration of disease (mo.) from first symptom	
			W. B. C. range	R. B. C. range	T.3	mc of P ₃₂	Other	W. B. C. range	R. B. C. range	mc of P ₃₂	Other	W. B. C. range	R. B. C. range	mc of P ₃₂	Other	W. B. C. range	R. B. C. range	To start P ₃₂	To death or 6/5/55			
1-O.L.S.																						
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these five cases after P^{32} was a little longer than the one obtained with X-rays and/or Fowler's solution.

The effect of P^{32} therapy in prolonging life of patients with chronic granulocytic leukemia cannot yet be ascertained. Yet the general view is that there is no significant change in the prolongation of life as a result of any of the most prevalent forms of therapy for this condition. Only the summation of data will eventually give us some indication to make such a crucial point clear.

CHRONIC LYMPHOCYTIC LEUKEMIA

We have had the opportunity to study only one case of chronic lymphocytic leukemia, who was directly assisted by a local physician for six months until his death. A summary of the clinical and therapeutic course of the case is below presented.

F.P.O. was a 58 year old white male, from Matto Grosso, Brazil, first seen on March 18, 1952. History of known splenomegaly for many years (which patient attributed to chronic malaria which is endemic where he lived). A symptomatic until February, 1952, when he suffered a fracture of the right clavicle. Being examined by the local M.D., the diagnosis of leukemia was established and he was referred to a physician in Rio de Janeiro. After a complete hematological study, including a sternal aspiration, the patient was considered as having chronic lymphocytic leukemia, probably of a long duration.

The hematologic and therapeutic data on this case are summarized in Table VII. It seems evident that this patient entered into a subacute and terminal stage at least from April 1952 on. He was given a fairly large dose of P^{32} (8.6 mc) in one single injection on May 14, 1952. In spite of a definite fall in WBC levels, the splenomegaly did not change thereafter. Patient was given numerous blood transfusions from July through October, when he died. During the entire course under the supervision of Rubens C. Mayall, M.D. (Rio de Janeiro), he was submitted to 23 blood counts and 1 sternal aspiration. No post-mortem examination was done.

No appreciable change in WBC differential counts was observed after P^{32} . During the last three months of life, patient had a severe anemia which was not ameliorated by blood transfusions. It became clear that the radioactive phosphorus could not diminish the size of the spleen (which was palpable, throughout the entire period of observation, about 14 cm below the left costal margin on quiet respiration). Patient never had appreciable lymph node enlargement and no symptoms whatsoever until May 1952, when he began to have fatigability, anorexia, dyspnea on exertion and progressive weight loss; fever and chills appeared in the last 3 weeks of life as well as a hemorrhagic tendency.

Obviously we cannot reach any conclusion from one case, presented here only as a matter of information to other workers interested on the field.

Table VII. Hematologic and Clinical Course of One Case of Chronic Lymphocytic Leukemia

Date	Million/mm ³	H.G.B. % of normal	W.B.C. per mm ³	W.B.C. differential	Observations
3/18/52	4.1	80	210,000	Blasts 3 Lymphocytes 96 Eo. 2 Seg. N. 2	Splenomegaly=14 cm below left costal margin.
4/14/52	4.8	85	336,000	Blasts 3 Large lymph. 6 Small lymph. 89 Band N. 2	P^{32} was given = 8.6 mc In March and April 1952 patient received 9 trans- fusions.
5/23/52	4.0	80	96,000	Large lymph. 1 Small lymph 97 Eo. 1 Seg. N. 1	Splenomegaly unaltered.
6/7/52				Blasts 2 Lymphocytes 93 Seg. N. 4 Mono 1 Eo. 1	
9/7/52	2.0	48	164,000	Blasts 3 Large lymph. 9 Small lymph. 86 Eo. 1 Mono 1	Splenomegaly unaltered.
10/6/52	1.8	42	182,000	Blasts 5 Large lymph. 10 Small lymph. 81 Eo. 1 Band N. 1 Seg. N. 2	Patient was given transfu- sions as follows: July 5; Aug. 4; Sept. 3; Aug 1. Died on 10/8/52. No autopsy.

Therapeutical Use of Radioactive Iodine in Rio de Janeiro

We have given I^{131} therapeutic doses varying between 4.5 mc to 8.2 mc in one single oral administration to eight patients with hyperthyroidism. No previous uptake test was done and the follow-up of the patients was incomplete as all the patients except two were assisted by other local physicians. These two were treated at the Hospital dos Servidores do Estado with definite amelioration of their hyperthyroid symptoms. However, as we are not yet equipped to study such cases, we have limited the use of this isotope to patients in whom the clinical situation forced us to try this form of therapy. Consequently we are not able to present any experience in the field.

We know that in São Paulo many cases have been adequately treated and followed but we were not able to receive the corresponding data on them.

SUMMARY AND CONCLUSIONS

A brief historic description of the use of radioactive isotopes in Brazil is presented. Complete data on fifteen cases of blood dyscrasias (polycythemia vera, five cases; chronic granulocytic leukemia, nine cases; chronic lymphocytic leukemia, one case) treated with P^{32} are reviewed.

The presented data seem to justify the following conclusions:

1. Radioactive phosphorus is probably the best form of therapy to use in the treatment of polycythemia vera. Complete hematologic remissions and almost complete disappearance of symptoms were induced by P^{32} , which may last from months to several years. No unpleasant complications resulted from its administration.

2. P^{32} therapy for chronic granulocytic leukemia is as useful as the other best forms of therapy: X-rays, Myleran. It is superior to Fowler's solution in view of the lack of side-effects of therapy, especially regarding G.I. disturbances and skin manifestations. It causes no radiation sickness which makes it more comfortable for patients than roentgen therapy.

3. It is not possible to establish whether P^{32} prolongs the life of patients with polycythemia vera or chronic granulocytic leukemia more than the other forms of therapy do.

4. There is a remarkable variation in the sensitivity of patients to the same dosage of radioactive phosphorus, in relation to the blood-forming tissues' depression which the isotope induces. Following patients with daily counts, we have observed a slight rise in WBC levels from the first to the fifth day after the P^{32} injection; thereafter it causes a more or less evident depression in all three series of blood corpuscles.

5. X-ray therapy is undoubtedly more effective in causing a rapid reduction in the splenomegaly than P^{32} . Similarly it is advisable to use roentgen therapy instead of, or paralleling, P^{32} for relieving compressive symptoms on nerve roots or other vital structure, if present.

ACKNOWLEDGEMENTS

The authors wish to express their indebtedness to Rubens C. Mayall, M.D., J. Maia Mendonça, M.D., and Reynato S. Borges, M.D., Rio de Janeiro, Brazil, who referred patients to us or gave their data to be analysed by us. Special reference should be made to Dr. Vera R. L. Ribeiro, who collected information of the University of São Paulo Laboratory of Isotopes at personal sacrifice of time and convenience.

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Artificial Radioisotopes in Nylon Ribbons for Implantation in Neoplasms

By U. K. Henschke, USA

DESIGN OF THE NYLON RIBBONS

Figure 1 shows a drawing of the nylon ribbon which we have developed for the implantation of neoplasms with radioisotope sources. This nylon ribbon is prepared by stretching and flattening of a nylon tube. On the one end of the nylon ribbon, a short piece of tubing is left. Through this end, the radioisotope sources are inserted and pushed to the desired position with a wire. The sources are firmly held in place without spacers by the elasticity of the hollow nylon ribbon. Beyond the radioisotope sources, a length of the ribbon of about one meter is provided. This end is attached to an implantation needle which is inserted through and around the tumor, while the sources remain in a protective lead container. After all needles have been properly placed, the loaded ends are quickly pulled into position. Any number of sources can be cut off at the time of implantation in order to adapt the active length to the tumor size. It is also possible to change the position of the sources within the nylon ribbon.

Compared with the usual interstitial implantation with radium needles or radon seeds, the nylon ribbon provides for (1) more accurate implantation, (2) more comfort to the patient and (3) less exposure to the operator.

The nylon ribbon is a further development of the flexible carrier method, which was first used by Hames in 1937 in form of silk tubing loaded with radon seeds. In 1951, Morton, Callendine and Myers at the Ohio State University Medical Center reported on the use of nylon tubes, into which small radioactive Co^{60} sources were inserted and held in position by aluminum spacers. The main advantage of the nylon ribbons over the older carriers lies in the ability to adjust the number of the radioactive sources at the time of implantation. This feature permits economical centralized preparation of the nylon ribbons and eliminates the need for special isotope facilities where ribbons are used.

RADIOISOTOPES FOR USE IN NYLON RIBBONS

Table I lists the important properties of the radioisotopes which we have used in the past for implantation in nylon ribbons. Ir^{192} has proven to be the most suitable of all available radioisotopes. Due to its high cross section, it can be produced so economically that recovery and reuse is not worth-

while; it has a gamma radiation which is hard enough to avoid increased bone absorption but is still easily shielded with lead; and it has a half-life, which permits storage over two months, while presenting a smaller hazard in case of accidental loss than the long-lived radium and Co^{60} . The Ir^{192} sources now in use are small wires, enclosed in copper tubing for absorption of the beta radiation. They measure 3 mm in length and 0.6 mm in diameter.

For dose calculations with Ir^{192} sources, a calibration sheet is provided which gives the gamma activity of one Ir^{192} source in milligram-radium-equivalent for each day. In this way, the same tables and curves as for radium implants can be used for approximate dose calculations. More accurate dosimetry is possible by actual measurements on the patient with direct reading instruments equipped with small ionization chambers or scintillation crystals. Instruments of this type suitable for clinical use are becoming available commercially and will help greatly to improve the dosimetry with gamma-ray sources in interstitial implants as well as in intracavitary and surface applications.

THE STANDARD NYLON RIBBON SET

Figure 2 shows the nylon ribbon set, which we suggest as standard for shipping to hospitals which want to use this technique. One set contains 16 nylon ribbons. Each ribbon is loaded, as shown in Fig. 1, with 12 Ir^{192} seeds spaced at one centimeter intervals. Thus, the set contains a total of 192 Ir^{192} seeds. The gamma radiation of each seed corresponds to one-third to one-half milligram of radium. The total ac-

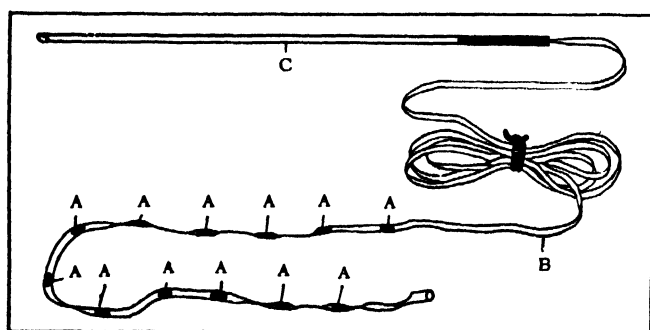


Figure 1. Nylon ribbons for the implantation of neoplasms with radioisotopes. (A) radioisotope sources; (B) nylon ribbon; (C) implantation needle

Table I. Radioisotopes Used for the Nylon Ribbon Technique

Radioisotope	Symbol	Cross section	Half-value layer in Pb	Max. beta energy, kv	Half-life
Radium	Ra ²²⁶	—	7.9 mm	3198	1590 yr
Radon	Rn ²²²	—	7.9 mm	3198	3.8 d
Cobalt-60	Co ⁶⁰	34 barns	10.4 mm	310	5.3 yr
Gold-198	Au ¹⁹⁸	96 barns	2.7 mm	970	2.7 d
Iridium-192	Ir ¹⁹²	750 barns	2.3 mm	670	74 d

tivity of such a set is therefore equivalent to 66 to 100 milligrams of radium.

The loaded ends of the nylon ribbons are inserted into a small cylindrical lead container. Due to the low half-value-layer of the Ir¹⁹² in lead, a wall thickness of 12 mm is sufficient to satisfy all postal regulations for the safe shipping of radioisotopes. The lead container therefore weighs only a little more than one kilogram (2.2 lb), is inexpensive and therefore dispensable.

For shipping and storage, a tin can of the same size and design as used for packing one pound of coffee is used. In this can the lead container is suspended by a metal bridge. This bridge serves for shock absorption during the transport and holds the conical lid tightly in place. The loaded ends of the ribbons cannot be pulled out of the container unless the two screws, which hold the metal bridge to the lead container, are loosened.

The tin can is filled with a solution, which sterilizes the whole set and keeps the nylon ribbons smooth and pliable. The tin can also contains buttons and clips for the fixation of the nylon ribbons after implantation and a small screw driver for loosening the bridge. The whole self-contained, nonreturnable set weighs only 2½ kilograms (5.5 lb) including the shipping box. It can be sent anywhere in the world by mail for less than \$2.50 postage and is ready any time for immediate use without further preparation.

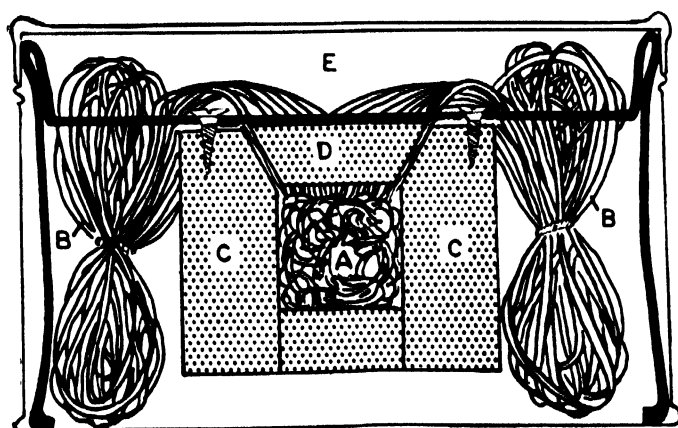


Figure 2. Shipping can with standard set containing 16 nylon ribbons. (A) loaded ends of the 16 nylon ribbons; (B) unloaded ends of the 16 nylon ribbons; (C) lead container; (D) conical lid; (E) metal bridge

IMPLANTATION TECHNIQUE

Figure 3 shows schematically the important steps in the implantation of nylon ribbons. The upper drawing shows the insertion of one of the needles with attached nylon ribbon through the neoplasm. Accurate implantation is greatly facilitated if the needles are at least twice as long as the length of the implanted volume. In most locations, we prefer hollow needles 25 cm long; they can be bent if required. Up to this time, the loaded ends of the nylon ribbons remain in the lead container and no radiation exposure occurs to the operating personnel. The implantation of the needles can therefore be carried out without hurry and their position can be corrected until a satisfactory pattern has been accomplished.

The middle drawing shows the placing of the active ends of the nylon ribbons with the Ir¹⁹² sources. For this step, the two screws shown in Fig. 2, which hold the metal bridge to the lead container, are first loosened a few turns. This gives some play to the conical lid of the lead container and the ribbons can now be pulled out one by one. Next, the operator, from a distance of at least 20 cm from the implanted volume, pulls the loaded ends of the ribbons into position. A button and a tubular stainless steel clip, which come attached to the loaded end of each ribbon, prevent the ribbon from being pulled through completely. The tubular stainless steel clip, in turn, cannot slip off, because it is stopped by the round end of the nylon ribbon.

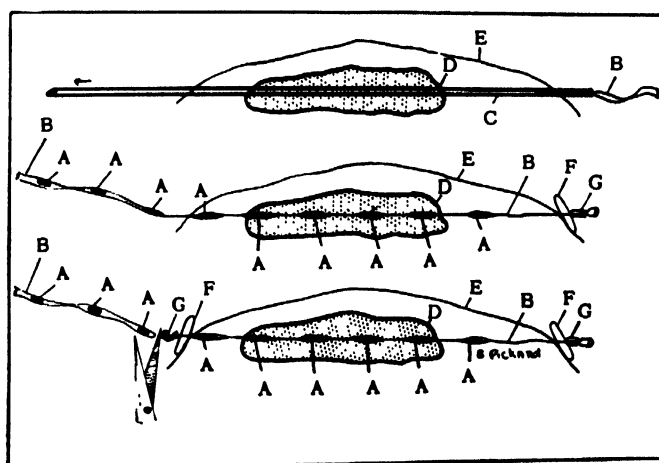


Figure 3. Steps in the implantation of nylon ribbons. (A) Ir¹⁹² sources; (B) nylon ribbon; (C) implantation needle; (D) neoplasm; (E) skin; (F) button; (G) clip

Table II. Types of Implants in the First 182 Nylon Ribbon Implants

Intra-oral implants	56
Neck implants	45
Breast implants	30
Skin implants	17
Hemangioma implants	17
Intra-abdominal implants	15

The lower drawing shows the fixation of the nylon ribbon and the cutting off of the superfluous seeds. For the fixation, one button and one tubular clip are guided along the nylon ribbon from the left side, until the button is close to the skin. The tubular clip is now squeezed with a special forceps and thus anchored securely on the nylon ribbon. Finally, the ribbon is cut with long scissors right behind the clip and a simple dressing is applied.

With practice, it takes only a few minutes from the time of pulling the active sources out of the lead container until the completion of the implantation. Furthermore, by using long instruments, the hands of the operators can stay away at least 20 cm from all sources during this time. Measurements with finger ionization chambers have shown that even with two implants daily, the permissible dose to the hands is not exceeded. The total body radiation to the operator is usually not more than 10 mrad per implant, about one-fifth of the daily permissible dose.

The technique illustrated in Fig. 3 is used in neck tumors, breast tumors, skin tumors and in all other tumors, where normal skin is present on both sides of the tumor. For implantation of intra-oral tumors, especially of carcinomas of the tonsil and of the base of the tongue, the ribbons are implanted in form of an inverted "U". The loop of the "U" lies over the mucosal surface of the lesion while the open ends pass through the main lymph node drainage area of the neck. For intra-abdominal and intrathoracic tumors, the buttons and clips are not used for fixation, because in this location, the ribbons remain in the implanted position due to the bead-like structure of the ribbon and the absence of major movements. By these modifications, the standard nylon ribbon set has become suitable for implantation of most tumors.

The nylon ribbons have also proven useful for the preparation of surface and intracavitary applicators. They can be prepared quickly and with negligible exposure from sponge rubber, into which the nylon ribbons are implanted in the same way as shown in Fig. 3. Due to the pronounced absorption of the Ir^{192} gamma radiation in heavy elements, lead inserts for selective shielding are much more effective than for radium or Co^{60} .

CLINICAL EXPERIENCE

Table II gives the number of the most frequent types of nylon ribbon implants which have been carried out from December 1952 until May 11, 1955.

In addition, nylon ribbons were used for the preparation of molds and intracavitary applications. Most of the patients had advanced and/or recurrent neoplasms and no satisfactory statistical evidence as to the effectiveness of the method can be therefore expected from this series. Clinically, the same tumor regression was observed as with comparable radium needle implants. However, our implants with nylon ribbons were more accurate than radium implants and larger areas and greater volumes could be implanted without undue exposure. These two features are hoped to lead to better results, since frequent causes of failure with radium implants are inaccurate positioning and recurrence adjacent to the implanted volume.

For illustration of the application of the nylon ribbon in various locations, one case from each group of Table II will be discussed.

Figure 4 shows an example of an intra-oral implant in two radiographic projections. Eight nylon ribbons with a total of 51 sources were inserted as a volume implant in form of an inverted "U" in the right tonsillar area for 5 days. Tumor dose was 6500 r. The loop of the "U" of each ribbon was placed over the mucosal surface of the tumor and the ends of each ribbon were brought out through the skin of the neck below the mandible. The patient was a 40-year-old housewife with a 3-cm diameter ulcerating squamous cell carcinoma of the tonsil. The tumor regressed completely and the patient appears to be free of recurrences and metastases 6 months after this implant.

Figure 5 shows an example of an implant in the neck. Fourteen nylon ribbons with a total of 144 Ir^{192} sources were implanted in one plane in the left

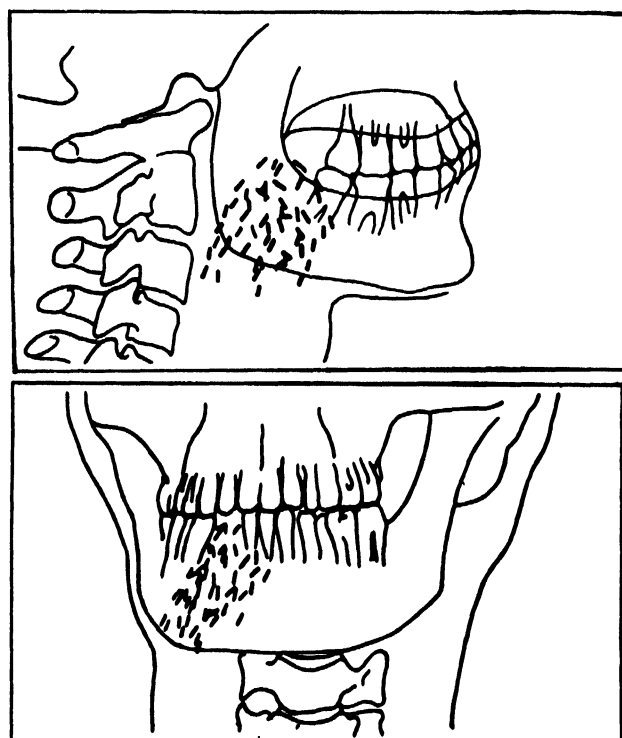


Figure 4. Intra-oral implant

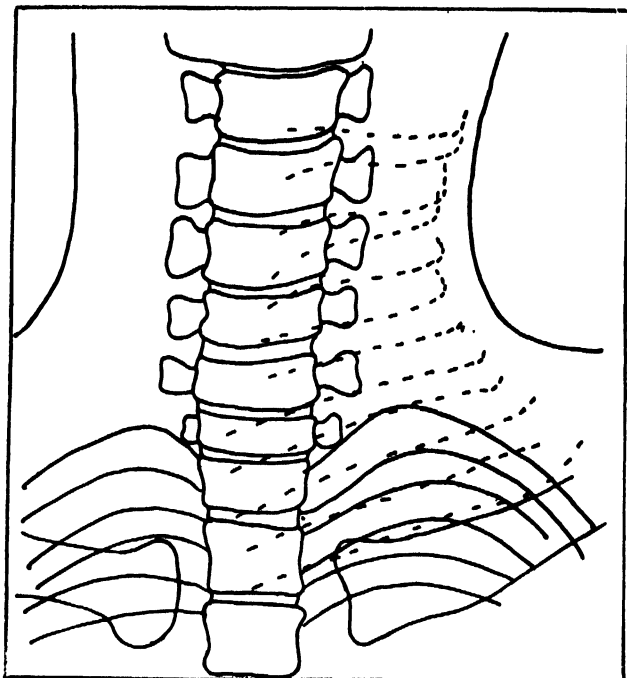


Figure 5. Implant in the neck

neck for 10 days. Tumor dose was 4500 r. The patient was a 78-year-old retired clerk, who had a recurrence in the left neck after radical neck dissection on this side 8 months ago. The primary was a squamous cell carcinoma of the right lower gingiva, which was first treated with X-ray 4 years ago and then resected by right hemimandibulectomy combined with right radical neck dissection 3 years ago. It was finally implanted for local recurrence with nylon ribbons 1½ years ago. The patient showed no evidence of disease 3 months after this implant.

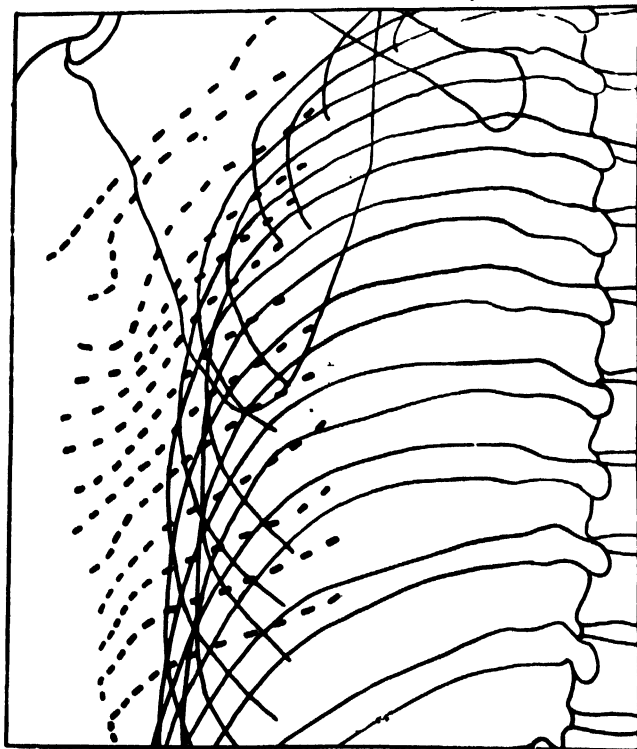


Figure 6. Breast implant

Figure 6 shows an example of a breast implant. Thirteen nylon ribbons with a total of 169 Ir¹⁹² seeds which were implanted for 7 days in one plane in the right lateral chest wall. Tumor dose was 4300 r. The patient was a 47-year-old housewife, who had a radical mastectomy for a well-differentiated adenocarcinoma of the right breast 15 months ago. Five months ago, she had a local excision of two skin metastases and 3 months ago a wide excision with grafting of the excised area for another skin recurrence. New skin nodules, which were proven metastases by biopsy, had developed adjacent to the skin graft. After the implant, the nodules disappeared and the patient is well without evidence of skin recurrences or distant metastases 8 months later.

Figure 7 shows an example of a skin implant in two radiographic projections. Six nylon ribbons with a total of 28 seeds were implanted for 7 days in one plane through the base of a squamous cell carcinoma of the skin, which measured $4 \times 4 \times 1$ cm³. Tumor dose was 7000 r. The patient was a 91-year-old retired farmer, who lived more than 100 miles away. The implant was done under local anesthesia and the patient was sent home immediately after the implant. Since the patient was only able to make one trip, the implant was removed after one week by his family physician in his home town. According to a recent report, the patient shows no evidence of recurrence or metastases 1½ years after this implant and the cosmetic results are excellent.

Figure 8 shows an example of an implant in a hemangioma in two radiographic projections. Eight nylon ribbons with a total of 42 sources were implanted for 3 days in the left side of the face around

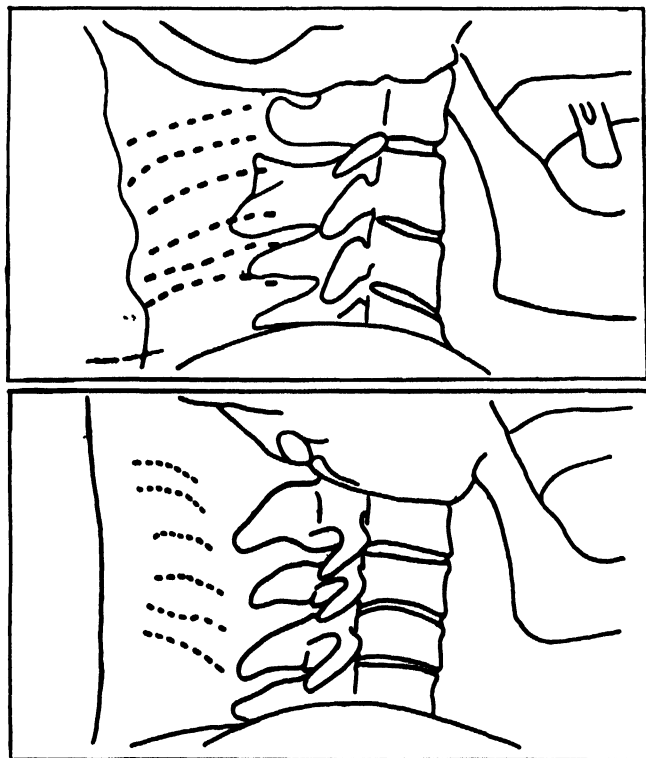


Figure 7. Skin implant

the ear. Tumor dose was 2000 r. The patient was a 3-month-old girl, who had a small hemangioma anterior to the left ear at birth. It had grown rapidly in spite of 3 X-ray treatments, involved now the whole left preauricular and postauricular area as well as the left earlobe and had ulcerated in the last two weeks. The implanted area was exactly adapted to the irregular form of the hemangioma; the implant plane was inserted in the middle of the hemangioma.

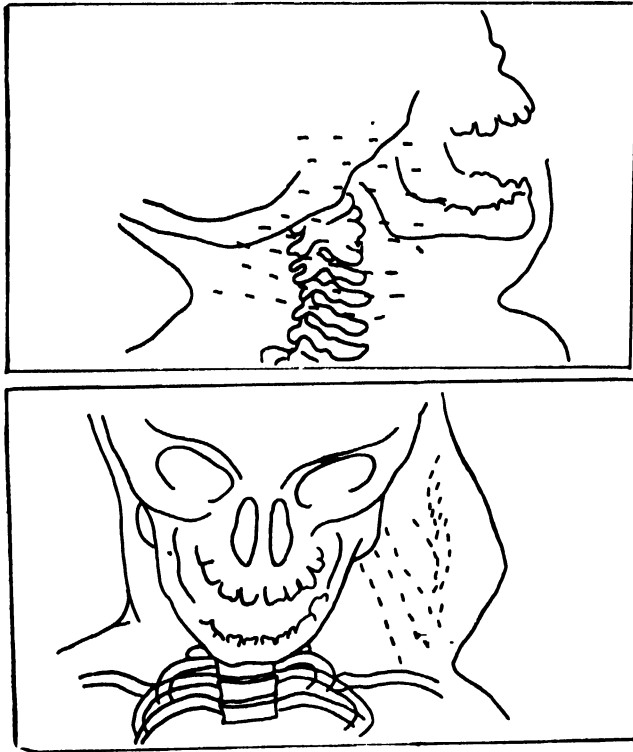


Figure 8. Implant in a hemangioma

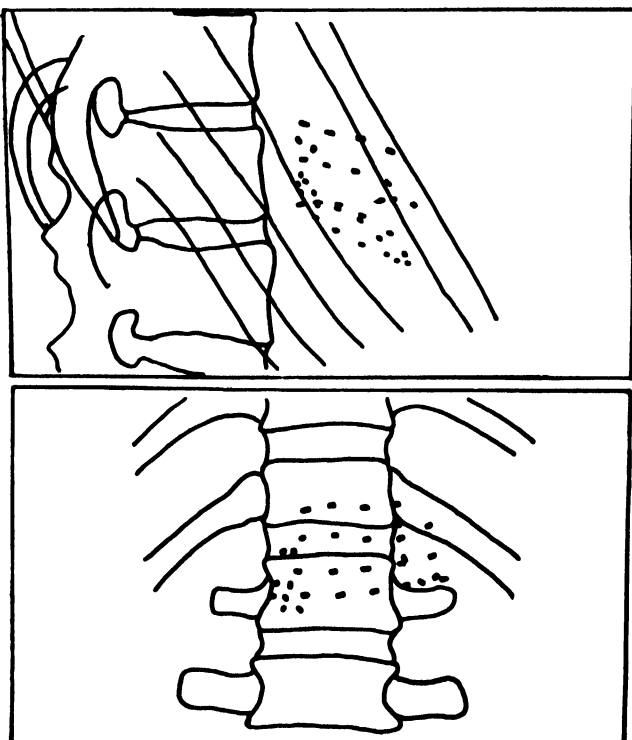


Figure 9. Intra-abdominal implant



Figure 10. Ulcerated breast carcinoma

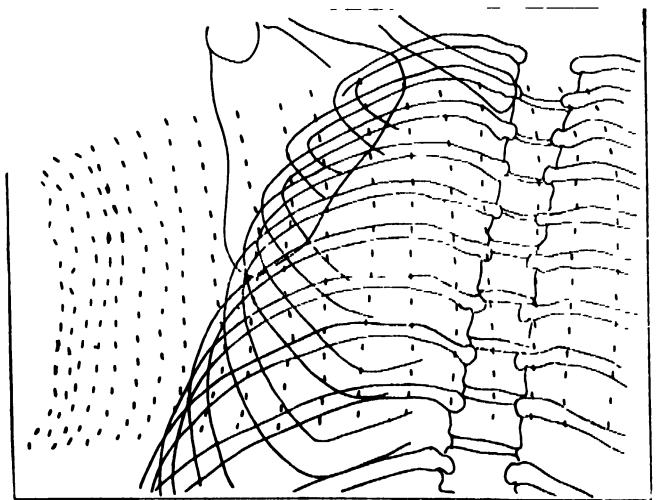


Figure 11. Implant in patient of Fig. 10

One year after the implant, the hemangioma had greatly regressed and blanched and the ulceration had healed. No further therapy was deemed necessary.

Figure 9 shows an example of an intra-abdominal implant in two radiographic projections. Six nylon ribbons with a total of 31 seeds were implanted for 10 days in several planes in a group of nonresectable nodes around the celiac vessels. Tumor dose was 7500 r. The patient was a 45-year-old clerk, who had had a resection of the colon for an adenocarcinoma of the cecum 7 years ago. He had developed excruciating pain not controllable by narcotics. At laparotomy, the only lesion was a metastatic mass around the celiac vessel, which was implanted. Three weeks after implantation, the patient's pain greatly decreased and he became able to resume his work. He shows no clinical evidence of local recurrence or other metastases one year later.

The last three pictures show the clinical response which may be accomplished with the nylon ribbon technique even in advanced carcinomas, when the usual treatment has been unsatisfactory.

Figure 10 is a photograph of a 38-year-old housewife who was treated in our hospital with a course



Figure 12. Patient of Fig. 10 1½ years after implant of Fig. 11

of X-ray therapy to an inoperable ulcerated breast carcinoma 2 years ago. After temporary regression, the tumor grew in spite of X-ray sterilization and testosterone treatment to the large size shown on the left. The patient at this time had been in bed for months and presented a difficult nursing problem due to the large foul smelling ulceration.

Figure 11 shows a drawing from the X-ray of the implant in the patient shown in Fig. 10. The implant consisted of 19 nylon ribbons loaded with a total of 285 sources. The ribbons were inserted in vertical rows spaced about 1½ cm apart. The implant stayed in place for 7 days. Tumor dose was 6800 r. The patient was then discharged to her home and was taken care of by her family physician. Hormone treatment was discontinued.

Figure 12 shows the result, which was accomplished 1½ years after the implant. The large ulceration had slowly healed and only a small area

was not yet covered by skin. No tumor was present in the implanted area but a few subcutaneous nodules were present adjacent to the implanted area. The patient was in good condition at this time and was able to take care of herself and her two children. X-ray surveys showed no evidence of distant metastases.

SUMMARY

For the interstitial implantation of radioisotopes in neoplasms, nylon ribbons have been developed, which are loaded with small Ir^{192} sources. The number of the sources can be adjusted at the time of implantation. A set of 16 of these ribbons is shipped in a small nonreturnable lead container. The ribbons can be used without special facilities or preparation at any hospital.

Compared with the usual implantation with radium needles and radon seeds, the nylon ribbons provide for more accurate implantation, more comfort to the patient and less exposure to the operator. The nylon ribbons loaded with Ir^{192} sources are also useful for the quick preparation of surface and intracavitary applicators.

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New Technique for the Therapeutic Application of Radioisotopes

By V. H. Franco, M. G. Quina and A. M. Baptista,* Portugal

The treatment of malignant tumors with radioisotopes has not corresponded to the hopes which rose with the availability of a great number of new radioelements. With the exception of a few cases where a selective absorption may lead to good therapeutic results irradiation of tumor masses has been done mostly by topical applications of the radioelements, as for example in the cases of radiocolloids, or elements which behave as such. Where a semispecific uptake is observed the degree of generalised radiation limits the activity we can use. This work is an attempt to further this limit so that we can have a therapeutic dose in a limited region without radiation damage to vital parts of the body.

PRINCIPLE OF THE METHOD

An arterio-venous circuit is established, limited to a certain body region, and a radioactive solution is injected intra-arterially. After some passages of the radioactive solution through the vascular territory the liquid is removed and the circulation re-established. In this way the radioactive atoms not assimilated by the tissues are taken out and the body is protected against the radiation effects.

The circuit, by the reasons we will give later, cannot be maintained a long while so the metabolic assimilation of the radioelement is significant only if:

1. The metabolic rhythm of the radioisotope is very fast at the tissue level we intend to irradiate.
2. The delicate tissue metabolism is not appreciably disturbed by the experimentally set conditions.

In order to get some information about the feasibility of the method the first results were obtained with somewhat simplified conditions. The radioisotope chosen was the P^{32} as sodium orthophosphate isotonic saline (Harwell) which satisfies the first condition above stated and is a pure beta-emitter with 14.3 days half-life; it is very convenient to handle. In fact, although the diffusion of the phosphate ion through the capillary walls into the extra-cellular space is somewhat slow—after an intravenous injection equilibrium is reached only about one hour¹ later—the plasmatic half-life is 30 minutes, that is, one-half of the phosphate ions of the plasm have gone

to the vascular system and are in the interstitial liquid.² Finally in this compartment the cell assimilation of the phosphorus is performed, the rhythm of this phenomenon being variable from tissue to tissue.

In the muscle, with the exception of the myocardium, the passage through the cell membrane is conditioned by an enzymatic process where intermediate compounds of the phosphorylation cycle are built before their uptake by the cell.³

In the bone an ionic exchange with the phosphorus atoms is observed, the half-life of this process being about three hours. The phenomenon in areas of rapid bone formation⁴ is different and still more active, as demonstrated by autoradiographs.

The results reported in the literature lead us to believe that we can attain a relatively high radioactivity level even if the time of contact of the radioisotope with the tissue is short.

TECHNICAL DESCRIPTION

Twelve young dogs of about 10-kg weight were used as experimental animals. The anesthetic was a mixture of barbiturates (Somnifen) intravenously injected (0.106 gm per kg). Then we proceeded as follows:

1. Exposition and separation of the femoral artery and the femoral vein.
2. Arterial puncture with a long needle, 0.7-mm diameter, or preferably a polythene tube.
3. Intra-arterial injection of 0.5 cm³ of heparin solution (Tromboliquine).
4. Circulatory interruption of this vascular territory. Immediately after the anti-coagulant injection the limb was ligatured in order to avoid the passage of the Tromboliquine into the general circulation. Tightening of the ligature was increased until the arterial pulse ceased.
5. Venous puncture with a needle similar to the one described above.
6. Establishment of the arterio-venous circuit.

Two syringes of 50 cm³ were connected to the needles through a polythene tube. The syringes were interconnected also by a polythene tube. By using clamps the connection between the two syringes or between each syringe and its needle could be stopped.

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A solution of sodium citrate and the active sodium orthophosphate isotonic saline was injected intra-arterially and simultaneously the venous blood was taken out by the second syringe.

Using the clamps, the blood was transferred to the first syringe which afterwards reinjected this blood.

The solution of blood was passed many times through the circuit during the operation for about 15 minutes.

7. Washing of the vascular territory.

Isotonic saline was injected intra-arterially and the liquid removed by the other syringe.

Washing ended when the change in the colour of the bloody liquid was very marked which indicated that almost all the blood was taken out.

8. Re-establishment of the circulation. The needle were removed and a local haemostatic (Spongostan) applied; the ligature was untied and the incision closed.

In all cases except one there were no anesthetic accidents.

The circulation was re-established, as the arterial pulse and a rapid increase of the limb temperature indicated.

The circuit described above does not correspond to the original plans which aimed at a blood arterial flow which would reproduce as closely as possible the physiological conditions. Practice, however, indicates that this type of circuit is very difficult to realize except by very complex apparatus.

Even the maintenance of a continuous flow by means of a conventional transfusion apparatus (Jouvet) or an insufflation apparatus with manometer did not succeed. The first device could not maintain a pressure sufficient to overcome the arterial resistance; the second one failed owing to the problem of the blood coagulation. Of course if the results are promising it would be worthwhile trying a more physiological method than the one we used.

The arterio-venous circuit as established changes the circulatory dynamics and originates a transitory anoxemia of the vascular territory.

There was some doubt if the mineral metabolism at the tissue level would not be disturbed by the experimental conditions.

It would be worthwhile also to try a more simple method, for example, the establishment of a venous extasis of the limb. For that reason with the circuit technique, we have used also different methods:

(A) Intra-arterial injection of the radioactive solution, the limb being ligatured to a pressure less than the maximum arterial pressure. The extasis lasts about 15 minutes and afterwards the blood is taken out through the femoral vein.

(B) Similar to the above method, that the ligature is replaced by a clamp tightened on the femoral vein. This could be a method of studying the collateral circulation.

(C) Simple intra-arterial injection without blood extraction.

RADIOACTIVE DETECTION

We follow the radioactive disintegration of the solution injected by surface measurements with an end-window G-M detector. Of course we can get some information only of the detectable volume near the skin since the range of the beta-particles of P^{32} in soft tissue is about 8 mm. However as we will show later there is a close proportionality between the activity as read by the G-M detector and the specific activity of the underlying tissues.

We used an end-window G-M detector, halogen quenched, 1.4 diameter and 2.5 mg/cm² wall thickness (Philips 18513), shielded laterally. The background was only 7 cpm. The counter was placed in contact with the skin after the hair has been shaved.

EXPERIMENTAL RESULTS

Figure 1 shows the time variation of the activity from tissue surface measurements over different body regions. The activity administered was 0.22 mc and method B was adopted, that is, an extasis of about 20 minutes and a clamp on the femoral vein. It must be said that with all the methods excepting method C the curves were identical.

Figure 2 shows the relation between activities measured in symmetrical parts of the limb subject to the experiment and the opposite one. Again we have not noticed any appreciable difference between the three methods.

Also the effective half-life in all cases was about 10 days, and the activity-time curves show two exponentials with about 6 hours half-life at the beginning and the 10 days half-life starting from about 2 days after the injection.

One of the points to be studied was the washing efficiency.

With method A, that is, a ligature establishing a pressure less than the maximum arterial pressure, we have noticed that although the femoral vein was completely collapsed at the moment of the injection of radioactive solution, the venous flow proceeded into the general circulation, easily detected by the radioactive measurement in different body regions.

The beginning of Fig. 3 shows this phenomenon, detected on the precordium. The curve shows that immediately after the re-establishment of circulation a slight inflexion downwards corresponds to the washing period (blood removed via the femoral vein).

This inflexion downwards can be explained, we think, by three mechanisms.

The first one is the diffusion of plasmatic phosphorus into the extra-cellular space and from here into the tissues. The second one is the stopping of the venous flow into the general circulation, made before by the collateral venous circulation, which was due by the now easier flow through the femoral vein during the washing period.

The third mechanism consists of the washing itself made with the animal's own blood. It was a real bleeding which removed 90 cm³ of radioactive blood.

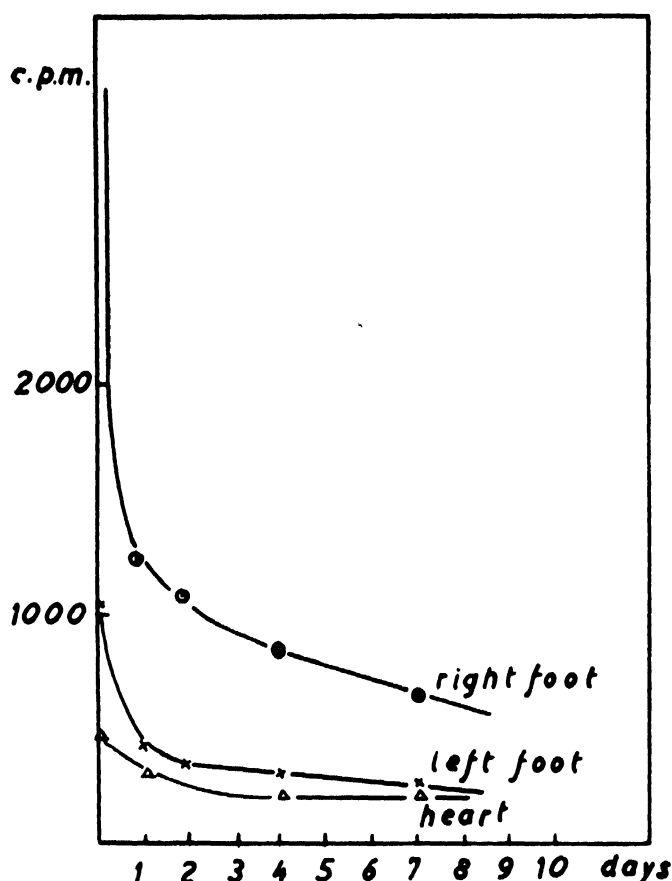


Figure 1. Activity-time curves in different parts of a dog after an extasis of 20 minutes in the right limb with a solution of sodium orthophosphate (0.22 mc of P^{32})

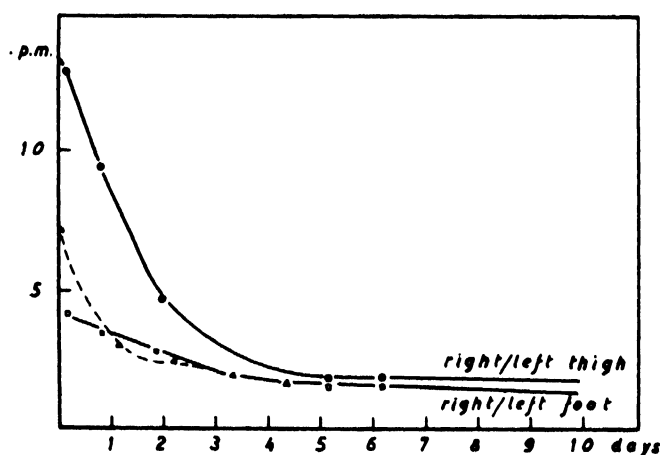


Figure 2. Activity relations in thighs and feet of a dog where an arterio-venous circuit was established in the right foot. The dotted curve was obtained in another dog where an extasis with ligature was made for 20 minutes

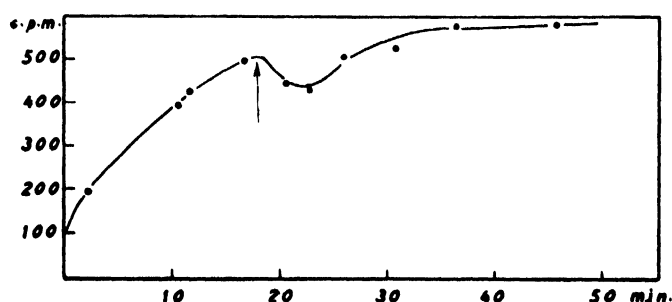


Figure 3. Washing efficiency. Extasis for 15 minutes with ligature and the activity measured on the pre-cordium. The arrow marks the point when circulation was re-established

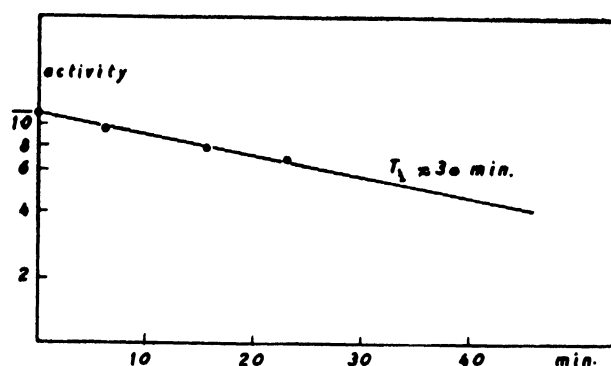


Figure 4. The half-life of the passage of the extra-cellular radio-phosphorus of the studied region into the general circulation

The efficiency of the washing is satisfactory since the general trend of the curve is not disturbed by the re-establishment of circulation.

Since the activity-time curve is horizontal after 40 minutes this shows an equilibrium between the extracellular and the total diffusion of phosphorus plus the flow from the region subjected to the experiment.

The radiophosphorus which flows from this region comes from the extracellular space where it went during the experiment. As soon as the circulation is re-established this phosphorus is taken by the tissue cells and the blood where the concentration is lower than in the extracellular space.

Figure 4 shows the initial part of this phenomenon with a 40-minute half-life.

By using method C, that is a simple intra-arterial injection of the radioactive solution, we noticed a slightly higher uptake of radio-phosphorus (1.3 times) in the limb subjected to the experiment, compared with the opposite one. This higher uptake was noticeable during various days.

As we said before it was important to know if there was a correlation between the tissue surface measurements and the specific activity of the tissues.

For this purpose two animals were killed after a radioactive injection and an extasis of 20 minutes (method B).

The results are presented in Fig. 5 where the activity of identical tissues in the opposite limb not subjected to the experiment is taken as unity.

Comparison of Fig. 5 with Fig. 2 shows that the tissue surface measurements may give some information about the specific activity of underlying tissues.

CONCLUSIONS

It is difficult to compare our first results and the ones reported by the physiologists. Ends and means are very different. Our data point out that there is an appreciable quantity of the radiophosphorus which went to the tissues of the region studied and remained there as an ordinary cell metabolite.

Our results were expressed as dynamic relations. This means that it was not possible to avoid passage of the radioisotope into the general circulation. This point deserves comment. The activity-time curves

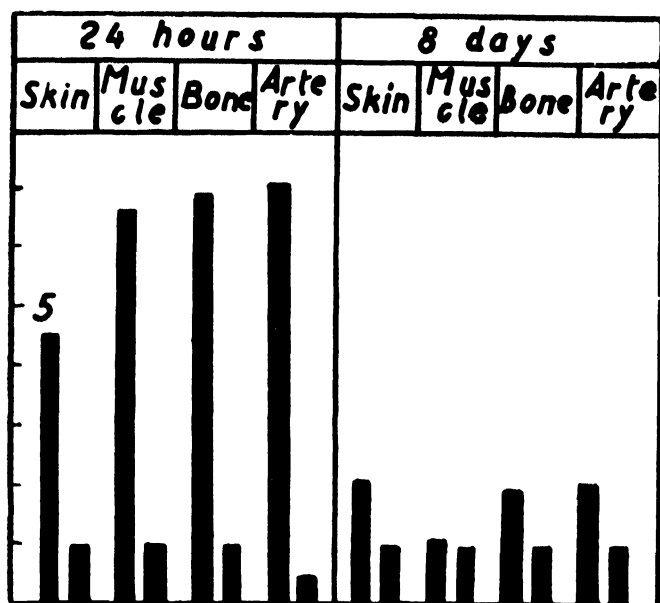


Figure 5. Specific activity relations between different tissues of two dogs killed 24 hours and 8 days after an extasis with ligature for 20 minutes. Specific activity of the tissues of the opposite limb is taken as unity

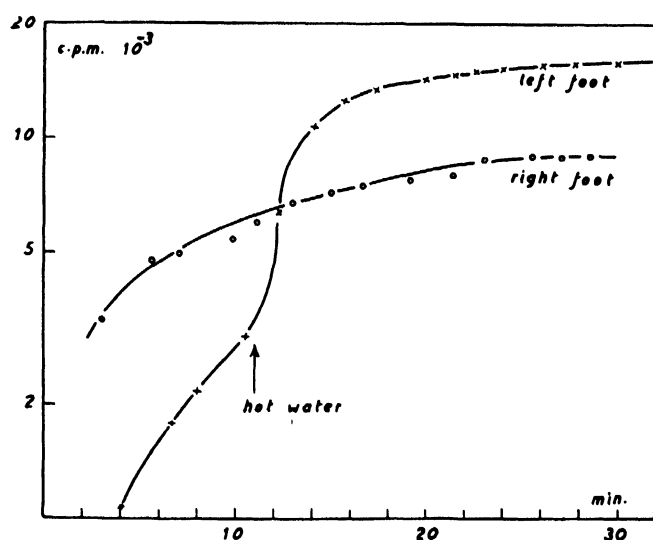


Figure 6. In a semi-logarithmic curve is shown the local heating effect on the radioactivity concentration of radiophosphorus in the left foot of a patient. Comparison made with the right foot

show that the transfer of radiophosphorus can be done by 4 mechanisms: (a) during the experiment due to an insufficient ligature pressure; (b) ineffective washing of the blood vessels and subsequent removal, as soon as the circulation is re-established, of the stored radiophosphorus; (c) passage of phosphorus from the extracellular space into the plasma at the end of the experiment; (d) by the cellular turnover of phosphorus.

We have seen that even if it is possible to avoid the passage of the radiophosphorus into the general circulation by the (a) and (b) mechanisms, the other two are independent of the experimental conditions and are always present.

We can consider, as the limit of a therapeutic application of a radioisotope by this method, the maximum dose that, applied to a certain vascular region where the tumor is, gives to the whole body a fraction not higher than the tolerance dose of the patient.

Our aim with the present work was limited to the intrinsic possibilities of the technique. We believe that the results can be effectively improved if in a therapeutic application we use a certain amount of apparatus and a radioisotope specifically chosen for the case at hand.

For example: an arterialization apparatus which not only could keep the physiological haemodynamic conditions but also could increase the time of contact between the radioisotope and the tissue, because in this case the therapeutic application is not so time-dependent.

It seems also from some results of this laboratory⁵ that we can artificially increase the intracellular radioisotope concentration.

So, for instance, Fig. 6 shows the almost two-fold tissue activity increase due to local heat application on the left foot of a patient after the administration of a therapeutic dose of radiophosphorus.

The effective half-life of the P^{32} in this region was 12.5 days, a value like that of the opposite foot.

A previous arteriography could inform us about the vascular dependency of the tumor. The possible introduction of a polythene tube up to the artery level which irrigates the neoplasia would allow a selective radioactive application.

Finally a radioisotope more adequate to the problem could be used.

For instance in bone tumors Ga^{72} or Sr^{90} could be used, which can be selectively concentrated by the bone in significant percentages of the administered activity.

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A Sr^{90} Surface Applicator Designed for Treatment of Corneal Lesions and Evaluation of Biological Effects

By H. L. Friedell, C. I. Thomas and J. S. Krohmer,* USA

Beta rays for therapeutic purposes about the eye have been utilized for a number of years and considerable experience has been gained with this form of therapy.^{1-5,8-12} The use of beta radiation rests upon the desire to exploit the unique physical characteristics of beta rays, one of which (rapid absorption in tissue) limits the radiation to a few millimeters of the surface structures, thereby avoiding damage to deeper tissues. Until recently, beta-ray sources were dependent entirely upon naturally occurring radioactive elements and were fashioned from radium and its daughters, most of the applicators being made from radon. With the development of nuclear piles or nuclear reactors, large amounts of artificial radioisotopes, some of which are particularly useful as beta-ray sources, have become increasingly available.

In our own institution we have, previously, utilized radon as a source of beta rays. However, we have considered the radon applicator unsatisfactory because of the short half-life and the subsequent need for frequent replacement and also because of the inherent difficulties in standardization of sources.

A careful review of the beta-ray emitters which were available either from natural sources or by artificial production in the nuclear piles was, therefore, made. The following table (Table I) lists those elements which have a half-life of at least two weeks and which appear suitable for this purpose.

In selecting an element as a beta-ray emitter, the following criteria appeared applicable:

1. Sufficiently long half-life to avoid frequent replenishment of the element.

2. Sufficient energy of the beta particle to permit effective penetration in tissue.

3. Absence of accompanying gamma radiation and ease of protective measures.

As a result of this survey, the isotope Sr^{90} , having a daughter product Y^{90} , was chosen. The long half-life (twenty years), the energetic particle (2.23 Mev peak), the absence of nuclear gamma rays, and the comparative ease of manipulation, seemed to satisfy the requirements which we had established.

Ru^{106} - Rh^{106} series, and the RaD - RaE series, also appear promising as beta-ray sources because of their long half-lives. However, the beta rays from RaE have an average energy of 0.33 Mev, corresponding

to a range of 0.9 mm in water. This limits the usefulness of this element since it can be used only for very superficial irradiation. The Ru^{106} - Rh^{106} series has a considerably shorter half-life than the Sr^{90} series, and also has associated with it an appreciable amount of gamma radiation.

PHYSICAL MEASUREMENTS

The present paper was prepared using data obtained from experience with two Sr^{90} applicators of significantly different construction. The first of these^{1,2,8} was constructed at this institution and was used for about six months before being discarded because of leakage. It was unique in that it had an inherent filtration of about 100 mg/cm², a filtration which absorbed a large percentage (50 per cent) of the emitted beta particles. The second⁸ was a pilot model applicator which was first used in October, 1949, and is still in use after more than five years. This latter applicator differs from the first mainly in inherent filtration, having a window thickness of only 20 mg/cm². In addition to these two applicators, several other commercial models were subjected to physical measurements and biological checks, but were not used clinically.

Initial physical measurements conducted upon the first applicator consisted of comparing film blacken-

Table I

Isotope	Energy in Mev		Half-life
	Beta rays	Gamma rays	
P^{32}	1.69	None	14.3 d
Rh^{86}	1.60		19.5 d
Sr^{89}	1.50	None	55 d
Sr^{90} series	0.65	None	20 yr
Y^{90}	2.23	None	62 hr
Y^{91}	1.53	None	57 d
Ru^{106}	Very soft	None	1 yr
Rh^{106} series	3.55 (82%) 2.30 (18%)	1.25 (1%) 0.73 (17%) 0.51 (17%)	30 sec
RaD	0.025	0.047	22 yr
RaE series	1.17	None	5 d
Rn	None	None	3.825 d
RaA series	None	None	3.05 min
RaB	0.65	None	26.8 min
RaC	3.15	1.8	19.7 min
UX1 series	0.13	0.092	24.1 d
UX2	2.32	0.802	1.1 min

* Western Reserve University.

Table II. Depth Dose

Depth, mm	Applicator					RaD + E (2)
	Ru ¹⁰⁶ (1)*	Sr ⁹⁰ (9)	Radon Burnham (3)	Radium (2)	Radon Hughes (2)	
0	100%	100%	100%	100%	100%	100%
1	55%	41%	31%	20%	8%	10%
2	30%	19%	19%	10%	2%	1%
3	17%	9%	6%	5%	1%	—
4	10%	4%	3%	2%	0.5%	—
5	6%	1%	1.5%	1%	0.3%	—

* Figures in parentheses indicate number of applicators measured. Depth doses shown are averages for all applicators measured.

ing in relation to the blackening produced by representative radon bulbs. All subsequent calibrations have been made using a specially designed extrapolation ionization chamber^{6,7} which allows calibration in terms of the rep (roentgen equivalent physical), a unit which is directly related to the roentgen. The first applicator was calibrated only once with this ion chamber, but the second has been measured at frequent intervals since its first use. The surface dose values obtained for the latter applicator (30 rep/sec) upon repeated calibration are in good agreement, having a spread of only ± 5 per cent from the mean. The depth dose curve for this applicator has been unvarying and is nearly identical to the curve obtained for the first applicator which had a much higher inherent filtration. The depth dose curves of other commercial Sr^{90} applicators have been measured and show essentially the same fall-off.

Depth dose measurements for the Sr^{90} applicator and other applicators are shown in Table II.

It should be noted that the depth dose for various applicators differs appreciably; Ru¹⁰⁶ having the most energetic beta rays is by far the most penetrating, RaD + E having the least energetic beta particles shows the greatest fall-off in depth.

For the clinical use of any of these applicators, a full understanding of the depth dose is essential. Obviously, various levels of the cornea will receive different doses for the same surface irradiation, and for the same exposure, lesions deep in the cornea receive much smaller radiation doses than lesions close to the surface. In our experience, slit lamp examination has been invaluable in determining the depth of the process and, therefore, estimating the amount of radiation necessary for adequate treatment.

BIOLOGICAL CALIBRATION

In order to understand the biological effectiveness of the Sr^{90} beta-ray applicator, an attempt was made to calibrate the applicator on various tissues. The effect on the skin appealed to us immediately since this had previously been used for various types of ionizing radiation and the general characteristics of erythemas of the skin are fairly well known.

Skin erythemas were produced with various beta-ray applicators as well as with the roentgen ray (44

kv Philips contact unit).⁶ The beta-ray applicators which were included were Sr^{90} , RaD + E and Ru¹⁰⁶. These tests have shown that the skin reaction for equal radiation dose (as determined by independent means of calibration) using any of these sources is quite comparable. The energy of the beta particles from these sources differed considerably and the results would therefore suggest that differences in beta-ray energies in this general range had no effect on the biological reactions of the skin. It would indicate further that the extrapolation calibrations are valid and that dosages recorded in reps for the beta-ray applicator appear to approximate the dose in roentgens of a low voltage roentgen ray.

Skin studies alone would not make it possible to predict the results of radiation of the normal or vascularized cornea. However, the skin studies help considerably in relating the beta radiation dosage used for various eye conditions to radiation dosage used in general therapeutic irradiation. Therefore, the effect on the normal cornea as well as vascularized corneas was also studied. Our experience with these tissues is much more limited. However, we were able to establish definite changes in the cornea after irradiation. The radiation effects on corneal tissues must be interpreted with great caution. Like all tissue that is avascular and high in collagen content, the stroma of the cornea is very resistant to radiation and can be exposed to extremely high doses without obvious evidence of injury. It is for this reason that the normal cornea appears unsatisfactory for evaluating the biological changes at comparatively low doses of radiation. The cornea will withstand high levels of radiation without injury and then at the highest doses, very abrupt changes with marked corneal effects will occur. F. M. Wilson¹¹ has shown that the minimal inflammatory reaction is noted only after exposure to 35,000 rep. We have made essentially the same observation. However, in all our studies, the epithelium showed radiation effects at considerably lower dosage levels, and the limbus, which often received comparable doses to the cornea, also showed marked cellular effect.

Vascularized corneas were also studied. The data here are preliminary but the response to radiation appeared much earlier than in normal corneas. In this situation there was an extensive infiltration of fibro-

blasts and young vessels into the substance of the cornea, producing a general rearrangement of the stroma and a separation of the corneal fibrils. In vascularized areas, the vessels, after a comparatively short latent period, would suddenly break up with punctate extravasations of blood. The cornea would show no effects at this time, indicating that the vascular tissue was much more sensitive to radiation than the cornea proper. Our studies, both in humans and animals, lead us to believe that infiltrations of young fibroblasts and vascular buds are far more sensitive indicators of radiation effect. Unfortunately, this change is not easy to produce and to measure in experimental animals. It is our opinion that although excellent studies of both normal and vascularized cornea may be made, this structure is not responsive enough for biological calibration.

CLINICAL DATA

The method of treatment of corneal lesions consists simply of anesthetizing the eye with 1% Pontocaine, immobilizing the lids, and applying the applicator directly with full contact to the corneal process and has been discussed previously.^{1,3,2} In corneal disease, treatment is given along the limbus to affect the vessels near their origin. It is imperative that the center of the cornea be avoided whenever possible. Fractionation was practiced in every instance with the radiation given at weekly intervals. The output of our applicator was 30 rep per second, thus delivering 1800 rep in 60 seconds.

In this institution, treatment of eye lesions has been a cooperative effort between the Departments of Ophthalmology and Radiology in an attempt to obtain the best evaluation of the treatment. The ophthalmologist is of the greatest assistance in the use of the slit lamp for estimation of the depth of the vascularization and the character of the vessels. In addition, the appearance of various eye lesions to the uninitiated is confusing and requires the careful attention of someone familiar with corneal disease.

Fractionation of the radiation dose is one of the cardinal tenets of general radiation therapy, and we adopted this procedure for use in the treatment of eye lesions as well. Because of the problems of continually replenishing and refabricating our original radon source, fractionation was highly impractical and was not followed and the desire to fractionate the dose was one of the important reasons for development of the Sr^{90} applicator.

In the treatment of our cases, one week was usually employed as a fractionation interval, but there is no evidence to indicate that this is an optimal one, and it may well prove that shorter periods between treatments are superior. Fractionation permits one to assess the effectiveness of the radiation and to terminate the treatment if the response is prompt, or to prolong and increase the treatment if the response is slow. This permits some individualization of each case and generally provides for the lowest effective dose.

The difference in the sensitivity of various pathological processes also deserves consideration. This, too, is one of the important principles of radiation therapy and an evaluation of the radiosensitivity of the process to be treated is extremely desirable. It may be stated that on the whole, poorly differentiated, rapidly growing structures are more sensitive to ionization radiation of all types, and newly formed vessels and young fibroblasts which appear in many corneal processes fall into such a category. Combining an estimate of the radiosensitivity of the corneal process and the fractionation of the radiation dose generally permits the smallest practicable dosage levels.

The following table (Table III) summarizes the cases treated, the results of treatment and the radiation doses employed.

In assessing the usefulness of the applicator, in our hands it has had the most value in controlling vascularization both prior and following keratoplasty. Unless the vessels are controlled, the keratoplasty will not succeed. Following corneal transplantation, the newly formed vessels and fibroblasts which invade the cornea are effectively treated with comparatively small radiation doses and herein appears the most important application of the device.

The next most frequent use of the applicator has been in the case of pterygia and with this process the applicator has proved most successful when used post-operatively. Pterygia may be treated with beta rays alone, but this should be reserved for thin, comparatively new lesions. A long standing chronic well established pterygium requires unusually high radiation dosages and surgery plus beta radiation to prevent recurrences appears to be the method of choice.

New growths, such as papillomata, seem to respond exceptionally well to radiation as does Bowen's disease. However, malignant lesions that are fairly thick, as basal cell carcinoma of the lid, should not be treated with this applicator. Ordinary radiation would appear to be preferable.

Vernal catarrh responds very well to radiation and comparatively small doses are necessary. The applicator appears very useful in this condition and seems to control the follicles quite well. In long-standing chronic cases of vernal catarrh, or where the process is continually recurring, considerable attention should be paid to the mounting radiation dose which is often required. If the lesion does not respond to comparatively modest doses, other forms of therapy should be introduced.

We have observed no complications of any consequence with this applicator. The possibility of producing a cataract must always be kept in mind. We have estimated that the surface of the lens would receive less than 10% of the dose delivered to the cornea. If one calculates the average dose given to the lens, assuming the lens to be 3 millimeters in diameter, it proves to be approximately 3% of that given to the corneal surface. The Sr^{90} applicator has

Table III. Summary of All Cases Treated Over a Period of 5 Years with Sr^{90} Applicator

Diagnosis	No. of cases	Dosage			Results*			
		Low	High	Average	0	+	++	+++
1. Vascularization								
a. Superficial	37	325	12,000	6000	7	6	22	2
b. Deep	12	1950	16,575	10,800	1	2	8	1
c. Mixed	16	2975	21,500	10,800	0	4	11	1
2. Pterygium								
a. Beta radiation only	8	325	16,000	12,000	3	1	4	0
b. Surgery and post-operative beta radiation	12	1300	14,800	4000	0	0	8	4
3. Vernal catarrh								
a. Palpebral	11	1800	16,950	9000	1	1	3	6
b. Limbal	4	3250	9600	6000	0	0	2	2
4. Tumors								
a. Papilloma	11	450†	21,600	6000	1	0	2	7
b. Hemangioma	3	1320	3000	2400	0	2	1	0
c. Basal ca. of lid	1	3900	—	—	0	0	0	1
d. Bowen's disease	1	1950	—	—	0	0	1	0
e. Chalazion	5	650	5300	2600	0	0	5	0
5. Infection								
a. Tuberculous sclerokeratitis	5	1800	7375	5400	0	0	0	5
b. Chronic conjunctivitis	1	17,800	—	—	0	0	1	0
c. Epidermic keratoconjunctivitis	1	2400	—	—	1	0	0	0
d. Disciform keratitis	1	650	—	—	1	0	0	0
6. Miscellaneous								
a. Filamentary keratitis	3	1950	2275	2000	3	0	0	0
b. Irritable scar	2	4500	5700	5000	0	0	1	1
c. Epithelialization of anterior chamber	1	2825	—	—	0	0	1	0
d. Epithelial dystrophy	1	650	—	—	1	0	0	0
e. Conjunctival cyst	1	5200	—	—	0	1	0	0

* Results: 0 No change, + Fair, ++ Good, +++ Excellent.

† Lesion disappeared with single small treatment.

been used for only four years and it is still possible that lenticular opacities might appear later. However, with our radon applicator which was previously in use for over ten years and which gave much larger doses to the lens, no evidence of cataract formation has been observed. It has been estimated that for equal doses to the corneal surface, the radon applicator would deliver twice as much to the lens as the Sr^{90} source. This is due to the additional gamma rays from radon.

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Biological Studies on Calcium, Strontium, Lanthanum and Yttrium

By D. Laszlo,* US

Many factors have contributed to the great surge of interest in the metabolic fate of stable and radioactive metals in man. The advent of tracer techniques has opened new avenues for investigating minerals and trace metals present in the human body. The anticipated widespread use of atomic energy for industrial purposes created a demand for the study of new occupational hazards due to radioactive metals. Furthermore, the availability of many artificial radioisotopes has stimulated medical investigators to search for the application of isotopes in diagnosis and treatment, mainly of cancer. An extensive investigative program has been conducted for several years at this institution on the metabolism of stable and radioactive metals in man. Some of the results obtained have been recently published. Selected data on calcium metabolism using Ca^{45} as a tracer, on the metabolism of the fission product radiostrontium, on biological applications of radioactive rare-earth elements, and on the effect of chelating agents upon radiometal metabolism will be presented here.

I. UTILIZATION OF DIETARY CALCIUM AND CALCIUM HOMEOSTASIS

Data on the plasma disappearance rate, on the urinary and fecal excretion and on the uptake of radiocalcium administered orally or intravenously to normal subjects and to patients with various bone disorders have been previously reported.^{1,2} A technique was recently described to measure the utilization of dietary calcium, to partition the stool calcium into an endogenous fraction (digestive juice) and exogenous fraction (unabsorbed food) and to estimate the total digestive juice calcium in man.³

This technique was applied to two normal subjects on whom a three months metabolic study was conducted. A standard low calcium diet containing approximately 150 mg calcium/day was given throughout the entire study. The calcium intake was then raised by the addition of calcium gluconate approximately 5-fold in the second month, and 12-fold in the third month of the study. At the start of the experi-

nent a tracer dose of Ca^{45} was administered orally to both patients. Data obtained by metabolic balances and by the radiocalcium technique are summarized in Table I.

The calcium balances were slightly negative in both patients as is usually observed on a low calcium intake. With the increase in calcium intake the balances became progressively more positive and were +610 mg and +874 mg/day at the highest intake level for the two patients respectively. The urinary calcium excretion of the two subjects was in the normal range. While the urinary calcium increased only slightly at higher calcium intake levels, the stool calcium content increased significantly and progressively. Whether this high stool calcium content was due to food calcium which passed unabsorbed through the digestive tract or due to larger amounts of calcium excreted with the digestive juices into the intestinal canal was clarified by the radiocalcium technique. The figures in column 8 of Table I are the fraction of stool calcium contributed by the digestive juices: endogenous fecal calcium. The values for the two subjects are: 91 and 118 mg on low calcium intake, 91 and 67 mg on medium high, and 73 and 93 mg on high calcium intake. Therefore, the increase of dietary calcium did not significantly change the endogenous fecal calcium so that the high stool calcium is due to unabsorbed food calcium.

The utilization of dietary calcium was calculated as follows:

$$\frac{\text{absorbed food calcium}}{\text{calcium intake}} \times 100$$

whereby absorbed food calcium = calcium intake — (fecal calcium — endogenous fecal calcium).

For Patient B the calcium intake was 107 mg/day during the first month, the absorbed calcium was 47 mg/day or 44% of the intake; on a medium high intake 41% and on the high intake level 54% were utilized by this patient. The corresponding values for Patient L were 67, 69 and 61% respectively.

If amounts of calcium are offered which are in excess of the requirements, the body has several mechanisms to deal with this excess load: it can reduce intestinal absorption or it can increase renal calcium excretion or calcium excretion with the digestive juices. Since neither the urinary calcium nor the endogenous fecal calcium increased significantly in

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Table I. Utilization of Dietary Calcium. Metabolic and Ca^{45} Studies in Two Normal Subjects

Calcium mg/24 hours				Digestive juice calcium				
Intake	Urine	Stool	Balance	S.A.* stool S.A.* plasma	Total	Re- absorbed	Endogenous fecal calcium	Utilization, % Ca^{45} -technique
Patient B.								
107	90	151	-134	0.60	160	69	91	44
529	85	408	+ 36	0.23	158	67	91	41
1446	103	733	+610	0.10	160	87	73	54
Patient L.								
135	51	162	- 78	0.73	365	247	118	67
638	66	278	+294	0.30	273	186	67	69
1740	92	774	+874	0.12	237	144	93	61

* S.A. = Specific activity. The balances and Ca^{45} -studies represent averages of 4 six-day metabolic periods for each calcium intake level.

the two subjects when the calcium intake was raised over 10-fold, it may be assumed that the calcium homeostasis is maintained in part through a mechanism which regulates the rate of calcium absorption from the intestinal tract. Information is available that in certain physiologic and pathologic conditions such as growth, aging and malnutrition, the utilization of dietary intake may vary according to the requirements of the body. For instance, in osteoporosis of the aged, the utilization seems to be considerably lower than in young adults.

II. METABOLISM OF STRONTIUM (Sr^{85}) IN MAN

Carrier-free Sr^{85} was administered by the intravenous and by the oral route in doses varying from 0.2–4.0 $\mu\text{C}/\text{kg}$ body weight to six human subjects.

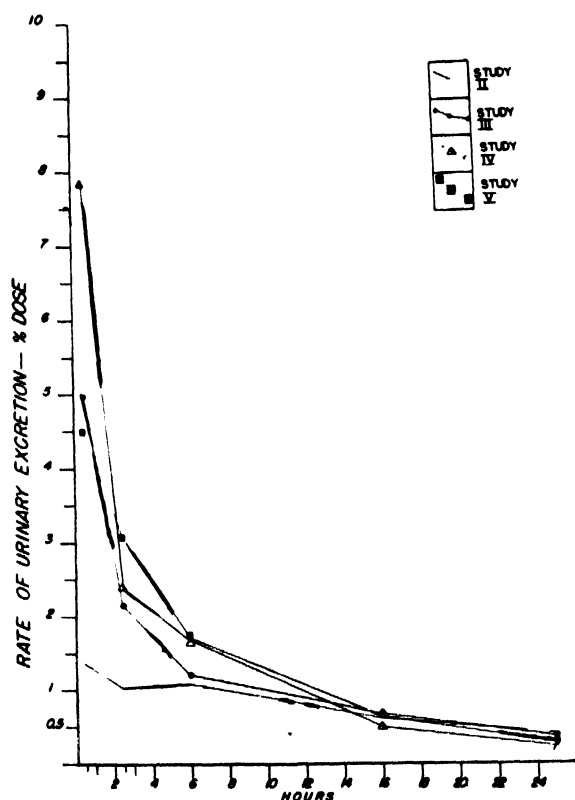


Figure 1. Effect of calcium upon the rate of urinary strontium-85 excretion. Calcium gluconate administered in studies III, IV, and V

In this report the data of five consecutive studies will be described which were performed on the same subject in order to minimize biological variations. In each of these studies the dose of strontium was approximately the same, namely, 0.2 $\mu\text{C}/\text{kg}$ body weight. In studies I and II the dose was rapidly injected intravenously through the distal end of the tubing of an infusion set through which 250 ml of 5% glucose in water were administered in two hours, the tracer being injected at the mid-point of the infusion. In studies III, IV and V the experimental arrangement was the same except that calcium was added to the infusion in order to study its effect upon strontium excretion. In study III, the infusion contained 50 ml of 10% calcium gluconate (469 mg Ca^{++}), in study IV, 100 ml 10% calcium gluconate (938 mg Ca^{++}). In study V, 100 ml 10% calcium gluconate were infused in six hours, the tracer being injected at the end of the first hour of the infusion. Blood and urine samples were taken at frequent intervals during the first 24 hours while subsequently the twenty-four hour urinary Sr^{85} excretion was determined and only one blood sample was obtained daily. The duration of the studies varied from 8 to 15 days at which time the levels of radioactivity had declined considerably. The total urinary strontium excretion of the first day and the Sr^{85} content of the total plasma volume at the end of this day, expressed in percent of the administered dose, are listed in Table II. The urinary excretion rates of Sr^{85} of the first 24 hours in studies II–V are graphed in Fig. 1. Study I is omitted because the results were similar to study II. Table II and Fig. 1 show that the simultaneous administration of calcium resulted in marked enhancement of the rate of urinary strontium excretion in the first 24 hours of the experiments. No enhancement of fecal strontium excretion was noted.

III. TREATMENT OF PLEURAL AND PERITONEAL EFFUSIONS WITH Y^{90} IN CANCER PATIENTS

Biological studies on stable and radioactive rare earth compounds have been carried out for several years at this institution. The interest in the fifteen

rare earth elements arose from several considerations: they behave similarly chemically while their numerous isotopes have a broad spectrum of different radiation characteristics. If any one of these elements could be shown to localize in a specific area, the most suitable radioisotope could then be chosen to deliver internal radiation. Furthermore, Hammarsten reported in 1924 that lanthanum formed a complex with nucleic acids which is insoluble in the physiological pH range.⁴ An interaction of lanthanum and other rare earth elements with nucleoproteins of rapidly growing cells such as of tumor cells would be of considerable interest.

The desoxyribonucleic acid complexes of lanthanum and of other rare earth salts were investigated and the data were previously reported.⁵ Inhibition of bacterial growth by stable lanthanum was observed.⁶

The effect of lanthanum on respiration and glycolysis of ascites tumor cells was investigated.⁷ The distribution and excretion of lanthanum compounds administered in ionic and chelated form were studied in normal and tumor-bearing animals and in cancer patients.^{8,9} It was observed that lanthanum when administered in ionic form in the presence of a sufficient amount of carrier remained localized at the site of the injection. The conditions which are most suitable for the localization of intraperitoneally injected lanthanum were studied in normal and ascites tumor-bearing mice: the amount of carrier was shown to have a profound effect upon the localization of La^{140} and only small quantities of radiolanthanum escaped from the injected cavity into the general circulation. Good localization was noted for Y^{90} by Kyker¹⁰ and by Andrews *et al.*¹¹ The formation of radio-colloids of rare earth elements with tissue constituents and good localization was also noted for Lu^{177} , Ho^{166} , Y^{90} and Y^{91} by Kyker.¹² These authors observed that "heterogeneous" carriers of the rare earth group were as effective as "homologous" carriers in localizing the isotope. The elements which these authors investigated were Lu^{177} , with lanthanum, yttrium and samarium carrier; Ho^{166} with lanthanum and yttrium carrier; and Y^{91} with neodymium carrier.

Since good localization of rare earth isotopes was noted on intraperitoneal injection into ascites tumor mice, its effect upon the growth of this tumor was

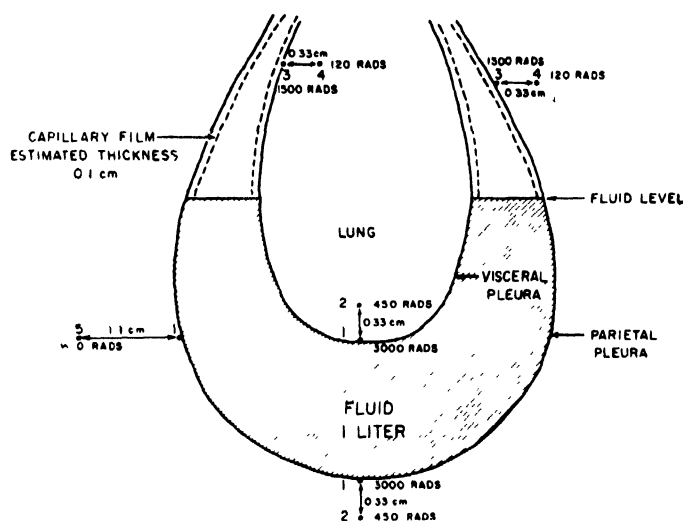


Figure 2. Integrated dose delivered to the walls of the pleural cavity following administration of 30 mc Y^{90}

investigated. A significant prolongation of the survival of these animals, inhibition of cell multiplication and of fluid formation were noted.⁹ The localization of the isotope in the injected cavities was also confirmed in cancer patients.

Since Y^{90} , although strictly not a rare earth element, has more favorable radiation characteristics for intracavitary use, than La^{140} , having a 64 hour half-life, a pure β -spectrum and a maximum penetration of 10 mm, tracer studies similar to those with lanthanum were carried out. Favorable localization of Y^{90} in the presence of carrier was noted.^{13,14,15} Y^{90} , 0.3–4.0 mc and 100 mg Y^{+++} as the carrier was injected as the chloride intrapleurally to 11 cancer patients. Blood levels, urinary and fecal excretions were measured up to twenty days. Y^{90} concentrations in the fluid of the injected cavity and in other body fluids and tissues, whenever available, were determined. It was found that the blood levels were low, that only insignificant amounts were excreted and that the concentration of the isotope was high in the fluid of the injected cavity.¹⁶

Since these tracer studies with Y^{90}Cl_3 have indicated good localization of the isotope in the injected cavities of cancer patients a trial with therapeutic doses was instituted. The radiation dose delivered by Y^{90} to the walls of the injected cavity was calculated as illustrated schematically in Fig. 2; 9–32 mc Y^{90} with 100–200 mg Y^{+++} as carrier were administered to fifteen cancer patients. No significant side reactions attributable to radiation or to the presence of yttrium carrier were noted. The evaluation of therapeutic effects was based upon the frequency and upon the amounts of fluid removed prior to and subsequent to the isotope therapy. Only eleven patients have been followed long enough to permit evaluation of therapy; seven of these eleven patients derived definite beneficial effects (see Table III).

The favorable radiation characteristics and the favorable therapeutic results suggest that Y^{90} -therapy may have its place in the treatment of cancer pa-

Table II. 24-hour Urinary Sr^{85} Excretion and Sr^{85} Content in Plasma 24 Hours after the Injection of the Tracer*

Study	% dose of Sr^{85} in	
	Urine	Plasma
I	18.2	3.30
II	18.67	3.33
III	27.07	2.73
IV	29.54	2.80
V	30.69	2.72

* Calcium gluconate was administered in studies III, IV, and V.

Table III. Studies on Patients with Cancerous Pleural Effusions

<i>Dose</i>	<i>Number of patients</i>	<i>Type of study</i>	<i>Follow-up studies Number of patients</i>	<i>Palliative results Number of patients</i>
0.3-4 mc Y ⁹⁰	11	Stable yttrium toxicity	11	Tracer studies only
100 mg Y ⁹⁰ Y ⁹⁰		Y ⁹⁰ localization. plasma levels, excretion, tissue distribution.		
9-32 mc Y ⁹⁰	15	Radiation toxicity	11	7
100-200 mg Y ⁹⁰ Y ⁹⁰		Fluid accumulation		

tients. The greater safety to the patient, the decreased hazards to personnel handling large amounts of isotopes represent advantages as compared to other methods currently being used for intracavitary therapy.

IV. THE EFFECT OF CHELATING AGENTS UPON THE DISTRIBUTION AND EXCRETION OF RADIOISOTOPES

The effect of ethylenediamine tetra-acetic acid, EDTA, upon calcium metabolism in man was previously reported.¹⁷ Marked enhancement of urinary calcium excretion was noted following the slow infusion of this agent. When infusions of EDTA were given to patients who had previously received Ca⁴⁵ a very significant rise of the excretion of calcium and of radiocalcium was noted.¹ When the calcium salt of EDTA, Ca-EDTA, was injected to humans who previously had received a tracer dose of radiocalcium the exchange of the stable calcium of Ca-EDTA with Ca⁴⁵ in the body could be measured.

When tracer doses of La¹⁴⁰ were injected intravenously as the chloride to human subjects, the urinary excretion of La¹⁴⁰ was less than 5% of the dose. When the strong metal chelate La¹⁴⁰-EDTA was injected in tracer amounts, the urinary excretion of La¹⁴⁰ was only slightly higher indicating that the metal chelate was largely dissociated by physiologic competitors within the human body. This dissociation was in part prevented if, simultaneously with the administration of La¹⁴⁰-EDTA a large excess of Ca-EDTA was given. The effectiveness of EDTA in enhancing the urinary excretion of La¹⁴⁰ decreased with increasing time interval between the administration of La¹⁴⁰-EDTA and the injection of excess Ca-EDTA.¹⁸

In order to gauge the effectiveness of body competitors to dissociate metal chelates of known formation strength, the plasma levels and urinary excretions and the body load of lanthanum and of yttrium were studied following the administration of La¹⁴⁰-EDTA, Y⁹⁰-EDTA, a stronger metal chelate, and Y⁹⁰-NTA (nitrilotriacetic acid), a weaker metal chelate. The cumulative urinary excretions following the intravenous administration of the Y⁹⁰-compounds are shown in Fig. 3. Approximately 90% of Y⁹⁰ was excreted when Y⁹⁰-EDTA was injected together with a large excess of Ca-EDTA, while only 50% was excreted when Y⁹⁰-EDTA was injected

without Ca-EDTA. The excretions of Y⁹⁰ following the injection of Y⁹⁰-NTA and Y⁹⁰Cl₃ were much lower than following the injection of Y⁹⁰-EDTA.

In order to study the distribution and the transfer of yttrium from the vascular to the extravascular compartment, simultaneous measurements of urine and plasma levels of yttrium were performed. In Fig. 4 the cumulative urinary excretion and the plasma content of yttrium are plotted in per cent of the administered dose. The difference between the injected dose and the amount accounted for in urine and plasma represents the amount of yttrium retained in extravascular sites. This figure illustrates that large amounts of yttrium remain in the plasma following the injection of yttrium chloride; approximately 40% of the dose is still present in the plasma three hours after injection, while less than 1 per cent has been excreted in urine by that time. In contrast, plasma content of yttrium decreased rapidly after injection of Y-NTA and Y-EDTA chelates.

This figure also illustrates that although the yttrium plasma levels are similar for Y-NTA and Y-EDTA, the urinary excretions and the yttrium concentration in the extravascular compartment are different. Although both chelates diffuse readily from the vascular to the extravascular compartment, only

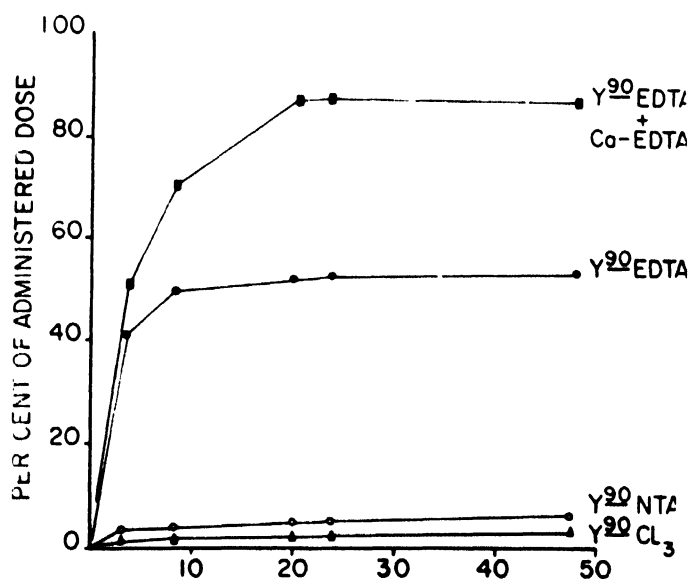


Figure 3. Cumulative urinary excretion following intravenous administration of yttrium-90 compounds

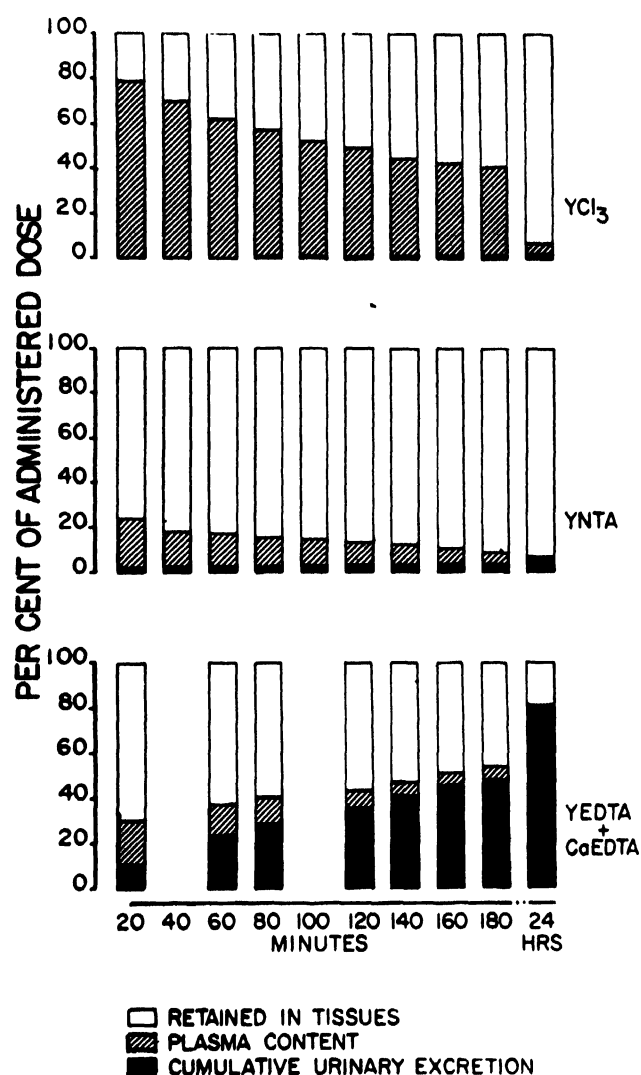


Figure 4. Cumulative urinary excretion and plasma content after intravenous administration of yttrium-90 compounds

Y-EDTA seems to be able to re-enter the vascular system in significant amounts, and thus be excreted. This is shown by the observation that, although as much as 70 per cent of Y-EDTA enters the extravascular compartment within 20 minutes, only 20% is retained in the tissues after 24 hours. No such reversal of net transfer is seen for Y-NTA or YCl₃.

These data illustrate the profound effect of chelating agents upon the disappearance rate of metals from the blood stream, their urinary excretion and their transfer from the vascular to extravascular sites into tissues. Chelating agents, such as EDTA, are currently used for the forced removal of stable and radioactive metals¹⁹ which entered the body either accidentally or due to occupational hazards. Good therapeutic results have been obtained in lead poisoning.²⁰ This agent is also being investigated for the treatment of hypercalcemia.²¹ However, the observations described in this section indicate a wider scope of usefulness of chelating agents for the purpose of medical diagnosis and therapy. For instance, one may select a metal chelate of such binding strength that it will resist dissociation by body competitors, permeate freely the cells and re-enter the interstitial and vascular compartments and

be promptly excreted. Or one may estimate the strength of body competitors by their ability to dissociate metal chelates. Finally, it may be possible to select a radiometal chelate which may pass through the capillary walls and membranes of certain tissue cells. The metal chelate may become dissociated within these cells and the radioisotope fixed to intracellular constituents. Guided by the formation constant and the pH dependence, one may find a metal chelate which concentrates its radioisotope within tumor cells preferentially. Experiments currently performed with several chelates of Y⁹⁰ in normal and tumor-bearing animals strongly indicate the feasibility of this approach.

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Teletherapy Devices with Radioactive Isotopes

By Marshall Brucer,* USA

Before 1947 discussions on the use of Co^{60} visualized attempts to mimic the historical radium pack at 5 or 10 curies of activity. A few persons contemplated the use of machines that would use up to 100 curies. Sometime before 1950 it was found that kilocurie sources were a practical possibility. From 1950 to 1952, for the most part, our thinking emphasized the kilocurie range. There have been a few exceptions to this trend but usually the exception was made because kilocurie sources were not available. In 1952 a reversal of this trend began with the investigation of the potentialities of a hectocurie machine. A cutaway diagram of the hectocurie prototype is shown in Fig. 1. Since this design has been

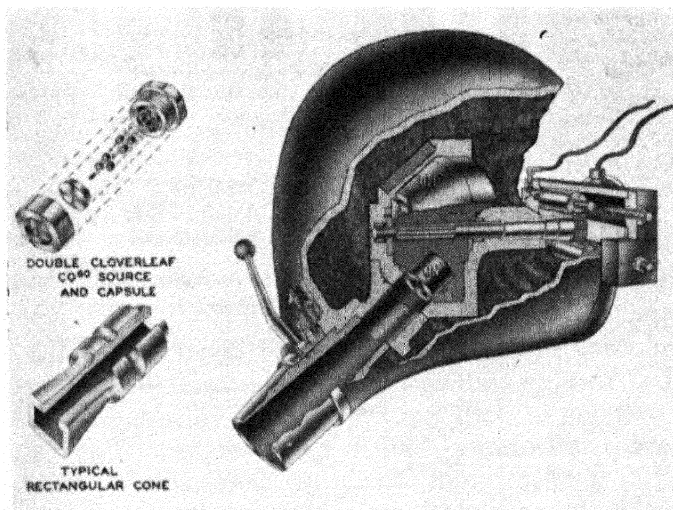


Figure 1. A cutaway diagram of the hectocurie prototype design (Teletherapy Evaluation Board)

tested, it has become increasingly apparent that there is a place in therapy for Co^{60} machines of all curiages.

Although this review emphasizes the use of Co^{60} , it is not the only isotope available for teletherapy uses. A full discussion of the potentially useful isotopes for teletherapy¹⁻⁵ has been published.

Cs^{137} is probably the outstanding teletherapy source. It produces gamma rays at a true super-voltage energy (0.661 Mev). At this energy most of the skin-sparing effects and the depths of penetration of Co^{60} are maintained. From Fig. 2 it can be seen that there is an increased efficiency of absorption of the cesium gamma rays in high Z mate-

rials. This means that, although the energy is much the same insofar as treating patients is concerned, the problems of shielding the source and building barriers in a teletherapy room will be markedly reduced with the use of Cs^{137} .

The 33-year half-life of Cs^{137} is an obvious advantage. Therapists now using Co^{60} are recalibrating their treatment times about four times a year. If a similar schedule were applied to Cs^{137} , a recalibration of treatment times would be necessary every 1½-2 years. One of the disadvantages of the half-life of Co^{60} is illustrated by the fact that, even in its short history, a number of therapists in the United States are already considering their second Co^{60} source. With the use of Cs^{137} sources, such an economic problem will arise only once or twice in the life of a radiotherapist.

Cs^{137} is a relatively new isotope.⁶ It was not thoroughly investigated until 1950. It is a fission product and as such will be a waste product of every nuclear reactor. With the increasing plans for the

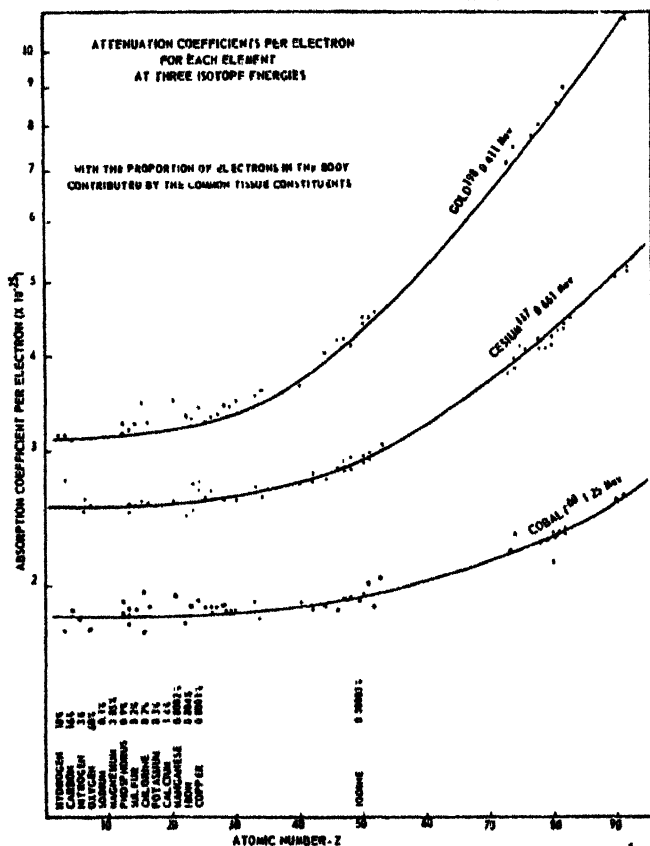


Figure 2. The relationship of Z to the absorption of radiation from Cs^{137} , Co^{60} and Au^{198}

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use of power reactors there will be, in the near future, an ever increasing volume of fission-product cesium available for teletherapy use. This fission-product cesium is a true waste material in that the first step in its separation must be performed in order to maintain the function of a nuclear reactor. Reactor fuels must be purified periodically. Once the fuel elements have been extracted from the fission products, the most serious problem of storing fission waste is with a few of the long-lived isotopes; among them cesium is most prominent. The problem of disposal of waste material is aided by the purification of gross fission products. The purified waste product, Cs^{137} , is a valuable source of radiation.

There has been much speculation during the past five years on the possible use of gross fission products and the potential efficiency of recovering Cs^{137} as sources of radiation. It has already been amply demonstrated that in such things as teletherapy and food sterilization, gross fission products are impractical because of their rapid nonlinear decay, their corrosion properties, the changing energy, the production of gases, and the difficulties of handling large volumes of radioactive liquids. Gross fission products are an expensive and an uneconomical source of radiation.

In the interest of demonstrating the practicability of careful separation of fission products, the first kilocurie cesium production was started in 1952 as an experimental procedure and has recently been completed. Two 750-curie pellets of separated Cs^{137} have been manufactured. These two sources were combined and are now installed in a teletherapy machine.

There are also some disadvantages to the use of cesium. The "rhm" value of Cs^{137} is about one fourth that of Co^{60} . Cs^{137} teletherapy sources will be larger than Co^{60} sources. This is not an insuperable problem in the design of teletherapy machines, but the design must take into account the necessity for longer collimating distances. This is complicated by the decreasing radiation intensity with increasing distance, but teletherapy machines can be designed with this in mind.

Probably the major disadvantage of Cs^{137} has been the fact that it has not been available. However, a pilot plant is being constructed in Oak Ridge that will make over 200,000 curies of cesium available each year starting in 1957.

$\text{Eu}^{152,154}$ is another source that appeared, at first, to be of great practicality in teletherapy. Its half-life of about $12\frac{1}{2}$ years has a distinct advantage over Co^{60} . The energy given off by europium is about the same as that of Co^{60} . An attenuation study of europium through lead indicates that approximately 83 per cent of europium has an average energy close to one million volts. This is probably due to at least four spectral lines ranging from 900 to 1400 kev. The 17 per cent of low energy (filtered out by approximately 3 mm of lead) consists of about seven spectral lines averaging between 200

and 400 kev. The rhm value of europium is approximately half that of Co^{60} and twice that of Cs^{137} .

The first short-term neutron irradiation studies of europium indicated an extremely rapid build-up so that very high specific activities would be available. In longer range studies it was found that a number of other reactions occur in the parent europium. A short-lived isomer of Eu^{152} decays to gadolinium, which has a high neutron cross-section. Also, the short-lived isotope itself has a cross section that is in direct competition with the production of the desired isotope. Further, Eu^{152} has a competing high cross section so that as the desired daughter is produced it is quickly burned up in the reactor. The same thing occurs with the production of the Eu^{154} isotope, although it has a lower cross section and therefore its build-up is somewhat slower. The total picture of europium production is that of a build-up to a peak specific activity reached very early during the radiation. Thereafter there is a loss of specific activity if the europium is kept in the reactor. The position and height of the peak varies with the flux of the reactor. In general, however, the maximum specific activity available from $\text{Eu}^{152,154}$ is approximately 18 to 25 curies per gram; this specific activity is reached within a period of three to four months of irradiation.

A specific activity of 20 to 25 curies per gram is better than was possible in the first Co^{60} machines, and the specific activity does not necessarily cause us to discard europium. However, another factor enters into the economic picture. Europium is one of the rarest of the rare earths. The parent isotope for irradiation consists of 48 per cent Eu^{151} and 52 per cent Eu^{153} . The pure metal has never been separated but is available as an oxide. During the process of separation of rare earths, the samarium element is separated from the europium element with difficulty. Samarium has an exceedingly high cross section in a nuclear reactor, and if there is a significant amount of samarium present in the europium oxide, the samarium will seriously compete for neutrons. Europium has no other uses in commerce and industry; hence, it is expensive. Therefore, the economic problem of obtaining pure europium oxide has been a significant one.

Recent work by one of the distributors of rare earths has indicated the possibilities of obtaining an almost pure europium metal deposited on a carbon contaminant. This material would assist in increasing the volume specific activity but it, too, is expensive and does not answer the economic problems of the europium sources.

A further use of $\text{Eu}^{152,154}$ has been in the production of very small sources of radioactivity. In these very small sources the europium oxide or the carbon-contaminated europium metal is mixed with aluminum powder to form a stable metallic pellet. This pellet can be easily irradiated in low flux reactors to millicurie levels. Although this procedure is valu-

able in the production of brachytherapy devices, the costs of production mount rapidly when it is followed for teletherapy sources.

It would appear from our studies of $\text{Eu}^{152,154}$ that there is a useful place for it in brachytherapy devices.¹⁶ There may also be a useful place in teletherapy devices, particularly in the small machines. Two machines which will use europium as sources are now under construction. One is a 10 rhm machine similar to the radium pack; another is a small office-model teletherapy machine which will have a maximum output of approximately 50 rhm. The shielding requirements for these machines will be approximately ten per cent less than for Co^{60} .

Other sources have been more fully discussed elsewhere^{1,4,9} but there are some changes in opinion during the past year that might indicate an increasing interest in the shorter half-life sources. The use of iridium in teletherapy has been well demonstrated in Cambridge, England. In a geographically small country, where extended periods of reactor production time is at a premium, it may be feasible to arrange for quarterly or even monthly replacement of sources. Our experience with the loading of hectocurie and kilocurie Co^{60} sources in hospitals at long distances from the reactor have indicated that this loading can be done in a matter of minutes and with exposures to personnel uniformly less than a total of 10 mr. A standard source capsule (Fig. 3) is now in use in the United States and Canada. This

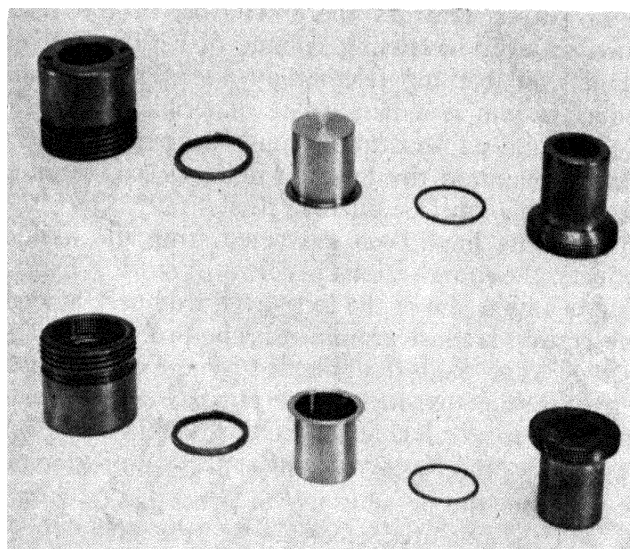


Figure 3. A standard source capsule for Co^{60} teletherapy sources adopted by the United States and Canadian manufacturers of teletherapy equipment

capsule allows the replacement of sources and their interchangeable transfer between most teletherapy machines.^{7,10,13} The biggest problem in field loading of teletherapy sources has been that of transportation of multiton containers. Therefore, the use of short-lived radioisotopes for teletherapy will be impractical if the distances from the reactor to the teletherapy treatment room are sufficiently long to pose transportation problems.

Table I. The Major Characteristics of Various Teletherapy Machines ($\text{Co}^{60} = 5.2 \text{ yr}$; $\text{Eu}^{152} = 12.5 \text{ yr}$; $\text{Cs}^{137} = 33 \text{ yr}$)

Name	Curie		Multicurie			Hectocurie			Kilocurie		
Machine no	1	2	3	4	5	6	7	8	9	10	11
Approximate treatment (rhm)	5	10	20	40	80	150	300	600	1200	1800	2400
Approximate curiage											
Co^{60}	5	10	22	42	83	165	330	660	1320	1980	2640
Eu^{152}	12	24	53	100	200	400	800				
Cs^{137}	15	30	60	180	378	740	1510	3807	10,000		
Approximate r/minute at SSD (cm)											
100							5	10	20	30	40
70.7						5	10	20	40	60	80
50					5	10	20	40	80	120	160
35.5				5	10	20	40	80			
25			5	10	20	40	80				
17.7		5	10	20	40	80					
12.5	5	10	20	40							
8.8	10	20	40								
6.3	20										
Practical source diameter (cm)											
Co^{60}	0.3	0.3	0.5	1.0	1.0	1.5	1.5	2.0	2.0	2.0	2.0
Eu^{152}	0.3	0.5	0.8	1.0	1.0	..	..	..	..	..	..
Cs^{137}	1.0	1.0	1.0	1.0	1.3	1.8	2.5	3.0	4.0	..	..
Magnitude of source cost (\$)											
Co^{60}	400	500	600	700	1000	1200	3000	4000	8000	16,000	24,000
Eu^{152}	800	1000	1400	2000	4000	..	..	..	..	..	..
Cs^{137}	2500	5000	10,000	..	..	unknown	..	..	..	..	..

Co^{60} still represents the source with which we have had the most experience. This discussion of the range of Co^{60} sources can with modifications apply equally well to other sources. The following tables have been prepared to show a wide range of these machines with some approximate dose rates in various sizes of machines. Some of these machines are already under construction.

Table I shows the maximum output at the useful source-skin-distance (SSD) of various Co^{60} teletherapy machines. The machines are numbered from 1 through 11. The first two machines can be called curie machines since only a few curies of the isotope are used. The next three machines are called multi-curie machines since they use many, but less than 100, curies. The machines in the third group are in the hectocurie range. The fourth group is made up of the machines in the kilocurie range.

Row 3 of Table I shows the maximum treatment output in roentgens per hour at a meter (rh/m). These range from 5 rh/m in the smallest machine to 2400 in the largest machine. Except for machine No. 10, each machine is the next largest by a multiple of two. Although the curiage necessary to give this output is a varied one depending upon the size and shape of the source, very roughly the order of magnitude can be estimated for Co^{60} at 1 curie equals 1 rh/m.

Since the treatment output (rh/m) is measured in terms of a relatively narrow beam geometry, this figure will not be correct for estimating shielding requirements or purchase prices. In considering the amount of radiation applicable in shielding calculations, one must use a broad beam geometry. Also, the purchase price for cobalt is determined with a broad beam geometry measurement.^{4,5,13} The details of the experience on 12 units recently installed are shown in Table IV.¹² In a standard capsule and with a standard cone size, one curie of Co^{60} yields from 1.1 to 1.4 rh/m. The data for Cs^{137} are from "Teletherapy Design Problems I".¹ The one Cs^{137} source that has been installed checks well with the theoretical calculations.¹⁴ The europium data are estimated from studies on a 2-curie source.

The middle section of Table I shows the roentgens per minute at various treatment distances. This is roughly arrived at from Co^{60} data and is only an order of magnitude figure. In order to determine the usefulness of the machine, the assumption has

been made that an output of less than 5 r/minute at the treatment distance is inadequate for teletherapy purposes. At the other end of the scale, the shielding size will limit very large machines in terms of close-in treatment distances. Many of the kilocurie machines cannot be used at even 50 cm SSD but most could be designed for this treatment distance. In the hectocurie range, the present 600 rh/m machine is designed to be used at 35 cm. Shorter cones, however, have been designed to go as close as a 17.7 cm SSD.

The fourth section of Table I gives the smallest practical source size to furnish the required maximum treatment rh/m. This has been determined for the currently available Co^{60} . However, if a 2-cm diameter source is used as a constant source size, it is possible in the smaller machines to insert a pin-hole device that will allow the source size to be effectively reduced without too great a decrease in the rh/m output. There will, however, be a moderate increase in penumbra over that of a truly small source.

The last section in Table I shows the magnitude of cost of an encapsulated source necessary to produce the stated output and the minimum specific activity that can be used in such a machine. A full description of the factors entering into the Co^{60} cost figures is given in "Teletherapy Design Problems III".⁴

Table II shows the broad beam absorption coefficients used in the calculation of the sizes of Co^{60} machines with various outputs. These are given for Co^{60} only, since the experience with other isotopes is confined to one machine and very small sources. Under the specific conditions of teletherapy use, these broad beam absorption coefficients are corrected to give a close approximation to the absorption and scatter attenuation in a 4π geometry. Machines calculated from these coefficient will give a minimum protection. Corrections for cracks and joints and openings in the shields must be added to the basic dimensions arrived at by using this table.

Table III shows the approximate sizes of machines made of various materials. In the most commonly used internally-rotating-wheel shutter design these are the dimensions for the source in the "off" position. The protection is calculated to reduce the radiation to an average of 2 mr/hour at one meter from the source. When the source rotates to the "on" position less shielding is necessary. Here, the radiation need be reduced only to a level equal to that

Table II. Corrected Broad Beam Absorption Characteristics Used in Calculations for Co^{60} Teletherapy Machines

	Narrow beam I_n	Broad beam I_b	HVL cm	TUL cm
Tungsten alloy	0.92	0.84	0.825	2.75
Lead (sb)	0.64	0.58	1.20	4.00
Brass or bronze	0.43	0.39	1.78	5.93
Steel	0.40	0.36	1.93	6.43
Marble	0.16	0.14	4.80	16.00

Table III

Name	Curie		Multicurie			Hectocurie			Kilocurie		
Machine no.	1	2	3	4	5	6	7	8	9	10	11
Approximate treatment (rhm)	5	10	20	40	80	150	300	600	1200	1800	2400
Approximate (radius cm) thickness of primary shield for Co ⁶⁰ (Eu ¹⁵² is about 10% less):											
Tungsten alloy	9.4	10.2	11.0	11.9	12.7	13.5	14.4	15.2	16.0	16.5	16.8
Lead	13.8	14.8	16.0	17.2	18.5	19.7	20.9	22.1	23.3	23.9	24.5
Bronze	20.4	22.0	23.8	25.5	27.4	29.2	30.9	32.7	..	..	..
Steel	22.2	23.9	25.8	27.8	29.8	31.8	33.7	..	..	..	..
Approximate (radius cm) thickness of primary shield for Cs ¹³⁷ :											
Tungsten alloy	5.6	6.1	6.6	7.1	7.5	8.0	8.5	9.0	9.5	..	..
Lead	7.2	7.8	8.4	9.1	9.7	10.3	10.9	10.5	11.1	..	..
Bronze	14.3	15.6	16.8	18.1	19.4	20.5	21.7	23.0	24.3	..	..
Steel	15.6	17.0	18.4	19.8	21.1	22.4	23.7	25.1	26.5	..	..
Thickness of primary barrier for direct beam:											
Marble { Co ⁶⁰	48.0	52.8	57.6	62.4	67.2	71.5	76.3	81.0	85.8	88.6	90.6
{ Cs ¹³⁷	36.6	40.3	43.9	47.6	51.2	54.5	58.2	61.8	65.4	..	..
Lead { Co ⁶⁰	12.0	13.2	14.4	15.6	16.8	17.9	19.0	20.2	21.4	22.2	22.6
{ Cs ¹³⁷	6.3	7.0	7.6	8.3	8.9	9.5	10.1	10.7	11.3	..	..

scattered from the patient.¹¹ With Co⁶⁰ the 90° angle scatter from the patient is about 0.1 per cent of the direct beam. The maximum radius of the machine around the source in the "on" position therefore is about three tenth-value-layers of material. If tungsten alloy is used the radius is 8.25 cm; with antimony lead it is 12.0 cm, with bronze 17.8 cm, and with steel 19.3 cm.

The protection necessary directly around the useful beam is provided by the collimating cones of the

machine. Although an international congress set the diaphragm thickness at a reduction to 2 per cent of beam strength, most of the designers in the United States have attempted to make cones about two tenth-value-layers thick. This automatically sets the minimum source-skin-distance of the machines at 5.5 cm when tungsten alloy is used. It is 8.0 cm for lead, 11.9 cm for bronze, and 12.9 cm for steel. These figures are for Co⁶⁰ radiations. The figures for Cs¹³⁷ and Eu¹⁵² are tentative calculations.

Table IV. Summary of Co⁶⁰ Installations

University or hospital	No. of curies	rhm	rhm per curie	Size of treatment room	Cost of unit \$	Cost of source \$	Cost of instal- lation \$	Special problems in remodeling
Louisville	979	1350	1.4	16' × 16'	19,700	8037	3965	Temporary location
Mt. Sinai	306	420	1.4	9.5' × 13'	20,800	1830	10,000	Densely populated area
Emory	294	360	1.2	10' × 12'	10,200	2800	2000	Remodeling of large room
Wayne	341	360	1.1	15' × 19'	19,700	2205	15,905	Complete remodeling of old building
Tennessee (1)	311	315	1.0	9' × 18'	20,000	2055	900	Remodeled 250 kv room
Tennessee (2)	309	309	1.0	14' × 16'	20,000	2045	450	Changes to new construction
Arkansas	1089	1350	1.2	9' × 13'	19,700	9012	717	Remodeled 400 kv room; temporary
New York	599	762	1.3	15' × 15'	20,000	4632	100	Remodeled radium pack room
City of Hope	744	945?	1.3?	12' × 19'	32,000	6200	11,624	New construction
Southern California	1066	1440	1.4	13' × 13'	10,000	8820	42,000	Special protection for other work
ORINS Medical Division(1)	209	232	1.1	14' × 24'	Experimental source in unit below			
ORINS Medical Division(2)	963	1160	1.2	14' × 24'	26,000	19,000	22,000	
ORINS Medical Division(3)	454	360	0.8	14' × 24'	Experimental source in prototype hectocurie machine			

Many other designs might be feasible for use in more limited areas of teletherapy. For example, a number of machines using extended sources are being studied.¹⁴ The initial interest in the use of a single source was to a certain extent a copy of the previous history of X-ray therapy. With the metallic sources it is now possible to use 2, 10, or even 100 individual sources to accomplish a build-up of radiation intensity inside a body. With the cesium isotope absorbed onto an ion exchange resin, it is possible to completely depart from the isolated discrete source and to extend the sources in planes, over hemispheres, or in enclosed envelopes such as were discussed by Van Roojen in the 1930's.¹⁵ Such sources would not be expensive; in fact they would be cheaper to construct and to maintain than the present day Co^{60} sources. Such sources would not be milligram radium sources that Van Roojen investigated but could be brought up to hectocurie or even kilocurie levels. Cosine surfaces with Cs^{137} have already been accomplished on an experimental scale.

Very small sources in the range between the 5 rhm teletherapy machine and various brachytherapy devices have been suggested by various people for intracavitary use. Many of the serious disadvantages of Cs^{137} , Eu^{152} , and Co^{60} disappear completely with such very small devices.¹⁶ Also it is practical to consider much lower energies when the treatment device is brought close to the treatment area. With lower energies the transportation-shielding requirements are lessened and isotopes with shorter half-lives can be considered. One of the very useful isotopes in this range should be Ce^{144} . This is also a fission product of high yield and very high volume specific activity. Eu^{155} is another fission product that has promise for short distance therapy. A machine is now being designed to make use of such sources of radiation.

With the advent of many machine designs and many sources of radiation, the physical measurements necessary for adequate treatment techniques have become more complex. To assist the therapist

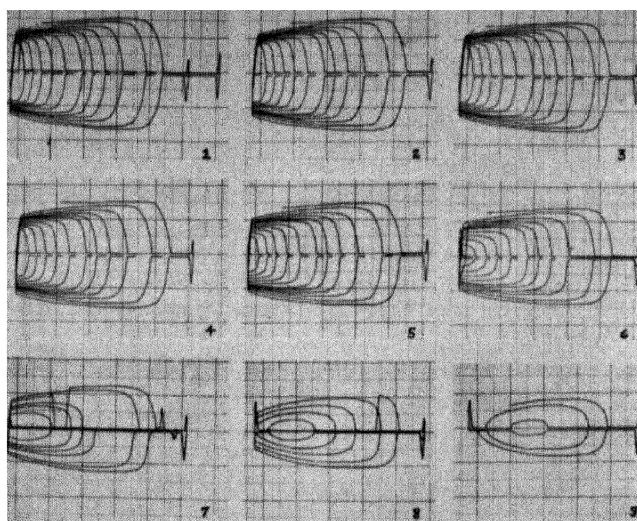


Figure 5. A set of isodose curves through nine planes of a treatment volume

in visualizing his treatment beam, an automatic isodose plotter has been designed. Figure 4 shows this plotter. It will determine the 2-dimensional isodose charts and 3-dimensional isodose volumes for each of the various kinds of machines. An example of a series of plots through nine planes of a circular cone is shown in Fig. 5. From these plots, tables of isodose are complete for Co^{60} at SSD's between 25 and 70 cm with all field sizes. These have been published in "Teletherapy Design Problems IV".⁵ Isodose charts for selected fields and distances are partially complete for the Eu^{152} and Cs^{137} isotopes. A series of isodose charts comparing 250 kvp X-ray, Cs^{137} , Eu^{152} , and Co^{60} has been prepared. Along with the isodose charts, a series of measurements of the total integral dose to selected tumor and body phantoms is available. These measurements were obtained by chemical dosimetry and ion chamber isodose techniques. They allow for an approximate prediction of integral dose with changing isotope, field size, body size, and tumor size.

One of the economically important factors in the installation of isotope machines has been the cost of installation and the incorporation of protective devices. Detailed plans and cost surveys have been made for 11 currently operating installations with the Co^{60} kilocurie and hectocurie machines. A survey of these installations is shown in Table IV, and a complete description of the problems involved in the installation of these first units is available in the published minutes of the Teletherapy Evaluation Board.^{8,12}

It is apparent that the radioisotope teletherapy devices are practical machines that can be used wherever the X-ray generators have previously been used. It is also apparent that they have a potential usefulness far beyond that of the present devices.

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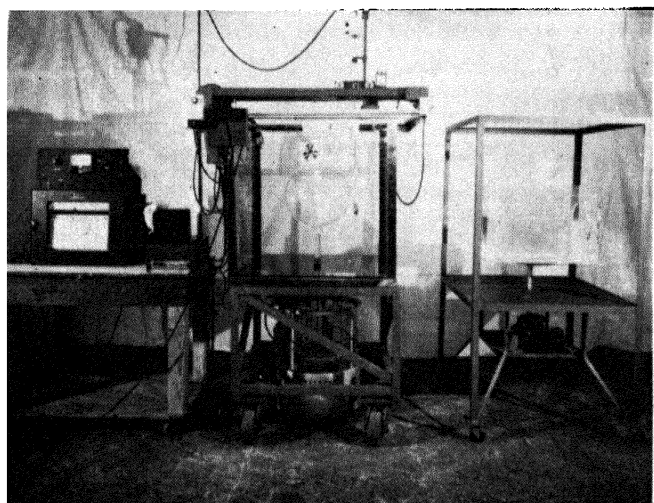


Figure 4. An automatic isodose plotter for measuring 2- and 3-dimensional isodose curves of teletherapy machines

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Kilocurie Revolving Cobalt-60 Therapy Unit

By L. H. Lanzl and L. S. Skaggs,* USA

Teletherapy units using radium have been in use for many years, but the source activity has been of the order of a few curies. As a result, the source-to-skin distances have been small and the treatment times long. The recent availability of kilocurie sources of radioactive cobalt has allowed a new approach to the teletherapy problem.¹ In an attempt to make the fullest use of these possibilities, we have designed and installed a new Co^{60} therapy unit at the Argonne Cancer Research Hospital. Because of the distinct advantages of rotation or revolution therapy in regard to the dosage distribution obtainable, it was decided to incorporate the moving beam feature in the hospital unit. Other features are:

1. The use of uranium for shielding results in a small, compact unit with large clearances.
2. A small, high specific activity source yielding a small penumbra.
3. Precise positioning of a recumbent patient.
4. A high degree of reliability and safety in use of the machine.
5. A convenient and safe reloading system.

The revolving type of motion is obtained by having the source shield mounted on the periphery of a pair of large rings that are held rigidly together, and having the rings turn about their common axis. During treatment, the patient remains in a fixed position while the source revolves in a vertical plane around him. The center of the tumor mass is placed at the center of rotation, 81.6 cm from the source.

Figure 1 is a photograph of the complete unit. The unit consists of several parts: (1) the Co^{60} source; (2) the main shield, including the source shutter, light localizer, and collimating system; (3) the large rings that support the shield; (4) the drive mechanism by which the shield is moved around the patient; and (5) the control console. These parts, as well as source preparation, irradiation, loading, and several measurements are discussed below.

Co^{60} SOURCE CONSIDERATIONS

In many respects, the design of the whole unit is dependent upon the physical size and the intensity of the source of radiation. As a result of a study of source considerations in terms of present-day radiological therapy standards of intensity and penumbra, including the not-so-standard feature of revolution,

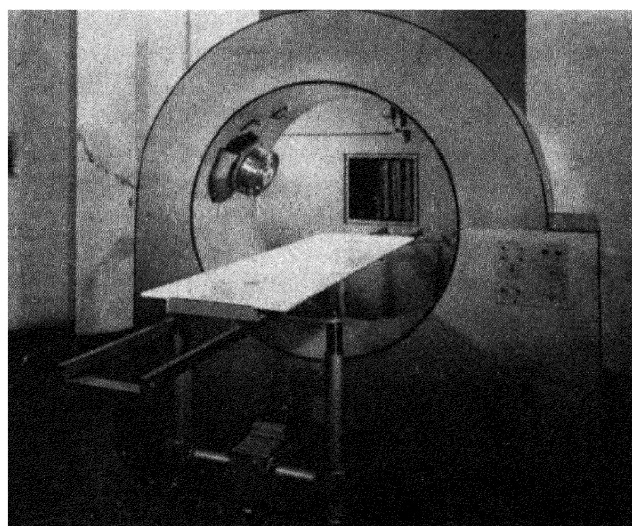


Figure 1. Photograph of the Co^{60} revolution therapy unit. The source shield, treatment cot and rails, and auxiliary control panel are visible. The viewing window appears in the background

it was found reasonable to use a source, in the shape of a right cylinder, 3-cm long and 0.75 cm in diameter. The central ray of the useful beam is taken along the axis of the cylinder, although for this geometry, the self-absorption of the useful part of the gamma-ray output is greater than for a source of larger cross-sectional area and smaller length. The advantage of less penumbra is considered to outweigh these efficiency considerations.

In a unit in which a collimating applicator can be brought to the surface of the skin, the size of an extended source is not as important a consideration as in the case of the moving beam, where the collimator has to be placed at some distance from the patient to allow clearance between collimator and patient. From the point of view of activation, a source as small as this can be produced in a reasonable time with sufficient curie content to be useful in therapy, if the highest available neutron fluxes are procured.

The highest neutron fluxes available to date in the United States are those from the Materials Testing Reactor in Idaho. Although peak fluxes of 5×10^{14} thermal neutrons per cm^2 per second exist in certain reflector positions, average fluxes of 2×10^{14} thermal neutrons per cm^2 per second and 1×10^{14} fast neutrons per square cm per second are available.²

Co^{60} has a thermal neutron capture cross section of 34.8 barns. Assuming this cross section, a half-

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life of 5.3 years for Co^{60} , a flux of 2×10^{14} thermal neutrons per cm^2 per second, and no burn-up of Co^{60} , a specific activity of 150 curies per gram can be produced with an activation time of eight months. This figure is valid for very small, but not for large samples because of the effects of self-protection and flux depression. Since it is difficult to calculate flux depression, measurements of this quantity as well as of self-protection for a piece of cobalt, 3-cm long and 0.75 cm in diameter, weighing 11.7 gm, were made at our request by Drs. J. S. Levin and D. J. Hughes at the Brookhaven National Laboratory. These measurements have been reported in detail by them.³ With experimental conditions similar, but not identical to those of the Materials Testing Reactor, their results gave a factor of 0.77 for self-protection and 0.58 for flux depression. The net effect is that the activity of the cobalt would be $0.77 \times 0.58 = 0.45$ of the activity calculated on the basis of a uniform, undepressed thermal neutron flux. Thus, assuming the above conditions; it would take about 18 months to produce a source of 150 curies per gm. There would be less self-protection and flux depression if the source were divided into smaller units, activated at various places in the reactor. However, because of handling, encasing, and reactor-loading problems, it was decided not to take advantage of this possibility at present.

A source of the above size, shape, and specific activity would give a dose of 35 roentgens per minute in air at 82 cm as seen along the axis of the cylinder. This dose is calculated using the value⁴ of 13.0 roentgens per hour per millicurie at one cm for Co^{60} and the tables of the self-absorption function $F_p(\mu_a)$, as given by Dixon.⁵

In the planning stage of source acquisition, the above calculations were used as the basis for (1) the strength of the final source, i.e., 150 curies per gm or 1750 curies total, and (2) the source-to-tumor distance of the entire unit. (At present the strength of the source within the unit is 1225 curies.)

SOURCE SHIELD

A sketch of the source shield is given in Fig. 2. The shielding material is natural uranium in metallic form. Uranium is one of the best shielding materials available because of its high density and high atomic number. A lead shield of equivalent shape and with an equal Co^{60} gamma-ray attenuation would weigh almost four times as much. The thickness of uranium in the shield between the source position and the surface is 14 cm. Using a broad beam absorption coefficient⁶ of 1.045 cm^{-1} , this thickness gives an attenuation factor for Co^{60} gamma rays of 2.3×10^6 .

The source is held rigidly in the source shield by means of a silver-plated uranium plug, which is held firmly in place within the source shield by a steel retaining plate backed by a thin neoprene disk.

The gamma-ray beam is controlled by a shutter that is an integral part of the source shield. It consists of a thick uranium drum with a conical-shaped

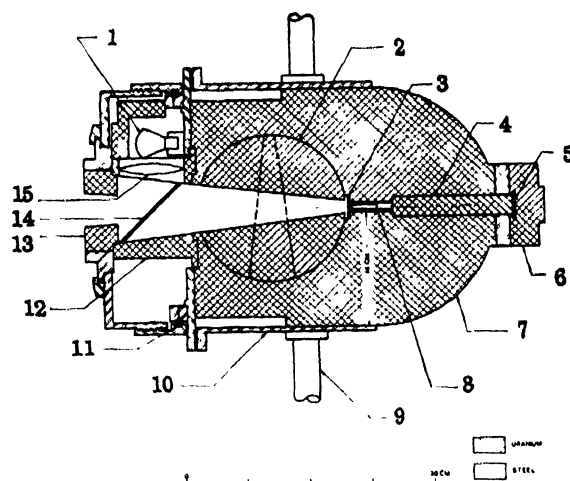


Figure 2. Schematic cross-sectional drawing of the source shield. The shutter is shown in the open position; the closed position is indicated by dotted lines. (1) light source; (2) shutter; (3) thin aluminum disk; (4) plug; (5) neoprene; (6) retaining plate; (7) shield; (8) cobalt source; (9) support; (10) steel cradle; (11) ball bearing; (12) cone; (13) changeable fixed collimator; (14) mirror; (15) lens

aperture whose axis is along a diameter in the mid-plane of the disk. "On" and "off" positions of the shutter are obtained by 90° rotation of the disk. In its "off" position, the minimum thickness of uranium provided by the shutter is 14 cm. In the "on" position, the shutter leaves the beam completely free of absorbing material. The shutter is motor-driven through a mechanical gear train containing two springs, a mechanical stop, and a magnetic clutch. During an opening cycle, the clutch is energized and the motor provides the necessary work to overcome any off-balance of the shutter as well as work against the springs. During a normal closing cycle, the springs supplement the motor.

However, during a power failure or emergency shut-off, the clutch is de-energized, and either one of the springs has sufficient potential energy to close the shutter. Thus, the shutter would be closed under the unlikely condition of simultaneous power and single spring failure. The shutter is provided with a positive mechanical stop in the open position and, in the closed position, with a combination spring and pneumatic shock absorber. Immediately adjacent to, and on the exterior side of the shutter is a thin mirror that forms a part of the light localizer.

The uranium shield is cradled in a steel shell onto which is welded a pair of trunnions. The shield consists of two separate castings that are bolted together and, in turn, to the steel cradle. The total weight of the uranium in the source shield, excluding the collimator, is 361 kilograms.

Two types of collimating systems have been designed and built. One is a set of 11 fixed collimators giving a variety of square and rectangular field shapes. A cross-sectional view of this type of collimator is shown in Fig. 2: The second type is a variable collimator that is composed of 2 pairs of interlocking leaves. The opening between each pair can be adjusted independently, thus giving either square

or rectangular field shapes. With these collimators, the maximum field size obtainable at the tumor area is a 15×15 cm². The uncollimated beam gives a field that is the circumscribed circle of the square.

Both types of collimators are composed of a 4 cm thickness of uranium, which reduces the intensity of the primary beam to 1.5 per cent. The collimators are easily interchangeable and can be rotated about an axis that lies along the central ray of the gamma-ray beam. A scale calibrated in degrees is provided for this motion. The weight of the collimators rests on ball bearings (see Fig. 2).

MOTIONS OF SOURCE SHIELD AND TREATMENT COT

The source shield is capable of two kinds of motion. One of these is the revolving motion in a vertical circle (see Fig. 3). The motion of the shield during treatment can be any number of complete revolutions or a sector of arbitrary angular size over an arbitrary portion of the circle. It can also be used at an arbitrary fixed angle.

The second motion is an angulating one of the source shield about its trunnions. This motion occurs about an axis that lies in the plane of the vertical circle and is perpendicular to the gamma-ray beam (i.e., an axis parallel to the tangent of the vertical circle). The maximum obtainable angle is 45 degrees with the vertical.

The treatment cot can be positioned by one rotational and three orthogonal linear motions. The rotational motion is about a vertical axis. The cot can be rotated up to 44 degrees in both directions from the position normal to the large rings. The three linear motions are: along a pair of rails in the direction of the long axis of the cot, perpendicular to the rails in a horizontal plane, and vertical. The cot and its supporting rails can be seen in Fig. 1. The length of the rails, combined with the overhang of the cot beyond their ends, allows a total movement of 212 cm in the direction of the rails. This rather large movement permits the placing of any portion of a patient in a position within the gamma-ray beam.

The transverse motion of the cot with respect to the rails has a range of movement of 25.5 cm. The range of the vertical motion of the cot is 30.5 cm.

The rotational and linear motions of the cot, as well as the revolution and angulation motions of the shield, are calibrated in metric units and degrees. Thus, the patient line-up can be reproduced with speed and accuracy from day to day.

SUPPORT STRUCTURE AND DRIVE MECHANISM

Figure 4 is a schematic drawing showing the various components of the support structure and drive mechanism for the source shield. The large rings, on which the shield is mounted, consist of 2 steel hoops joined by 10 cross members to which they are welded. Each hoop is 10.2-cm wide, 5.1-cm thick, and has an outside diameter of 170.2 cm. The hoops are 66.0 cm apart.

A counterweight is fastened on a pair of cross members of the large rings, diametrically opposite the main shield, so that the rings rotate with a minimum of power.

The weight of the shield, counterweight, and large rings is supported by a steel structure composed of plates strengthened by webbing. This structure is welded to a nearly circular steel base plate (Fig. 1).

As mentioned previously, the large rings revolve about their common axis. The rings move on a set of eight rollers, four for each ring, which are mounted on the steel support structure (labeled "bottom support rollers" in Fig. 4). In addition, there are 8 spring-loaded side rollers, which serve to keep the rings properly centered within the unit. One pair of the bottom support rollers friction-drives the large rings while the remaining six are idlers.

The drive rollers are connected by a gear train to a $\frac{1}{8}$ -horsepower Thymotrol Drive Unit, which gives any desired speed of the large rings from 0 to 2 revolutions per minute in either direction. The speed may be varied during a given treatment if desired. The gear train includes a fail-safe magnetic brake as well as a clutch. Electrical power for the various com-

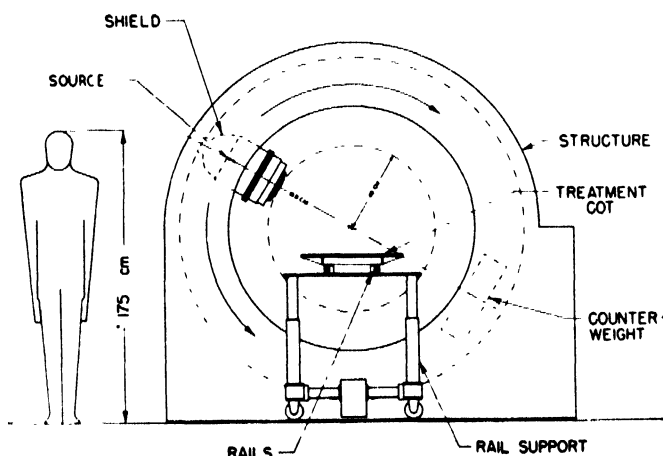


Figure 3. Sketch of unit indicating revolution of the source shield. The silhouette of a man gives a size comparison

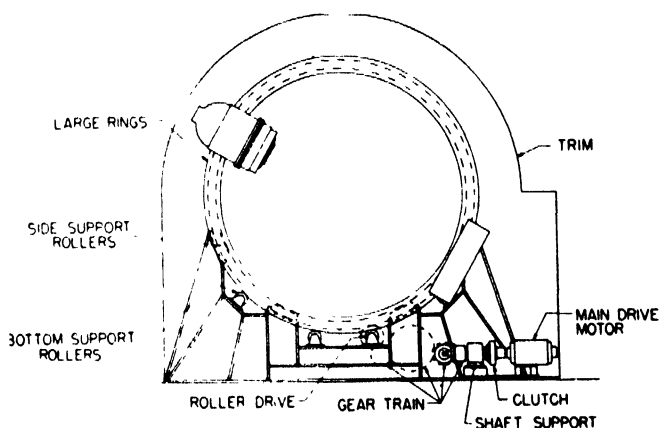


Figure 4. Diagram of main drive gear train and support structure of large rings. The fail-safe magnetic brake that is near the shaft support is not visible

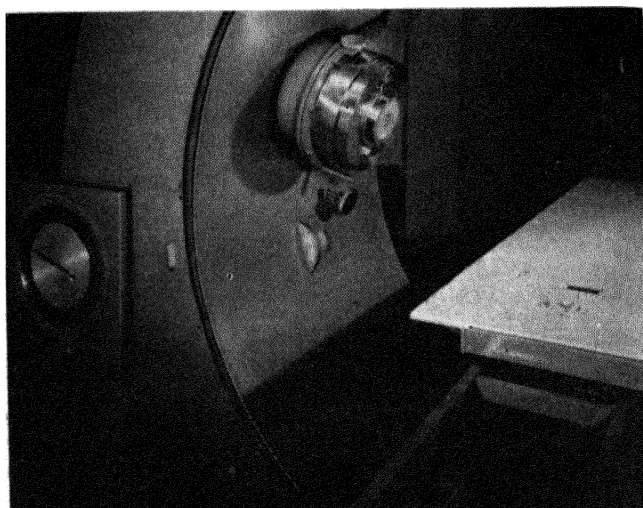


Figure 5. Photograph showing, on the extreme left, dual angular position controls with reversing switches for sector irradiation, source shield position indicator, revolution direction and jogging controls and vertical motion controls for the treatment cot; in the center, the source shield with angulation controls and indicator. The treatment cot is visible to the right

ponents of the source shield is provided through a set of 8 slip rings that are affixed to the large rings.

As can be seen in Fig. 1, the entire support structure, including the large rings, is enclosed in sheet metal trim. The trim is stationary except for the section on the inner circular surface, which is rigidly fastened to the large rings and contains an opening for the shield. (See also Figs. 4 and 5).

It was mentioned previously that a treatment plan may be used in which the source moves through a sector of arbitrary angular size, over an arbitrary portion of the circle. For use in this type of treatment, a mechanically coupled gear train extends from the large rings to a dual limit switch device. Each limit switch may be set independently, and when the main shield reaches the angle of either of them, the motion of the large rings is reversed.

The two limit-switch indicators as well as a pointer that indicates the position of the source shield on the vertical circle are shown on the left-hand side of Fig. 5. This figure also shows the revolving part of the trim and, below the shield, the dial that indicates the angular position of the shield.

CONTROLS

The unit is controlled from a console that is located outside the treatment room. However, some of the adjustments are made within the room from auxiliary panels located on the unit itself.

The sequence of operations is as follows: (1) a three-position selector switch is set at revolution, sector, or fixed-beam irradiation; (2) if either revolution or sector irradiation is chosen, the speed of the main shield is preset; (3) the dose selector is set; and (4) the final step is the pressing of the treatment start button. The shutter then opens, the source shield revolves, and a synchronous motor starts the dose computer (the dose computer is described be-

low). A pilot light on the control console indicates when the shutter is fully opened. The direction of the revolving motion of the source shield is usually unimportant, but can be predetermined. The direction in which the shield will turn is indicated by one of two pilot lights.

The control console also is equipped with a dose selector and a scattered radiation meter.

The dose computer is a computer of the type used in most gasoline pumps in the United States. The "rate wheels" for this application are set to correspond to the air dose rate at the center of revolution of the source shield. The "quantity wheels" of the computer then indicate the treatment time in minutes, and the "money wheels", the dose in roentgens. Each week, the dose rate setting is decreased corresponding to the decay of the source. The computer eliminates the need for a time decay chart and concomitant calculations, but does not, of course, eliminate depth dose considerations. The computer is powered by a synchronous timing motor that is actuated by the opening of the shutter.

The dose selector is connected mechanically to the dose computer in such a way that power is derived from the timing motor of the computer. The dose selector, as its name implies, is a device to set the required dose so that the more standard procedure of setting the required time of treatment is not used. It should be pointed out that the value to be set on the dose selector is the air dose adjusted for depth and tissue dose correction factors, rather than merely the air dose. The actual setting is accomplished by turning three independent wheels, each corresponding to one digit, in hundreds, tens, and units.

As the treatment proceeds, the dose selector gives an ever-decreasing number, corresponding to the number of roentgens still to be delivered. When it reaches zero, i.e., when the desired dose has been delivered, the dose selector provides an electrical signal that automatically closes the shutter and stops the motion of the shield. At all times during treatment, the dose delivered as well as the time of irradiation appears on the computer. At the conclusion of the treatment, the computer thus provides a check on the total dose and time of irradiation.

The scattered radiation meter gives the reading of a halogen-filled Geiger counter that is located in the treatment room and serves as a monitor.

SOURCE ACTIVATION

Among the problems of high specific activation of cobalt is that of providing sufficient cooling for it during activation. The heating of the cobalt is due primarily to its absorption of reactor gamma rays, the capture gamma rays of Co^{60} , beta rays of Co^{60} , and gamma of Ni^{60} . The most important of these is the absorption of the capture gamma rays. These gamma rays are emitted almost instantaneously following neutron capture. The maximum gamma-ray energy emitted per neutron capture is 7.7 Mev.⁷ This

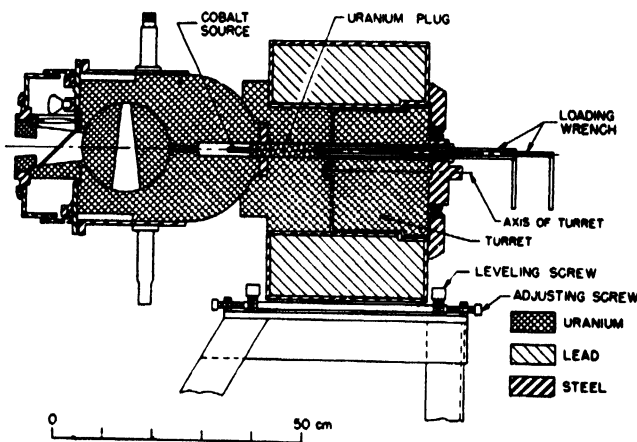


Figure 6. Schematic drawing of the transfer shield in position for loading a source into the source shield. The leveling and adjusting screws are needed to align the axis of the port in the transfer shield with the plug hole in the source shield

energy may or may not be confined to a single photon. Actually, there is a spectral distribution of photons, with a peak at 1 Mev, which causes more heating than if all were emitted at 7.7 Mev. This heat is dissipated by passing water over the source. Since, however, activated cobalt is likely to be covered with radioactive cobalt oxide dust, the source is permanently encased in an aluminum jacket. To insure intimate contact between the cobalt and aluminum, a shrink-fitting procedure is used in jacketing. The jacket is welded closed.

After a source reaches the desired level of activity, it is removed from the reactor and then affixed to the uranium plug (see Fig. 2) by means of a master-slave manipulator and hot cave.

TRANSFER SHIELD AND LOADING PROCEDURE

A transfer shield was designed and built to facilitate the handling and loading of the cobalt into the source shield. By its use, a dry loading procedure is

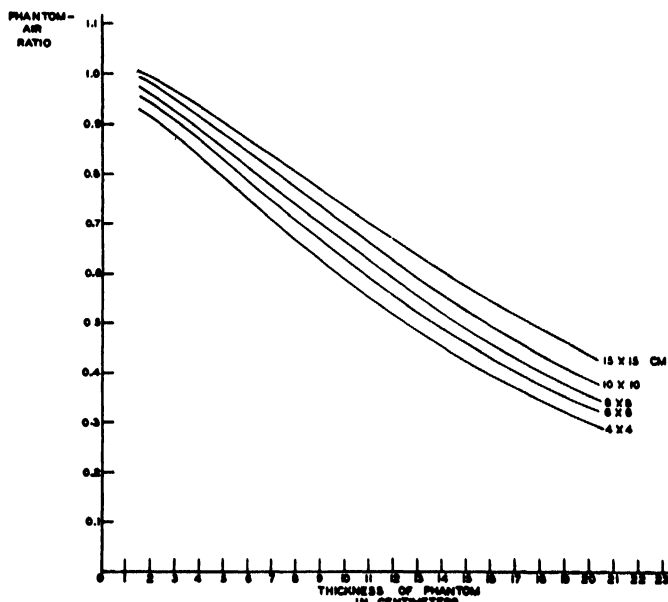


Figure 7. Ratio of phantom to air dose plotted against thickness of phantom. Measurements were made with a fixed source to detector distance of 81.6 cm. Phantom material is Masonite

accomplished. The rear part of the transfer shield consists of a turret with 3 identical and equally spaced holes with diameters just slightly larger than the uranium plug. The purposes of the 3 holes are: one for line-up with the main shield, one for a new source, and one to accommodate a decayed source. Figure 6 is a cross-sectional drawing of the transfer shield placed adjacent to the source shield. (In this view, only one of the holes is visible; the others are not in the plane of the drawing.) The front portion of the transfer shield has only a single hole, through which a source is placed in or removed from the source shield. The turret rotates about a horizontal axis in such a way that any one of the three holes in the turret can be aligned with the hole in the front section.

The loading procedure is as follows: The uranium plug with the source is placed into one of the turret holes to such a depth that the source is near the center of the transfer shield. The rear of the uranium plug is bolted to the turret. A solid shipping plug, which serves to reduce the external radiation, is placed into one of the unused turret holes with its forward end extending into the front portion of the transfer shield.

The shield is then delivered to the hospital site. There it is placed on a movable table and aligned with respect to the source shield with the aid of leveling and adjusting screws. The old source is then removed from the shield by means of a hand-operated loading wrench. The turret is rotated, placing the new source in the loading position. The new source is then pushed into place in the source shield. The transfer shield is removed, and the steel retaining plate (see Fig. 2) is replaced to secure the uranium plug and source. During loading, the source shield remains in place in the large rings. However, a section of the trim is removed to facilitate the loading procedure. The total time required for loading has not exceeded 2 hours.

RADIATION MEASUREMENTS

Measurements of the radiation field in the immediate vicinity of the source shield have been made with the shutter closed, using a 650-curie source. The weakest place in the shield is just behind the steel retaining plate (see Fig. 2). On a trim surface directly behind the plate, the reading was 3 mr per hour. On either side of the unit at points on the trim adjacent to the shield, the values ranged from 0.8 mr per hour near the front of the shield to 0 near the rear. At various points in front of the shield, the results varied from 2.5 mr per hour to 0.

Depth dose measurements, using an ion chamber, have been made using Masonite as the phantom material. Figure 7 gives some of the results obtained in which the source to detector distance is held fixed at 81.6 cm, that is, the position of the center of rotation of the unit. This is not the usual way of displaying depth dose data, but it is the way indicated by rotational therapy. The abscissa gives the thick-

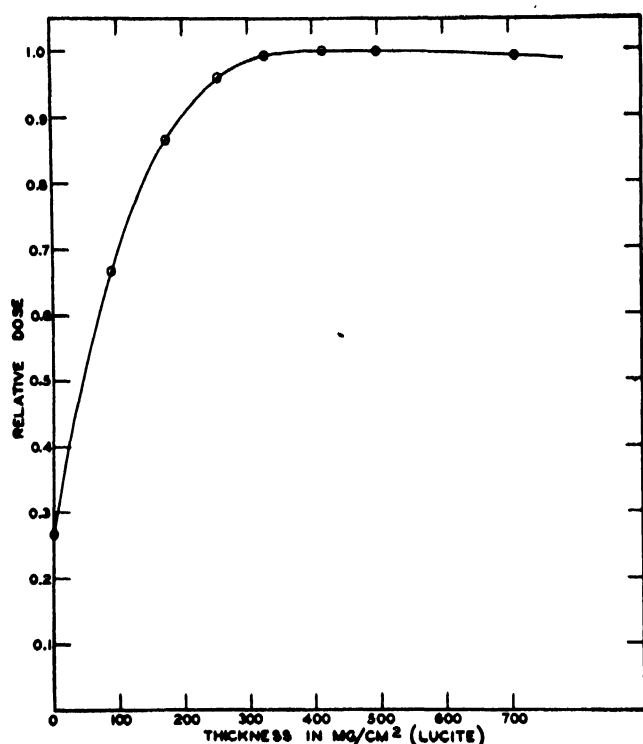


Figure 8. Dose plotted against thickness of added front wall on a pill box type ion chamber. Dose measurements are normalized to the maximum dose. Surface dose equals 26.7 per cent of the maximum

ness of Masonite between the source and detector. In all cases, the detector is backed by Masonite. The ordinate gives the ratio of the dose as measured in the phantom to that in air. The chamber that was used was calibrated for Co^{60} by the United States Bureau of Standards. The field sizes are those measured at the position of the detector.

Figure 8 shows data obtained with another ion chamber shaped like a pill box with a diameter of 3 cm and a thickness of 0.5 cm. Its axis is placed along the central axis of the gamma-ray beam. The top wall consists of 0.0012 cm Mylar film. The abscissa gives the thickness of Lucite placed on the chamber between it and the source. The ordinate shows the build-up of the ionization due to Compton electrons that were produced in the Lucite. These data are normalized to the peak dose that occurs

at 425 mg per cm^2 of material. The small surface dose indicates clearly the reason for the minimal skin reaction of patients treated with Co^{60} .

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* The above list of references is not to be taken as a complete list of the various designs of Co^{60} units.

Cobalt-60 Teletherapy Isodose Patterns for Combined and Non-Uniform Longitudinal and Transverse Source Motions

By C. B. Braestrup and R. T. Mooney,* USA

This investigation is an extension of previously reported studies¹ of the dose distribution obtained with a kilocurie rotating cobalt therapy equipment.² With this type of equipment the source of radiation may move over a large part of the surface of a hypothetical sphere with the beam of radiation always directed at the center as shown in Fig. 1. The advantage of this form of circumaxial moving beam therapy³ is that the patient is irradiated in a recumbent position insuring better fixation and duplication of position as well as greater comfort to the patient.

With Co⁶⁰ rotation therapy the skin is no longer the limiting factor in delivering an adequate dose to deep seated tumors. This has been indicated by phantom ionization measurements and confirmed clinically over a period of two years.

UNIFORM SOURCE MOTION

There still remains the important problem of minimizing the relative dose to normal, critical organs near the tumor. This objective may be accomplished partially by varying the source motion from complete rotation to scanning over a limited arc. It should be realized, however, that any decrease in source motion increases the relative skin dose within the scanning arc. This is illustrated in Figs. 2 and 3.

These dose patterns were obtained with the beam moving in a vertical plane, transverse to the longitudinal axis of the patient. The relative dose was determined photometrically using a contour Masonite phantom and industrial films, type M. The exposure was so adjusted that only the straight portion of the film calibration curve was used. The resulting

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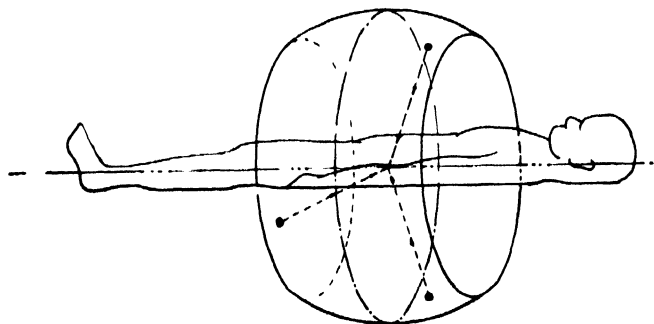


Figure 1. Beam direction obtainable with spherical source motions

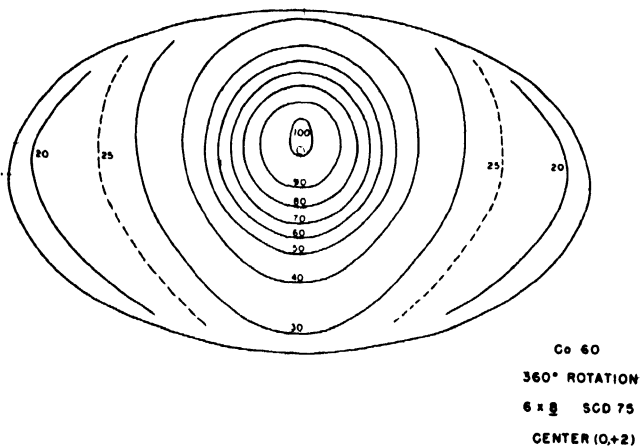


Figure 2. Isodose pattern for 360-degree rotation of source. The figures indicate percentage of center dose

isodose patterns check closely with those calculated from ionization measurements made in water phantom with stationary beams.

NON-UNIFORM SOURCE MOTION

It will be observed that there is considerable difference in the dose pattern for full rotation and for that where even a small arc, such as 60° is not utilized. Often the optimum dose distribution lies somewhere between those shown in Figs. 2 and 3; that is, between full rotation and scanning over 300 degrees. Such a dose pattern may be obtained by using 360 degrees rotation and increasing the speed of the source motion over the arc where reduced dose contribution is required.

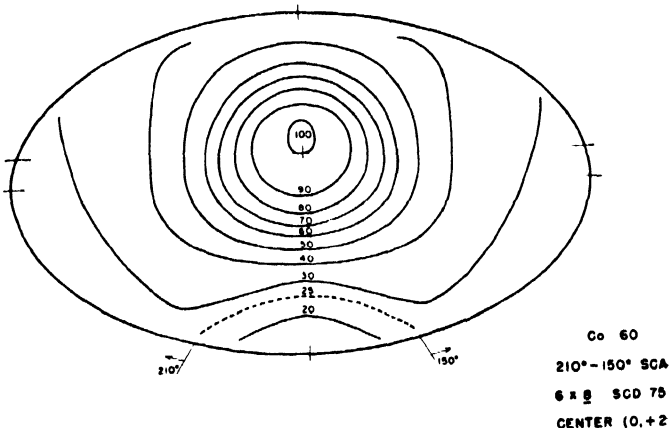


Figure 3. Isodose pattern for scanning from 210 to 150 degrees

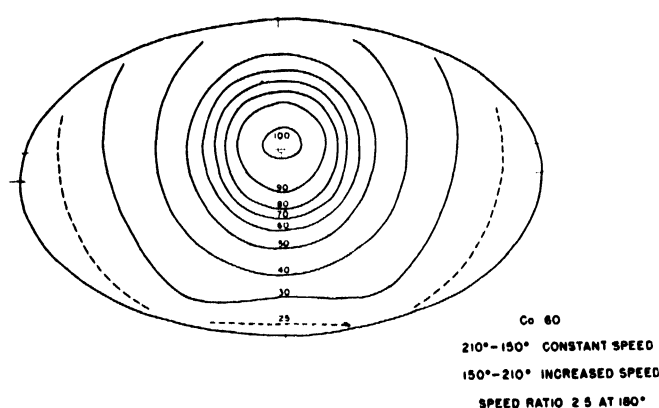


Figure 4. Isodose pattern obtained by rotating the source at constant speed from 210 to 150 degrees, and increased speed from 150 to 210 degrees. The maximum speed ratio was 2.5 and occurred at 180 degrees

The non-uniform speed is obtained by means of cams attached to the main drum shaft. The cams control the setting of a potentiometer which regulates the slip in an eddy current clutch thus changing the speed. Cams of different shapes can be attached to the shaft and so arranged that non-uniform speed can be accomplished for any arc up to 360 degrees.

A typical pattern obtained with this technique is shown in Fig. 4. A uniform speed of 0.5 revolution per minute was used from 210 to 150 degrees; from 150 degrees the speed was increased gradually until it reached a maximum of 1.25 revolutions per minute at 180 degrees. The speed was then reduced gradually, returning to 0.5 revolution per minute at 210 degrees.

It is evident that this technique permits a more flexible control of the dose distribution in the patient. The greater the relative speed over a given arc the more nearly is the dose pattern equal to that obtained by scanning over the remainder of the circumference; conversely, the more nearly uniform the speed the more closely does the dose pattern approach that of complete rotation. It is clear, however, that with this method there still is a definite limit to the dose differential between the tumor and adjacent tissue.

ROTATION WITH OBLIQUE BEAMS

It is possible to further reduce the dose to intermediate tissue by using two transverse planes of source rotation with the beam moving obliquely to the longitudinal axis of the patient; this is shown in Fig. 5. With this technique the beam is directed alternately through the upper and lower part of the critical organs while still aimed at the tumor; thus the tumor dose remains nearly the same while the dose to intermediate tissue is reduced materially. The dose pattern obtained with this arrangement is shown in Fig. 6. By comparison with Fig. 2 it is obvious that the dose falls off much more rapidly with distance from the axis of rotation; nearby tissue thus receives less radiation for the same tumor dose.

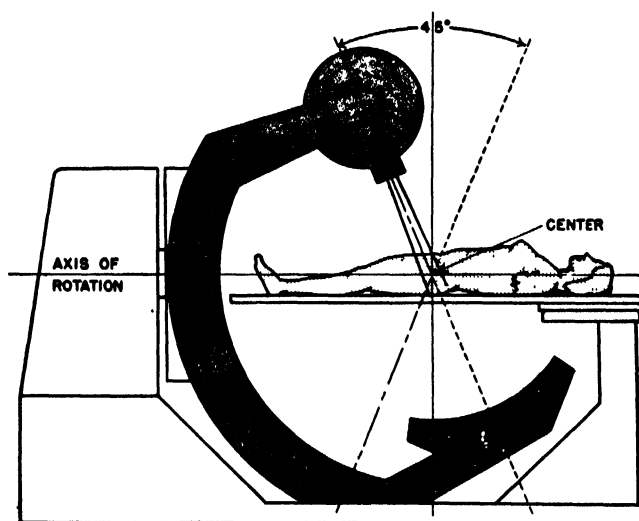


Figure 5. Schematic diagram illustrating the two source motions used to obtain the dose pattern shown in Fig. 6. The dash-dot lines indicate the conical surface of revolution generated by the central beam when the source is rotated in a vertical plane displaced to the left of center. The dotted lines apply when the displacement is to the right

DISCUSSION

There has been a tendency to use conventional X-ray techniques with cobalt teletherapy equipment. This study indicates clearly that new and more effective techniques are available with Co^{60} . Perhaps the outstanding advantage of cobalt teletherapy is the compactness of the radiation source which makes it uniquely adaptable for moving beam therapy.

Originally rotation therapy was employed primarily for the purpose of sparing the skin when irradiating deep-seated lesions. With cobalt moving beam therapy, a low relative dose to intermediate tissue is of particular importance. A large variety of techniques is available for this purpose. In selection of the optimum pattern in a given clinical case consideration must be given to the effects of the various controlling factors. These factors include: (1) size of field; (2) penumbra; (3) arc of rotation (complete rotation or scanning); (4) uniformity of source mo-

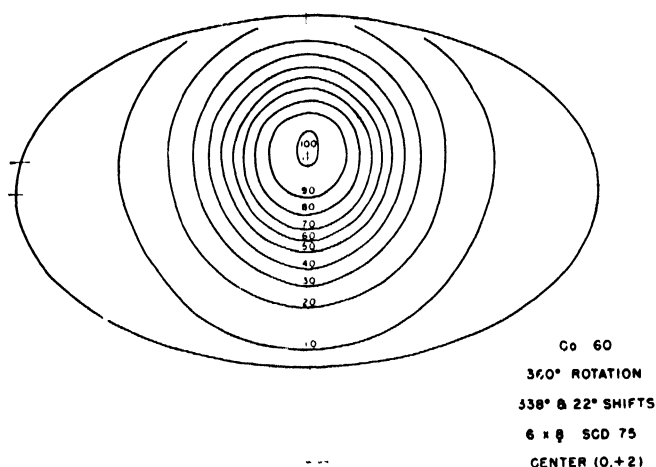


Figure 6. Isodose pattern obtained by 360-degree rotation with source shifts of 22 degrees to the left and right of center. The plane of the shifts is perpendicular to that of rotation as shown in Fig. 5

tion; (5) angle between beam direction and longitudinal axis of patient.

Isodose patterns have been prepared to indicate the effect of these factors and typical examples are included in this preliminary report. It obviously is possible to produce innumerable different isodose patterns; of these, however, only a limited number may be expected to have clinical usefulness. Such patterns must provide an adequate dose differential between the tumor and nearby normal critical organs, without excessive irradiation of other healthy tissue.

Extensive clinical investigations are needed to establish which patterns best meet these requirements.

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Use of the Betatron for the Treatment of Cancer of the Oesophagus and of the Broncho-Pulmonary System

By M. Tubiana,* J. Dutreix,* B. Pierquin* and P. Jockey† France

Since October, 1953, we have been using, for the treatment of thoracic tumors, a betatron (made by Allis Chalmers Establishments) which produces X-rays having a maximum energy of 22 Mev. Quite apart from the problems connected with the installation, and with protection of the medical personnel end of the untreated regions of the patient's body, we were led, by this work, to look into a certain number of questions.

DOSAGE PROBLEMS

Isodose Curves — The Change from Plexiglass to Water

With an ionization chamber, it is a simple matter to make a study of the distribution of the dose in a water phantom. However, the isodose curves are determined much more rapidly with photographic film, and lend themselves better to a study of uncommon occurrences which may require special therapeutic conditions. This, however, makes it imperative to use solid phantoms, made, for instance, of plexiglass. Now, the density and atomic composition of plexiglass are different from those of water, so that it is necessary to find a correction coefficient which makes it possible to change from the one to the other as needed.

Two curves showing the distribution of the dosage along the axis of a beam of rays were plotted experimentally, for water and plexiglass, with identical ionization chambers having a volume of 0.5 cm³. If the lengths are expressed in such a way as to allow for the number of electrons per cm³ (3.34×10^{23} for water and 3.84×10^{23} for plexiglass, namely a ratio of 1.15) and an inverse square law distance correction is made in order to get the curves for a parallel beam, it is found that, within the precision limits allowed by these measurements (3%), the two curves can be superimposed.

Thus, it is possible, allowing only for geometrical conditions and the number of electrons present in the two materials, to change from the depth distribution of the dose, as computed for one medium, to that found for the other.

Dose Due to the Photons Created by the Annihilation of Positrons

When electron pairs are formed, the negative electron, after it has lost its kinetic energy, behaves like a free electron, while the positive electron is annihilated, at the end of its path, by becoming bound to a free negative electron. Their masses are transformed into energy, and there appear two 0.5 Mev photons. The dose due to these annihilation photons can be computed, bearing in mind that each annihilation is equivalent to the disintegration of two radioactive atoms, each of which emits one 0.5 Mev photon. The number of annihilation photons, per cm³ of plexiglass which has received a dose of 1 r (as defined by the Mayneord convention), would correspond to a concentration of 1.65×10^{-5} mc/hr of an element which emits one 0.5 Mev per disintegration.

The dose received at any point of the irradiated volume is $D = kcg$, in which k is the dose delivered in one hour at 1 cm from a 1 mc point source for 0.5 Mev photons, $k = 3$ r/mc/hr, and g is the geometrical factor (in the case of a cylinder having a height of 34 cm and a radius of 14 cm, $g = 165$ at the center of the cylinder and 90 at the center of its base).

In the case under discussion, the dose D due to the annihilation photons thus amounts to about 1% of the total dose at these points.

Dose Due to the Bremsstrahlung

The fast electrons emit X-rays by bremsstrahlung. The number of electrons in each energy range which go through a 1 cm² surface in the mass of irradiated water having been established (by the method of Cormack and Johns), surface integration provides a means of computing the radiative energy loss per unit length (cm) covered by the electrons, and thus the photon flux emitted.

Assuming that these photons are directed forward, and that their average energy is equal to $\frac{1}{3}$ that of the electrons, it is computed that, for a flux corresponding to 1 r at the surface, the dosage due to the bremsstrahlung at a depth of 35 cm is 0.01 r, or less than 2% of the total dose at that point.

By adding the doses arising from the absorption of annihilation photons and bremsstrahlung due to neutrons found in the X-ray beam, to neutrons

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released during a photonuclear reaction of the (γ, n) type and to the radiation emitted by the radioactive body formed by these reactions, a total is reached of approximately 5% of the dose due to the secondary electrons excited by the Compton effect and the formation of pairs.

Role of Scattering

The yield in depth varies but little when the cross section of the bundle increases, in view of the facts that there is not much scattering (the ratio between the scattered energy and that absorbed is much smaller than for less energetic X-rays), and also that the direction of the scattered photons is not markedly different from that of the incident photons. However, this scattered radiation may alter the spectral composition of the beam, and it struck us as worthwhile to measure the contribution of these scattered photons to the depth dose.

1. By interposing a lead shield 25 cm thick in the axis of the beam, it is possible to stop completely the central part, or core, of the said beam. An ionization chamber placed in such a phantom, along the axis of the beam, thus measures only scattered radiation.

2. By leaving in a fixed position an ionization chamber having sufficiently thick walls to ensure electronic equilibrium, and by moving a plexiglass phantom along the axis of the beam, the flux of primary photons which reaches the chamber does not vary. Under those conditions, the increase in the observed dose, when the distance between the plexiglass phantom and the chamber decreases, is due to the radiation scattered by the plexiglass.

It can also be shown that, under the usual therapeutic conditions, 20% of the dose may be due to scattered radiation. The table below shows, for instance, the fraction of the dose due to scattering, on the axis of the cylindrical bundle of radius r , behind a thickness y of plexiglass.

y (cm)	$2r$ (cm)		
	10	20	30
10	3%	5%	6.5%
20	6%	10%	12%
30	8%	14%	18%

Absorbed Energy

1. To pass from the number of pairs of ions formed in a thin wall chamber having a known volume to the energy absorbed by mass unit, it is necessary to know the ratio between the energies absorbed locally by unit length of an electron path, in the gas, and in the phantom, respectively. This ratio, which is designated by the letter ρ is equal, as a first approximation, to the ratio between the relative stopping powers, with respect to electrons, of the gas which fills the chamber, and of the irradiated medium.

ρ varies with the energy of the electrons, which led us to compute the energetic spectrum of the elec-

trons which circulate in the mass of the irradiated volume and the various corresponding values of ρ .

Computation also shows that, in bone, 0.67 rad are absorbed for an ionization of 1 esu/cm³.

For the same flux of 1 r, bone absorbs 0.77 rad and water 0.90 rad. The soft tissues surrounded by bone received the same number of rads as the bone itself.

2. By using an ice calorimeter for the purpose of measuring the energy absorbed locally, and a set-up which makes it possible to measure the absolute value of the ionization created in a very thin wall chamber, we found that, for 1 esu/cm³, a dose of 0.94 ± 0.15 rad was delivered to water.

3. We computed the theoretical ratio of ionizations in water and bone respectively, for a given flux of photons: this ratio is 1.14. We verified experimentally the value of this figure, and thus confirmed the results of the computation of the theoretical parameters used for bone.

The results obtained for water have shown that, under our experimental conditions, the use of Gray's formula ($E = JW$) is justified, and that the margin of error is $\pm 15\%$, and we have applied the same indeterminacy to the results obtained in the case of bone. Thus, the quantity of energy absorbed by bone is 0.67 ± 0.10 rad for 1 esu/cm³.

TECHNICAL PROBLEMS

Improvement in the Spectral Composition of the Beam

The usefulness of a filter rests in its ability selectively to stop low energy radiation while showing little absorption for the most energetic photons. In such a range of energies, we thus must use a body of low atomic weight. Theoretical computation shows that, under 62 cm of filtration through graphite, the spectrum is considerably shifted toward the high energies, the maximum energy passing from 5 to 17 Mev.

However, it is found experimentally that the distribution of the dose in depth is but little modified: the maximum passes from 4 cm to 6.5 cm but the subsequent attenuation remains the same. The advantages are too limited to justify the important reduction in beam intensity connected with such a filtration.

Electron Scattering — Shield in the Beam

The dose at the surface of an irradiated medium depends on the number of electrons present in the beam of photons, both at the level considered, and coming from levels which have been gone through before.

If the shield is interposed in the beam at some distance from the phantom, the electrons which are set in motion in the shield increase the dose at the surface of the phantom, as well as the ratio between the surface and maximum doses respectively, thus reducing one of the major therapeutic advantages of the high energy beam. Indeed, if the electrons are thrown forward at zero time, interactions in the shield modify

their directions and, as they come out of the shield, the direction of the electrons makes a substantial angle with that of the photons.

We have studied, under the conditions customary for radiotherapy, this dispersion of the secondary electrons and its influence on the additional dose created by a shield in the first centimeters of the phantom. By measuring the electron flux in air behind a radiating screen, it is noted that this flux decreases rapidly and that, at 30 cm it doesn't exceed 10% of its initial value as measured immediately behind the screen.

This decrease which is all the more rapid as the beam is thinner, follows a law of the d/r form (d = distance from the shield to the point where the measurement is made, r = radius of the cross section of the beam at the level of the screen).

A study also has been made of the influence of the secondary electrons emitted by the screen on the depth distribution of the dose in a phantom. When the distance D from screen to phantom is small, the point of the phantom at which the dose is maximum is very close to the surface. As D increases, the scattering of the electrons causes the dose which they bring at the level of the field of entry to drop very rapidly, and the maximum is displaced in depth. The influence of the screen is no longer perceptible on the transition curve of the irradiated medium starting from distances which were found to be equal to 10 cm, 20 cm and 40 cm for beams having diameters of 4.5, 7.5 and 15 cm (for a screen made of plexiglass or aluminum, the thickness of which is greater than the thickness which ensures electronic equilibrium).

To the extent that this minimum distance can be preserved, it is thus feasible to insert, in the path of the beam and ahead of the patients: (1) a mirror for centering of the anterior aspect; (2) compensation wedges to homogenize the dose delivered to the target volume when certain arrangements are adopted (T shaped fields, for example); (3) a compensating cone, which can be placed outside the magnetic field, thus avoiding the need to have it laminated; its distance from the source gives it a more rounded form, which facilitates centering and aligning with the beam.

THERAPEUTIC USE

Method of Irradiation

The sharpness of the geometric limits of the beam, along with the small amount of lateral scattering, accounts for the fact that a point some centimeters outside the beam is not irradiated; this requires very accurate ballistics for the proper treatment of a tumor.

Localization of the Tumor

In view of the inconstancy of the geometric relationships of the tissues and viscera which make up the human body, it appeared necessary, in order that the relative position of the area of the tumor should

remain constant with respect to the neighboring and peripheral tissues, from the very first moment of tumor localization to the last treatment sessions, that the patient should always occupy the same position.

The indices used to define this position cannot be chosen in the soft tissues, and the skin, in particular, moves too readily, with respect to the deep planes, to lend itself to use in that fashion. In order to make localization easier, and to immobilize the irradiated segment, we mold it in a rigid envelope made of a plastic substance.

While identification of the tumor limits on the plates is easy (for instance, the oesophagus following opacification), it is convenient, in order to determine its position, to use a reference system outside the organism. The segment under study is placed between two parallel plexiglass plates. A network made of thin parallel and equidistant lead wires is embedded in the plexiglass and makes it possible to effect localization, on the X-ray picture, of a tumor projected on these two plates.

Using plates taken from two directions, one can readily reconstitute the position and volume of the tumor in space.

The contours of the organism in one or several planes passing through the tumor then are defined by means of a special measuring device, the sliding rods of which are brought into contact with the mold.

In the case of the lung, the contours are not so clear, and such localization becomes difficult. We prefer to make axial tomographic sections of the patient already in the mold, on which lead markers determine the levels of the sections. These plates supply a distortion-free enlarged picture, which can be brought up to the actual size of the corresponding section of the thorax, showing the limits and relations of the tumor with neighboring organs and the general outline of the body.

Centering of the Beams

The choice of the number, size and direction of the beams, is determined on the section. This allows for the position and size of the target volume, for its anatomical relations, for the radiosensitivity of the peripheral tissues, and for the risks entailed by the irradiation, as well as for the yield in depth of the X-ray beam used.

Index marks are necessary to give the beams the desired orientation during the courses of therapy. The points of entrance and exit of the axis of the beam are determined on the cross sections and transferred to the mold with a compass. If these points alone were used to define the beam, the patient could pivot about this axis. In order to rule out this uncertainty as to the position of the patient, it would be enough to take the position of a third point, but it is simpler, at times, to locate it during treatment, moving the patient with respect to the reference system in the position which he occupied when the tumor was local-

ized and the beams centered. This ensures reproducibility of the patient's position.

Setting Up and Immobilization

The patient is first positioned with respect to his seat and points of support. The seat then is brought before the betatron without moving the patient with respect to it.

In order to visualize the X-ray beam, we used two optical devices, one of which traces the outline of the entrance field of the beam on the patient, while the other, by means of the projection of a reticule, shows the center of the exit field. Thus verification of the adjustments is rapid, whatever the direction of the equipment.

During irradiation, it is essential to make sure that the patient does not move. So that the operator is aware of a possible displacement, a circular photoelectric cell is placed at the center of the exit field; four quadrants balanced two by two, have been traced on the cell, and they are lit by the optical system used to determine the center of the exit field. If the patient changes position, one of the quadrants is less brightly lit than the others, the equilibrium is broken, and this displacement of the patient appears as the variation of a galvanometer spot. This safety device proved useful in the case of tired or uncooperative patients, particularly during the first sessions.

Transmission Through Bone and Lung

The computation of the depth dose and the tracing of the isodose curves are carried out by assuming that the irradiated medium is homogeneous and equivalent to water. Heterogeneity of the tissue (bone and lung in particular) requires important corrections of these computations. These corrections are exceedingly complex at 200 kv, difficult with a radiation of Co^{60} , and relatively easy with a beam produced by the betatron, the small amount of lateral scattering makes it possible to assume that the dose, at this point, is, in the main, a function of the primary radiation which reaches it, and of the attenuation which it suffers by going through tissue.

The experimental evaluation of the attenuation in the various tissues is effected by measuring the transmission along an axis by means of ionization chambers which are placed on this axis ahead of, and behind, the patient. The thicknesses of the various tissues gone through are measured for each direction with the help of stratigraphic plates, and reduced to scale.

Repeated measurements on the same axis and on the same patient, on the occasion of different sessions of irradiation, give results which are comparable within $\pm 10\%$.

We studied 8 fields of irradiation (in five different patients). For each field, the measurements were repeated two to four times, while, for each measurement, transmission was measured along the five axes.

Study of the results shows that the attenuation, for 1 cm of bone, is equivalent to that of 2 cm of water, and that of 1 cm of lung to $\frac{1}{2}$ cm of water. The correction factor used in order to allow for such considerations when evaluating the tumor dose may reach 20%.

Choice of Patients

1. Since it is still difficult to evaluate the therapeutic effect of such an energetic radiation, it appeared preferable to irradiate at first types of cancer for which the classical methods gave but mediocre or poor results.

2. Conversely, it seemed useless to treat patients for whom the extent of the malignant process ruled out any hope of a final cure.

3. The deepest tumors are those which are most benefited by so penetrating a radiation. For instance, in the case of a patient having a thorax 28-cm thick along the axis of the radiation flux, with cancer of the oesophagus 14 cm away from the skin, a skin dose of 5 r, corresponds to 1 r delivered to the tumor with 200 kv X-rays, and 10 r with a betatron. In this case, the volume to be irradiated was a cube having a side 6 cm, treated by means of 4 opposite fields arranged 2×2 and crossed at right angles, giving 1 rad at the tumor site. The total dose delivered to the healthy tissues is 260 gm-rad with the betatron and 560 with 200 kv, while the total dose applied to the tumor then is 80 gm-rad with the betatron and 70 with 200 kv.

In order to fulfill this group of conditions we have treated, with the betatron, mainly two types of tumors: Cancer of the oesophagus and bronchopulmonary cancer.

First Therapeutic Results

Cancers of the Oesophagus

The tumor dose, in all cases, was 6000 rad, at the level of the tumor, and treatment lasted 6 weeks on an average. It always was perfectly tolerated; we never observed any anemia, leucopenia or radiation sickness. It would seem, on the other hand, that a belated reaction might take place in the months which followed the end of the treatment. In nearly all cases, dysphagia had disappeared or much decreased during the weeks which followed the end of the treatment. Thus, the immediate symptomatic improvement was constant.

Since November, 1953, 41 cancers of the oesophagus were treated. For 15 of these, more than a year elapsed since the course of treatment. Four are alive, 1 died at the very beginning of the treatment, and 10 have since died. Out of the 18 patients treated for over 6 months and less than 1 year, 8 died, 9 are alive, and contact was lost with 1.

Six post-mortems were carried out. In 4 cases, there was persistency of the cancer tissue at the level of the irradiated tumor: in 2 cases no lesion could be found at the level of the primary tumor (1 case of

pancreatic recurrence, 1 case of recurrence at the level of the oesophagus). In 2 cases, bronchial perforations were found, and in 3 more cases, there were lesions of the myocardium with extensive adipose infiltration.

Lung Cancer

Twenty cancers of the lung have been treated with the betatron. One tumor dose of 5500 to 6000 rad was delivered in 6 weeks. This treatment, generally speaking, was tolerated perfectly. Even though we have not had time to evaluate the therapeutic results, we are in a position to indicate that, while, in some cases, the dose is sufficient to sterilize the tumor (in one case we have not found any cancer tissue at the level of the lung, although there were metastases), in many other cases, the tumor continued developing despite irradiation. Belated accidents have been observed, particularly a case of total paralysis con-

nected with medullary lesions (the dose at the level of the spinal column could, in this case, be evaluated at some 4000 rad).

CONCLUSION

The practical conditions under which a betatron developing 22 Mev is used lent themselves perfectly to the treatment of thoracic tumors and make it possible to irradiate the region of the malignancy accurately and intensely.

The determination of the optimal tumor dose remains difficult. For cancers of the oesophagus, as for those of the lung, doses varying between 5500 and 6000 rad do not always provide sterilization of the tumor, although, in this case, the irradiation of the neighboring healthy tissues is not devoid of risk, and certainly demands the exercise of a great many precautions.

Pre-Therapeutic Considerations for 70 Mv X-Rays

By Gail D. Adams and Robert S. Stone,* USA

In July, 1952, a 70 Mev synchrotron, built by the General Electric Company and installed in this Laboratory by the US Atomic Energy Commission, was accepted in working order. In the conversion of that basic unit to one capable of continued use suited to cancer therapy, a number of interesting points have been uncovered. This paper is concerned with the highlights of these developments and preparations.

THE SYNCHROTRON

The synchrotron¹ is a circular magnetic electron accelerator the design² of which has been described elsewhere. Some of the Radiological Laboratory synchrotron characteristics are displayed in Table I.

Table I. General Characteristics of the Synchrotron

Energy rating, electrons at internal target	70 Mev
Pole diameter	29 in.
Line power requirements	50 kva
Circulating energy in resonant circuit	4500 kva
Suspended weight	17 tons

This particular unit has been suspended from an overhead truss the elevation of which can be adjusted by hydraulic rams. The whole magnet structure can also be rotated about a horizontal axis passing through the target position so that the X-ray beam produced can be angulated and elevated to suit a proposed treatment schedule. As delivered, the unit is capable of producing an X-ray beam in the range of 20 to 70 Mev. Because of low X-ray intensity at reduced energy and because of technical difficulties to be mentioned below in the utilization of more than one energy, the only X-rays for which clinical use is anticipated are those generated from 70 Mev electrons. Ultimately the electron beam will also be extracted and put to clinical use. It might be difficult to justify coping with the technical difficulty of controlling 70 Mv X-rays were it not for the eventual availability of a variable energy electron beam.

X-RAY BEAM SHAPE

The 70 Mv X-ray beam produced by the synchrotron does not have uniform intensity in all directions; it is highly peaked³ in the direction which the electrons had as they approached the target. The full angular width of this beam is about two degrees of

arc measured between points of intensity one-half of maximum. It was considered that a beam of such great non-uniformity would not be applicable to radiobiological experimentation nor to cancer therapy. Compensation to flatness (uniformity) is achieved by the introduction of a suitably shaped absorbing filter. In selecting the actual filter size it was necessary to effect a compromise between field size and unabsorbed output. The choice made produces a reasonably uniform field at the peak of the depth-dose curve (Fig. 1) for a 15-cm diameter field and having an ionization indication of about 30 esu/cm³-min, all at a distance of 2 meters from the target. It is the existence of the depth-dose peak at approximately 10 cm depth which makes attractive the utilization of 70 Mv X-rays in cancer therapy.

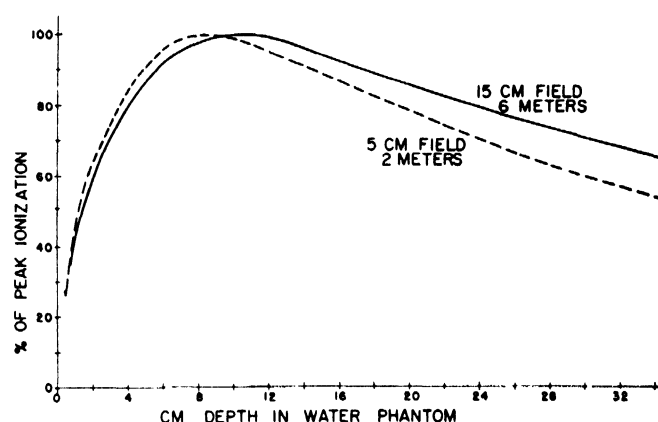


Figure 1. Typical central-axis depth-dose curves for 70 Mv X-rays. Taken with an ionization chamber in a water bath

The statements above pertaining to measurements on the beam lack precision because rigorous statements and measurements are difficult. No sooner are these measurements started than the whole question of X-ray intensity and absorbed dose rate is posed. Does a given detector respond to either one alone or to an arbitrary mixture? Which quantity is really needed for a given situation? The tissue is complex but a number of examples have been identified, some of which will be mentioned in the balance of this section.

X-ray film of types exhibiting a linear increase in photographic density with exposure have been used in a Pressdwood phantom to examine beam shapes. It is thought that at least the major part of the blackening is proportional to local energy absorption,

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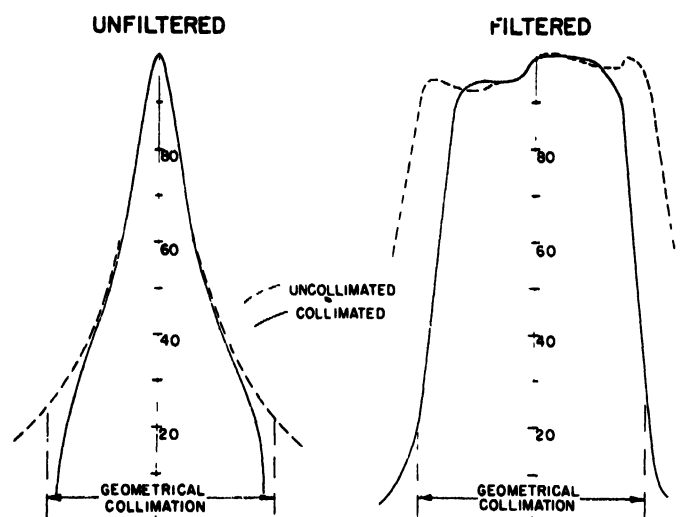


Figure 2. A comparison of the effect of introducing a collimator for compensated and uncompensated beams of 70 Mv X-rays

that is, to absorbed dose. Figure 2 illustrates the per cent density variations across the beam for four situations, all at the customary 2 meters from the target. On the left are compared the natural X-ray beam with and without the 15 cm field defining collimator. The contribution to film density near the shadow of the collimator due to X-ray intensity beyond the shadow is clearly seen. The comparison on the right is perhaps more striking. A field was approximately compensated with the collimator removed and then the change upon insertion of the collimator demonstrated the real contribution of secondary radiation from relatively distant primary radiation. Quite obviously, further filtration was necessary to compensate the beam to the desired 15-cm diameter. In fact, for best results, it will probably be necessary to construct a separate filter for each field size and shape to be used. One might point out that the variations in the "flat" region are undoubtedly due to irregularities in the X-ray film. By multiple exposures of the same field it has been found that peak relative density fluctuations can be as small as 2 or 3 per cent in favorable cases but usually is 5 per cent and occasionally is as high as 15 per cent.

In the course of a calorimetric calibration of the synchrotron, it was found convenient to use a field the diameter of which was 5 cm. This was achieved by adding a suitable collimator to the existing 15 cm nearly flat beam without any change in the compensating filter. By so doing, the peak of the depth-dose curve was moved towards the surface from 10 cm to perhaps 8 cm, the ionization rate at the peak was reduced about 22 per cent, and the beam shape was changed as shown in Fig. 3. Moving the water phantom in which these ionization rates were measured back to 6 meters from the X-ray target so that the field diameter was again 15 cm, essentially the same ionization was collected as would have been predicted from the 15 cm field at 2 meters using only the inverse square law for distance conversion. In fact, the ionization was about 4 per cent higher than expected

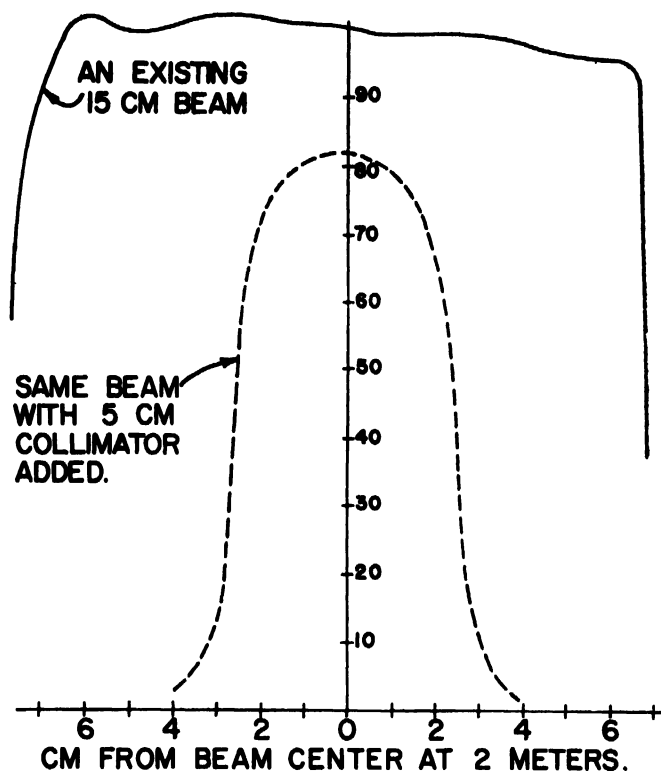


Figure 3. The effect of introducing a collimator to produce a 5 cm beam of 70 Mv X-rays at 2 meters from the X-ray target. The ordinate values have been corrected to demonstrate the loss in ionization at the depth-dose peak by this introduction

but the ionization peak had moved back to 11 cm, all of which is probably due to smaller divergence of the primary radiation at the larger distance. This again demonstrates the importance of secondary radiation to absorbed dose at points lateral to that at which absorption of primary radiation occurred.

A simple experiment was designed to demonstrate approximately the relative appearance of X-ray exposure and absorbed dose using the same geometry except for the detector. The nearly compensated X-ray beam, 15 cm in diameter and at 2 meters as usual, was directed towards a Pressdwood phantom. An X-ray film was exposed and the density measured is regarded as substantially proportional to absorbed dose. Subsequently, a copper sheet was inserted in the same position as the film had been and was irradiated for about 20 minutes. The sheet was then placed in contact with X-ray film to expose the film in proportion to the radioactivity which had been induced. The induction of activity depends primarily on the absorption of photons, and most of the photons in the energy range which efficiently produced this activity should still be primary despite the absorption processes preceding the position of the sheet. Quantitative distinction has not been made because neither detection scheme is pure as to intensity or absorbed dose rate. That these two quantities are not proportional everywhere is clear from Fig. 4.

COMPENSATING FILTER POSITION

Perhaps the most difficult mechanical problem found was that of providing an adequate support for

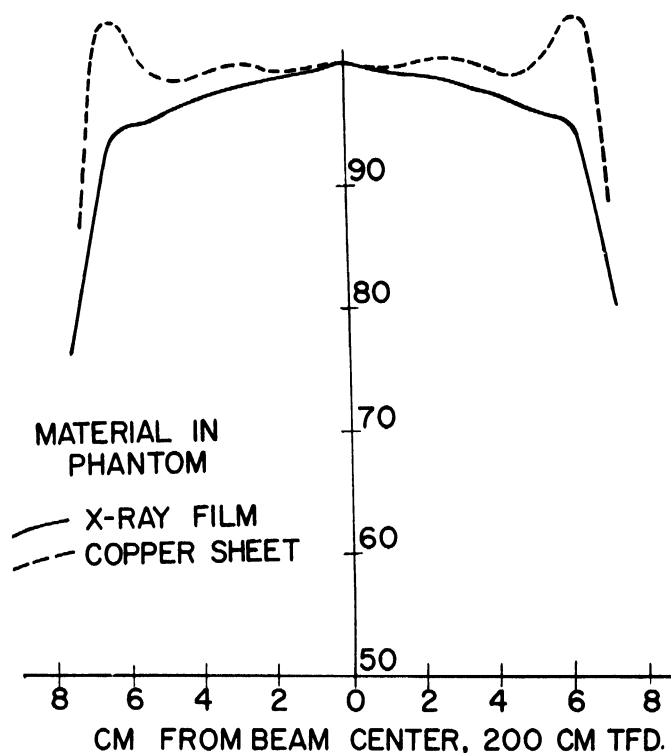


Figure 4. An approximation to the distinction between intensity and absorbed dose rate for 70 Mv X-rays. In one case, X-ray film is exposed directly in a Presswood phantom; in the other, a copper sheet is "exposed" at the same place and the induced activity is allowed to blacken another film

the field compensating filter. The compensating filter which is used is approximately conical in shape, is 1.25 in. in diameter at the base and has an altitude of 1.68 in. In the center of the beam the absorption is about a factor of five. This large central absorption and the highly peaked beam make the precise align-

ment of this filter with the beam rather critical. The problem concerning the support can be stated as follows: Assume a filter has been shaped and positioned coaxially with the X-ray beam so as to produce the flatness desired. Then a translation of the filter perpendicular to the beam direction produces a change in the relative height of the shoulders of the previously flat beam shape. The rate of change of heights of these shoulders with translation is about 32 per cent per millimeter. Apparently owing to the mass of the lead blanket across the face of the synchrotron, no structures were found which were immobile and to which the filter could be attached. Intensity variations of a few per cent would not be objectionable, but some of the structures show relative motions of at least 1.5 mm between horizontal and vertical positions of the X-ray beam.

Despite the additional complication, a servomechanism has been developed and installed. The operation of this device may be explained as follows, referring to Fig. 5: A quadrant transmission ionization chamber is used to sense any variations from a previously determined condition of flatness. The quadrants are connected in pairs through gain-stable dc amplifiers to Brown amplifiers. The Brown amplifiers are chopper amplifiers which have an ac output, the phase of which is determined by the sign of the dc input signal. Each such ac output is fed into a two-phase motor so that the direction of rotation of the motor shaft depends on the sign of the dc signal. Actuated by the motor rotation is a piston, the motion of which displaces oil in a closed system. At the other end of the oil lines is a similar piston. This piston is moved by the oil displaced by motor rotation. It is connected

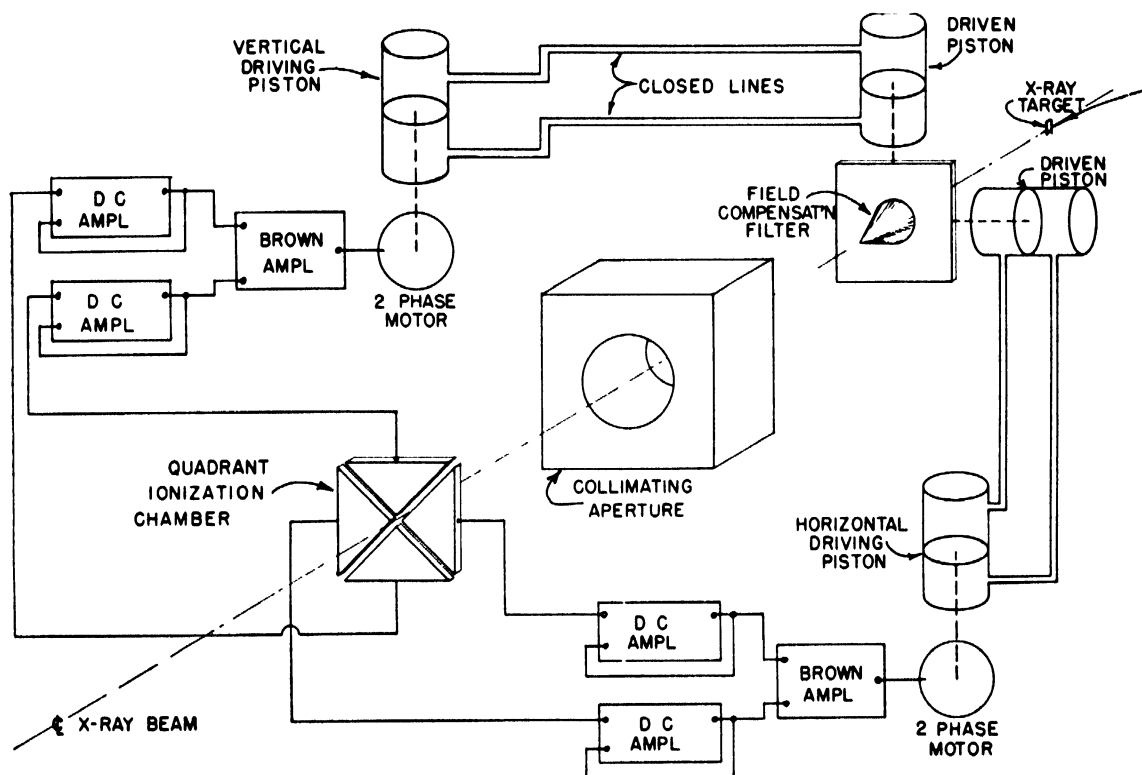


Figure 5. The servomechanism to maintain alignment of the compensating filter with the 70 Mv X-ray beam

to the support for the compensating filter. This support is mounted in independent horizontal and vertical ways. Overall, then, dissimilar signals from a pair of quadrants cause the filter to be moved in such a direction as to minimize the difference between signals. The unit responds to differences of about 1 per cent at the level used and appears to function reliably over substantial periods.

EXPOSURE INTEGRATION

In conventional X-ray generators the tube current can be measured conveniently. This is not the case with the synchrotron. In consequence, accurate measurements of total exposures delivered cannot be made employing "milliampere-seconds." The X-rays from the synchrotron appear in pulses of short duration. A properly designed⁴ transmission ionization chamber may prove to be a reliable indicator of intensity. However, a part of the current collected will be the result of ionization from electrons which are not secondary to local absorption of photons, and another part will represent spurious currents such as leakage due to insulator conductivity. Mainly for these reasons it was believed that proportionality between chamber current and X-ray intensity would have to be demonstrated and could not be assumed. Consequently another method for integration of total exposures delivered was sought.

Induced radioactivity appeared to hold considerable promise. It was known⁵ that the most efficient production of artificial activities by photons occurred for photons of energy between 10 and 30 Mev. A previous use⁶ of induced activity was especially difficult since the peak energy actually fell midway across the efficient activity-induction span. This required exceptionally close control of the peak energy (that to which electrons are accelerated). However, using X-rays in the neighborhood of 70 Mv, the amount of activity induced per unit exposure varies slowly with X-ray energy. As a matter of fact, closer control of energy is required to match the existing shape of the compensating filter than for maintaining the calibration of induced activity for exposure integration.

Activities induced in titanium, zinc, magnesium, nickel, and tantalum were investigated for suitability. The element finally chosen was magnesium and the principal contributing activity is $_{11}\text{Na}^{24}$ which is apparently formed by $_{12}\text{Mg}^{26}(\gamma, p)_{11}\text{Na}^{24}$. Graphical analysis of half-lives formed in magnesium has repeatedly shown the following values to be present together with a tentative assignment of the production reaction: $_{12}\text{Mg}^{26}(\gamma, p)_{11}\text{Na}^{24}$, 15.0 hr; $_{12}\text{Mg}^{24}(\gamma, 3p3n)_{9}\text{F}^{18}$, 112 min; $_{12}\text{Mg}^{26}(n, \gamma)_{12}\text{Mg}^{27}$, 9.5 min; $_{12}\text{Mg}^{26}(\gamma, p)_{12}\text{Na}^{25}$, 62 sec; and $_{12}\text{Mg}^{24}(\gamma, n)_{12}\text{Mg}^{23}$, 12 sec.

Of these, the only unusual one is that producing F^{18} . The reaction type is shown as $(\gamma, 3p3n)$ only to show the necessary number of particles emitted. The actual reaction may be as elegant as $(\gamma, a d)$. The

production of this isotope from 70 Mv X-rays has been reported recently.⁷ It is surprising that it is produced in noticeable amount compared with Na^{24} , for example. Correcting the observed counting rates for half-life, decay during production, and relative abundance of the parent isotopes, the ratio of cross sections for production of F^{18} relative to Na^{24} appears to be about 1:400.

A fixture has been arranged on the face of the synchrotron to hold these foils in reproducible positions for irradiation. After irradiation, a waiting period of 30 minutes is allowed to elapse before starting a standard counting interval of 30 minutes. The waiting period allows the short half-life activities to decay to the point where they contribute little in the standard counting interval.

The count recorded in the standard interval requires correction for decay during production. If only one isotope were contributing to the count in this interval, then the form of the correction function for irradiation time t_1 would be

$$\lambda t_1 (1 - e^{-\lambda t_1})^{-1}$$

λ being the decay constant for that activity. This function gives a number by which the observed standard count can be multiplied to obtain a count which is proportional to the exposure, given during t_1 . When n activities are present this function takes the more complicated form:

$$F(t_1) = \left[\sum_{i=1}^n \frac{\rho_i A_i}{\rho_i \lambda_i t_1} (1 - e^{-\lambda_i t_1}) \right]^{-1} \quad (1)$$

where λ_i = decay constant of the i th activity, ρ_i = relative apparent production cross section, $A_i = e^{-\lambda_i(t_s - t_1)} - e^{-\lambda(t_s - t_1)} = e^{-30\lambda_i} - e^{-60\lambda_i}$.

This formula was derived for the case of constant intensity. The case of intensity increasing linearly during t_1 was also examined. The instance of a 20 per cent increase was compared numerically with that of constant intensity at the average value of the increasing intensity instance. The correction to the standard count for the non-uniform intensity amounts to only 0.014 per cent so the relatively simple form of Equation 1 is used.

The relative apparent cross sections, ρ_i , which include counting geometry, isotopic abundance, and the like, must be determined experimentally. This was done from analysis of activities and half-lives induced for a graded series of seven exposures from 2 to 60 minutes in duration. A Victoreen ratemeter was connected to a Brown strip chart recorder to normalize the activities for actual exposure. The probe of the ratemeter was located at the peak of the depth-dose curve in a Pressdwood phantom at about 2 meters from the X-ray target. The location was not disturbed during the series. The correction function $F(t_1)$ in practice is normalized to be unity at $t_1 = 0$ and rises almost linearly to 1.09 for $t_1 = 80$ minutes.

The counting system is very reliable but represents the end of a developmental study. End-window Geiger counters were used at first but have been discontinued in favor of scintillation counters. The best Geiger counters used⁸ appeared excellent at first. They were judged by counting a standard long half-life sample each morning. At that time there were only a few exposures during any one day which required use of counters. Later, when some preliminary radiobiological experiments were in progress, the counters were used almost continuously each day. Exposure integral evaluations were found not to correspond to approximate evaluations from monitor indications nor to apparent biological effect. It was found, by counting the standard sample repeatedly all day following the pattern set during the experiments, that the sensitivity decreased monotonically at least 5 per cent during the day but nearly "recovered" overnight, averaging not more than 0.5 per cent decrease from day to day even in this service. A comparison was made between these counters and scintillators, using anthracene crystals with RCA 5819 and later with Du Mont 6292 multiplier phototubes. Initial results indicated superior performance. One drift was noticed which was traced to temperature of either the crystal or photocathode. Air-cooling this area increases sensitivity and enables uniform temperature and hence uniform sensitivity to be maintained. Quite contrary to the experience of others,⁹ these counters exhibit a standard count which is reproducible to about 1 per cent, the value obtained not fluctuating outside this limit for at least three months.

DOSIMETRY

Very often the doses delivered by X-rays to extensive media are estimated from the response of suitable ionization chambers immersed in the media. Interpretation based on the Bragg-Gray principle converts ionization measurements into values of absorbed dose. This Laboratory has undertaken experiments to establish the validity of this procedure under some circumstances.

Ionization measurements include spurious currents due to insulator conductivity¹⁰ and perhaps other extra-cameral effects. An attempt has been made to measure the resistivity of a series of insulators during and following irradiation with 250 kv X-rays. Figure 6 illustrates a typical behavior of polystyrene and Teflon. It should be noted that different samples of the same material will frequently exhibit quite different characteristics. For the determination of resistivity during irradiation, the sample was constructed and mounted so as to guard against collection of ionization or surface leakage currents. The resistivities found are volume resistivities; no attack has been made on the problem of surface leakage.

The Victoreen thimble has been found to have serious amounts of leakage following irradiation. The resistivities shown in Fig. 6 are estimated from observed leakage rates by noticing that the formal

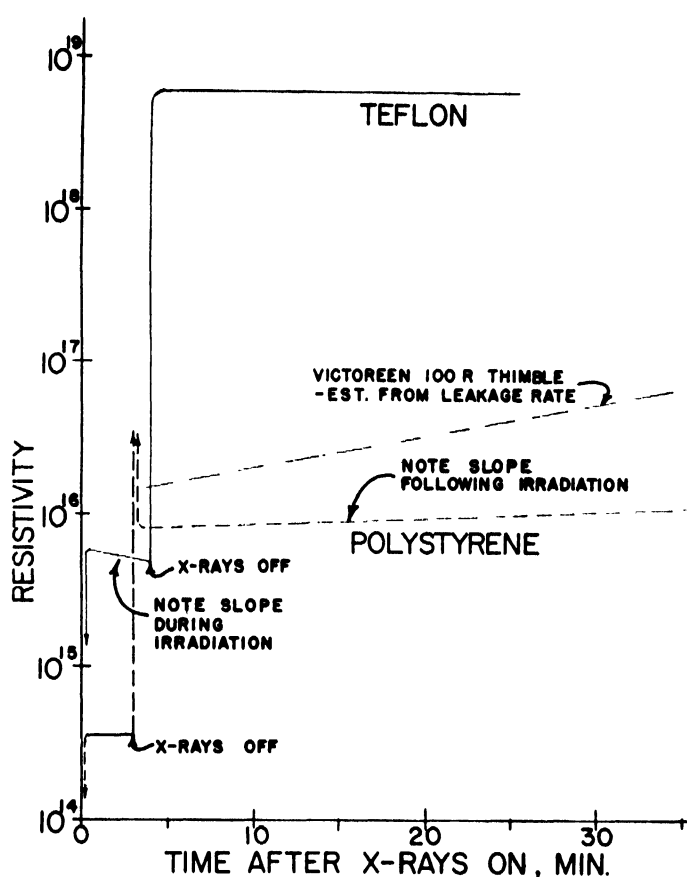


Figure 6. Apparent conductivity in polystyrene and Teflon during and following irradiation with 250 kv X-rays

expressions for resistivity and electrical capacitance have the same factor d/A . The electrical capacitance is approximately $50 \mu\text{mf}$. The question of correcting the readings obtained from Victoreen thimbles for various qualities of X-rays is receiving some attention. There appear to be two terms, one representing the total dose and the other depending on the exposure time. At this writing, the relative size of these two terms is not settled.

One promising method of dealing with insulator conductivity problems appeared to be supplying a guard electrode the potential of which might be continuously adjusted by the electronic instrumentation to equal the potential of the collector electrode. A small experiment to assess the potential difference allowable showed¹¹ that a current of at least 10^{-14} amperes flowed during irradiation in the absence of applied voltage. Depending on the geometry tested, 30 to 100 volts were necessary to balance this spurious current with leakage conductivity. The direction of this current is that of loss of electrons from the braid of a coaxial cable, as though the secondary electrons resulting from photon absorption there escape essentially to ground.

In order to compute doses in absolute energy units, a vacuum calorimeter has been built. The calorimeter alone can only measure energy content of an X-ray beam. However, by use of complete isodose determinations in a large water bath together with the calorimeter calibration of beam energy, the absorbed

dose can be determined. A calibrated ionization chamber is necessary to determine the amount of radiation leakage from the calorimeter absorbing blocks. The same chamber can be used to evaluate what part of the known energy entering the water bath is not absorbed in it. The balance must be absorbed. Assuming that the ionization found at each point tested in the water bath is proportional to the absorbed dose rate at those points, the integral of the units of ionization over the grams of the water bath gives a number proportional to total energy absorption. Since the total absorption is known from the calorimeter calibration, a simple division of this total by the above integral gives directly the absorbed dose per unit ionization measured. If the ionization chamber used is suitably designed, the above division is tantamount to an experimental determination of the quantity W/S of the Bragg-Gray principle.

SUMMARY

A 70 Mev synchrotron has been developed to the point of utilization in cancer therapy. This paper deals with some problems which are specific to the 70 Mev synchrotron and some which are general to multimillion volt X-radiation. A dosimetry scheme is proposed which enables absorbed doses to be measured with reasonable directness.

ACKNOWLEDGEMENTS

This report, written by Dr. Gail D. Adams, covers work done by himself and other members of the Physics Section.

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Irradiation Hypophysectomy and Related Studies Using 340-Mev Protons and 190-Mev Deuterons

By Cornelius A. Tobias, J. E. Roberts, J. H. Lawrence, B. V. A. Low-Beer, H. O. Anger, J. L. Born, R. McCombs and Charles Huggins,* USA

There is considerable evidence that the human pituitary gland is implicated in the pathogenesis of various serious diseases. In some of these conditions partial or complete removal of the gland may be of benefit. For the past few decades, attempts have been made by radiation and surgery to achieve complete hypophysectomy. It is only very recently that the surgical procedures have met with at least partial success.

Most of the difficulties in hypophysectomy are due to the central location of the hypophysis at the base of the skull, and to the sensitive structures of nerve, brain, and blood vessels that are surrounding and protecting it.

An account is given below of the initial use of accelerated high-speed protons in human therapeutic investigations: the rationale for proton hypophysectomy, the technique employed, and the initial physiological changes after proton hypophysectomy are given in detail.

REVIEW OF THE AVAILABLE TECHNIQUES FOR HYPOPHYSECTOMY

Radiation

Irradiation of the human pituitary by X-rays was reported as early as 1909.¹ It soon became clear that the hypophysis was fairly radio-resistant.² Because of limitations imposed by sensitivity of the skin and danger of injury to the brain, the doses by conventional X-radiation had to be limited. Nevertheless, attempts to suppress the physiological functions of the hypophysis by radiation has been a frequently used therapeutic approach in pituitary tumors,³ Cushing's syndrome,^{4,5} and acromegaly.^{4,6} Attempts have also been made to irradiate the pituitary in various other conditions, for example hypertension⁷ and malignant exophthalmos.^{8,9} Pituitary radiation by X-rays has also been attempted in malignancy in the human, particularly for prostatic carcinoma⁹ and mammary carcinoma.¹⁰ The highest doses were probably delivered by Kelly *et al.*¹¹ However, in three mammary cancer patients even 15,000 r to the region of the hypophysis was not sufficient to cause noticeable depression in hormone production and histopathological

damage, or remission of the disease. The technique of X-ray therapy is still being extended and improved, as exemplified by the recent work of Santos.¹²

Since the pioneer work of Lacassagne and his associates¹³ the implantation of radioactive seeds seemed to offer a method of delivering large local doses to the pituitary; indeed radon implantation did affect the pituitary in acromegaly and Cushing's syndrome.⁴ During the last few years, Brues and his associates¹⁴ have developed new methods for processing radioactive yttrium into seeds; this led to the therapeutic clinical investigations of Kennedy *et al.*,¹⁵ who implanted several yttrium seeds into the pituitary, and gave considerable doses of beta radiation in an attempt to stop hypophyseal function and to cause regressions in carcinomas. This study is still in progress at the University of Chicago and at McGill University in Canada.¹⁶

Surgery

Owing to great technical difficulties and associated severe trauma, it was only recently that complete surgical hypophysectomies were carried out with success, by Luft and Olivecrona in Sweden^{17,18,19} and by Perrault and associates in France.²⁰ Luft and Olivecrona assembled 34 cases of metastatic carcinoma of the breast and 12 cases of diabetes mellitus for surgical hypophysectomy. A significant portion of the patients in whom hypophysectomy appeared complete showed objective remissions and subjective relief, which lasted for periods ranging to more than two years. Currently Ray, Pearson, and associates, and other groups, are vigorously attacking the problems of surgical hypophysectomy in cancer^{21,22,23} and in diabetes mellitus.²⁴

PROTON IRRADIATION TECHNIQUE

General Properties of Particles

High-energy protons, deuterons, and alpha particles have great advantages over other radiations in producing localized radiation damage in a deep region in tissue as suggested by Wilson.²⁵ The accelerated and properly focused particles propagate in a well-collimated and straight pencil of beam, which may be directed to any portion of the body. As the particles penetrate, their scattering is so small (compared to electrons) that for practical purposes neg-

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ligibly small amounts of radiation fall outside the main beam to irradiate neighboring tissue areas. Further, the approximately monoenergetic particles penetrate to a uniform and well-defined range, producing their maximum ionization just before they stop.

A suitable aperture, made of brass or other metal, is used to determine the shape of the radiation field, and the background ionization may be kept to less than 0.1% of the beam.

These properties were demonstrated in biological experiments by Tobias, Anger, and Lawrence.²⁶

Biological Experimentation with 190-Mev Deuterons and 340-Mev Protons

Experience in the use of proton, deuteron, and alpha-particle beams was gained by investigating their biological effectiveness and mechanism of lethal action through localized irradiation of animal tissues.

The biological effectiveness of the high-energy portions of the deuteron and proton beam was found to be close to that of 200-kv X-rays. This could be predicted from physical data on the specific ionization and linear energy transfer of the particles. Experiments of this kind were done on mice,²⁶ yeast cells,²⁷ tradescantia microspores,²⁸ and fruit flies.²⁹

The use of high-speed particles allows one to produce radiation-induced lesions in the animal body with microscopically sharp demarcation lines.²⁶ In small animals, cylindrical lesions of one-mm diameter are easily produced. Since 1952, a sustained effort was made, in collaboration with the staff of the Institute of Experimental Biology, to study effects of the high-speed particles on the rat hypophysis.³⁰ Initial results were encouraging, and pituitary irradiation of young monkeys (in cooperation with Dr. Van Wagenen of Yale University) and of normal mature dogs (to be published) has been undertaken, with technique similar to that for humans (see below).

When large single doses of more than 5000 rad (1 rad is equal to 100 ergs absorbed/gm or 1.07 rep absorbed in tissue) deuterons or protons are given to the pituitary gland of animals the end result appears to be similar irrespective of the size of radiation dose: progressive atrophy of the entire gland results, accompanied by reduction in physiological function. The higher the dose, the sooner the physiological effects seem to manifest themselves. Single doses of the order of 30,000 rad are necessary to approximate the immediate effects of surgical hypophysectomy. To illustrate these statements, Fig. 1 shows the apparent onset in time of profound physiological changes resembling complete hypophysectomy. Data on rat, dog, and monkey follow reasonably well the same general relationship. Criteria for evaluation are chiefly rate of growth, thyroid function, and sizes of target organs.

According to the above reasoning, when single doses are applied, hypophysectomy can be achieved with relatively moderate doses, although it may not be observed until a fairly long time after the initial radiation exposure. The delayed onset of the effects

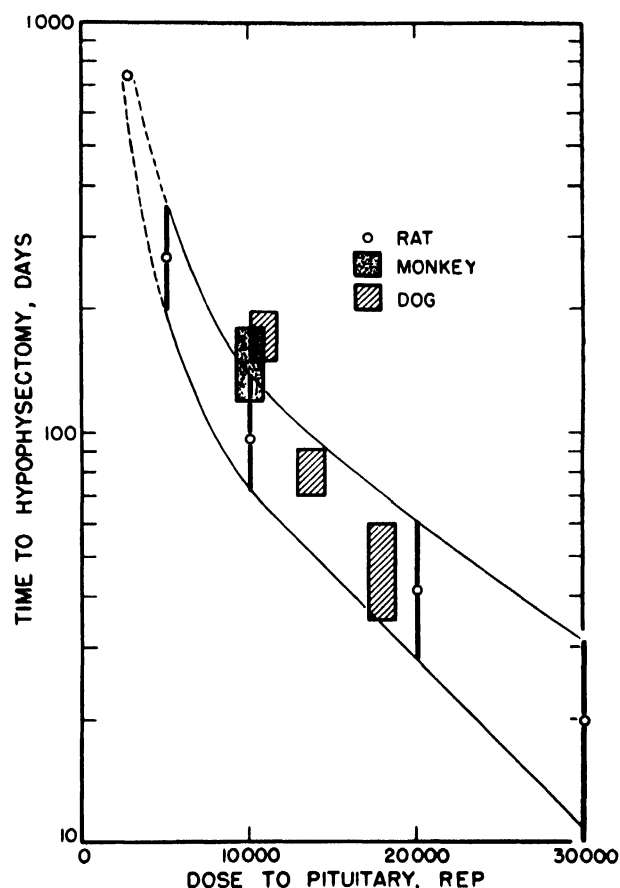


Figure 1. The time of onset of marked changes due to radiation hypophysectomy is plotted against dose. Data for rats, dogs, and a few monkeys are included

is a factor of great importance in deciding on the dose in humans.

The entire gland must be irradiated to achieve any lasting physiological changes in the target organs.

The effect of pituitary radiation on target organs does not progress simultaneously, but there is a definite pattern involved. In rats the first gland to show measurable functional changes is the thyroid. The ^{131}I uptake may decrease within a few weeks after even a moderate dose. Effects on growth and adrenal function follow, while regression of the sex glands, regulated by the follicle-stimulating hormone (FSH), is usually chronologically last. The histopathology of the irradiated pituitary exhibits changes that indicate a pattern (in the rat), though differential effects are difficult to detect. The acidophiles seem to be first to regress, followed by the basophils and, lastly, the chromophobes.

Long-term studies with doses up to 10,000 rad given to the young Long-Evans rat indicate that onset and frequency of spontaneous tumors are reduced if the animals are irradiated in the first month of life. Life span of the animals that received large doses did not significantly differ from the life span of surgically hypophysectomized animals.

Nerve and brain tissue is more sensitive than pituitary tissue. Lethal effects from pituitary radiation, where the cause could be definitely ascertained, could be ascribed to nerve and brain lesions, particularly to necrosis and hemorrhage.

Lethal effects of this kind were usually preceded by damage to the third, fourth, and fifth cranial nerves, which in the dog lie very close to the pituitary. The lesions led to inability of the animals to move their eyelids, to permanently dilated pupils, and to loss of corneal sensitivity. A series of experiments was carried out to test the dose-time relationship in dog cranial nerve. A characteristic dose-time injury curve resulted, which resembled the data obtained with the pituitary. The lower the dose, the longer the time required for the symptoms to appear, and—in this case—they usually proved to be less severe in degree. Since the data on dogs are available only over a period of 16 months, additional information is needed, particularly for long post-irradiation times. Figure 2 gives the data now available.

A temporary type of nerve damage was also observed in some of the animals; here the cranial nerves themselves had a low dose, but neighboring structures (pituitary and cavernous venous plexus) received heavy irradiation (in excess of 10,000 rad). The eye symptoms developed and increased in severity two to three weeks after irradiation, then partially or completely regressed six to eight weeks later. The symptoms were presumably due to pressure from edema of the nearby radiated structures.

When portions of the brain were given large doses along with the pituitary and cranial nerves (in dogs), in some cases coma and death followed the eye symptoms. At autopsy the heavily irradiated areas of the temporal lobes of the brain were found to be severely necrosed and liquified, with a remarkably sharp demarcation line between necrosed portion and healthy, normal-appearing tissue. The size of the necrosed area at time of death was about the same in different dogs; a typical brain is shown in Fig. 3, from a beagle dog that received 40,000 rad and died in two weeks. Necrosis begins in the portion receiving the highest dose, and progresses to areas with lower doses. The immediate cause of death in some cases appeared to be hemorrhage, with accumulation of fluid in the brain

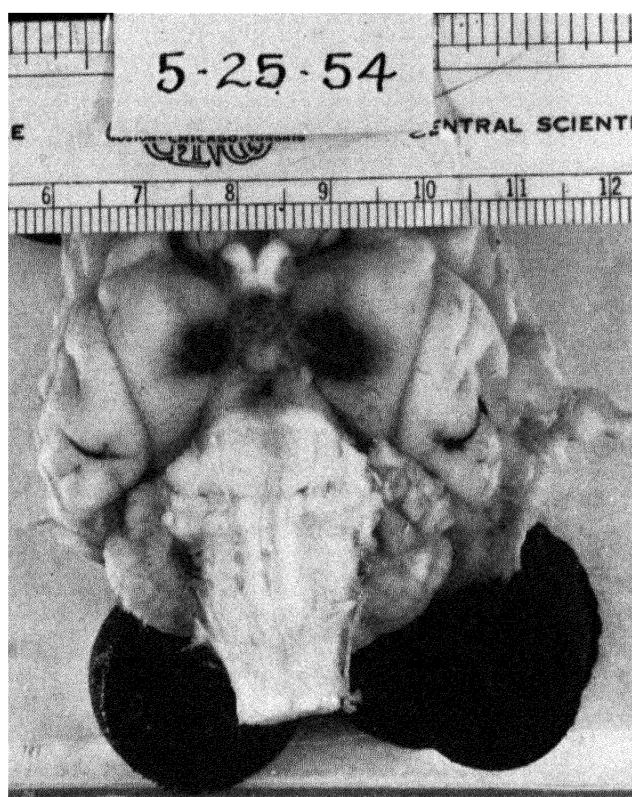


Figure 3. Photograph of the unstained brain of a fully grown male beagle dog after a single dose of 40,000 rad to the pituitary. Rotation of the head occurred in a single plane, so that the beam swept an arc of 90 degrees. Dose at the necrotic areas (black) is estimated to be about 30,000 rad. The animal died of brain hemorrhage two weeks after irradiation.

and high intracranial pressure. The relatively meager data indicate that brain damage is a function not only of dose and time but also of the volume of tissue irradiated. It is difficult to compare the literature on radiation damage to the brain with the results reported here; most data in the literature involve doses to massive areas of irradiated brain tissue. Perhaps most pertinent are recent observations by Arnold *et al.*,³² who observed delayed demyelination and radionecrosis in dose ranges of 1500 to 5000 r of high-energy X-rays, and in addition acute inflammation, hemorrhages, edema, and necrosis in the range of 3000 to 14,000 r, with increasing severity at higher doses.

Further studies are being carried out on the relative effectiveness of fractionated and single doses on the central nervous system.

LOCALIZED HYPOTHALAMIC IRRADIATION

The technique described in this report has been employed over the past two years for irradiation of hypothalamic areas of rats. The main problem of interest is the mechanism of hypothalamic control of hypophyseal functions. Because of the low mortality and high aiming accuracy this technique appears to show promise as an investigational tool. The results of this work will be reported elsewhere in detail. Already proton or deuteron irradiation of hypothalamus has produced a range of effects in rats. Among

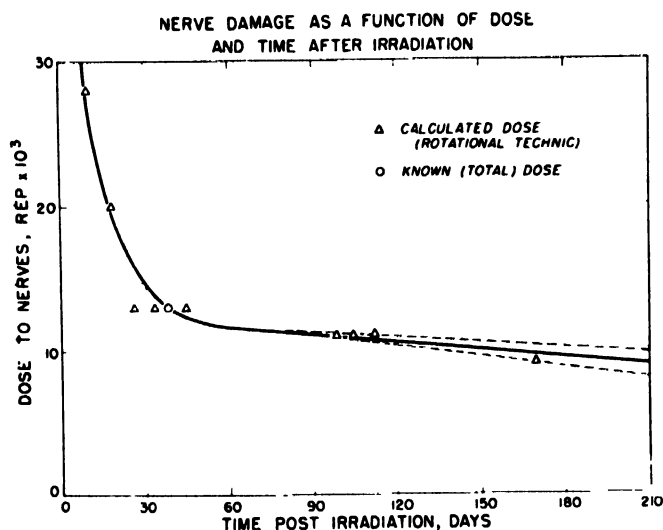


Figure 2. The occurrence of cranial nerve damage in dogs as a function of time after various single doses of deuterons.

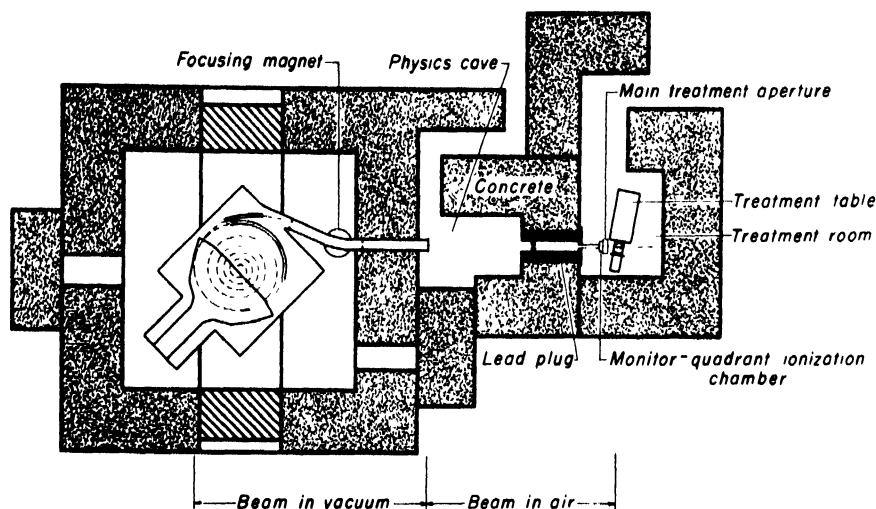


Figure 4. General layout of the 184-inch cyclotron and treatment rooms (not to scale). The deflected beam passes through a room for physics experimentation and arrives at the treatment room beyond

these are retardation of growth, diabetes insipidus, glycosuria, hypothyroidism, and obesity.

DESCRIPTION OF IRRADIATION TECHNIQUE IN HUMAN PATIENTS

The radiosensitivity of the brain and skin is greater than that of the pituitary. For this reason the proton irradiation of the human pituitary was combined with the well-known rotational irradiation technique. The position of the proton beam was kept fixed in space and directed to cross the pituitary as well as the entire head with uniform ionization. During irradiation the head was rotated around its own pituitary body. The plane of rotation was chosen differently each day of the fractionated therapeutic irradiations. In this manner the cumulative dose to the pituitary gland was considerable, while surrounding blood vessels, cranial nerves, lobes of the brain, bone, and skin received progressively less irradiation in the order of enumeration.

A contemplated further improvement in technique would involve stopping the proton beam in the pituitary fossa by making use of the high dose peak of the Bragg ionization curve and thus eliminating irradiation of one hemisphere of the brain. As yet this modification has not been employed in our patients.

Treatment Room

With the generous help of the staff of the Berkeley 184-inch cyclotron, a new treatment room (shown in Fig. 4) was constructed for the human irradiation program. The collimated beam passes through two rooms suitable for irradiation: the one closer to the cyclotron is mainly for physics and chemistry use, while the farther room, carefully shielded to avoid stray radiation, is for medical use.

Control of Proton Beam

The deflected beam passes along on an optical bench, and by means of electrical and mechanical techniques, it is brought to pass (within about 0.2

mm) along a line eight inches over the center of the optical bench and parallel to the tracks on the bench. The dose-monitoring ion chamber, main beam aperture, and focal spot of the diagnostic X-ray machine are all mounted so that the proton beam passes through their centers. The patient's head is held rigidly in an adjustable head rotator which is also mounted on the optical bench. Adjustment screws are provided to move the head holder in three directions *X*, *Y*, *Z*, with an index on each adjustment, so that on repeated treatments the index may be reset, and

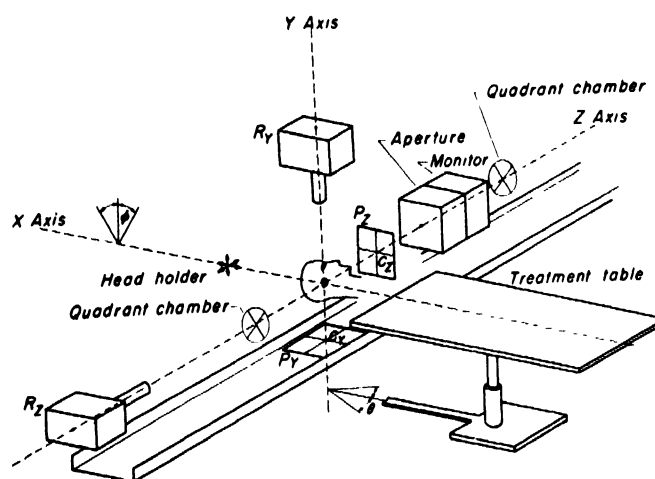


Figure 5. Schematic of the positioning, alignment, and rotation of the human head. The optical treatment bench is aligned with the beam, while the patient lies on an adjustable table at some pre-determined treatment angle. This angle is changed in 7.5-degree intervals on different treatment days. The head of the patient is held rigidly by a mask. Position of the head is checked by a lateral, horizontal diagnostic X-ray, taken by an X-ray machine rigidly mounted on the treatment bench. The sella turcica is aligned by moving set screws in the *X* (longitudinal) and *Y* (vertical) directions, until the center of the sella and the cross hairs marking the beam position are coincident. With the vertical X-ray machine, mounted above the treatment plane, one obtains a frontal diagnostic picture. The patient's head is moved by set screws in the *Z* direction until the center of his sella coincides with the center of rotation of the head rotator, as marked by cross hairs. Rotation of the head occurs around the longitudinal axis of the body (*X*); rotational limits of ± 30 degrees or ± 35 degrees are easily tolerated

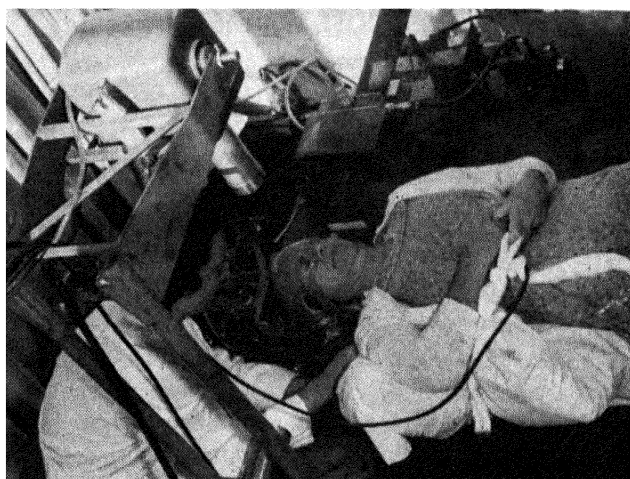


Figure 6. Photograph of the treatment room. The treatment bench occupies the upper part of the picture; the beam enters on the upper right side in a steel tube. The patient is lying in position ready for treatment, her head rigidly supported by a plastic mask. The plastic mask is fastened on the head positioner and rotator. Diagnostic X-ray machines used in the alignment are on the left side and at the top of the picture

the head placed each time into a very closely correct position. This is checked by X-ray films before each treatment. Figure 5 gives the schematics of the alignment and Fig. 6 shows the actual treatment room.

The success of the multiple-plane rotational-irradiation technique depended on providing a firm mask to hold the patient's head during alignment and therapy on repeated occasions. Very satisfactory two-piece individual plastic masks have been developed for this purpose, utilizing fiberglass cloth and polyester resin. The mask is tightened on the head with thumb-screws, until it fits tightly enough so that the patient cannot move his head, (or sella turcica) more than about 0.5 mm in any direction. The masks are comfortable enough to wear for two hours at a time.

Figure 7a shows the sella turcica of one of the patients in alignment with the cross hairs, while Fig. 7b shows the autoradiograph of the shaped beam and its alignment with the sella. This latter picture was taken by first exposing the patient to the X-rays, then to a small dose of protons. One should bear in mind that the sella appears enlarged due to the finite distance of the focal spot of the X-ray machine, while the beam spot is actual size.

Figure 8 shows a typical vertical X-ray, taken along the Y axis. Here, the center of rotation in the sella was ascertained by obtaining symmetry, on both sides of the X cross hair, of the faint line representing the base of the sella and the walls of the sphenoid sinuses. The anterior clinoid processes and the ocular cavity are also checked for symmetry. On the vertical X-ray it was necessary to use a diagnostic X-ray machine with 0.3-mm focal spot to obtain the necessary resolution.

Spatial alignment of both views of the sella was usually within ± 0.5 mm. Occasionally check X-rays were made with the patient forcibly trying to move

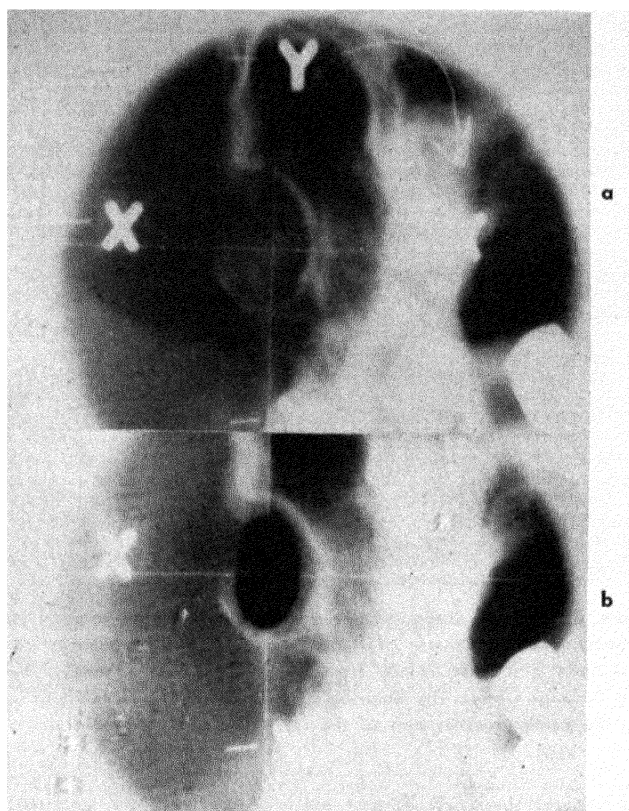


Figure 7. Above, diagnostic X-ray of a patient's head in position for treatment. The cross hairs mark the center of the beam. The outlines of the sella turcica are clearly shown. The anterior clinoid processes nearly overlap. Below, autoradiograph of the beam may be obtained on any of the diagnostic X-ray films by briefly turning the proton beam on prior to developing the film. The black spot shows the shape of the beam, adjusted to fit each individual patient. The beam spot is actual size, while the X-ray picture of the sella is enlarged by 20% due to the finite focal distance of the X-ray machine

his head out of alignment. These were always correct within 1.0 mm.

During the first treatment usually 6 to 8 sets of diagnostic X-rays were needed to bring the patient's head in alignment. On subsequent repeat treatments adjustment screws on the supporting head holder were preset to the positions that had previously given proper alignment, and usually one or two sets of diagnostic X-rays sufficed.

CONSIDERATIONS OF ANATOMY AND RADIOSENSITIVITY OF IMPORTANCE IN THE TREATMENT SCHEDULE

Relatively limited information is available concerning radiosensitivity of the brain and of the pituitary in the human. In addition to the animal data reported above, it is known that the brain is more sensitive to radiation than the pituitary and that 4800 roentgens of 50-kv X-rays given in a fractionated course over a large area may cause radionecrosis delayed by more than a year.^{31,32,33}

In the course of the present preliminary investigations the therapy was given in such a way as to minimize the dose received by radiosensitive tissues in the head.

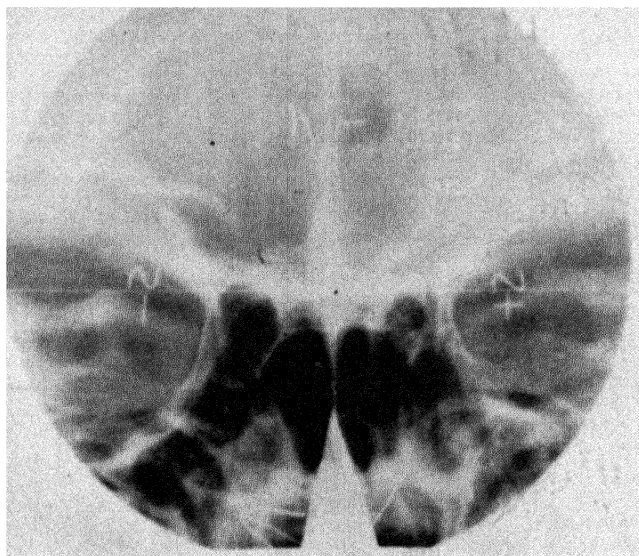


Figure 8. Vertical diagnostic X-ray of the skull (anterio-posterior projection). The symmetry of the sella turcica with respect to the cross hairs is checked chiefly from the location of the base of the sella (Grange's line). The anterior clinoid processes and positions of the ocular cavities and of the cristogale also are helpful

Figures 9a and 9b are scale drawings of sections through the sella turcica in the XZ and YZ planes. It is apparent that the most sensitive structures are right above the pituitary, particularly the optic nerve tract and the hypothalamus. These should be avoided by the radiation as much as possible. Laterally, the carotid arteries and the cavernous venous plexus are closest to the hypophysis. These structures are known to tolerate a higher dose. Of the cranial nerves the third, fourth, fifth, and sixth are closest; beyond the cranial nerves extend the temporal lobes of the brain; close to the hypophysis are the regions serving the sense of smell.

The limits of rotation of the head were chosen ± 30 degrees or ± 35 degrees from the Y axis. The limits of the angles were given by the requirement to completely avoid the eyes, so that cornea, retina, and lens are free from radiation. There are 11 planes of irradiation, each of which is about 7.5 degrees apart. On a given day rotation may be given in two of the planes, preferably 30 degrees apart. The choice of the treatment planes is such that the brain stem and the pars optica, mammillary bodies, tuber cinereum, and infundibulum get negligible doses, since the beam does not cross these.

DOSE SCHEDULE

At present 800 to 1000 rad is given in each of two to five treatment planes daily, three times a week, with the dose figure referred to the center of the pituitary. Owing to the rotation of the head, the daily dose at the cranial nerves is about 260 rad, and at the skin about 40 rad. The whole course of treatment takes 20 to 40 days, and doses of 14,000, 16,000, 18,000, 20,000, 22,000, 26,000 and 30,000 rad were given to the center of the pituitary.

ISODOSE CURVES

The elliptical beam apertures, multiple planes, and rotation make the evaluation of isodose curves a complex procedure. Two methods were utilized: measurement by photographic densitometry, and calculation from the known beam ionization profile. An autoradiograph of the proton beam as it crosses a solid piece of lucite is shown in Fig. 10.

A lucite phantom is used in place of the head and photographic film inside this phantom is exposed, using the same rotations as in patients. One complete set of rotations corresponding to the entire treatment schedule gave Fig. 11 (not retouched). The blackened area corresponds to the pituitary size.

Measured and calculated dose distributions are reproduced along the major axis XYZ in Fig. 12. The dose falls relatively slowly in the lateral Z direction where the hypophysis extends farther; towards the top of the head and to the front and back, the fall is very abrupt, insuring a considerable degree of protection for the optic chiasm and hypothalamus. Figures 9a and 9b have a typical isodose chart superimposed over the anatomical detail of structure.

Since the pathways of irradiation center in the hypophysis, it is useful to evaluate the average hypophyseal dose by integrating over the isodose surfaces. The average dose in the patients treated so far was between 55% and 75% of the peak dose at the center of the gland. The size and shape of the sella turcica varies considerably among patients, and several different apertures and rotations were used. Isodose curves for each are being accumulated.

DOSE RATE

Most patients received dose rates of 200 to 300 rad per minute; 800 rad is thus delivered in 4 to 2.7 minutes; in this length of time the head describes the 60-degree span of rotation 5.4 to 8 times. The beam is delivered in the form of microsecond pulses about 68 times per second; the instantaneous dose rate therefore is high.

BACKGROUND CONSIDERATIONS

Detailed measurements were carried out on the background dose distribution around the proton beam near the body of the patient and in the treatment room. The entire treatment room was shielded in such a manner that beyond a radius of 1.5 inches from the beam the whole body of the patient receives everywhere less than 0.1% of the peak dose. In a full course of therapy, the patient receives less than 20 rad total-body dose, most of which is radiation near minimum ionization. There is also a thermal neutron flux of 4000 neutrons/cm²-sec. Outside the shielded area, personnel delivering the dose and taking care of the patient receive a fast neutron dose of 420 Mev/cm²-sec near the treatment room door and 50 Mev/cm²-sec elsewhere while the beam is on high-energy gamma rays of less than 1 mr/hour, and about 100 slow neutrons/cm²-sec.

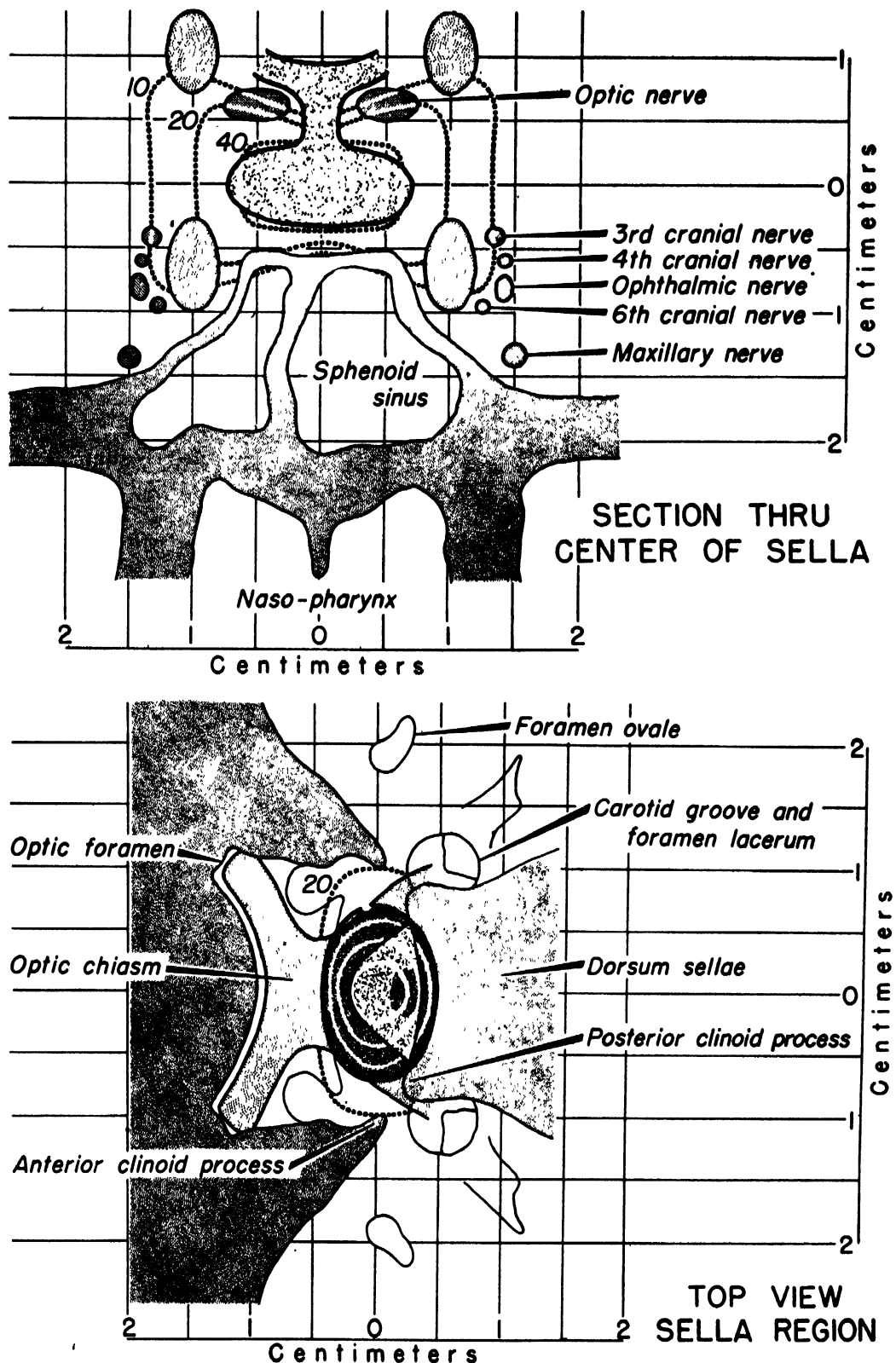


Figure 9. (a) Schematic drawing of a section through the sella turcica in the XY treatment plane, with isodose curves superimposed over the anatomical structures. Locations of the optic nerves, cranial nerves, and blood vessels are shown. (b) Schematic drawing of a section through the sella turcica in the YZ treatment plane indicating locations of the pituitary stalk and optic chiasm

CRITERIA FOR SELECTING PATIENTS FOR PITUITARY PROTON-BEAM IRRADIATION

It was felt that for the present time only patients with cancer who have had all other available therapy

should be exposed to protons. In addition, an attempt is being made to select patients who have a realistic chance of deriving benefit from pituitary ablation. Thus the criteria for acceptance now are as follows:



Figure 10. Autoradiograph of the 340-Mev proton beam as it crosses a 6-inch-wide piece of solid lucite. The beam enters from the left. Part of the loss of definition on the right comes from the slightly divergent nature of the beam, part from multiple elastic scattering

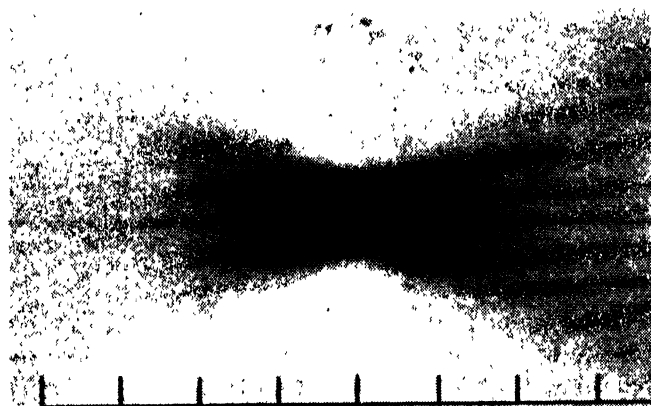


Figure 11. Autoradiograph of the proton beam on films placed in the lucite head phantom, exposed to a full set of rotations and position angles corresponding to the entire treatment schedule in human. The blackened area is the region of high dose. Note that the shape and size of this area closely correspond to the shape and size of the human hypophysis. (Rotation limits ± 30 degrees, treatment plane orientation angles ± 30 degrees, ± 22.5 degrees, ± 15 degrees, ± 7.5 degrees, 0 degrees. Beam aperture an ellipse with 8.5 mm major axis and 5.5 mm minor axis)

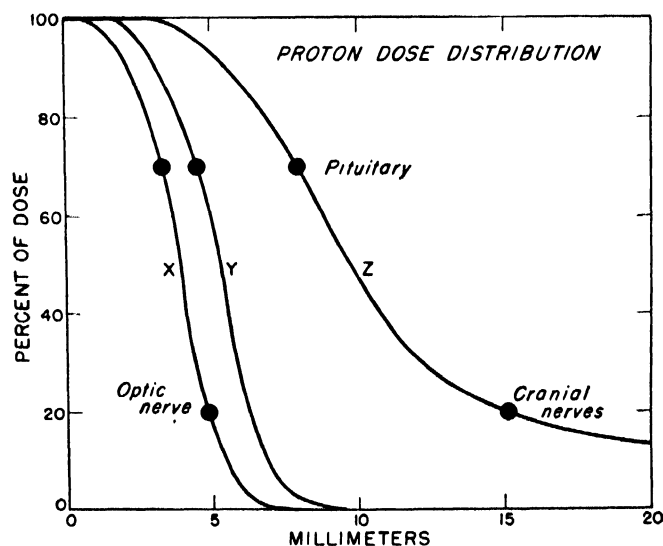


Figure 12. Dose distribution along the three major axes of the head, X (longitudinal), Y (A-P) and Z (lateral). Note that the dose level is 10% to 20% of the peak dose at the cranial nerves and temporal brain lobes. Parts of the optic nerves receive 20% to 30% of peak dose, while most of the optic chiasm and hypothalamus are essentially free from dose

(a) The patient should have mammary carcinoma. She should have had conventional surgical and radiological treatment.

(b) The patient should have received a trial of hormone therapy, or surgical or radiation oophorectomy and adrenalectomy.

(c) There must be objective evidence showing the presence of progressing metastatic lesions for which none of the above-mentioned procedures holds out further hope.

(d) The patient should be younger than 60 years of age and—apart from the carcinomatous process—in fair physical condition. Initially, desperate cases were selected who did not satisfy this criterion and were near terminal.

On the basis of experience with adrenalectomy by Huggins *et al.* it is felt that nondifferentiated carcinomas of the breast are controlled by the pituitary-adrenal-ovarian system to a lesser degree than the well-differentiated type.

CLINICAL STUDIES

Clinical studies and observations are being carried out in three separate fields in order to obtain as complete information as possible: (a) endocrinological tests, (b) neurological examinations, and (c) effect on tumor growth. Endocrinological tests include many phases:

Thyroid function tests are made by means of I^{131} uptake, protein-bound iodine determinations, and basal metabolism measurements.

Pituitary gonadotrophin activity is followed by urinary follicle-stimulating hormone determinations (FSH) and, where possible, urinary estrogen excretion studies.

Adrenal function tests are of no use since the patients thus far studied have been previously adrenalectomized and are receiving supporting steroids. Urinary ketosteroids are assayed, however, in most cases.

In view of the possible presence of a pituitary hemopoietic factor, blood-volume measurements and iron-turnover measurements are carried out as needed.

Water balance is studied by urine output and specific gravity measurements, as well as water-loading tests.

Sugar tolerance and blood-sugar determinations are carried out routinely.

Neurological examination includes tests for cranial nerve damage, visual-field studies, and tests for acuity of the sense of smell and touch.

Unfortunately there are no conclusive tests available for obtaining information about the state of proliferation of tumor cells. The Huggins serum coagulation test is being done periodically. In addition diagnostic X-ray examinations give information about bone and lung metastases; skin metastases are observed directly; biopsies are obtained if ad-

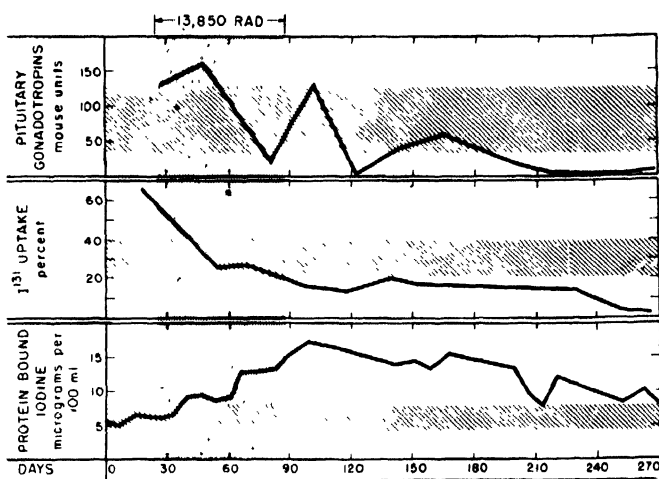


Figure 13. ^{131}I uptake, protein-bound iodine (PBI), and urinary FSH values in patient Mrs. M. W. (multiple skeletal and liver metastases), who received 13,850 rad in 63 days. Note the gradual decrease of ^{131}I uptake and FSH until in about 6 months they are in the hypophysectomy range. The PBI values somewhat unaccountably increased for a considerable period of time

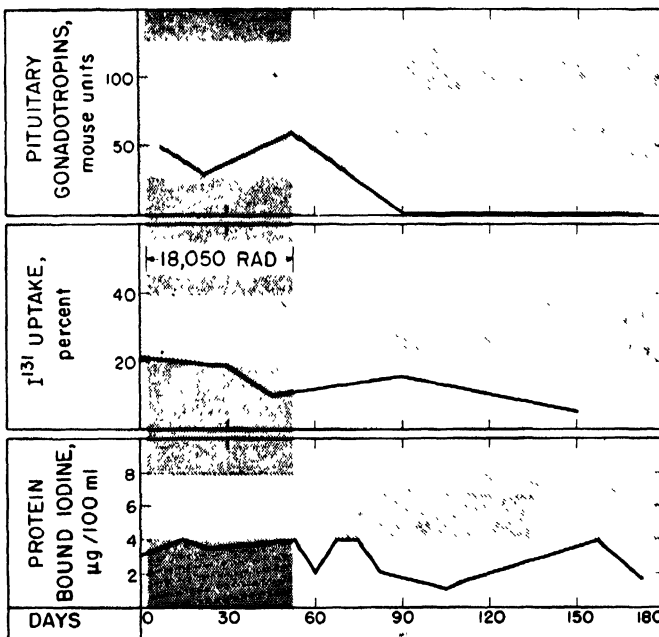


Figure 14. ^{131}I uptake, PBI and FSH values in patient Mrs. A. S. (extensive carcinoma metastases in cuirass and pulmonary metastasis)

visible. Serum alkaline and acid phosphatase levels and calcium excretion have been measured routinely. In addition all conventional clinical laboratory observations are made.

TREATMENT SCHEDULE

Early treatment schedules followed the present clinical radiotherapeutic practice of dose fractionation by administration of several repeat irradiations. Three doses were given weekly (Monday, Wednesday, Friday). This limitation was necessitated by the availability of the cyclotron. The dose at the center of the pituitary was 800 rad in a plane. Two or more planes were frequently used on a given day. The dose schedule was conservative and probably inadequate in the earlier cases. With accumulated experience and in the absence of contra-indications,

the daily dose was raised to higher values, until at present as much as 5000 rad may be given in a single day, in five different plane orientations of the body with respect to the proton beam, and ± 35 degree rotation of the head in each orientation. The dose at the center of the pituitary gland at the end of the treatment (usually 20 to 40 days) was from 14,000 rad to 30,000 rad.

CLINICAL OBSERVATIONS IN THE COURSE OF THERAPY

None of the patients treated so far has shown objective or subjective clinical signs of immediate physiological effects from the radiation. In the course of 200 individual exposures, severe headache was reported in two instances during the day following exposure; however this could not be definitely associated with the treatments. Radiation invoked no sensation; blood pressure and temperature remained normal.

No obvious evidence was found for secretion or dumping of hormones from the pituitary in the first 24 hours after exposure to protons. This effect was observed in rats by Mateyko and Edelman.³³

Undesirable skin effects, which may include erythema, epilation, and ulceration, were all completely absent during the first 5 to 9 months following exposure, no doubt because of the low doses delivered to the skin.

Neurological observations indicate likewise a complete absence of undesirable side effects on the cranial nerves, temporal lobes, or optic chiasm up to the date of preparation of this report. However, it will be necessary to continue these observations for a considerably longer time.

PHYSIOLOGICAL OBSERVATIONS IN THE POST-TREATMENT PERIOD

Significant depression of pituitary functions was observed in all patients during the post-irradiation period.

Thyroid Function

Over a period of five months there is a decline of thyroid function, as evidenced by the 24-hour uptake of radioactive ^{131}I administered orally. Two sets of data for individual patients are shown in Figs. 13 and 14. At the end of six months, iodine uptakes that had been in the normal and high range became as low as 3% and 5% respectively, in the same range (2% to 8%) as one obtains with surgically hypophysectomized patients. Values for protein-bound iodine, (shown also in Figs. 12, 13) decline, but in some patients show a curious and at present unexplained rise. Basal metabolic rates decrease, but less significantly. However, clinically, myxedema so far was absent or only mild.

There was indication that the iodine uptake decreased during the treatment period, increased again in the month following proton exposures, then slowly and progressively decreased again.

Urinary FSH

The initial FSH values were almost uniformly high, in the range of post-menopausal women. In each instance where measurement was possible, a decrease occurred; but great fluctuations are found in the FSH titer. After five months of post-treatment period, the FSH is too small to be measured, and (as far as our observations allow us to determine) stays at zero level. (See Figs. 12, 13.)

Urinary Estrogen

Only in one patient was measurable urinary estrogen level observed. This patient had incomplete adrenalectomy previously. In one month after completion of pituitary radiation the urinary estrogen fell to zero.

In order to obtain a general view of the endocrinological findings in the first 10 patients irradiated, Table I lists pre- and post-treatment iodine uptake and urinary FSH values. At 30,000 rad, the induced function changes begin to occur within the first 30 days and present a definite pattern. This is illustrated in patient M. S., Table II.

WATER TURNOVER

Because of the great fluctuation of water intake and urine output, cumulative curves were plotted, in which the total water turnover was graphically shown. In this fashion, one finds that water turnover of all patients is generally high. It is questionable whether a mild diabetes insipidus exists in the post-irradiation period. Blood volume and serum protein, serum cholesterol, sugar tolerance, and water-loading tests have so far not significantly aided in evaluating pituitary effects; these fluctuated in the normal range, except where the values were off normal because of complications already present owing to the disease process.

ALKALINE AND ACID PHOSPHATASE

In the presence of bone metastases alkaline phosphatase and acid phosphatase measurements are of some value; in some as yet not precisely determined way these depend on pituitary function. While tumor growth is actively infiltrating the skeleton the alkaline phosphatase is frequently low, and the acid phosphatase high. While deposition of new bone occurs the alkaline phosphatase is usually high. Thus high alkaline phosphatase is indicative of bone recovery, unless complicating factors are present. In several patients the pattern of alkaline and acid phosphatase has changed after radiation (see, e.g., Fig. 15). Complete evaluation of the significance of these findings will have to wait, however, until more data have been accumulated.

CALCIUM EXCRETION

Bone deposition sometimes depresses calcium excretion in the urine, and Figs. 15a and 15b show typical data obtained. This measurement is complicated by the fact that pituitary ablation and prolonged bed rest both cause decalcification of bone.

DISCUSSION OF THE PHYSIOLOGICAL CHANGES

One observes a definite depression of the physiological function of the pituitary gland in a period of six months, in the absence of undesirable side effects. While this result is encouraging and has perhaps not been attained previously, the authors feel it desirable to try to accelerate atrophy of the pituitary gland further by the application of higher doses and by further improved technique, in order to obtain more profound effects in a shorter time.

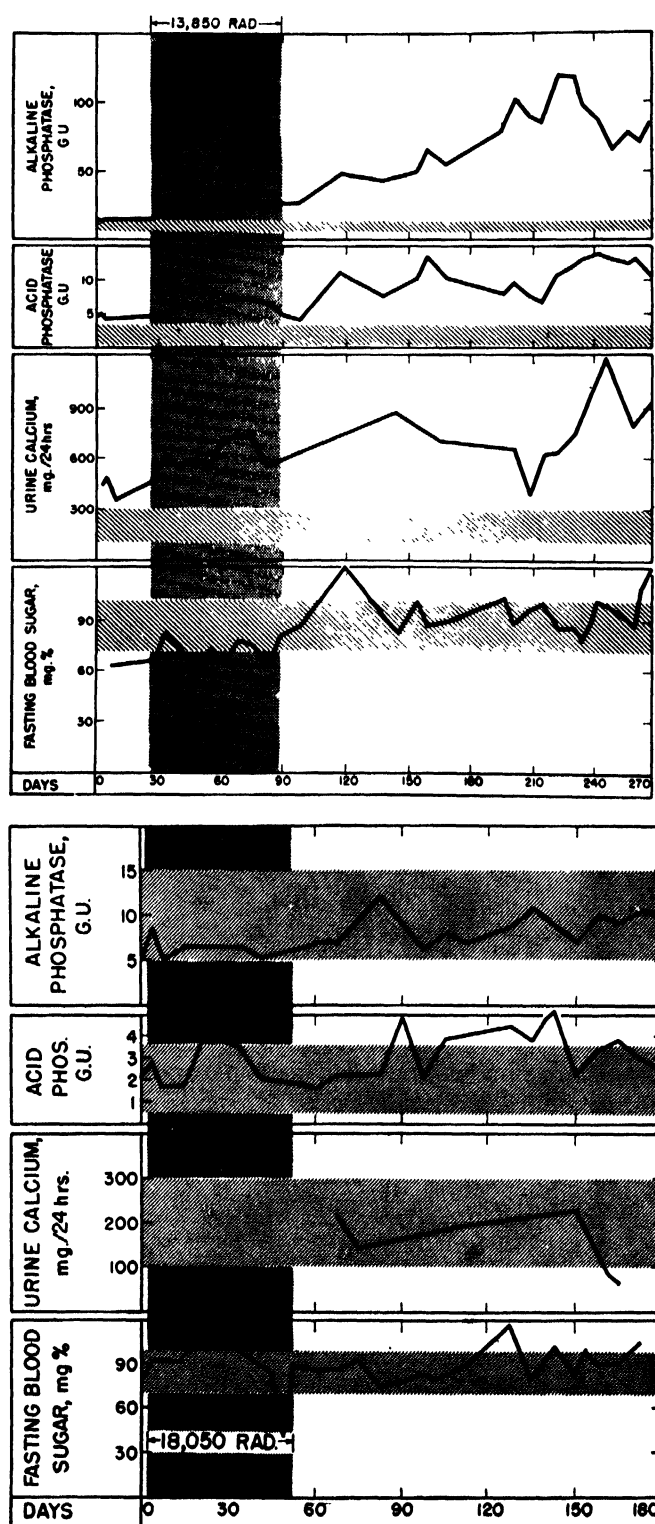


Figure 15. Alkaline and acid phosphatase, fasting blood sugar, and urinary calcium secretion for patients M. W. (above) and A. S. (below)

Table I

Patient	Peak dose radiation	Days	I^{131} uptake	PBI		FSH	
M.W.	13,850	245	66	5.4		>130	
			2.5	8.0		<5	
A.R.	12,880	122	23	6.0		>80	
			16.5	9.1		>100	
E.P.	13,200	28	30	4.3		>160	
			16	4.1		<80	
C.D.	16,280	105	30.5	8.9		>160	
			17	7.3		>120	
K.C.	16,400	141	30	7.3		>140	
			16.5	2.3		10	
A.S.	18,050	165	21	3.0		>50	
			6.4	3.2		<5	
C.H.	17,940	81	27	4.2		—	
			16	3.9		—	
A.T.	19,900	44	35	5.7		>200	
			24	4.3		<80	
B.G.	18,400	34	33	30		>80	
			35	22.5		<5	
E.H.	22,000	59	19	16.5		>80	
			13	17.0		>120	
R.H.	26,000	42	15	5.1		<5	
			19.5	3.8		<5	
M.S.	30,000	26	10	4.7		>250	
			6	1.2		<80	

Table II. Pituitary Proton Irradiation—Typical Laboratory Data.
Patient M. S., Multiple Bone Metastases

		Dose rad	Blood proteins			Phosphatase	
			Total gm%	Albumin gm%	Globulin gm%	Acid G.U.	Alkaline G.U.
Before	1 day	0	5.8	3.9	1.8	6	15.6
After	30 days	30,000	6.9	4.2	2.7	5.8	16.4
Normal range			6-8	4.5-5.5	1.5-3.0	0.5-4	5-13

		Urine		Thyroid		Urine		Blood	
		calcium mg/24 hr		uptake %/24 hr	PBI μ g/100 ml	gonadotropins mouse units		RBC million	Hemoglobin % grams
Before		1107		10	4.5	>160		2.4	53
After		689		6	1.2	<80	>10	3.2	57
Normal range		50-300		20-40	4-8	32-128		4-5	76-108
									11-15

CLINICAL MANAGEMENT OF PATIENTS FOLLOWING PITUITARY IRRADIATION

All the patients receiving pituitary irradiation have previously had bilateral adrenalectomies and oophorectomies and were on maintenance doses of cortisone ranging from 37.5 to 75 mg daily with NaCl supplemented and DOCA where postural hypotension existed. Following pituitary irradiation no additional substitution therapy, including pituitary extracts, has been necessary although in several cases the dosage of cortisone has been increased with subjective improvement to the patient. It was believed desirable to place several of the patients on 2 grains of thyroid extract daily. This, however, was on an empirical basis.

THERAPEUTIC EFFECTIVENESS OF PITUITARY IRRADIATION PROCEDURE IN CANCER

At the time of this writing it is too early to give an objective evaluation of the therapeutical usefulness

of this new method. Some evidence is already being obtained, however, that tumor proliferation and hormonal balance are intimately related. In order to obtain a reliable estimation of the value of this therapy much more time and experience will have to be accumulated to determine optimum dosage and timing. Report on this phase of the investigation is being deferred to a later date.

SUMMARY

1. Initial use of high-energy 340-Mev protons in a human therapeutic investigation is reported.

2. Localized irradiation of the human pituitary gland was achieved by a combination of multiple-port and rotational application of the high-energy particles. This procedure allows accurate delivery of high doses to the pituitary gland, with much lower doses to surrounding bone, brain, nerves, and skin.

3. The patients were advanced cases of metastatic carcinoma of the breast, who had previous mastec-

tomy, X-ray therapy, hormone administration, oophorectomy, and adrenalectomy.

4. Definite evidence of depression in pituitary hormone output and control of target organs was achieved with peak doses of 14,000 to 30,000 rad at the center of the pituitary. The effects were delayed and gradual in their onset. Undesirable side effects were absent.

5. So far only 9 months' experience on 12 patients is available. Full evaluation of the pituitary ablation and cancer-therapeutic effectiveness will have to be made at a later time.

ACKNOWLEDGEMENTS

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Destruction of the Hypophysis with Radioactive Yttrium Pellets in Metastatic Cancer

By P. V. Harper,* T. B. Rasmussen,† E. T. Yuhl,‡ and D. M. Bergenstal,‡ USA

It is a well-established fact that many cancers arising in the breast and prostate gland are subject to endocrine influence. Castration, adrenalectomy, and the administration of various hormones have produced prolonged and objective improvement in many of these cases. Hypophysectomy has followed logically as an extension of this approach to cancer therapy. Olivecrona¹ has shown that surgical hypophysectomy can be performed without serious risk and with encouraging results in many cases. However, in attempting to carry out this procedure, we have been uniformly unable to remove the hypophysis completely as shown by serial sections taken through the region of the sella turcica in a series of 9 cases (Fig. 1). Since the interpretation of the results of this important clinical experiment depends on the achievement of complete hypophysectomy, a more certain method of accomplishing this result was sought.

Destruction of the gland with injected radioactive colloidal material such as Au^{198} or $\text{CrP}^{32}\text{O}_4$ was considered, but it was decided, because of proximity of numerous important parasellar structures, that the destructive action of the radiation field could be better controlled by using discrete sources of beta radiation such as those described by Kisieleski, Svihla, and Brues.²

Y^{90} was chosen as the most suitable agent because of its strong beta radiation (2.3 Mev), short half-life, (62 hrs.) and adequate activation cross section for thermal neutrons (1.24 barns). Compressed pellets of Y_2O_3 powder were heated for an hour at 1650°C , which converted them to solid ceramic material. Small cylinders, 2 to 5 milligrams in weight, were formed in this way to fit into a No. 17 gauge spinal-puncture needle (Fig. 2). Activation of these pellets in a thermal neutron flux of 1×10^{13} neutrons/cm²/sec for 24 hours resulted in a specific activity of 0.4 mc/mg Y_2O_3 or 1 to 2 millicuries per pellet. Handling the activated pellets presented no problem since 11 millimeters of water offers complete shielding against the beta radiation. Under water, a pellet is placed in a curved spinal-puncture

needle whose proximal half is enclosed in a 2-millimeter lead shield (Fig. 2). This reduces the radiation dosage at the operator's finger tips to less than 20 milliroentgens per hour, so that the shielded needle may be handled at operation without haste.

While the maximum range of the beta radiation in tissue is about one centimeter, a significant radiation dosage is not reached until one approaches within four or five millimeters of the pellet. The dose gradient surrounding a representative pellet was measured using photographic film³ in a lucite phantom. Figure 3 shows isodose curves obtained by this method.

Yttrium pellets were implanted in the region of the hypophysis in monkeys. These became surrounded by a more or less spherical region of complete necrosis with very sharply demarcated borders (Fig. 4), as was expected from the steep gradient

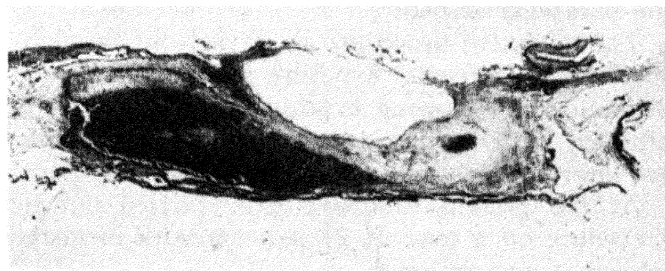


Figure 1. Low power photomicrograph of coronal sections through sella turcica following surgical hypophysectomy showing large amount of residual pituitary tissue

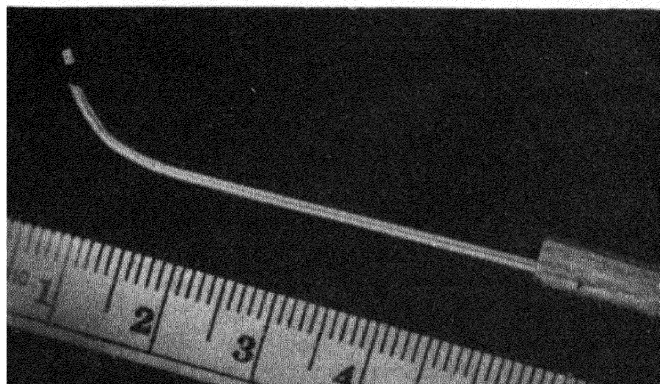


Figure 2. Seventeen-gauge spinal puncture needle with curved tip and lead shield surrounding proximal end. Y^{90} pellet is shown just beyond needle tip

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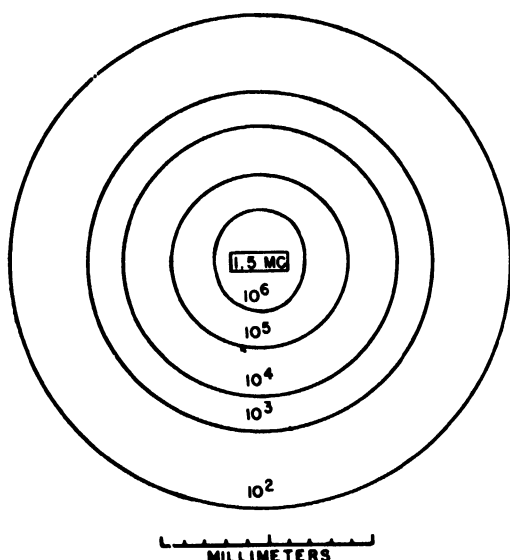


Figure 3. Isodose curves indicating total tissue doses in rep surrounding a 1.5 mc Y^{90} pellet

of the radiation field. The lesions reached their maximum diameter of 4 to 8 millimeters in 7 to 10 days, depending on the activity of the pellet. The radiation dosage at the border of the necrotic lesion varied somewhat for different tissues. The values measured for the hypophysis and other parasellar structures are shown in Table I.

On the basis of measurements on typical human pituitary glands it was believed that the whole gland could be destroyed by four properly placed one-millicurie pellets without risk of radiation damage to the parasellar structures.

The operative procedure is carried out through a standard right frontal exposure as in surgical hypophysectomy. Following exposure of the chiasm, the pituitary stalk is clipped and divided and the pellets are inserted.

At the present time, we have carried out this procedure on a total of 21 patients with metastatic cancer.

It became evident after the first cases that the four-pellet plan was inadequate. An error of a millimeter or two in the placement of the pellets left significant amounts of viable, undamaged pituitary tissue. In order to overcome this difficulty the number of pellets was increased gradually until we reached ten, the number we are using at present. The total quantity of isotope inserted remains constant, that is, 4 to 5 millicuries. Figures 5 and 6 show antero-posterior and lateral skull X-rays illustrating 7 pellets in the hypophysis. Figure 7 shows the degree of destruction obtained with properly placed pellets.

Table I. Radiation Dosage Required to Produce Necrosis

Anterior lobe of hypophysis	110,000 to 190,000 rep
Hypothalamus	60,000 to 120,000 rep
Optic chiasm and tract	60,000 to 140,000 rep
Oculomotor nerve	30,000 to 60,000 rep

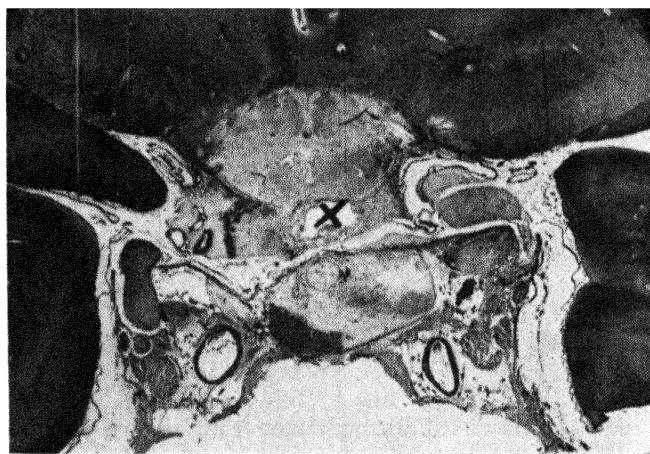


Figure 4. Low power photomicrograph of section through the hypophysis and hypothalamus of monkey. (X indicates location of 1.3 mc Y^{90} pellet) Sacrificed 31 days after implantation (By courtesy of W. B. Saunders Co.)



Figure 5. Antero-posterior view of skull showing distribution of seven Y^{90} pellets in pituitary

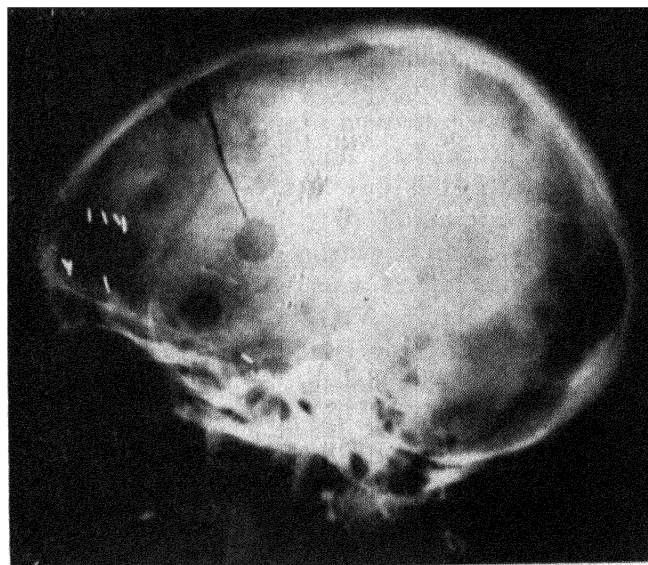


Figure 6. Lateral view of skull of patient shown in Fig. 5

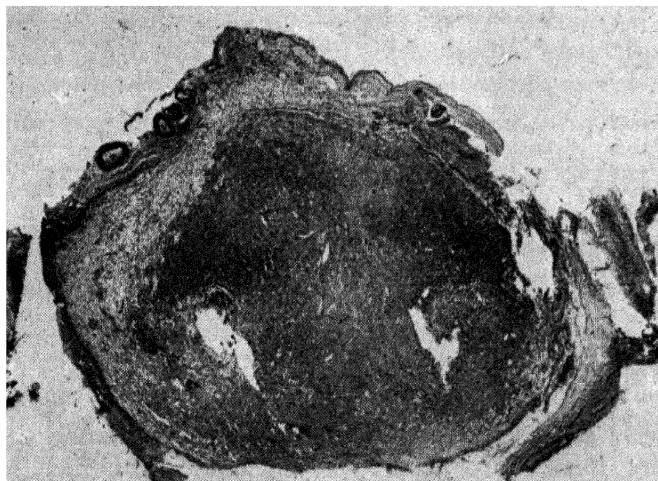


Figure 7. Low power photomicrograph of coronal section through sella turcica showing destruction of pituitary by properly placed Y^{90} pellets. Cephalad sections in this case showed residual gland

Complications attributable to radiation damage have been limited to four instances of third or sixth nerve paralysis due to misplacement of pellets, and two instances of transitory coma due probably to radiation of the hypothalamus by high lying pellets.

It is as yet too early to assess the clinical results in our series. However, it may be stated that even

patients with incomplete hypophysectomy, as shown at autopsy, nevertheless showed marked endocrine changes as evidenced by hypothyroidism and adrenal insufficiency.

While in none of our patients who has come to autopsy has there been complete destruction of the pituitary, the results have, in general, been better than in the surgical cases, and our recent cases, in whom a large number of pellets was used, are all still living.

At the present time we believe that the implantation of radioactive yttrium pellets in the hypophysis is as safe a procedure as surgical hypophysectomy, is much more rapid once the gland is exposed, avoids the danger of hemorrhage from the cavernous sinus, and probably offers a better chance for complete hypophysectomy than the surgical method appears to offer.

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Internal Radioactive Isotope Therapy of Neoplastic Diseases by Means of Radioactive Suspensions

By J. H. Muller,* Switzerland

The availability of artificial radioactive isotopes offers important means for the therapy of cancer and allied diseases. Besides high-intensity gamma-ray sources for external radiotherapy, there are other ways of utilizing isotopes, which are of even higher specific interest, since they approach or even fulfil the ideal of a selective internal irradiation.

When such an internal irradiation is attempted by means of artificial radioisotopes, in particular for cancer therapy, it is mandatory to localize the radioactivity within the diseased region, organ or tissue.

The localization of radioactivity through metabolism, which bears in itself the features of a scientifically ideal approach, has yielded but limited opportunities for tumor therapy. In fact only radioactive iodine (I^{131}) rendered possible so far the performance of really successful, i.e., curative assaults against selected types of metastasizing thyroid cancer, by means of the specifically selective incorporation of the radioactive atoms into the malignant cellular elements. A substantial number of human lives were thus already saved throughout the civilized world. As an example, we wish to quote 2 such cases of our own, both in their late twenties, which were treated $4\frac{1}{2}$, and $6\frac{1}{2}$ years ago with I^{131} , very successfully indeed. They have remained free of any symptoms of the disease, in spite of the fact that they had had, prior to treatment, thyroid carcinoma with extensive mediastinal and pulmonic metastases. One of these patients, a young woman, has given birth after the I^{131} -treatment to two so far healthy children.

Another approach for cancer therapy with artificial radioactivity is the isotope localization based on adequate conditions of what I suggested to call "micromechanics". For about ten years this therapy has proved successful enough to be medically acceptable. The general principles of this whole group of procedures, which are very typically medical ones, since they demand the integration of the physical problem involved with a good deal of clinical and even operative skill, are as follows:

The radioactive isotopes selected for convenience of their half-life and radiation spectra are used just as radiators, that is, deliberately leaving out of the

game their intrinsic biochemical properties. Hence they have to be used in a chemical form which is insoluble in the living media. The localizations of such materials within preselected parts of the organism demand, however, that they be used as injectable liquids, that is, in form of adequate suspensions of the insoluble particles. Highly useful degrees of localization can be reached by such methods, using anatomical aspects and also functional characteristics of the tissues. This means a fundamental and not merely technical progress in the field of radiation treatment.

THE CLINICAL UTILIZATION OF RADIOACTIVE SUSPENSIONS

Radiocolloids and sufficiently stabilized suspensions of larger particles, for the preparation of which radioactive gold (Au^{198}) is quite suitable and most frequently used, can be employed in three main ways. These are the intravascular, chiefly intravenous administration, the direct injection into tumors and surrounding tissues and the injection into the larger body cavities, that is intraperitoneal and intrapleural administration. The fact that a very substantial drainage of the thus deposited colloidal radioactive particles occurs into the regional lymphatics is quite important from the point of view of cancer therapy.

Intravenous Administrations

The intravenous injection of radiocolloids results in an almost quantitative pickup of the radioactive particles by the reticulo-endothelial system, mainly of the liver and spleen. This can be used for therapy of radiosensitive neoplastic conditions of these organs (leukemia, lymphosarcoma, visceral extensions of Hodgkin's disease) as advocated first by P. F. Hahn's group.¹ This therapy can also achieve, in the author's own experience, useful palliation in exceptional cases of carcinomatous liver metastases.

On the other hand, the author^{2,3} has also performed intravenous injections of radioactive zinc (Zn^{63}) and of radioactive gold (Au^{198}), absorbed on carbon, in the form of suspensions of much larger particles (about a thousand times larger than colloidal size), which result in a quite different distribution pattern, namely the almost quantitative localization within the lungs, through microemboliza-

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tion of these particles into the lung capillaries. By means of catheterisation of the heart, such suspensions (rendered sufficiently stable by the adjunction of pectin or dextran) can also be directed accurately into one lung only, or even "shot" into one particular lung lobe. This original method offers quite interesting possibilities both for the fight against primary cancer and early metastatic cancer of the lungs. This has been recently also investigated in England.⁴

Direct Injections into Tumor Areas

This direct injection of artificially radioactive colloids, which Mitchell⁵ called an "injection implantation" has already gained a substantial practical importance. A quite early original paper concerning this topic was contributed by the author,⁶ who, in 1946, first reported the clinical use of interstitial injections of semi-colloidal radioactive zinc suspensions in a small group of patients with cervical carcinoma of the uterus stage 3. This procedure was later strongly advocated by W. M. Allen and A. Sherman,⁷ using then colloidal gold, as a routine method for the treatment of cervix cancer. The author found it useful for special cases, but has not yet accepted the use of colloidal gold injections as a standard method for the treatment of this cancer location. The direct injection of colloidal gold with high specific activities is also an important procedure for the treatment of cancer of the prostate as shown by R. Flocks⁸ and his group. There is further an interesting contribution of P. F. Hahn⁹ on the utilisation of silver-coated radioactive gold colloids, as adjuvants in the surgical treatment of bronchogenic carcinoma.

Keeping in view the drainage of the colloidal gold particles into the lymphatics, the author has more recently used interstitial injections of the material performed by means of a special method for the preoperative interstitial irradiation of breast cancer, by means of intra-, retro- and paramammary injections of the colloidal Au¹⁹⁸. In all the investigated cases a substantial selective drainage of the radioactive gold occurred within the regional lymph nodes.¹⁰ Similar infiltrations of the interstitial spaces containing the lymphatic channels and glands were also performed by the author in other anatomical locations, with mostly very satisfying results (hitherto unpublished data).

Intraperitoneal and Intrapleural Application of Radioactive Colloids

The intraperitoneal and intrapleural administrations of artificially radioactive colloids were started by the author in 1945¹¹ and 1949¹² respectively. These procedures are now widely accepted as an efficient palliative treatment of malignant effusions. Moreover, it can be also considered as established, that such isotope administrations are likely to contribute even curative results, if performed early enough.

We started the intraperitoneal colloidal isotope injections because we were distressed about our many cases of ovarian cancer with malignant effusions in the abdomen and the poor results obtainable in such conditions with routine deep radiotherapy, even if the primary had been removed surgically. We thought that it would be logical to attack the tumor cells floating in these liquids, as well as the often innumerable secondary deposits within the peritoneal surface, by the internal administration of a radioactive compound, right into the cavity. The radioactive material would not be in a soluble phase, since it should not be absorbed.

In cooperation with the Cyclotron Group of Zurich, we were able in the late spring of 1945, to obtain samples of radioactive zinc (Zn⁶³), with activities of several hundred millicuries, which were prepared as semi-colloidal sulfide particles, of which sufficiently stable suspensions were made by the adjunction of pectin. Zn⁶³, mainly a strong positron emitter, has a half-life of 38.3 minutes only. These preparations, however, contained also a few percent of the isotope Zn⁶⁵ ($T_{1/2} = 250$ days), which contributed a substantial supplementary protracted irradiation.

In experimental animals these radioactive suspensions remained well localized within the abdominal cavity and the employment of this procedure was then commenced in human medicine. We had in our initial series, started in 1945, cases that did extremely well, for instance one patient with completely desperate abdominal ascitic carcinomatosis (of ovarian origin), who was expected to live only a few weeks, but who lived then three years more. This was obtained by means of a fractionated treatment with a total of 1 curie of radioactive zinc suspension administered intraperitoneally, which readily controlled the ascitic fluid production. Such total doses, however, involved a great deal of work and exposure, because we had to perform all the radiochemical separations and preparations in our own hospital department. Anxious to find a more suitable material for our method and knowing that radioactive gold would be good, we were then very happy to hear that the group of P. F. Hahn¹ had been able to obtain in the USA pile-produced radioactive gold (Au¹⁹⁸) and to make a classical colloid of it, which seemed, from animal experiments, suitable for applications in human patients.

As soon as preparations of this colloidal Au¹⁹⁸ were sent to me through the courtesy of the U.S. A.E.C. and of the Abbott Laboratories, we started to use it extensively for all cases of cancer of the ovaries with peritoneal extensions and in some other types of neoplasms with effusions. In 1949 I performed also the first intrapleural administrations of colloidal gold in cases of malignant pleural effusions,¹² which yielded satisfactory results. Shortly thereafter we submitted to the Isotope Division of the A.E.R.E., England, the suggestion to start also

the production of colloidal radiogold, which was then promptly turned into action. Our early letters on these treatments¹⁸ both to the Abbott Laboratories and to the British Medical Research Council contributed then to introduce these intraperitoneal and intrapleural administrations of the radioactive gold colloid at a broader scale into clinical medicine. These uses became readily accepted within a short period of time.

Up to the present time, the colloidal Au¹⁹⁸† has remained the most suitable radioactive material available for such treatments and its utilisation is still increasing steadily. Some other radiocolloids (in the authors' opinion less suitable) have also been tried for intracavitary application, such as colloidal radioactive chromic phosphate and colloidal radioyttrium, both pure beta-emitters.

The author and a number of clinical workers (for instance Smithers,¹⁴ Andrews *et al.*¹⁵ and many others) have consistently found that the intraperitoneal and intrapleural administration of colloidal Au¹⁹⁸ is a safe procedure, by no means hazardous for the patients and the operators if performed with sufficient care and appropriate technique. The actual "spilling" of colloidal radiogold into the reticulo-endothelial system of the liver, spleen and bone-marrow does not exceed a relatively small percentage, after intrapleural and intraperitoneal administration. On the other hand, however, an important aspect of these intracavitary administrations of colloidal Au¹⁹⁸ is its very substantial pickup by macrophages and migration into the lymph-nodes. Recent studies performed by the author on autopsy material collected from patients whose unrelated intercurrent death occurred about 10 days after such administrations, have definitely established that these highly desirable secondary distributions of the colloidal radioactive material are indisputable facts. Thus, approximate dosage assessments of the beta radiation based on conservative estimations provide figures of about 4000 rad for the visceral peritoneal serosa, 6000 rad throughout the omentum, 10,000–30,000 rad for all the retroperitoneal, paraoesophageal and paratracheal mediastinal lymph-nodes after intraperitoneal administrations of 145 millicuries of colloidal Au¹⁹⁸. About 750 rad of diffuse gamma radiation must be added for the intra-abdominal structures. Correspondingly, high activity deposits were also found after intrapleural administration within the pleural serosa and mediastinal lymph nodes.

These approximated figures, reliable, however, about the order of magnitude, are definitely of true practical significance, since they are well within the

range of therapeutically efficient radiation effects. Hence these observations suggest a strong encouragement for using these intracavitary colloidal Au¹⁹⁸ administrations at a larger scale, since many seemingly "prophylactic" applications are likely to be in fact curative ones, owing to the very great frequency of lymphatic involvement from intra-abdominal and intrathoracical neoplasms, even in the earlier "operable" stages of disease.

Clinical Results, with Special Consideration of Ovarian Cancer

Many miscellaneous neoplastic conditions can receive substantial benefit from the intracavitary radiogold therapy. We shall, as an important example, discuss our rather extensive clinical experience of the intraperitoneal administration of colloidal Au¹⁹⁸ for the treatment of ovarian cancer.‡ This is a particularly interesting field of action, since ovarian neoplasms respond to the intraperitoneal isotope therapy within the whole range of their extension stages, which renders possible a reliable study both of the qualitative and quantitative results obtained.

The author has thus, until the end of December 1954, treated 114 cases of ovarian cancer with colloidal radiogold, complete clinical and histological data being available. The majority of these patients were treated first by surgery and had then supplementary intraperitoneal colloidal radiogold, together with the standard postoperative radiation therapy, as used for many years. The latter consists of fractionated roentgen therapy resulting in a total depth dose of about 3000 to 4000 r within the pelvic cavity and the lower abdomen and of 1000–2000 r within the upper part of the abdomen. No such penetrating radiation, however, is dispensed upon the subphrenic structures. Most of these patients received further a vaginal radium application (from 1000 to 1800 mg el.h.). Since the great majority had either supravaginal or total hysterectomy only few intrauterine radium treatments were performed.

In order to insure a clearer analytical appreciation of the therapeutic results, a suitable classification of these patients is necessary, which the author found convenient as follows:

Stage I is the group in which a radical or seemingly radical operative removal of the tumor was possible.

The author, together with E. Held, has introduced since 1950 the "prophylactic" radiogold therapy of these cases, since it is well known that small peritoneal or subperitoneal deposits, hardly visible at operation, can then already be present. Such incipient deposits can be exceedingly well combated by intraperitoneal application of radiocolloids.

† The isotope Au¹⁹⁸ ($T_{1/2} = 2.69$ days)¹ is prepared from pure gold Au¹⁹⁷ by a neutron-gamma reaction, which has a very high capture cross section (100 barns) for slow neutrons, whence the production of Au¹⁹⁸ is quite economical. The disintegration scheme of this isotope involves mainly a strong beta-emission, with final production of extremely small amounts of Hg¹⁹⁸. The latter is without any practical significance.

‡ Intraperitoneal colloidal Au¹⁹⁸ administrations were recently also started by the author as a postoperative treatment in cases of cancer of the stomach. It seems quite probable that such a treatment would inhibit some of the lymphogenic relapses.

From the 21 patients of this group, 2 died from intercurrent affections (heart failure, bronchus carcinoma), but free of symptoms of the ovarian disease. The other 19 patients are all well and free of symptoms of the disease, four for more than $4\frac{1}{2}$ years and five for more than $3\frac{1}{2}$ years. This 100 per cent favorable preliminary result (not a single recurrence of the disease!) is quite encouraging and we shall proceed further with this type of a combined post-operative roentgen-radiogold therapy in stage I. 50 to 250 mc of Au^{198} were given intraperitoneally per patient of this group. §

Stage II group includes the patients in whom the one or double-sided ovarian cancer was still operable, but there were already pelvic tumor deposits beyond the ovaries, the removal of which was partially possible or even seemingly successful.

This group is represented by 16 patients, of whom 15 were primarily operated upon and then submitted to a combined roentgen-radium-radiogold treatment. One patient was first treated with radiogold and then operated upon. The doses given varied from 80 to 400 mc of Au^{198} per patient. Four of these patients died from the ovarian disease within the first year after treatment. The other 12 patients remained thus far free of symptoms of the disease, two after $5\frac{1}{2}$ years, one after $4\frac{1}{2}$ years, one after $3\frac{1}{4}$ years, two after $2\frac{3}{4}$ years, one after $2\frac{1}{2}$ years, the others after at least 1 year. Thus 75% of these patients remained free of symptoms, a preliminary result which is quite encouraging also. The average dosage of the radiogold might be advantageously raised somewhat in this group up to about 300 mc Au^{198} (twice 150 mc).

Stage III group includes the patients with carcinoma of one or both ovaries having extended to the omentum and to the peritoneum beyond the pelvic area, in whom the primary neoplasm and in some cases also the secondary deposits were at least partially resectable. ¶

In this group the disease has thus spread into the greater abdominal cavity, which means a considerable jump towards generalization involving *a priori* a much poorer prognosis. The 36 cases of this group were all operated upon, but none radically and then submitted to combined roentgen-radium and radiogold therapy with activities up to 450 mc Au^{198} . 15 cases (42%) remained thus far free of symptoms of the disease (one after $5\frac{1}{2}$ years, one after $4\frac{1}{4}$ years, one after $3\frac{1}{2}$ years, one after $3\frac{1}{4}$ years, one after $2\frac{1}{2}$ years, one after $1\frac{3}{4}$ years, the other between $1\frac{1}{2}$ years and 6 months). The other 21 patients died all from their ovarian cancer, between 4 months and $2\frac{1}{4}$ years after treatment. This prelim-

inary result of 42% patients free of symptoms is encouraging. There are here, however, obvious limitations since we have seen some cases in whom the carcinomatosis of the pelvis and of the peritoneum had disappeared, but who died later from metastases in the liver, spleen and other sites. In spite of this state of affairs there is still a reasonable chance for an increase of the over-all curative results in this stage III group by aiming at higher average total doses of the Au^{198} . We administer now in these cases at least 300 mc of it (2-3 applications of 120-150 mc).

The stage groups IV and V can be considered together:

Stage IV group includes cases of cancer of the ovaries with fully inoperable primaries and very extended deposits in the whole abdomen and in some cases also in the liver. No surgery is possible, but biopsies are obtained.

Stage V is the same as stage IV, but with further extensions of the carcinomatosis to one or both pleural cavities.

Palliative results only can reasonably be expected in such conditions from the Au^{198} treatment. Important, however, is the actual fact that in the great majority of these cases the often enormous production of ascitic fluid can be readily reduced to a minimum or completely stopped by means of the isotope treatment. The control of the pleural effusions is even more uniformly achieved. This often striking stopping effect of the intracavitary Au^{198} -applications is readily explained by the destruction of the innumerable tumor cells floating (and perhaps multiplying) in these malignant effusions. This effect is identical to that obtained in experimental work, especially with the ascites tumor of the mouse.¹⁶

Total doses of 90 to 560 millicuries of Au^{198} were administered intraperitoneally and of 40 to 250 millicuries intrapleurally. One patient with enormous extension of the disease had both intraperitoneal and bilateral intrapleural radiogold up to a total of about 800 mc and survived then for 2 years. These patients of stages IV and V were partly treated with radiogold only and partly also by means of supplementary X-ray and radium therapy.

We have in this series 29 patients of stage IV and 12 patients of stage V, thus a total of 41 patients. Two of these patients lived 3 years and one $2\frac{1}{2}$ years after the isotope treatment was started; one patient with a metastatic ovarian and abdominal carcinomatosis from a previously operated breast tumor lived $2\frac{2}{3}$ years; two patients lived $1\frac{3}{4}$ years, three from 1 to $1\frac{1}{2}$ years; fifteen other patients of this series died from 6 to 11 months after the isotope treatment was started.

It must be pointed out that all these patients were fully desperate cases, who would have died within a few weeks and months if they had not had this newly developed therapy, which plays indeed an important role in such conditions. Though it means a rather

§ Cases in which a cystic tumor burst before or during operation, with subsequent spilling of tumor masses into the abdominal cavity were also classified as stage I; they received, however, two Au^{198} treatments, with a total dose of at least 200 millicuries.

¶ Three cases of metastatic ovarian carcinoma from carcinoma of the uterine body were included in this series.

tough medical job to fight these formidable carcinomatous extensions, it is the right thing to do both from the medical and human point of view. If one takes as the minimum level for a valid palliative result six months of life prolongation after onset of the isotope therapy, a quotient of 59% palliation was obtained for stages IV and V together. It is reasonable to admit that such a result is worth the work and expenses involved. The radiogold therapy is thus an acceptable medical procedure for the palliative treatment of these unfortunate patients.

GENERAL CONCLUSIONS

As ascertained from the above reported and discussed data, the administrations of radioactive suspensions (in particular of colloidal radiogold Au^{198}) realize a new kind of internal radiotherapy, which has readily gained practical importance. These procedures are clinically safe and reliable and so are convenient for routine medical utilization.

Owing to the direct interaction of the radioactive material with the exposed structures, these methods offer some specific advantages that are not available from external deep radiotherapy nor from applications of closed radioactive sources. This is especially true of the migration of the radioactive particles into the regional lymphatic system and of their immediate destructive effects upon the tumor cells floating in the malignant effusions or deposited within the serous surfaces, the beta radiation being then the mainly efficient factor. On the other hand, the gamma radiation contributes substantially to the homogenization of the irradiation effect and thus plays also an important part.

It must be kept in mind, however, that this particular form of isotope administration is by no means a "new cancer cure", but rather a true progress in the field of radiation therapy. It has accordingly its proper indications and also its limitations, as outlined in the preceding pages. From the presented clinical data, which concern specially ovarian malignancies, it can be admitted that the intracavitary administrations of colloidal Au^{198} are able to contribute even curative results, if performed early enough.

SUMMARY

The localization of artificial radioactive isotopes through metabolism offers but limited opportunities for tumor therapy. Another important approach in this respect is the utilization of injectable suspensions of radioactive particles, down to the colloidal size, which can be administered in three main ways. These are the intravascular, chiefly intravenous injection, the direct injection into tumor areas, as well as the intraperitoneal and intrapleural administrations. The author reports on his encouraging clinical experience with these methods, which he has endeavoured to develop since 1945. A more detailed report is presented on the clinical results obtained by means of

the intracavitary application of colloidal radiogold (Au^{198}), performed routinely on an unselected group of over 100 cases of ovarian cancer. These preliminary results indicate that such isotope administrations must be considered as a true progress in this field of cancer therapy.

The interested reader will find more details and many illustrative documents in the printed publications indicated below.

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The Use of Colloidal Chromium Radiophosphate in the Treatment of Malignant Tumors

By André Chevallier and C. Burg,* France

Until now, surgery and the ionizing radiations have been the only means of treatment in use for malignant tumors. Radiation raises a certain number of problems, theoretical and practical.

When it comes to the local irradiation of neoplastic tissues, considerable progress should be made thanks to the industrial production of the radioisotopes which can be used, either as external, or else as internal, sources of radiation. In the latter situation, irradiation must fulfill, on principle, the following requirements:

1. The tumor-killing dose must be established homogeneously throughout the mass of the tumor, and yet not go beyond it and thus affect the neighboring tissues.

2. The half-life of the radioactive element must be such that the duration of irradiation will permit repair phenomena to develop, while at the same time accentuating the difference in radiosensitivity between the pathological and normal cells.

The half-life must be sufficiently short that there be no need to fear the "chronic irradiation" phenomenon on the affected region, with all the dangerous consequences which this may carry.

It may be though, in the absence of such strict specific affinity as is currently known only for radioiodine, with respect to thyroid tissue, that the application of an extremely fine suspension of a radioisotope insoluble in the tumor tissue may provide an adequate local irradiation dose. It also would seem that, if the isotope used emits β -particles of the highest possible energy, without any γ -rays at all, for a half-life of some 10 days, the above-mentioned conditions will have been fulfilled ideally. This is the program which we endeavored to carry out.

In the present state of our knowledge, it would seem that P^{32} is one of the elements which meet the above requirements fairly well, provided however, it be used in a strictly insoluble and stable form, as for example when it is combined with chromium: anhydrous $CrPO_4$.

Since 1950, we have been studying, at one and the same time, the preparation of such a product, its

behavior in the system and its therapeutic action in the case of malignant tumors.^{1,2} We shall draw attention here to a few points which seem to be of special interest.

EXPERIMENTAL ASPECTS

When the solution used is colloidal, the particle size plays a very important part in the distribution of the product through both the blood and lymphatic vessels. With very small granule dimensions, smaller than 100 Å, a notable proportion of the $CrPO_4$ is distributed throughout the entire organism from the point of injection, both by passing through the walls of the blood vessels and by transport in the lymphatic system. For truly larger dimensions (close to 1 μ), the fraction diffused is very small and is reduced to a very small amount of lymphatic transport. Between those two extremes, we find all the intermediaries. Since the lymphatic migration is of special interest, we give, in percentage, the changes in activity of the

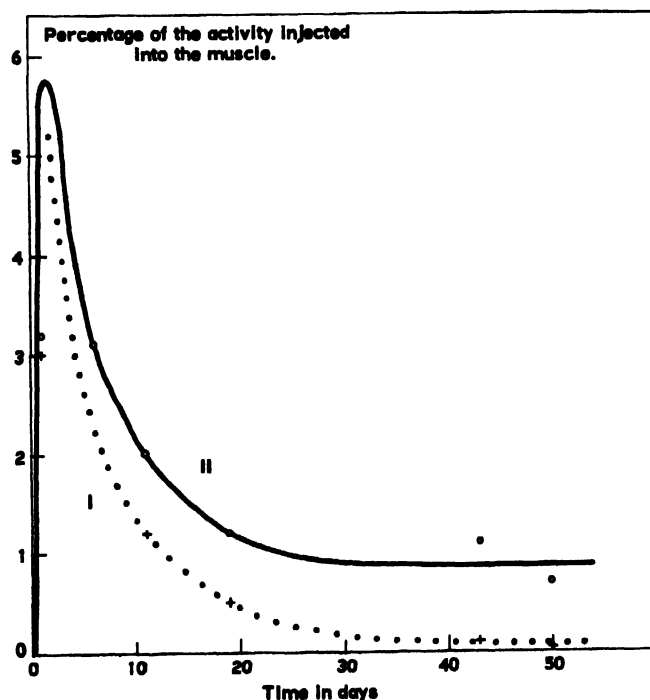


Figure 1. Variation of the activity as measured (I) and the activity reduced to time 0 (II) for the lumbar lymph node

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lumbar lymph node in a rat which received a dose of 50 microcuries of CrPO_4 in the thigh muscles:

Activity of the lymph node	24 hr	40 hr	6 d	11 d	19 d	43 d	50 d
Right lumbar node measured activity	3.0	5.2	2.34	1.2	0.48	0.14	0.06
Activity reduced to 0 time	3.2	5.70	3.14	2.02	1.2	1.12	0.68

It will be noted that some measure of excretion takes place after the first forty hours. This, however is not quantitative, and a substantial fraction of CrPO_4 (approximately 30%) remains in lymph node tissue. This quantity is adequate to bring about the production of a radiation, equivalent to a dosage of up to several tens of thousands roentgens (rep) throughout the volume of the lymph node. Naturally, the specific activity of the product used plays an important part in the phenomenon.

THERAPEUTIC ASPECTS

We applied this technique to the treatment of approximately 150 patients afflicted with a wide variety of malignant tumors.^{3,4} The time elapsed and the number of cases are not sufficient for us to make any sort of accurate evaluation of the method. At the present time, however, some conclusions may be brought out.

1. With the product we used, which contains no trace of any soluble radiophosphates, and with granule sizes which do not permit any appreciable blood diffusion, no systemic disturbances were observed in the patients treated. The doses used have been very high at times: they ranged from 10 to 100 millicuries for very extensive and recurring cancers, and the differential blood count of the subjects so treated remained practically normal, while the patient's general condition was not affected by the treatment.

2. The sclerosis phenomena which follow the use of high activities always remained very limited, and restricted to the immediate vicinity of the substance.

Following extensive experiments on dogs, either on the brain or on the mediastinum, it was determined that the sclerosis brought about by the injection of therapeutic doses of chromium radiophosphate (several millicuries) did not extend to more than 10 millimeters from the point of injection. This fact shows that the spatial extent of the tissue reaction exceeds but little the effective path of P^{32} radiation in the tissue, the length of which ranges from 4 to 5 millimeters.

This finding was made in a large number of animals and after variable periods (to 14 months) following injection.

In view of the above, no remotely located reactions are to be feared due to the application of this technique, and the surgical procedure which may follow is not made more difficult.

It is indeed our belief that, on the plane of therapeutics, the method not only does not rule out the use of external radiotherapy or of surgery, but rather that it is a very good complement to either one.

The destruction of a small volume of tumor tissue by irradiation is of no interest if the tumor could have been removed surgically without any risk.

The injection or implantation of thin plates of gelatin which contain a suspension of chromium radiophosphate may make it possible, prior to the operation, to charge the lymphatic system with the active product. Then, following excision, the same technique may make it possible to achieve cellular sterilization of the operating field.

Most of our therapeutic applications were carried out under such conditions, whether we were dealing with cancer of the breast, of the rectum or of the thyroid, or again with muscular sarcomata or cerebral tumors.

Our experience is most extensive in the applications of the method to cancers of the urinary bladder, both as regards the number of cases and the time elapsed. These results appear to be very satisfactory, the patients make no mention of any painful reactions, and healing of the cystostomy takes place within a normal time^{5,6,7}. In the case of one patient, we were able to achieve histological control five months after the operation. This showed that the neoplastic tissue originally present had disappeared.

It is quite evident that each localization and each type of cancer raises its own specific problems. Only following prolonged experience will it be possible more accurately to define the indications and limitations of the method.

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Versatility of Radioactive Gold in the Treatment of Malignant Disease

By P. F. Hahn,* USA

Much of the early therapeutic use of isotopes was predicated on availability of materials which could be produced by cyclotron bombardment and which showed a predisposition for some degree of selective uptake by the tissues which were desired to be irradiated. Both P^{32} and I^{130} satisfied these requirements and as a result were used in the treatment of malignancy fifteen years ago. The availability of chain-reacting pile products altered the economics of isotope production very markedly. Today P^{32} is obtained by an (n, p) reaction on sulfur in the pile whereas I^{131} has come to substitute for its shorter lived isotope and is obtained primarily as a by-product of uranium fission. More important however is the wide variety of other isotopes, which may be economically produced, offered to the investigator for potential therapeutic use. Metabolic concentration of elements, as exemplified by the relatively specific uptake of iodine by the thyroid, is not common in mammalian organisms and one is therefore forced to consider other mechanisms whereby concentration of the radiating material may be attained. With over 1000 isotopes from which to choose it may seem surprising that relatively few have proven satisfactory for therapeutic use in the nine years during which they have been available. Elsewhere ^{1,2} in some detail we have enumerated the criteria by which one could choose an isotope for treatment of malignant disease. Briefly these criteria may be summarized as follows:

1. The isotope must be of class A type, i.e., its identity and characteristics must be well established.
2. The chemical reaction of the elements should be well understood in order that separation and purification procedures can be effective.
3. The biological behavior of the material should be known in order that its degree of localization, its excretion, its metabolic interchanges, etc., can be recognized and dealt with.
4. The element must have a high cross section to thermal neutron capture or be produced by transmutation reaction in order that the specific activity may be high and toxicity thus minimized.
5. Correlative of the last criterion the efficiency of the production should be good in order that the isotope may be produced economically on a wide scale.

6. The half-life should not be so short that production, handling and transportation become unnecessarily detrimental and hazardous problems.

7. The half-life should not be so long that the agent itself might be carcinogenic. An upper limit of about 10 days is suggested.

8. The question of spectrum is a moot one at the present time, there being little agreement as to whether a pure beta or mixed beta and gamma spectrum is more desirable. In the use of isotopes having a pure beta spectrum less damage is encountered by the nearby normal tissues and fewer unfortunate sequelae to therapy are likely to be encountered. On the other hand a gamma component may be highly desirable inasmuch as it facilitates assay of the original material administered, allows simple assay of losses due to excretion as well as those encountered as a result of administration, due to seepage, etc., and external measurements of the material *in situ* are made possible by employment of the proper directional counting or scanning equipment. This last provides a check on the localization of the material following administration. Also the gamma component affords radiation of regional nodes in some instances.

The Au^{198} isotope satisfies these criteria to a remarkable degree. Its chemistry has been known for centuries. The metallic colloid is physiologically inert, sets up no foreign body reaction and, since it does not participate in metabolic processes, there is no problem of radioactive excreta involved in its use. Its half-life of 2.7 days is nearly ideal since the treatment response is easily "titrated" within a reasonable length of time. Its mixed spectrum of an 0.96 Mev β particle and 0.45 Mev gamma emission represents good compromises.

One must not lose sight of the fact that the common malignancies encountered are those of the breast, cervix, prostate, stomach, intestine, lung, etc. It is among these conditions that our efforts toward the application of radioisotopes should be more generally directed. It was with this in mind in addition to the various criteria summarized above that led to the choice of radioactive colloidal gold as an agent for the selective irradiation of body tissues. Its employment in a wide variety of conditions is based generally upon two processes, i.e., its propensity to undergo phagocytosis by the tissues desired when

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administered under certain conditions and its tendency to remain at the site of instillation when administered by the interstitial route.

At the present writing approximately 20 curies per week of Au¹⁹⁸ are being made at the Oak Ridge National Laboratory facilities alone. A small quantity is routinely also produced at Brookhaven National Laboratory. About 80 per cent of all this gold is being used for palliative purposes, largely in the control of peritoneal ascites and pleural effusions by intracavitary administration. In 1947 we reported the localization of gold colloids in the peritoneal cavity following intraperitoneal administration³ of this material in two cases of hypernephroma. Subsequently Muller, who had worked with a short-lived zinc isotope in the suppression of fluid formation, employed Au¹⁹⁸ colloids for the same purpose and found them to be satisfactory in the performance of this function.^{4,5,6} At the present time it has become a widely accepted procedure for control of such fluid production in metastatic carcinoma of the thoracic cavity and peritoneum. A consensus of published reports would indicate its effectiveness in this regard in from 50 to 75 per cent of the cases treated.⁷ Employed for the same purpose, X-ray has been rather uniformly disappointing probably due to the undesired inevitable irradiation of normal structure and viscera especially as exemplified by induction of pleural adhesions and unnecessary irradiation of the mucosa of the gastro-intestinal tract respectively. The administration of gold colloids by the intracavitary route is a very simple one. Its chief disadvantage is its cost. The dosages commonly employed range from 75 to 100 millicuries to one side of the chest by intrapleural route and from 125 to 200 millicuries when used intraperitoneally.

We originally employed colloids by the intravenous route for the treatment of various forms of leukemia and Hodgkin's disease.^{2,8,9} In view of the unfavourable prognosis of the disease large doses were risked in the treatment of acute leukemia in children. As a result in several cases extremely marked leukopenia occurred necessitating the liberal use of antibiotics in supportive therapy of these patients. However in two instances survivals of 9 and 12 months respectively were obtained and it is felt that perhaps further study of gold colloids in this condition in combination with the use of chemotherapeutic agents should be attempted. In the chronic leukemias we have been quite well pleased with the results obtained following intravenous gold colloid administration. Remissions lasting from 5 to 18 months have been obtained, the longer ones in the cases of older individuals in which the disease process seems to be more benign in its manifestations. Here the dosage by the vein is approximately 0.7 mc per kg body weight although remissions have been obtained in some instances with considerably lower doses. Also it might be mentioned that the colloid dosage tolerance is quite considerable since twice the

amount has been administered with successful results and without the establishment of marked leukopenia. In most cases of chronic leukemia such treatment is followed by reduction of smudge forms in chronic lymphogenous leukemia to negligible or absent quantities and leukocyte counts to near normal levels. In myelogenous leukemia the number of blast forms in the peripheral circulation is either markedly reduced or eliminated entirely with reduction of leukocyte levels to normal values and seldom below normal. In both instances there is usually an increase in the number of circulating red cell elements to normal levels without resort to transfusion. Since when given by this route gold is essentially all taken up by the liver and spleen the therapeutic effect is probably obtained by irradiation of these organs suggesting a humoral response to irradiation rather than a direct effect on the hematopoietic system.

Our experience with the various leukemias embraces a group of approximately 80 patients. In general it might be said that following therapy there was: (1) Reduction of total leukocyte count to normal or near normal levels. Leukopenia occurred only when the patient was drastically treated or over treated. (2) Alteration of the differential leukocyte values to normal or near normal. (3) Improved sense of well being. (4) Loss or diminution of anorexia. (5) Reduction in hepato-splenomegaly if pre-existing. (6) Restoration of erythrocytic elements toward normal levels without recourse to transfusions. (7) Occasional, although not uniform, reduction of lymphadenopathy.

Among the most interesting findings following therapy was the logarithmic rate of decrease of white cell elements in the peripheral circulation,¹⁰ thus indicating that the effect of radiation was one of a first order reaction. This would suggest that the site of the irradiation was not on the stem cell. Furthermore, inasmuch as most of the material is concentrated largely in the liver and spleen a humoral effect on the peripheral preformed white cell elements must be seriously entertained. Parenthetically, it might be mentioned that during relapse in the leukemic state the leukocyte count increases at a logarithmic rate. Such a first order reaction, indicating that the rate of increase of white cells is a function of the leukocyte level, suggests that the aberrant process is an uncomplicated one.^{10,11}

Treatment of patients with Hodgkin's disease was for the most part undertaken during our first two years of use of radiocolloids.⁹ The patients were unselected and in many instances represented terminal cases. The total number treated was 30. Material for study was obtained from a 200 mile radius and as a result there were no adequate follow-ups in at least eight instances. Furthermore another eight patients had had extensive X-ray therapy previously and were therefore far from ideal subjects on which to study the effects of a new form of irradiation. Six patients were definitely in a terminal state. Two were

not treated by intravenous administration but by interstitial injection of enlarged nodes alone. Since the use of intravenous colloids by vein was new, nothing was known concerning the dosage and it was felt that in at least 10 instances, in an attempt to be conservative, the patients were undertreated. It is felt that from fair to excellent results were obtained in about $\frac{1}{3}$ of the patients treated. In 3 of these the results were quite dramatic. These latter patients who were bedridden, emaciated and subject to exhausting fever and sweats were, for a period of some months at a time, returned to normal life. Indicative to successful therapy were reduction in size of enlarged lymph nodes in the neck, axilla and inguinal regions; increase in appetite and strength; gain in body weight; disappearance of Pel-Ebstein type fever; reduction or disappearance of hepatosplenomegaly where it had previously existed. On the negative side disadvantageous results were transient leukopenia, and in some instances thrombocytopenia. In these latter cases it was felt that it was due to a swing in the opposite direction following realization of previous undertreatment with the result that rather over-zealous attempts were made to manage the condition. The use of colloids in Hodgkin's disease was discontinued at the time of introduction of nitrogen mustards in the treatment of this condition in view of the enthusiastic claims for this chemotherapeutic agent. It was not felt justified to continue experimental type of therapy if claims for mustards were well founded. In the ensuing years the experience of clinical investigators suggested that the initial enthusiasm for nitrogen mustard therapy had been only partially justified. Perhaps one of the main objections of this drug is the closeness of the therapeutic to the toxic range of dosage. As stated earlier, most of these patients with Hodgkin's disease were included in our first two years of therapy with colloids and consequently many of them received the Mn^{52} containing colloid. Therefore Au^{198} colloids have probably not had adequate trial in this condition. We are therefore desirous of extending this series with the use of the latter agent alone and perhaps in combination with some chemotherapeutic agents. In a new series it would also be of value to determine whether Hodgkin's granuloma, paraganuloma or sarcoma was being treated since the prognosis might be greatly affected.

In summarizing the advantages of intravenous use of isotopes as contrasted to external irradiation procedures in the treatment of leukemias and Hodgkin's disease:

1. The ambulant, unhospitalized patient is treated by a single intravenous injection. This is in contrast to from ten days to two weeks daily splenic therapy by X-irradiation in the case of chronic leukemias and therefore results in a considerable saving in hospitalization time both to the institution and to the patient.

2. Inasmuch as the radiation is delivered over an integrated period there is a considerably lesser amount of nausea and anorexia.

3. Skin erythema is no longer a limiting factor in dosage.

4. Elaborate and expensive equipment is not necessary permitting bedside therapy on an extra-institutional basis.

In 1947 we reported on preliminary observations following the interstitial administration of radiogold colloids.³ This included cases of leukemia cutis; fibrosarcoma; squamous cell carcinoma; carcinoma of the cervix; etc. Since that time we have employed such therapy in conditions ranging from the injection of superficially enlarged lymph nodes; metastatic post-operative breast cancer; neurogenic sarcoma; lymphosarcoma; carcinoma of the stomach, prostate, palate, vagina, and a wide variety of other conditions.²

In skin cancer there is little reason to employ colloids by such administration inasmuch as the cure rate by X-ray and/or by surgery is of the order of 95 per cent. In such instances one is not justified in jeopardizing the welfare of the patient by exploratory new procedures. In certain instances of breast tumors, however, where the use of X-ray might cause pleural and pulmonary complications one is more than justified in using these colloids by the interstitial route. Under such circumstances it is surprising to find the tremendous degree of tolerance of subcutaneous tissues to irradiation procedures when the superficial skin need not be considered, as is necessary in external irradiation procedures.

The longest continuous and largest series of patients treated by the interstitial route in carcinoma of the cervix is that of Allen, Sherman and co-workers. Employing radiogold colloids of high specific activity (produced at Brookhaven National Laboratory) and injecting each of the parametria with 50 millicuries of the material they have, by combination with endo-cervical use of radium followed by operation several weeks later shown a marked increase in cure rates in stage 0, 1 and 2 carcinomas of the cervix. They find that following intraparametrial injection satisfactory quantities of the colloid move to the regional lymphatics and assist in combating metastatic involvement at these sites of metastases.^{12,13,14} Our group is at present investigating the use of silver-coated radiogold colloids in cervical tumors.

Carcinoma of the prostate is an increasingly common type of tumor in males, especially in view of the fact that the older age segment of our population is on the increase. The widest application of radiogold in this direction has been carried out by Flocks, Elkins, Kerr and Evans at Iowa City. They have recently reported on over 300 cases of carcinoma of the prostate with early metastatic spread and find the results quite satisfactory. Improved technique for administration of the material, making use of the

fascial planes for distribution within the prostatic capsule and seminal vesicles has resulted in satisfactory results and fewer undesirable secondary reactions have occurred than were first obtained (rectal and bladder complications).^{15,16} Our own early attempts at treatment of prostatic carcinoma were largely concerned with trans-urethral and rectal approaches¹⁷ and were not attended with as good success as that of Flocks and his collaborators. This we attribute to the inability to obtain uniform distribution when administered by these two routes and subsequent studies in which we used the supra-pubic approach indicated that this latter was the desirable method.

Bronchiogenic carcinoma is generally considered to be on the increase in its incidence. By surgical procedures with or without attendant X-irradiation the cure rate is of approximately only 5 per cent. In order to provide irradiation of the thoracic lymph nodes which constitute the first line of defense as well as the usual site of metastases we studied the intrabronchial administration of radiogold colloids.¹⁸ It was found that adequate drainage of the radioactive material by the lymph nodes required from 10 days to 2 weeks. Due to the short half-life of the radioactive isotope it was thus impossible to attain the desired irradiation effect. We therefore turned to Ag¹¹¹ containing colloids and found that uptake by the lymph nodes of these was quite prompt. However there are technical difficulties involved in the production of short-lived radioactive silver, thus precluding its general use for such purposes at least at the present time. We therefore developed colloids in which the core consisted of Au¹⁹⁸ and a superficial coating of non-radioactive silver was superimposed. These colloids are simply prepared by "seeding" a solution of silver nitrate with gold colloids of high specific activity (prepared in a high neutron flux at Brookhaven National Laboratory). Subsequently the silver is reduced by the addition of an excess of cevitic acid in an alkaline medium containing a small amount of gelatin used as a protective colloid. Such material allows us to make use of the desirable radiation spectrum of Au¹⁹⁸ and the physiological behavior of the silver coating. Such silver-coated gold colloids have been found to concentrate in large quantities in the mediastinal lymph nodes following their introduction by the bronchial route.^{9,19} It is suggested that 50-75 mc be administered approximately two weeks prior to planned pneumonectomy in order that the desired irradiation effect can be accomplished prior to operation and sufficient decay of the radioactive material undergone in order to minimize exposure to the surgeons.

More recently we have studied another approach to the adjunct treatment of bronchiogenic carcinoma. It was found that following pneumonectomy in dogs the injection of silver-coated gold colloids into the empty chest cavity was followed by a satisfactory uptake of the radioactive material by the regional

lymphatics.²⁰ Therefore we have in 5 cases injected these colloids into the empty hemithoraces of patients two weeks following pneumonectomy. It is of course too early to say anything concerning the results obtained. Ultimately the preoperative bronchial instillation followed by the postoperative intrathoracic use may be indicated.

When Au¹⁹⁸ colloids are injected into the brain ventricle we have shown that the material is concentrated to a considerable degree by the choroid plexus and this suggests the use of these colloids in the treatment of hydrocephalus. On the other hand when the gold colloids are injected directly into the brain substance the material remains at the site of injection throughout its radioactive decay and provides a direct means of treatment of a variety of brain tumors.²¹

SUMMARY

In the nine years since we first introduced the use of radioactive gold colloids there has been a steadily increasing realization of their value as part of the armamentarium of the therapist. The bulk of the material being used at the present time is for palliative purposes. Nevertheless, there is apparent an awakening of their usefulness to the broader problems of treatment of the commonly encountered tumors when still in a curable state. Their continued successful application presents a challenge to the investigator as well as the therapist.

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Therapeutic Usefulness of Radioactive Colloids: Comparative Value of Gold-198, Chromic Phosphate (P^{32}), Yttrium-90 and Lutecium-177

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Within recent years there has been increasing clinical use of radioactive isotopes prepared as colloids or as suspensions of larger particles. These materials have been used in several quite unrelated ways. The material is usually injected directly into the area to be treated, and there has been little utilization of active physiological mechanisms such as determine the localization of radioiodine. However, phagocytosis by reticuloendothelial cells plays a part in the distribution of colloidal materials; the importance of this depends largely on the route of administration.

The present paper attempts to review the current status of the medical use of these radioactive colloids. Emphasis will be placed on the clinical studies performed in the United States, with illustrations chosen chiefly from the work done at the Medical Division of the Oak Ridge Institute of Nuclear Studies.

CHARACTERISTICS OF COLLOIDS

When the various elements, stable or radioactive, are prepared as colloids they lose most of their individual pharmacologic properties and take on a pattern of behavior characteristic of colloids in general. This is not a completely uniform pattern for all colloids but tends to follow certain general outlines. Variations in behavior depend more upon the particle size and other colloidal characteristics than upon the element itself. Since the colloids play little part in metabolic processes, the elements do not produce the effects that would be expected if they were administered in soluble form. This means, among other things, that toxic effects of the stable elements are at a minimum, and if they do occur they are probably due to the small amounts in true solution.

Many radioisotopes can be prepared as colloids and therefore the therapist is offered a wide choice for clinical use. He will choose the best possible one on the basis of radiation characteristics, availability, and colloidal properties. There is no reason to believe that any one colloid will be ideal for all clinical applications. Further experience is likely to show that the choice depends upon the specific use.

In trying to improve his diagnostic tests and therapeutic results with radioactive colloids, the clinician has two obvious approaches at the present time: (1) to determine the isotope with the ideal quality of radiation and half life for the particular application, and (2) to attempt to obtain a better distribution of the material in the body by varying the properties of the colloid; for example, to change the relative amounts in liver and marrow after intravenous administration by changing particle size. At present it appears that the former approach, the choice of radiation characteristics, offers promise of definite progress in the near future. The second approach is somewhat less encouraging since it has been possible to produce only limited variations in behavior of these materials (except by using very large particles which can be lodged by capillary embolism after arterial injection).

Table I gives several types of information on four colloidal materials of current importance. The subsequent discussion will in most instances be based on work with colloidal Au^{198} with some additional data on the other colloids.

The gold Au^{198} colloid is the most familiar. Its preparation presents little difficulty. Evidences of stable gold toxicity are very rare or nonexistent, although the amounts of the element used, if given in soluble form, might be expected to produce toxic effects in some patients. Because of the gamma emission, Au^{198} presents a significant radiation health hazard and various devices have been reported for handling it.¹⁻¹³

Chromic phosphate containing P^{32} has been used for some years.^{14,15,16} Its preparation presents some difficulties and the final product may be unsatisfactory because of the presence of some very large particles not in true colloidal suspension and also because of some exchange of the P^{32} with ionic phosphate. Recent renewed interest in this material is indicated in clinical reports by Jaffe¹⁷ and others.¹⁸

Our own interest in yttrium and rare earth elements has been stimulated by G. C. Kyker and associates whose fundamental work, performed as part of the same research program, has preceded our clinical trials.^{19,20,21} Other recent reports on the

* Oak Ridge Institute of Nuclear Studies.

behavior of these materials are available.^{22,23} It has been shown that when given as chlorides these elements behave as colloids in the body. An important factor in their behavior is the amount of stable carrier; carrier-free preparations show much less tendency to remain localized after intracavitary or interstitial injections than do preparations with added carrier. Animal studies indicate that toxicity of the stable elements is slight.

Earlier work with yttrium and the rare earth elements was based on the *in vitro* production of colloids. Yttrium, zirconium, columbium, and lanthanum were prepared and studied by the group at Berkeley.^{24,25} In a report chiefly concerned with bone-marrow changes, Berlin and Lawrence²⁶ mention the use of Y⁹⁰ in the treatment of leukemia.

Several colloids other than those listed in Table I have been used. One of the first was prepared from Zn⁶³ an isotope of very short half-life.²⁷ Manganese-52 has also been used²⁸ and, as compared with Au¹⁹⁸ tended to show a much lower concentration in the spleen after intravenous administration. Silver-111, an isotope difficult to obtain in adequate amounts, appears to be taken up readily by lymph nodes and may have potential value.²⁹ Hahn has studied silver-coated gold colloids in an effort to take advantage of this behavior.^{30,31,32,33} Muller has used large particles, which lodge in capillaries, particularly for treatment of lung lesions by arterial injection.³⁴ The

various clinical uses of colloids can best be considered in relation to the route by which they are introduced into the body.

When injected into tumors or normal tissues colloids tend to stay at or near the site of injection. This is presumed to be due to adherence of the substance to cells and intercellular material, as a result of reaction with protein, without the presence of any very active or dynamic physiological process. Some of the material is soon engulfed by phagocytes, but it is not known how important these cells are in determining the amount that leaves the tissue of injection and reaches lymphatic channels or the blood stream.

When these colloidal materials are injected into body cavities a large fraction is soon found to be unevenly deposited on the surfaces of the cavity (Fig. 1). As with the interstitial injection small and variable amounts reach the lymphatics and the blood stream.

Intravenous injection of colloids is followed by rapid removal from the blood stream due to phagocytosis in the reticuloendothelial system. The liver usually concentrates the greatest share of the dose; lesser amounts are found in the spleen and bone marrow with small fractions in other tissues. This same pattern of distribution is also characteristic of the fraction that reaches the blood stream after interstitial or intracavitary administration.

Table I. Four Isotopes Used as Colloids*

Isotope and half-life	Preparation as colloids	Characteristics of the colloid	Average beta energy, Mev	I γ 1 cm, r/mc-hr	Personnel and shipping hazard
Gold (Au ¹⁹⁸) 2.69 d	Gold chloride reduced with ascorbic acid in presence of gelatin (Sheppard <i>et al.</i>)	pH 3-5—large particles up to 200 m μ pH 8-10—small particles approx. 20-40 m μ Particles from 10-40 m μ depending on preparation	0.32	2.36	++++
Phosphorus (P ³²) 14.3 d	Gold foil dissolved in HCl, reduced with yellow phosphorous (Zilversmit)	Particle size depends upon method of preparation but in general particles are large	0.695	—	+
Yttrium (Y ⁹⁰) 65 h	Prepared as chromic phosphate by reaction between chromic and phosphate ions with subsequent drying, burning and grinding	Particle size depends on such factors as rate and volume of injection—biological behavior seems to suggest a relatively small particle size	0.90	—	+
Lutecium (Lu ¹⁷⁷) 6.7 d	Injected as chloride, forms colloid in body fluids	Particle size depends on such factors as rate and volume of injection—biological behavior seems to suggest a relatively small particle size	0.14†	0.42†	++

* All of these isotopes are reactor produced. In addition Y⁹⁰ may be obtained from the reaction $\text{Sr}^{90} \xrightarrow[25\gamma]{\beta} \text{Y}^{90}$.

The Sr⁹⁰ is a readily obtainable fission product.

† These values are tentative assignments.

DIRECT INJECTION OF TUMORS

Experimental and Theoretical Background

There are certain distinct theoretical advantages in delivering destructive doses of radiation to tumors by means of infiltrating them with colloidal suspension of radioisotopes. Early work on this procedure was done by Hahn and associates.^{35,36,37} Among these advantages is the possibility of delivering very high doses to the tumor with low doses to normal tissues. As compared with the use of implanted discrete sources of radium or other radioisotopes, the directly injected colloid requires no subsequent removal. Furthermore, if the injected suspension could be evenly distributed in the tumor, its many tiny point sources would utilize the beta radiations and give a more uniform gamma dosage than could be obtained with the larger encapsulated structures. If a suitable amount of the colloid were carried to lymphatics draining the lesion, this might be still another advantage.

Unfortunately, certain major difficulties have greatly interfered with the successful use of this therapy. One of these is the extreme nonuniformity of distribution of the colloidal material in the lesion, even when the injection is done with great care (Fig. 2). Another major problem is that, since the isotope has no selective affinity for neoplastic tissue, the complete injection of the tumor requires either knowledge of its exact borders or treatment of a wide surrounding area of normal tissue. The fact that the

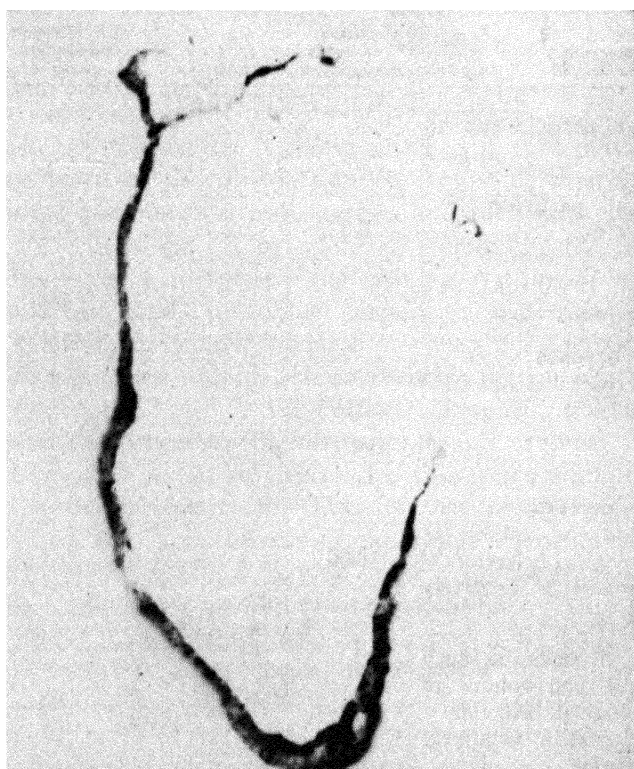


Figure 1. No. 210203. Gross autoradiogram of slice of lung 16 days after 78.4 mc of Au^{198} was injected intrapleurally. The width of the area of activity is partly explained by the fact that the pleural surface was not perpendicular to the film but was folded and flattened against film. Actual tissue penetration was less than it appears

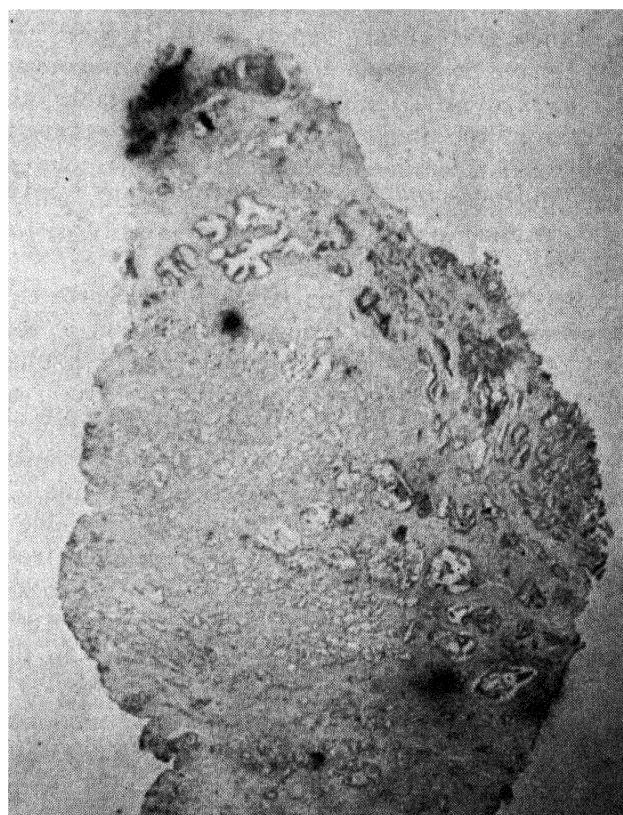


Figure 2. No. 410436. Microscopic autoradiogram of a transurethral prostatic biopsy 11 days after direct injection of 35 mc of Au^{198} through 14 needle injection sites. Note the irregular distribution of the radioactivity. $\times 20$

injection of the deep areas of a tumor is done without visualization leads to obvious limitations of usefulness in this respect. A further disadvantage of this form of therapy may be excessive transport of the isotope to distant reticuloendothelial tissues, of which the bone marrow is more important clinically. Indeed, it is difficult to appreciate that, with these handicaps, there is any real value in such therapy as compared with surgical excision. However, the fact that injection treatment minimizes shock and hemorrhage and does not leave raw neoplastic surfaces exposed gives some reason for using it in the palliative treatment of certain non-resectable lesions.

Clinical Use

Hahn reports good palliative effects in some patients with lymphoma and inoperable neoplasms treated by the direct injection of colloidal Au^{198} . One of the advantages noted was a hemostatic effect on ulcerated tumors. Certain lesions were so friable that they would not retain the isotope and so could not be treated. Dosages required for a good clinical effect were much larger than preliminary calculations would suggest in relation to dosages of externally administered radiation.

Flocks and associates have reported distinctly favorable palliative results with the use of colloidal Au^{198} injected directly into tumors of the prostate.^{38,39,40} This treatment is recommended for patients who have lesions that have extended beyond the

capsule of the prostate but have not shown evidence of distant metastases. The injections are made by means of a supra-pubic incision extended through the wall of the bladder so that needles can be directed into the lesion. A heavily shielded syringe is used and the material is injected into multiple sites in the tumor. Ordinarily the doses used vary from 1 to 3 mc per estimated cm^3 volume of tumor, the smaller dose per gram being used in the larger tumors where the larger total dose leads to a more significant effect to the tumor from the gamma radiation. Postoperatively, the bladder is drained suprapubically until the immediate reaction subsides. Shrinkage of tumor with necrosis of malignant cells and subsequent fibrotic reaction occurs in many cases along with symptomatic improvement (Fig. 3). Residual areas of tumor are sometimes treated by additional injections made through the perineum. It appears that the value of this therapy, at least as a palliative procedure, is well established.

A similar use of chronic phosphate containing P^{32} has been reported by Jaffe⁴¹ with favorable preliminary results. Yttrium-90 given as the chloride with added carrier appears suitable for consideration for intraprostatic injection, and in a single patient treated by our group the immediate response of the tumor was favorable.

Little is known about the relative merits of the different isotopes used for direct infiltration. If

therapeutic results with the P^{32} and Y^{90} preparations are equal to, or better than, those with Au^{198} , the former isotopes will have a distinct advantage. Being pure beta emitters they will offer relatively less personnel than Au^{198} . Although with proper equipment intracavitary and intravenous injections of gamma-emitting isotopes can be given with very little radiation hazard to personnel, the direct injection of tumors presents a problem in this respect, especially if considerable time is spent to achieve a meticulous distribution of the isotope.

Animal studies with Y^{90} used for direct infiltration of prostates and parametria of dogs show a very high concentration in lymph nodes.^{42,43} Lutecium-177 with its soft gamma and weak beta may have distinct advantages for interstitial injection of tumors. In one patient given 16.2 mc of Lu^{177} with 6.1 mg carrier in 5 ml volume by direct injection into a pulmonary tumor, it was found that 37.5 per cent of the dose was in the liver and 1.2 per cent in the spleen at autopsy 20 hours later. Additional preliminary data in other patients given Y^{90} and Lu^{177} also suggest that transport from the site of injection may be excessive. This is in contrast to intracavitary injections of these materials which stay well localized even if only a small amount of carrier is added. Current investigations involve consideration of the role of larger amounts of stable carrier in affecting the distribution after direct tumor injections.

There has been much confusion about the radiation dosage following interstitial injections, especially with Au^{198} . Statements about the relative beta and gamma doses determined for certain geometrical situations have sometimes been applied inappropriately to quite unrelated patterns of distribution. It is probable that the gamma dose is more important than has been appreciated. This is particularly true because of the very uneven distribution of the interstitially injected material. The clinical therapeutic result is dependent more on the dose at the points of least radiation than on the dose at the points of heaviest radiation, and the former dose may be largely from gamma rays unless the distribution is very even. It is important to consider that at focal points of high concentration of the isotope there may be levels of radiation many fold over those required for cell death. The additional superlethal spotty radiation at sites already necrotic is of no biological significance, and it is misleading to attempt to describe the situation in terms of an unrealistic average dose based on a postulated uniform distribution. This set of factors probably explains the requirement of very excessive doses of calculated beta radiation to produce clinical effects.

RADIATION OF LYMPHATICS

Experimental Background

Radioactive colloids have been used clinically as a means of radiating lymph nodes. This effect may be achieved along with the radiation of the tumor



Figure 3. No. 410487. Photomicrograph of prostatic capsule four months after direct injection of 135 mc of Au^{198} for adenocarcinoma of the prostate. Note hemosiderin in the acellular fibrous stroma. A nest of preserved cancer cells is in a perineural lymphatic space.

× 40

when lesions are treated by direct infiltration. However, lymph node irradiation may also be achieved by injecting the colloidal isotope into normal tissues drained by these nodes.

Several general points about the lymph node concentration become important:

1. What per cent of the injected dose gets into lymph nodes and what per cent stays at the site of injection? Often too much of the material stays localized and does not reach lymph nodes. If the injection site is a tumor or an organ that is subsequently to be removed, the heavy radiation at the injection site may offer no disadvantage. It appears likely that the preparation of colloids with different particle sizes and properties may help to control the amount reaching lymphatics. If Y^{90} and Lu^{177} are used the amount of carrier may influence this lymphatic distribution.

2. There is a great inequality of deposition in various lymph nodes of the same chain. With some exceptions⁴¹ studies in normal animals demonstrate this fact and data from patients support it.^{45,46,47,48,49}

3. The distribution of the isotope in a normal lymph node is non-uniform. Nevertheless, it is possible by this method to produce complete necrosis of lymph nodes (Fig. 4).

4. The distribution of the isotope in lymph nodes containing metastatic neoplasm has been the subject of much discussion, although it has generally been assumed that the area of neoplasm would fail to

contain any of the colloid, since the reticuloendothelial tissue would undoubtedly be destroyed by the tumor. Information on this problem is contributed by Mellgren *et al.*⁴⁰ Figure 5 offers confirmation in the human being. The fact that nodes completely replaced by tumor will not be able to concentrate colloids does not necessarily preclude the clinical usefulness of this type of radiation. A very early metastasis in a node might well be destroyed by cross fire or imprisoned by fibrosis. The general effects of such irradiation of nodes on subsequent spread of tumor in the area are not known

Clinical Use

The most extensive effort to make clinical use of this type of lymphatic radiation has been made by Sherman and Allen who have injected colloidal Au^{198} into the parametrial areas of women with carcinoma of the cervix.^{50,51,52,53} The isotope injection is not directed at the primary lesion and is always followed by radiation or surgical treatment of the latter. Preliminary results suggest that the parametrial injection procedure improves the outlook in patients with stage I and stage II carcinoma of the cervix

Another study has been done on the irradiation of the retroperitoneal lymph nodes by means of presacral isotope injection.⁴⁵ Also, it has been shown that in dogs the injection of colloidal Au^{198} into normal bronchial mucosa by means of a bronchoscope is followed by a high concentration in the mediastinal lymph nodes.⁵⁴ In a smaller number of patients we have attempted to duplicate this procedure, but external studies with the scintiscanner have indicated that most of the isotope remains in or near the site of injection into the bronchus. If lymph nodes were reached they were only those very close to the site of injection.

INTRACAVITARY INJECTION OF COLLOIDS

The intracavitary injection of radioactive colloids has offered a form of therapy that is receiving wide acceptance.⁵⁵⁻⁶⁴ Mechanisms of action of this procedure still present most puzzling fundamental problems. The initial use of this route of administration for practical treatment was reported by Muller.^{65,66}

Intracavitary treatment is used in at least two distinct ways: (1) as a method of control of pleural effusions and ascites in palliative treatment of patients with extensive neoplasm, and (2) as prophylaxis against the spread of malignant cells in serous cavities, especially at the time of operation.

The first of these uses seems firmly established as having definite but limited value. The second is based on animal research and theoretical considerations but has not been clearly shown to be effective in patients. One further application for the procedure, which has been discussed, is in the treatment of patients with intractable effusions of non-neoplastic origin. We have very little data on this problem. In two patients with cirrhosis of the liver given intraperitoneal colloidal Au^{198} there was no



Figure 4. Courtesy of Dr. William Christopherson. Photomicrograph of axillary lymph node from dog injected with Au^{198} into the mammary tissue. The draining lymph node has been destroyed by the radiation

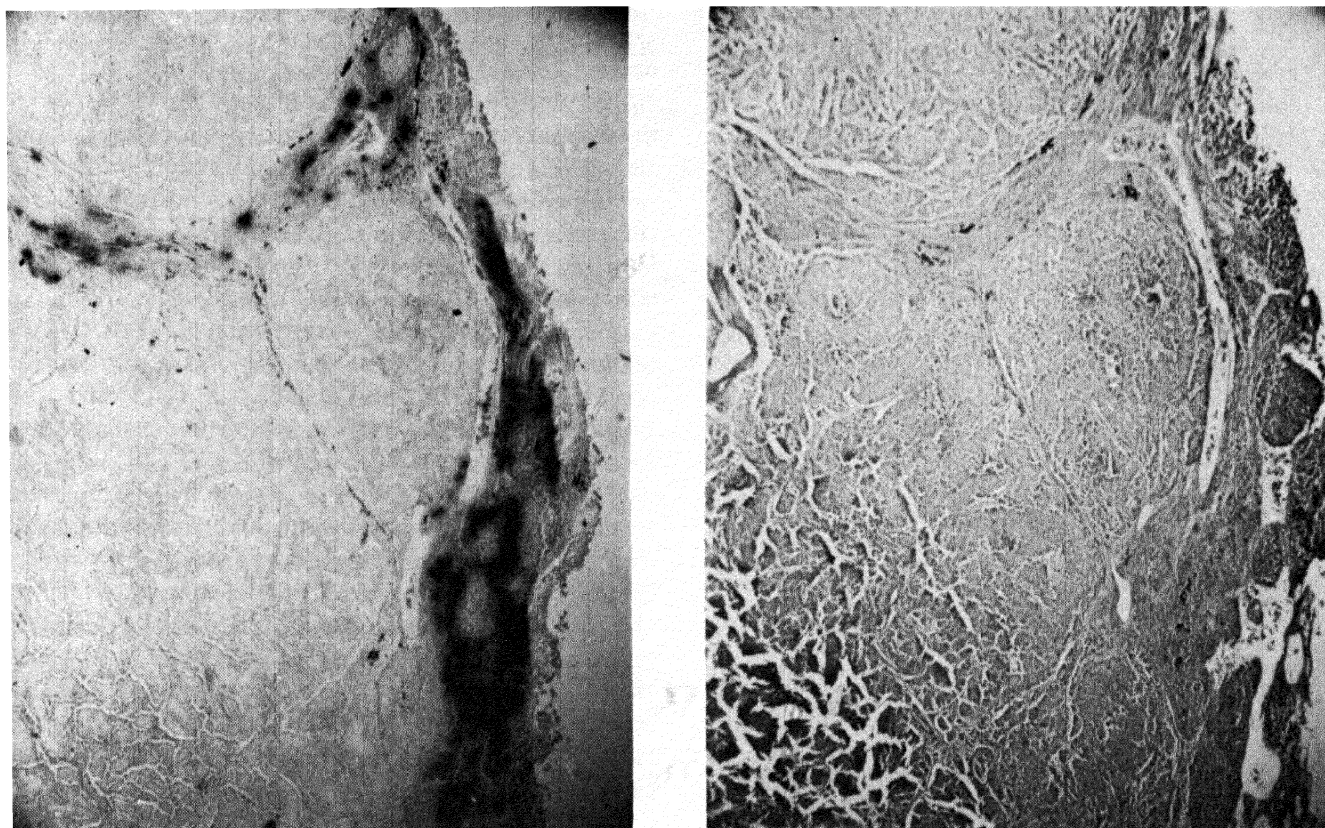


Figure 5. No. 510539. (A, at left) microscopic autoradiogram of mediastinal lymph node, and (B, at right) histologic photomicrograph. A direct injection of bronchiogenic carcinoma with 16 mc of Lu^{177}

was made 21 hours before death. Note concentration of radioactivity in the normal remnant of lymph node and failure to pick up the colloid by the tumor tissue. $\times 15$

distinct therapeutic effect. One patient died with a portal vein thrombosis a few days after the treatment and the other one continued to collect fluid with no striking change in rate.

The use of intrapericardial colloidal Au^{198} has been reported.^{50,57} A scintiscan record on such a patient from our own experience is shown in Fig. 6. The injection of this material into the subarachnoid space has also been reported.⁶⁸

Our own work has been largely on the palliative control of effusions and several aspects of this problem will be considered.

Selection of Patients

Clinical criteria are very helpful in determining which patients may be helped, while histologic type has seemed less clearly related to results. Favorable features are: (a) absence of symptoms due to neoplasm other than those related to effusion; (b) duration of effusion for several months before treatment; although this seems a paradox, it appears to relate to variations in the course of neoplastic disease with early death of patients who have rapidly progressive lesions; (c) absence of masses or lesions palpable or visible by X-rays; and, (d) absence of severe anemia and malnutrition.

Dosage

Little reliable evidence is available on this problem. Single and multiple doses have been used. Perhaps two or more doses considered as a single frac-

tionated course of treatment allow a better chance for even distribution of the isotope on the wall of the cavity than does a single dose. It appears that the pleural cavity requires less dosage for therapeutic effect, although it will tolerate at least as much as the peritoneal cavity without excessive systemic radiation reaction.

The following intracavitary doses have been tentatively suggested for single treatments or short courses of treatment: colloidal Au^{198} , 50 to 250 mc; chromic phosphate (P^{32}), 5 to 25 mc; Y^{90} chloride (with carrier 50 to 250 mg), 5 to 20 mc; Lu^{177} chloride, 50 to 100 mc.

The treatment (or brief course of treatment) can be repeated in cases where there is a poor response. We have usually waited six to eight weeks before concluding that the first treatment was ineffectual and that a second should be given, since this time may be required for improvement to become manifest. Subsequent courses may be given and very large total doses have been administered to some patients—up to 600 mc or more of Au^{198} .

Clinical Response

A favorable result may be shown by spontaneous disappearance of effusion or by failure of the fluid to reaccumulate after paracentesis. In a very small number of patients there may be an associated distinct general clinical improvement. The control of the effusion may be maintained for a few weeks or months or until the patient's death.

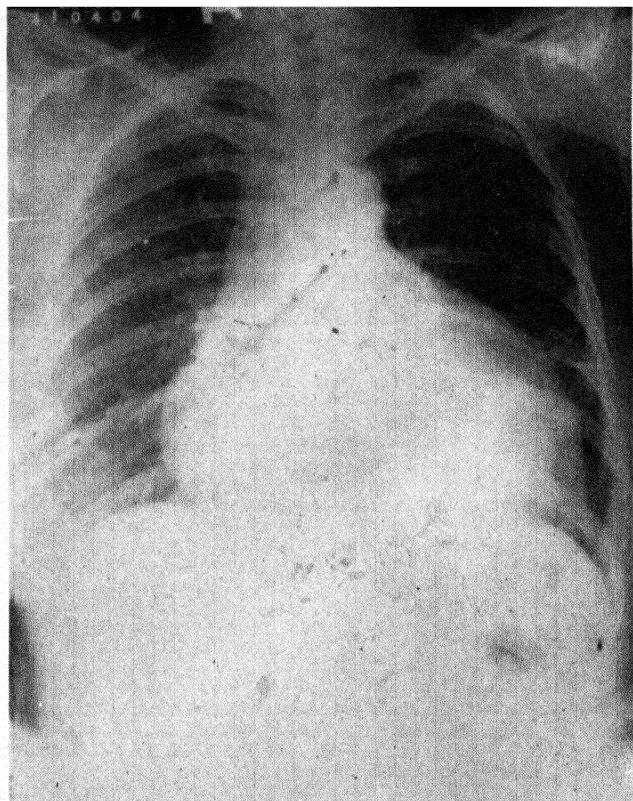


Figure 6. No. 310404. X-ray and scintiscan tracing following injection of Au^{198} . The tracing records the localization of the isotope to the pericardial space

In evaluating results one is impressed chiefly by the fact that many treated individuals die of other manifestations of neoplasm before there is any opportunity for evaluation of the effect of the isotope on the effusion. This is particularly true of patients with abdominal carcinoma who may develop neoplastic

intestinal obstruction, which is not influenced either favorably or unfavorably by the isotope therapy.

Complications of Therapy

At the time of administration there may be extravasation of the isotope into the body wall or into a viscus inadvertently punctured. This can be largely eliminated by the use of a polyethylene tube leading into the body cavity instead of injecting the material directly through a needle (Fig. 7).

Hematologic damage has been slight with intracavitary therapy and unnecessary confusion has been created by failure to emphasize the route of administration in assessing blood and bone marrow effects.⁶⁹

Nausea, vomiting, and, occasionally, diarrhea have been noted, especially following intraperitoneal administration. These have never been prolonged or serious complications of the therapy itself. It may be that damage to the mucosa of the gastrointestinal tract with associated symptoms will be more prominent with the strong beta energy of Y^{90} intraperitoneally as suggested by Muller;⁷⁰ our experience with a very small number of cases suggests that this is true.

Fate of the Isotopes

After intracavitary administration most of the colloidal material stays in or near the cavity⁷¹ (Fig. 1). It is perhaps surprising that while red cells injected intraperitoneally soon reach the circulation,⁷² the much smaller colloidal particles do not. We have been interested in studying the concentration of the colloid in the fluid of the cavity and in many cases have inserted an additional polyethylene tube from which serial samples can be drawn (Fig. 7). The concentration of the colloid is influenced, of course,

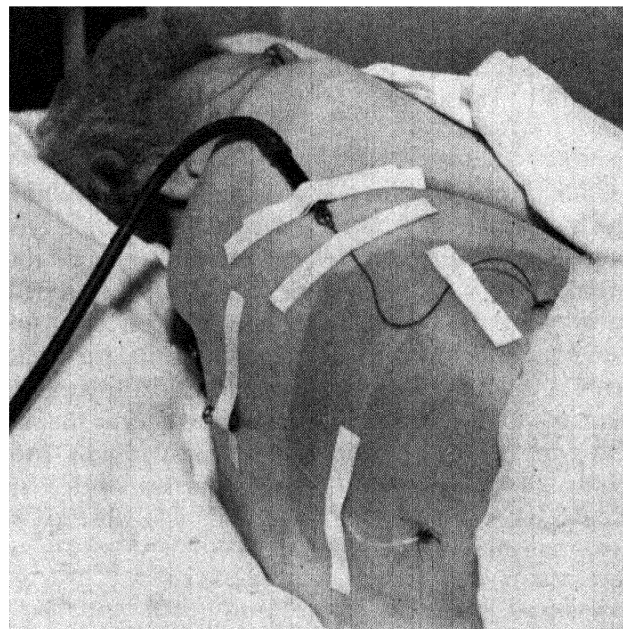


Figure 7. Photograph of patient during injection of colloidal Au^{198} into the pleural cavity. The additional polyethylene tube is for withdrawal of samples for study of pleural fluid after treatment

by rate of mixing in the cavity, removal or re-entrance of the isotope, and variations in fluid volume. The individual curves show irregular variations for a few hours presumably due to mixing. This phase usually is over in six hours and seems always complete in 24 hours. After mixing there is a rather persistent and fairly rapid decline in the concentration of the isotope in the fluid (Fig. 8). Dilution by an increasing volume of the effusion is not believed to explain most of this change. Some of the colloid in the fluid becomes associated with various types of cells and particulate matter and may be separated by gravity. This is particularly true of the chromic phosphate preparation¹⁸ (Fig. 9). Most of the decline in concentration is due to deposition of the material on the walls of the cavity. This is believed to occur by simple process of adsorption, although there is some active phagocytosis by wandering cells near the surfaces. While external radiation measurements may suggest that this distribution is fairly uniform (Fig. 10), a more discriminating method shows the pattern of deposition to be extremely uneven (Fig. 11). The reason for the pattern of deposition on the wall of the cavity is obscure. It may be related to the normal paths of egress of fluid from the cavity. Areas of tumor do not appear to have any special affinity for the colloids. Significant but variable concentrations of radioactivity are found in the lymph nodes adjoining the cavity; studies on this are fragmentary. Blood levels, while extremely low, may account for transport of significant amounts of the substance and we have estimated that as much as 15 per cent of the dose of colloidal Au^{198} may reach reticuloendothelial organs by this route (Figs 12 and 13). Since this transport occurs over a period of many days during which radioactive decay is continuing, the radiation dose to these organs is relatively less than this figure might suggest.

Pathology and Changes in Fluid

Pathologic findings show no consistent pattern.⁷³ The surfaces of treated cavities often have a bluish discoloration or are streaked with a gray-black pigment. Occasionally, a short time after treatment there are serosal hemorrhages. The degree of fibrosis that develops is variable but extensive adhesions attributable to radiation have not been seen. Radiation damage to the tumor is not prominent and frequently viable appearing cancerous tissue will be found growing on the treated serosal surfaces. In other instances there is a superficial zone of necrotic tumor. Evidence of damage to adjacent normal tissues or organs has not been seen in general, although leakage of the radioactive colloid around the site of injection can lead to local destruction of tissue.

We have studied the cellular elements in effusion after intracavitary isotope therapy.⁷⁴ No special inflammatory cell response is evoked, and few or many cells may be present. Some of them are phagocytic

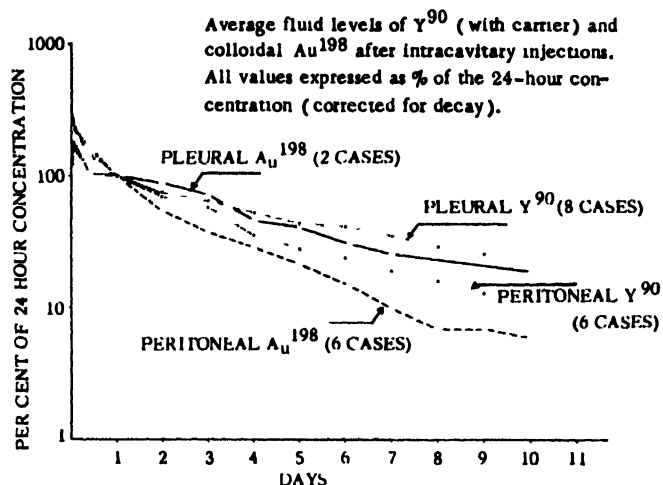


Figure 8. Mean concentration of radioactivity curves for Au^{198} and Y^{90} show a somewhat more rapid decrease of the Au^{198} in the fluid of the cavity

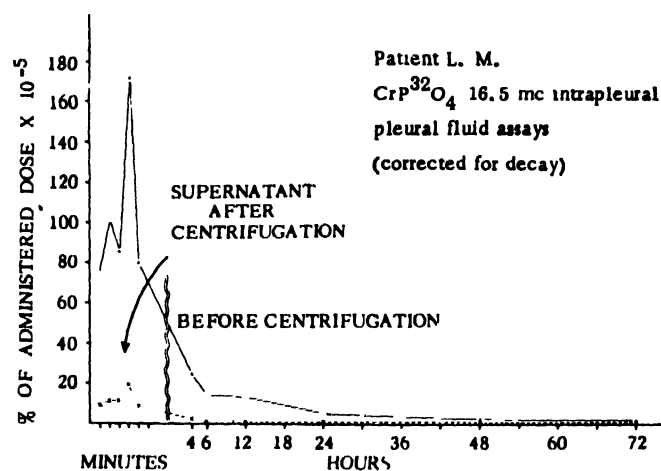


Figure 9. Concentration of radioactivity in pleural fluid containing $\text{CrP}^{32}\text{O}_4$ before and after centrifugation. Most of the radioactivity is found in the sediment.

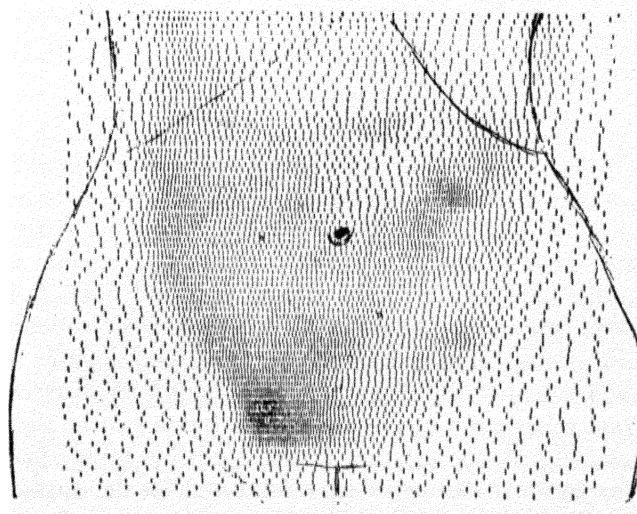


Figure 10. Scintiscan record of abdominal cavity following intraperitoneal injection of colloidal Au^{198} . Note diffuse distribution of the radioactivity

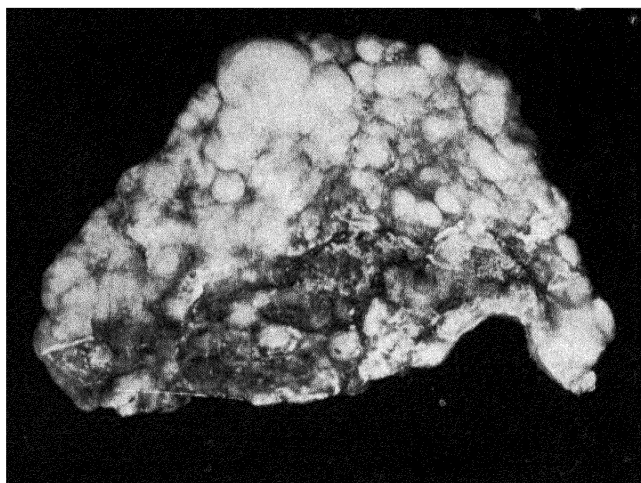


Figure 11A. Photograph of area of peritoneum with widespread carcinomatosis treated with colloidal Au^{198}

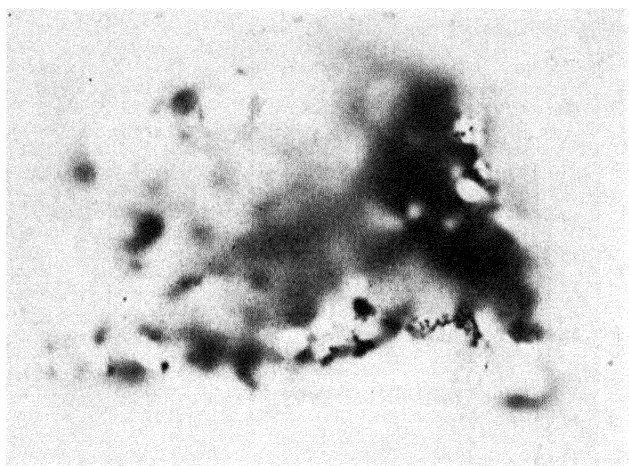


Figure 11B. Autoradiogram of surface shown in Fig. 11A. The uneven surface prevented uniform contact with the film accounting for the indistinct image. Note the uneven distribution of the Au^{198} ; there is some suggestion that the neoplastic zones hold less of the isotope than the normal

forms containing black pigment, aggregates of gold, that measure about one micron in diameter. It is noteworthy that viable-appearing malignant cells have sometimes persisted in the fluid, even in the presence of a favorable clinical response.

Mechanism of Action

In spite of the efforts expended on clarifying the behavior of these materials, there has been little advance in our knowledge of the mechanism of effect. Animal studies with ascites tumors, reported by Goldie and Hahn^{75,76,77,78} and others,⁷⁹ have indicated the effectiveness of intraperitoneal colloidal isotopes in controlling the disease, presumably by radiation destruction of the malignant cells. In contrast to this the fact that in most human cases there are solid plaques of tumor not accessible to adequate irradiation from an isotope deposited on the surface. Furthermore, histologic study of these tumors after intracavitary isotope therapy shows no convincing evidence of even superficial control of lesions, and

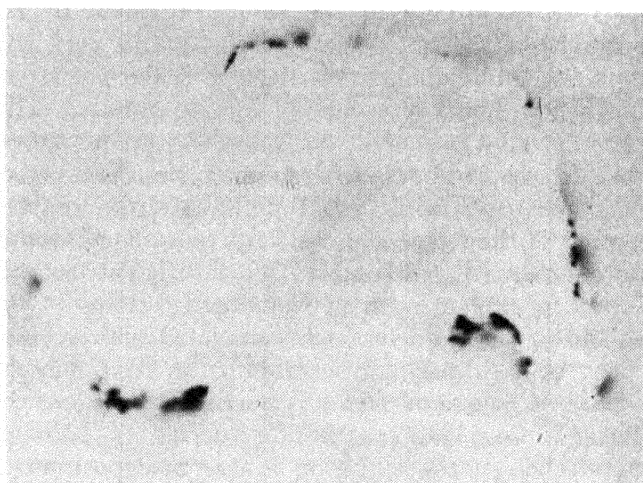


Figure 11C. Autoradiogram of the under surface of the specimen. The tissue shields the film from the superficially placed radiogold and the darkening is chiefly at the edges where some folding allows contact of the peritoneal surface with the film

cell studies of the fluids after therapy fail to show uniform evidence of damage to free cells. Recent studies of effusions^{80,81} have brought radical changes in knowledge of these fluid accumulations with the demonstration of the rapid formation and removal of the fluid and exchange of protein. Thus, it seems unlikely that the fluid can be accounted for on the basis of simple exudation from a neoplastic lesion in the wall of the cavity. These findings lead to conjectures that the colloidal isotopes may influence the rate of fluid ingress and egress in some way other than through a simple and direct effect on neoplastic cells.

Studies with Y^{90} and Lu^{177}

Preliminary clinical work with these isotopes indicates a satisfactory colloidal behavior when they are given with added carrier. The effect of added carrier is illustrated in Fig. 14. When injected into body cavities these isotopes behave much like colloidal Au^{198} but the concentrations in the fluid fall somewhat more gradually (Fig. 8). Deposition on surfaces of the cavity appears also to be much the same as with colloidal Au^{198} . Preliminary clinical studies reported by Seigel *et al.*⁸² indicate a favorable therapeutic effect on effusions from Y^{90} in a small number of cases studied. The isotope with the best radiation characteristics and half life is yet to be determined.

Distribution of Y^{90} and Lu^{177} after intracavitary administration is shown in Tables II and III. The preliminary data on distribution suggest that clinical trial will be worth while. It is expected that differences in clinical effect as compared with Au^{198} will be largely attributable to differences in radiation characteristics.

Prophylactic Intracavitary Use

Several investigators have discussed the prophylactic use of intracavitary colloids for the purpose of preventing carcinomatosis from seeding, particularly

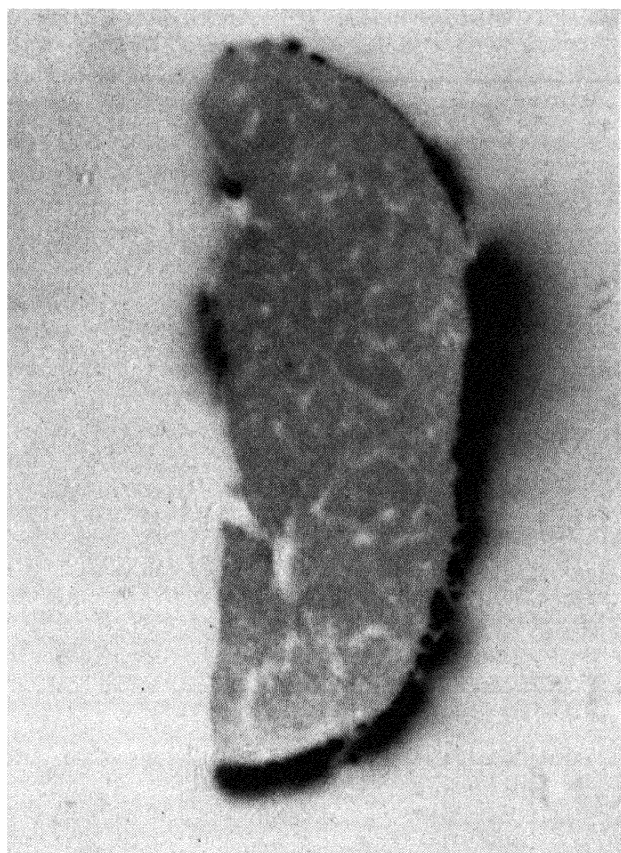


Figure 12. No. 110132. Gross autoradiogram of slice of spleen of patient 14 days after 187 mc of Au^{198} was given intraperitoneally. This shows the superficially deposited isotope on the surface of the organ and also diffusely spread blood-borne radiogold throughout the parenchyma

during laporatomies. Ruptured cystic ovarian tumors seem most suitable for this therapeutic trial. Animal studies on ascites tumors suggest that such an attempt might be worth while.^{75,76,77,78,79} One preliminary clinical report suggests a favorable effect.⁸³ The possible late hazard of radiation in patients who may already have been cured by surgery is to be considered. Our experience suggests that the isotope can be given immediately after operation without interference with normal healing. Long range, carefully controlled studies will be needed to determine whether such treatment has clinical value.

INTRAVENOUS USE OF RADIOACTIVE COLLOIDS

The intravenous injection of radioactive colloids has been the subject of some informative studies. Used in this way the substances bring into play some highly dynamic features. This has made possible some diagnostic as well as therapeutic applications.

Removal from the Blood

The rapid removal of these colloidal isotope preparations from the blood was early recorded by Shepard *et al.*⁸⁴ A large share of the material is removed in the first passage through the liver,^{85,86} variations in reticuloendothelial activity apparently being less important in the over-all removal pattern than variations in blood flow. Dobson *et al.*⁸⁷ have used chronic

phosphate (P^{32}) to measure liver blood flow, and Vetter *et al.*⁸⁸ have used colloidal Au^{198} for this purpose. It is believed that the early rapid phase of removal of particles from the blood may be most important in indicating liver blood flow. The later, more gradual removal may, in part, reflect the fact that particles of small size are not so rapidly phagocytosed. Our own autopsy studies have indicated that a significant share of the intravenous dose is removed by the bone marrow, but we do not know how much this influences the very early part of the disappearance curve and thus introduces an error into the liver blood flow estimation (Fig. 15).

Diagnosis of Liver Metastases

A quite different diagnostic application of intravenously administered colloids is the use of external counts or scanning devices to show the location and pattern of the liver and spleen after intravenous gamma-emitting colloids have been given. It is clear that the general outline of the liver can be clearly shown with the scintiscanner. On the basis of autoradiographic studies on autopsy material, from our patients with metastatic neoplasms of the liver, we have become interested in the distribution of various isotopes (Figs. 16, 17, 18, and 19) and in the possibility of attempting to delineate metastases by external counting studies which might show the area

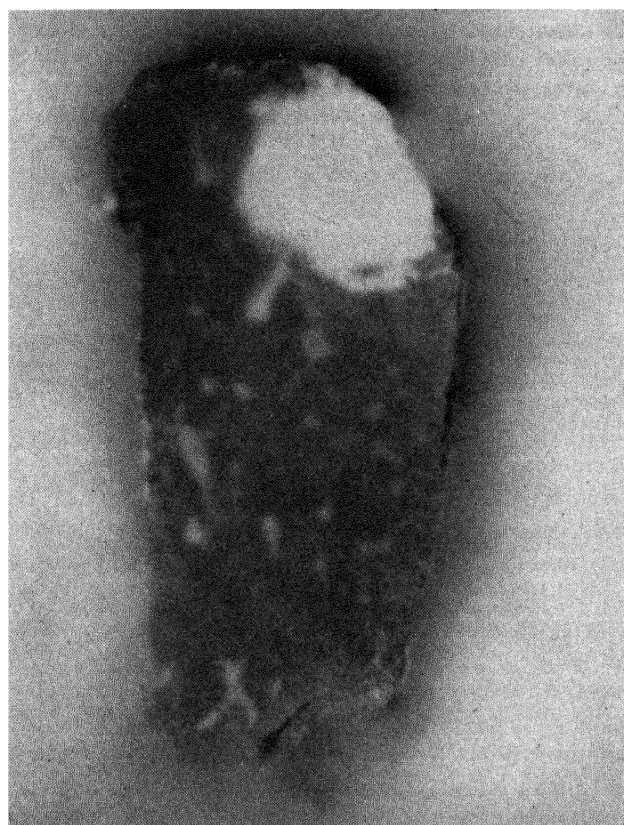


Figure 13. Gross autoradiogram from a slice of liver six days after 10 mc of Au^{198} was given intraperitoneally. There is an uneven deposition on the surface. Within the organ there is a more uniform distribution resulting from the blood-borne fraction of the radiogold deposited in the reticuloendothelial cells. The pale area in the upper part of the autoradiogram is at the site of a metastasis

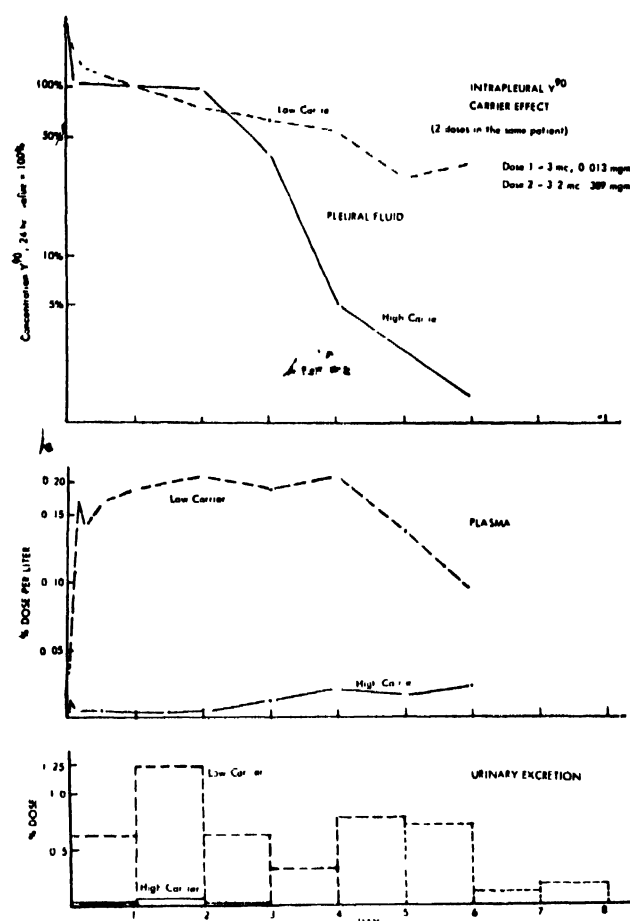


Figure 14. Carrier effect on distribution of intracavitary Y^{90} after two comparable intrapleural injections of Y^{90} in the same patient. The values have been superimposed on the same time scale. Note that increased carrier reduces blood levels and excretion

of decreased uptake in the liver (Fig. 20). Early in 1953 we attempted clinical studies with the scintiscanner. We were able to demonstrate some large metastases but could not show small ones that were not clinically suspected. Studies with the scanner, on 1 cm thick slices of liver obtained at autopsy and containing neoplasm, showed that the instrument was quite capable of delineating small metastases under these circumstances. In the patient, however, the ability to outline these lesions was poor chiefly because of the large size and irregular shape of the liver, the fact that most small metastases were partially or completely surrounded by normal liver tissue, and the interference of the respiratory movements of the patient. Much more favorable results, which we cannot duplicate, have been reported.⁸⁰ Although we believe that this procedure has limited practical value at the present time, much better results can be anticipated with the development of scanning devices having improved collimating heads and pulse height analysis to minimize the recording of scattered radiation.

Therapeutic Use of Intravenous Colloids

Since these materials concentrate in reticuloendothelial tissue (Fig. 21), the possibility of therapeutic use was early considered. As a basis for therapeutic use the effects in normal animals are

Table II. Intraperitoneal Y^{90} . Approximate Liver and Spleen Distribution

	High carrier 4 patients				Relatively low carrier 1 patient
	(E.S.)	(G.F.)	(I.M.)	(M.H.)	(M.A.)
Dose	4.9 mc	4.0 mc	11.1 mc	3.8 mc	29 mc
Carrier	70 mg	176 mg	200 mg	178 mg	3 mg
Interval	26 hr	9 d	16 d	16 d	28 hr
Liver	0.04%	1.1%	0.83%	3kgd.	0.053%
Spleen	0.006%	0.13%	0.083%	0.13%	0.0024%

Table III. Intraperitoneal Lu^{177} with Yttrium Carrier

	(E.T.)
Dose	7.7 mc
Carrier	200 mg Y 37 mg Lu
Interval	3 d
Liver	0.99%
Spleen	0.08%

of considerable importance. It appears that acute death produced by massive doses of intravenous colloidal Au^{198} is due to hematologic effects more directly than to acute liver damage. The more chronic damage produced by repeated lower doses is one of cirrhosis and this may be a useful method of producing this disorder experimentally.^{90,91}

Hahn has reported the usefulness of intravenous colloidal Au^{198} in the treatment of leukemia and lymphoma.⁹² More recently our own group has also studied the treatment of chronic granulocytic leukemia with this technique. Our own experience in a small number of carefully studied cases has led us to several preliminary conclusions.

1. The early response in untreated cases is quite good with striking improvement in red cell values. The latter may reflect a contracting blood volume due to shrinkage of liver and spleen.

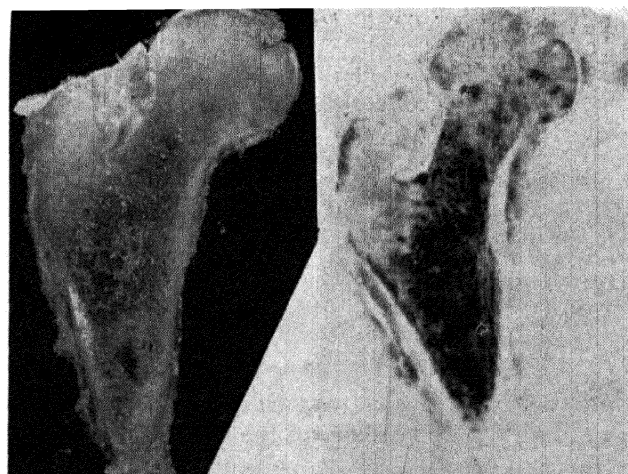
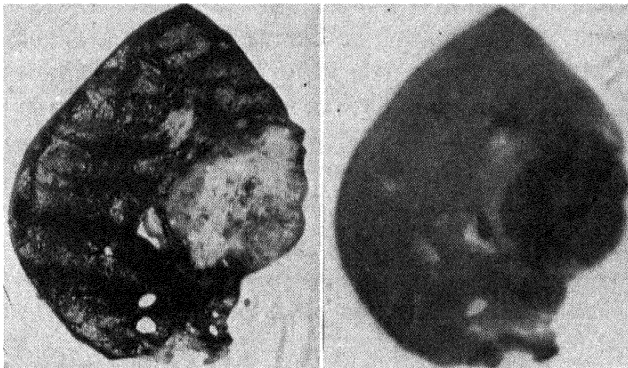


Figure 15. (left) A slice of femur, and (right) gross autoradiogram five days after 23.3 mc of Au^{198} was given intravenously. The areas of activity correspond to the sites of active cellular red marrow

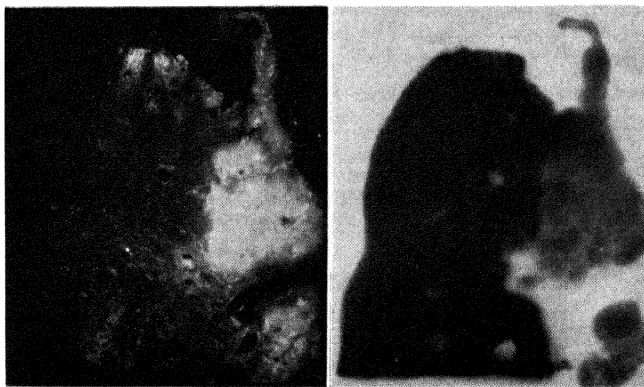
2. Liver functional tests do not show impairment and, in fact, in some cases improve after treatment.
3. Maintenance of uniform control of the hematologic status requires quite frequent treatment.
4. Late in the disease splenic enlargement may be inadequately controlled.



A

B

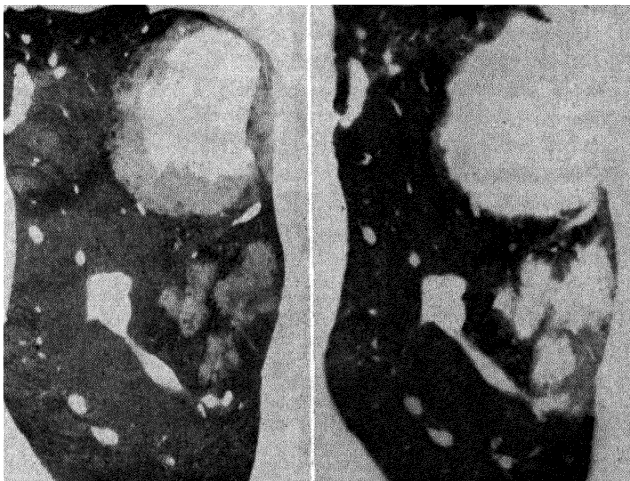
Figure 16. (A) Photograph of slice of liver, and (B) autoradiogram following a dose of P^{32} as sodium phosphate. The neoplasm has a higher concentration of P^{32} than the normal liver parenchyma



A

B

Figure 17. (A) Photograph of slice of liver, and (B) gross autoradiogram after intraperitoneal administration of colloidal $CrP^{32}O_4$. There is concentration in both liver parenchyma and neoplasm with the greater amount in the normal liver tissue



A

B

Figure 18. (A) Photograph of liver slice, and (B) gross autoradiogram after intravenous Au^{198} . The autoradiogram shows no uptake of the isotope in the areas of metastatic carcinoma

5. Acute leukemic terminations have been seen but we do not know the incidence as compared with that in other forms of therapy.

Our preliminary estimate is that this form of therapy is not significantly better than other forms of treatment for this disease. Another colloid containing Y^{90} has been used by Berlin and Lawrence²⁶ in the treatment of this disease.

Although Hahn has reported fairly satisfactory results in the treatment of lymphocytic leukemia and Hodgkin's disease with colloidal Au^{198} , we have been hesitant to use it because autopsy studies in a variety of types of patients have shown very little concentration of the colloidal isotopes in lymph nodes after intravenous injection. In one patient with an unusual lymphosarcoma, characterized chiefly by diffuse involvement of the liver, we have seen a very good response.

Attempts to treat discrete metastatic lesions of carcinoma in the liver have been given up because of failure to achieve an adequate dose in the lesions.⁹⁴

Intravenous Use of Y^{90} and Lu^{177}

These isotopes, when given as chlorides intravenously, are removed much less rapidly than is colloidal Au^{198} . The fall to 50 per cent of the initial venous level of concentration requires something in the neighborhood of 30 to 60 minutes as compared to three or four minutes for colloidal Au^{198} . This may suggest that the yttrium and lutecium isotopes are behaving as colloids of small particle size. As yet we have not shown any important effect of car-

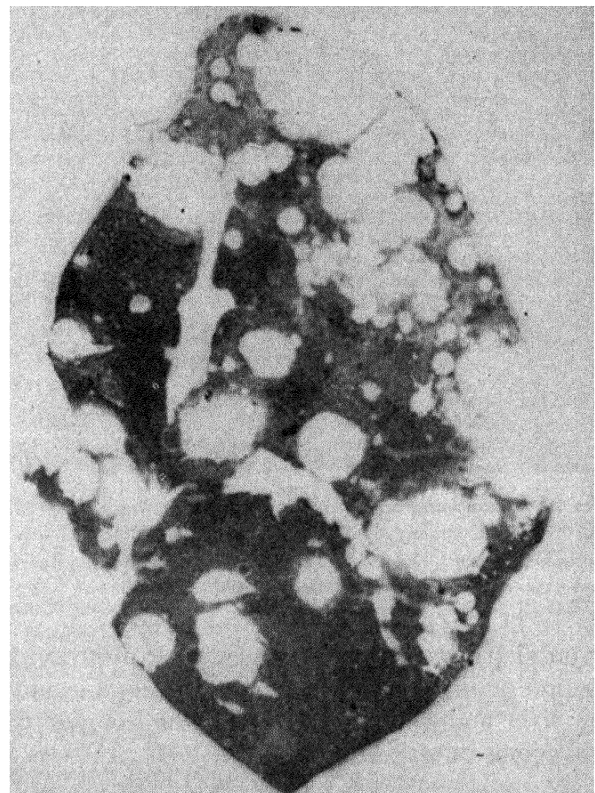


Figure 19. Gross autoradiogram of slice of liver following intravenous Lu^{177} . The autoradiogram shows no uptake of the isotope in the areas of metastatic carcinoma

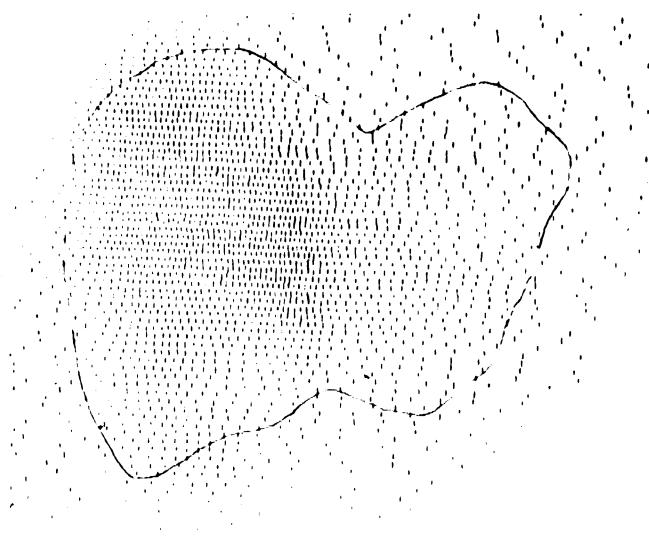


Figure 20. Scintiscan tracing of whole liver following removal at autopsy. Intravenous Au^{198} had been given in an attempt to outline during life areas of metastasis in the liver by external radiation measurements



Figure 21. Photomicrograph of liver. The patient was given 105 mc of Au^{198} (approximately 82.5 mg of stable gold) intravenously 23 days before death. Note the black particles in the Kupffer cell; these are aggregates of gold. $\times 1080$

rier on distribution such as is seen after intracavitary injection or infiltration of tumors. Tables IV and V show liver and spleen content of these isotopes after intravenous injection.

SUMMARY

The therapeutic uses of radioactive colloids are diverse and therefore generalizations concerning the

choice of material and mechanism of action should not be attempted. We have reviewed the clinical uses under four headings: interstitial injection of tumors, radiation of lymphatics, intracavitary use, and intravenous administration. Each of these appears to have limited but definite value when applied in selected situations. Further clinical investigations are needed to delineate the relative usefulness for each specific application of the various isotopes available. There is in addition reason for anticipating value of these materials in basic research on the reticuloendothelial system and in diagnostic tests.

Table IV. Intravenous Y^{90} . Approximate Distribution of Total Dose

	High carrier * 3 patients		Low carrier 1 patient	
	(W.Y.)	(R.M.)	(R.W.)	(L.A.)
Dose	4.7 mc	1.0 mc	11.2 mc	0.98 mc
Carrier	50 mg	85 mg	66 mg	0.004 mg
Interval	14 hr	6½ d	17 d	3½ d
Liver	66%	53%	78%	34%
Spleen	8%	4%	10%	3%

Table V. Intravenous Lu^{177} . Approximate Distribution of Total Dose in Two Patients

	(W.D.)	(E.L.)
Dose	3.44 mc	3.4 mc
Carrier	3.03 mg Lu	0.91 mg Lu
Interval	16½ hr	3½ hr
Liver	57.5%	20.18%
Spleen	6.63%	5.35%

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Isotope Therapy for Intra-Abdominal Tumors

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The radium volume implant technique developed by Paterson and Parker¹ produces in the treated region a relatively uniform localized field of radiation. The development and clinical trial of a flexible extension of this method forms the basis for this report.

The use of radium, cobalt, or radon implants for intra-abdominal tumors has certain inescapable disadvantages. Plans for having the isotope available must be made well in advance of its use; hence, unexpected situations usually cannot be dealt with at the primary operation. The operator is subjected to a substantial radiation dosage in the course of making the implantation and closing the abdomen. It is necessary to remove the implant, at the termination of treatment (except for radon seeds), and the possibility of loss and the consequent dangerous search must be considered. Postoperative complications such as wound disruptions, which require surgical intervention, can lead to very uncomfortable situations for the surgeon.

In order to circumvent these difficulties, the following technique was devised. Very fine polyethylene tubing (Clay Adams PE-10, outer diameter 0.61 mm, inner diameter, 0.28 mm), which is kept sterile and available at all times in the operating room, is threaded around and through the tumor, following as closely as possible the Paterson-Parker radium rules. Three-quarters of the tubing is evenly distributed on the periphery of the tumor and one-fourth is placed evenly through the volume. The tubing is spaced about 1 cm between turns, and careful measurements are made of the length of tubing comprising the implant and of the ends leading from it. The ends of tubing are brought out through the wound and left long, one end being longer than the length of tubing in the implant. During this process the tumor is disturbed and handled as little as possible. The tubing may be filled with mercury, and the distribution checked by X-ray before closing the abdomen. This permits careful, painstaking placement of the implant without exposing the surgeon to radiation. Nearby vulnerable organs may be displaced surgically away from the implant to whatever extent is feasible.

After the patient has recovered sufficiently from the operation, roentgenograms of the implant with

mercury in the tubing are made, and from these, the volume of tissue to be irradiated is calculated.

I^{131} is used as a source of gamma rays. The tubing wall filters out a portion of the beta radiation, and the tissue immediately adjacent to the tubing filters out the rest. This tissue is exposed in any case to a very intense gamma-ray field so that little additional damage is done by the beta radiation. Radioactive iodine was chosen because it is cheap, readily available, and has suitable chemical properties for concentration to the necessary small volume, and because the short half-life makes removal of the isotope unnecessary.

The air dose rate at 1 cm from a 1-mc point source of I^{131} is 2.28 r per hr $\ddagger$ as compared with 8.3 r per hr for 1 mg of radium shielded by 0.5 mm of Pt. Thus 3.65 mc-hours of I^{131} correspond to 1 mg-hr of radium. The linear absorption coefficients in tissue for radium and I^{131} gamma rays are both about 3 per cent per cm³ so that this same proportion should be valid at various distances from the sources in tissue. On this assumption, 1 mc of I^{131} , allowed to decay completely, gives the same tissue dose as 76 mg-hr of radium. Using this factor, the Paterson-Parker radium tables may be used directly to calculate the number of mc that are needed to produce the desired total dose of radiation.

A quantity of I^{131} that is about 20 per cent in excess of the required amount (usually 5 to 10 cm³ of solution) is placed in a conical centrifuge tube. One or 2 mg of KI are added as carrier, and the iodine is precipitated as AgI with a 50 per cent excess of AgNO₃. The solution is acidified with HNO₃, digested at 70° for an hour to flocculate the precipitate, and then centrifuged. The supernatant is decanted, and the precipitated AgI is dried with a stream of air in the dark at room temperature. The supernatant is surveyed to check the completeness of the precipitation, which is usually about 99 per cent. The centrifuge tube containing the isotope is handled at all times in a lead-shielded container to minimize exposure. The total-body exposure to the operator, determined by a pocket dosimeter, during the processing of 200 mc of I^{131} , without taking extraordinary precautions, was 7 mr.

The silver iodide is prepared for placement in the implant by dissolving it in a sufficient volume of

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$\ddagger$ Calculated from the data of Bell and Graham,² using the method of Marinelli *et al.*³

saturated KI to give the correct number of mc per cm of tubing. This is accomplished with sufficient accuracy by filling an appropriate length of the polyethylene tubing with saturated KI and discharging it onto the AgI precipitate, which dissolves rapidly.

The isotope is then brought to the patient's bedside. The tubing in the patient is cleaned with distilled water and dried with alcohol, ether, and air and filled with clean dry mercury. Particular care is taken to displace all air bubbles.

The tubing is marked as shown in Fig. 1, $AB = CD$, $DE = FG$. The distance DE is measured at the time of surgery, and CD is the difference of AD measured at surgery and AC . The long end of the tubing, on which the distance FG is marked, is placed in the centrifuge tube containing the isotope solution, which is drawn up by suction into the tubing until the solution reaches point F . The end of the tubing is then withdrawn from the centrifuge tube, and the isotope is drawn into the implant until the head of the column of isotope solution appears at point C beyond the tumor. The syringe is then removed from the tubing and the mercury is allowed to drop back to point B , forcing the isotope solution back to point D . When this procedure is carried out properly, the isotope is in the tubing within the tumor while the two leads going down to the implant contain air or mercury. The ends of the tubing are sealed with heat and pressure and left long. This entire procedure usually takes about a minute, and exposure to the operator is of the order of 10 mr.

If it is necessary to re-explore the patient, the isotope may be removed and later replaced without difficulty. Similarly, the isotope can be removed prior to autopsy.

The tubing is left in place after the decay of the isotope. The ends are cut short, sealed, and allowed to retract beneath the skin, where they cause no difficulty.

Several lines of investigation are in progress in connection with this method. First, an effort is being made to check independently the calculated radiation dosage. Measurements with Sievert chambers

in a phantom indicated approximate agreement between the measured and calculated dose. However, since the Sievert chamber is energy sensitive, the calibration for the I^{131} gamma rays was in some doubt.

Measurements have been made in a water phantom with X-ray film that had been calibrated against a standard Victoreen chamber. Preliminary results indicate that the measured dose is somewhat lower than the calculated dose.

The treatment of carcinoma of the pancreas has been undertaken as a clinical experiment using this method. Radical surgical treatment of this disease has proved to be ineffective, and the palliative and possible curative effects of properly applied local irradiation are being explored. The procedure has been attempted in 7 cases, successfully in 5. The following are case abstracts in chronological order:

1. A.S. NUMBER 57-76-16

This 56-year-old man had a 150-gm tumor of the body of the pancreas which was first discovered at operation. This was wrapped with tubing as described above. In the process, the hepatic artery was perforated twice. The tubing was withdrawn and bleeding ceased promptly and there were no apparent ill effects. A cholecystogastrostomy was performed. The patient at first did well after the operation, but soon became severely depressed, refused to eat, and finally died of inanition 3 weeks after the operation. No autopsy was obtained. No isotope had been placed in the tubing.

2. L.M. NUMBER 58-65-08

This 55-year-old woman had a mass in the pancreas that was discovered in May, 1953, during cholecystectomy. She was re-explored at the Mayo Clinic in August, 1953, for continued back and epigastric pain, and the tumor was found to have extended around the root of the mesentery. She was again re-explored at this hospital on November 5, 1953. At operation, a large tumor, weighing about 135 gm, in the head and body of the pancreas was wrapped with tubing. It was impossible to place the core of the implant symmetrically because the superior mesenteric artery and portal vein traversed the implant. The common duct was transplanted to the greater curvature of the duodenum, and the pylorus was transected and an anterior gastroenterostomy was performed. These procedures removed the bile duct and stomach from the field of irradiation. One week after operation, 135 mc of I^{131} were placed in the tubing. This gave a calculated dose of 5000 r to the lowest point in the implant. The patient tolerated this well, without evidence of radiation sickness. Her appetite remained good. Four weeks after isotope administration she developed a severe anemia (hemoglobin of 80 gm per cent) and leucopenia (white count, 1900). This responded during the next 3 months to repeated transfusions and supportive therapy. The patient's epigastric pain and back pain were much improved so that she was able

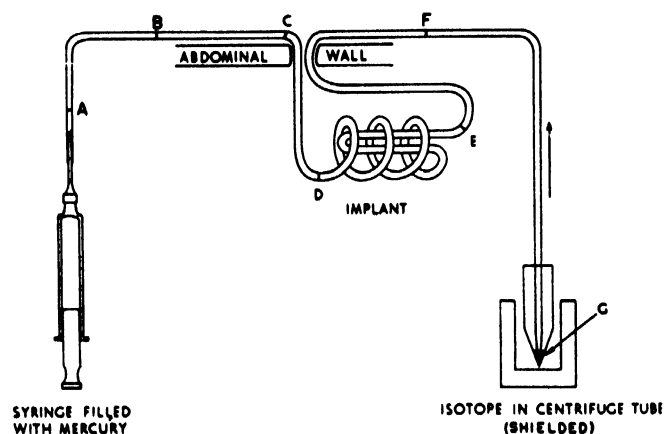


Figure 1. Apparatus for placing isotope in implant. (By courtesy of W. B. Saunders Company)

to discontinue completely the use of narcotics. Her tumor mass decreased slightly in size, and the skin over it became tanned from radiation coming from the implant. Six months after treatment she began to fail, then numerous abdominal masses became palpable, and the patient became severely jaundiced and died on June 8, 1954. No autopsy was obtained.

3. M.S. NUMBER 58-90-08

This 66-year-old woman had a history of back pain, weight loss, and anorexia for 9 months and had a moderate obstructive jaundice for 2 weeks. She was explored on December 5, 1953, and a small tumor (15 gm) of the pancreas or distal common duct was wrapped with tubing. The common duct was transplanted to the greater curvature of the duodenum to relieve the obstruction. On December 10, 10.6 mc of radioactive iodine were placed in the tubing, and 5 days later, this was removed and replaced with 21.3 mc which gave a calculated total radiation dose of 9200 r. The patient's jaundice disappeared but she continued to have some pains in her back. She did fairly well for 3 months. There was no apparent adverse effect from the radiation. She was hospitalized again in January 9, 1954, when she complained of nausea and vomiting. Roentgenograms of the duodenum adjacent to the implant did not reveal any abnormal conditions. Further studies revealed a marked uremia. The patient expired on March 23, 1954. At autopsy, the cause of death was attributed to chronic pyelonephritis. Grossly, there was not much evidence of tumor, but microscopic studies revealed that tumor was infiltrating the peripancreatic tissues. In the region of the implant, the tumor showed marked regression. There was some evidence of fibrosis in the duodenum adjacent to the implant.

4. H.T. NUMBER 59-30-17

This 42-year-old man gave a 5-month history of abdominal and back pain and a 50-pound weight loss. The character of his stools had changed to an oily amber liquid that frequently floated on top of the water. Physical examination was not remarkable. Laboratory data showed 1+ reduction in the urine, and the glucose tolerance curve was markedly diabetic. Roentgenograms were negative. On February 1, 1954, an exploratory laparotomy was done at which time a carcinoma was found that involved almost the entire pancreas and the surrounding tissue. There was some local lymph node involvement and a few small metastases to the under surface of the liver. A gastrojejunostomy, cholecystoduodenostomy, and splanchnectomy were done, and the tumor was implanted with polyethylene tubing. The patient made an uneventful recovery. On February 11, 1954, 176 mc of iodine were placed in the tubing. The radiation dose in the implant was difficult to estimate because of the peculiar elongated shape of the tumor. The volume was estimated at about

150 cm³, and the radiation dose at about 8600 r. The patient was discharged on February 19, 1954, and returned to work 3 weeks later. His back pain was nearly gone, and his only complaints were persistent diarrhea and inability to gain weight. Four weeks after the isotope administration he developed marked leucopenia and anemia, which required no particular treatment and regressed spontaneously. He continued to do well until about May 1, 1954, when he was readmitted for splanchnic blocks because of the recurrence of pain. At this time, a hard mass was palpable in the epigastrium. Splanchnic block gave some relief from pain. The patient expired suddenly on June 19, 1954. An autopsy revealed complete destruction of the pancreas in the region of the implant, without significant damage to any nearby structures. The portal vein was patent. The tumor, however, had spread extensively to the liver and to the gastrocolic omentum. The cause of death was apparently biochemical.

5. R.H. NUMBER 59-93-87

This 50-year-old man had a carcinoma of the head of the pancreas that was discovered at laparotomy. The pancreas at this time was extensively mobilized, and cholecystjejunostomy was performed. Four weeks later, he was re-explored at this hospital. The operation presented extreme technical difficulty because of the extensive fresh adhesions from the previous surgery. While the pancreas was being mobilized for the second time, the portal vein was torn, and the resulting hemorrhage could not be controlled. The patient expired and no autopsy was obtained.

6. J.B. NUMBER 60-10-62

This 59-year-old man complained of severe burning abdominal pain for 1 year and lost 25 pounds in the 6 months prior to admission. He also had recently developed a steatorrhea, and in the course of his work-up, he was found to be diabetic. Physical examination was otherwise negative, and roentgenograms revealed a pyloric lesion of undetermined nature. On May 21, 1954, the patient was explored, and a large tumor mass of about 120 gm was found in the head of the pancreas. Anterior gastrojejunostomy following transection of the pylorus, cholechojejunostomy, and cholecystectomy were performed, and the tumor was implanted with polyethylene tubing. Following this operation the patient became jaundiced and was re-explored 1 week later, at which time an obstruction of the cholechojejunostomy was relieved, and a transjejunal catheter was left in the common duct. The patient made a good recovery from the second operation except for a slight wound dehiscence, and on June 2, 1954, 97.3 mc of iodine were placed in the implant giving a total dose of 8900 r. The core of this implant was displaced somewhat by the portal vein and superior mesenteric vessel (Fig. 2). The patient got along well

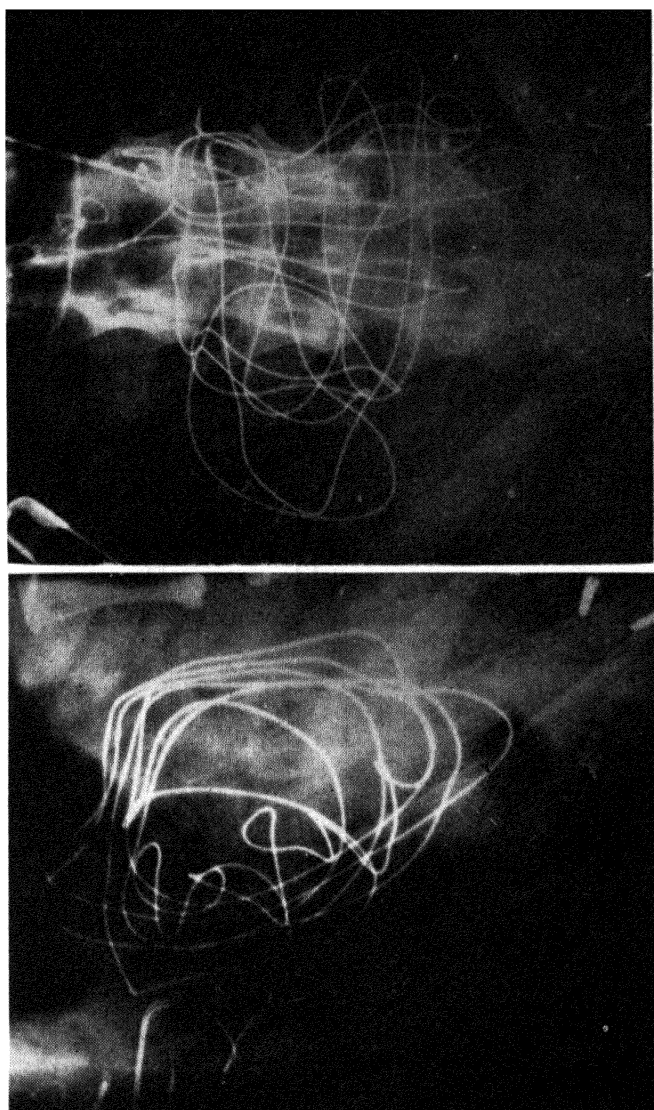


Figure 2. A-P (Above) and later (Below) views of the typical implant that was placed in patient J. B. The volume is 121 cm³ and 97.3 mc of I¹³¹ gave a calculated dose of 8900 r. The core of this implant is displaced somewhat by the portal vein and superior mesenteric vessels

and did not show evidence of radiation sickness. He developed a leucopenia to the extent of 1900 cells per mm³ 4 weeks following the administration of the isotope but recovered spontaneously.

Following removal of the common duct catheter 1 month after operation, a persistent jejunal fistula remained, which subsequently communicated with the opening in the abdominal wound. The patient had several febrile episodes that responded to Gantrisin and were interpreted as being caused by cholangitis. He gradually became weaker and became jaundiced on August 14, 1954, and died on August 16, 1954. Throughout his postoperative course he was free from pain. Autopsy revealed marked regression of the tumor with no evidence of further spread or radiation damage to other organs. The fistula from the jejunum communicated with the implant which was infected.

The cause of death was a septic thrombophlebitis of the portal vein.

7. T.D. NUMBER 60-43-91

This 49-year-old woman complained of weakness for 3 months, a 20-pound-weight loss in the previous 2 months, anorexia for 6 weeks, and increasing jaundice for 6 weeks. Physical examination was negative except for jaundice, and laboratory examination revealed an obstructive type of jaundice. On July 2, 1954, the patient was explored and a carcinoma of the distal common duct was found to be causing the obstruction. A gastrojejunostomy and cholecystjejunostomy were performed, and the tumor mass was implanted with polyethylene tubing. The patient made a rapid recovery, and on July 8, 1954, 28.5 mc of iodine were placed in the tubing. She has had no adverse effect from the radiation, continues to do well, and has been gaining weight. The total dose delivered to the tumor was 7400 r.

CONCLUSIONS

It appears that radiation may be delivered to intra-abdominal tumors in amounts that if delivered externally would be intolerable to the patient. This is probably because of the relatively small volume of tissue irradiated, and because it is possible to move vulnerable organs away from the field of radiation. In all the cases in which pain was a problem, some relief was achieved. Cases 3 and 6 were done unexpectedly without prior planning. The great need for being able to do the implantation at the primary exploration is illustrated by case 4. It seems feasible at the present time to attempt to deliver cancericidal doses to early small carcinomas of the pancreas since the results from such treatment can certainly be no worse than those from radical surgery. Small implants of dose levels ranging from 20,000 to 40,000 r are being made in experimental animals to investigate the dangers and limitations of the method's application.

The use of isotopes other than I¹³¹ is also being explored and will form the basis for later reports.

SUMMARY

An extension of the conventional radium implant technique is presented that permits much more flexible application of the method and the use of various artificial radioactive isotopes. Clinical experiments using this method are yielding encouraging results.

ACKNOWLEDGEMENT

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Radioactive Isotopes in Hematologic Therapy

By John H. Lawrence,* USA

In view of our lack of understanding of the true nature of many hyperplastic and neoplastic conditions involving the hematopoietic system, and also our relatively ineffective treatment of these conditions, it seemed important to the author in 1935, when artificially radioactive elements first became available from the 37-inch cyclotron in Berkeley, to investigate the potential role of these new tools in the treatment of several syndromes of the hematopoietic system. Success would depend on the ability to localize radiation-emitting isotopes in the hyperplastic or neoplastic tissues. The present article is a summary of our experiences in this direction and the results achieved after twenty years. The first section will be devoted to the isotopes with which we have had extensive experience over a period of many years, and which have been used in the treatment of a sufficiently large number of patients, so that it is possible to discuss the findings with some confidence. The second section will concern isotopes or compounds of more recent or less extensive use. Their place in therapy cannot yet be so accurately assessed.†

One of the earliest available radioisotopes was radiophosphorus or P^{32} . It was predicted and found quite early in animal experiments¹⁻⁵ that P^{32} injected or fed would be metabolized and remain for a period of time in relatively high concentration in rapidly metabolizing or growing tissue such as, bone marrow, liver, and neoplastic tissue, and also that it would eventually concentrate in the bone. With the beta rays emitted one could obtain five or more times greater irradiation to some tissues than to others with safety to the patient. Because of the relatively great penetration of the P^{32} beta rays (8 mm in tissue), it also was clear to the author in those early days that normal cells would always receive some irradiation from P^{32} and beta irradiation from P^{32} could not be used in sufficient quantities to destroy neoplastic or leukemic cells. Consequently,

in the therapy of diseases of the hematopoietic system, isotope radiations have been used for inhibition of growth rather than complete destruction of the neoplastic tissue.

MULTIPLE MYELOMA

Since X-ray is believed to prolong life in some cases of multiple myeloma and since no form of therapy, including X-ray, has yet provided a satisfactory answer to this disease, it seemed worth while to learn if better therapeutic results could be obtained using internal radiation from radioisotopes known to localize in bone. The most intensive study was given radioactive phosphorus, since it seemed best suited for this purpose, but radioactive strontium and radiocolloids of yttrium and chromic phosphate also show a high uptake in the reticuloendothelial tissues, especially bone marrow, and these have been tried too.^{6,7,8}

A series of 24 cases was studied⁹ of whom 15 had received prior X-ray therapy. Eleven cases were treated with P^{32} alone, nine received combined P^{32} and Sr^{90} , and one received colloidal Y^{90} only. One patient had associated polycythemia and diabetes mellitus and had not yet undergone therapy when these data were analyzed; one patient was terminal and died before treatment could be instituted; and one patient had a severe thrombocytopenia and anemia which precluded any form of radiation therapy.

The dosage was individualized according to the general status of each patient. In general, however, if red cell, white cell, and platelet values were satisfactory, 1 millicurie was given once or twice a week over a period of four to six weeks, for a total course of 5–10 mc. Three to four months after the last dose had been given, another course was instituted when necessary if the hemogram remained at normal levels. No benefit was observed in seven cases treated with P^{32} alone or in combination with Sr^{90} or in the case treated with colloidal Y^{90} . Eight patients reported subjective improvement, but the progress of the disease did not seem markedly influenced. Five patients showed a good response either on the basis of a dramatic relief from pain or significant extension of life. For 21 deceased patients who had received isotope therapy, the duration of disease from onset to death ranged from six months to nine years, with an average of approximately three years. This is slightly longer than observed in series treated by

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† The present report does not give a complete review of the literature on radioactive isotopes in hematologic therapy. This has been covered in other publications by the authors, cited in the bibliography. The studies on leukemia and polycythemia in the present paper have been reported previously in great detail; whereas, the material on Hodgkin's disease, lymphoblastoma and radiocolloids is presented here for the first time. All of the work reported in this paper was started in 1936 and has been carried on continuously since that time.

others with X-ray or stilbamidine. There was no evidence that a combination of Sr^{89} with P^{32} was superior to P^{32} alone.

It is difficult to evaluate these results since the disease is rare, and it is difficult to find comparable data. The rate of progression of the disease and the radiosensitivity of the myelomatous tissue are variable. As would be expected, favorable results are often observed in cases of solitary myeloma, and some observers believe the duration of life would approximate seven years in patients with solitary myeloma adequately treated with local X-ray.¹⁰ In a review of 83 cases treated by various methods, the average duration from onset in multiple myeloma was found to be 19 months.¹¹ Some workers have reported good results following treatment with urethane and with stilbamidine, but prolongation of life has not been demonstrated. Tracer studies of C^{14} -labelled stilbamidine have shown uptake by myelomatous tissue, but its localization in the liver is proportionately much greater so there can be no therapeutic application of this compound as a source of radiation.¹²

It seems likely that radiosensitive cases may be influenced equally well by X-ray or radioisotopes. For solitary myeloma one might consider local X-ray superior since a larger dose could be given the one area with less effect on the total hematopoietic tissue. For widespread disease, one might consider P^{32} to have advantages over X-ray in that administration of the radiation is easier and there is no associated radiation sickness. Since striking improvement is sometimes seen, P^{32} deserves a place in therapy of this disease, and it is our experience that a combination of these two forms of radiation therapy, perhaps with the chemotherapeutic agents as well, may be necessary to achieve the optimum results in an individual case.

LYMPHOBLASTOMAS

While there have been a few cases of dramatic improvement in cases of lymphoblastomas following radiophosphorus therapy, local X-ray to tumor masses continues to be the best therapeutic approach especially when the involvement is relatively localized. P^{32} has been shown to effect palliation of symptoms in widespread disease, but it is evident that over-irradiation of the marrow must be carefully avoided.

Approximately 30 cases of Hodgkin's disease have been referred to us for radiophosphorus therapy, in most cases after X-ray had become less effective in controlling the disease. Some degree of favorable response was observed in about one third of these patients, and two of the patients are still living more than 15 to 20 years from onset. But the latter two received combined X-ray and P^{32} therapy, and both had the advantage of diagnosis and treatment early in the course of the disease.

The most favorable results in this group of dis-

eases have been observed in giant follicle lymphoblastoma. A series of 14 patients receiving isotope therapy was studied, and follow up data maintained to death of all patients. The average length of life was 6.3 years and the maximum was 11 years. This is slightly longer than that reported for other series.²⁰ Pertinent data are presented in Table I. It will be noted that nine of the 14 showed some beneficial response following P^{32} therapy, three of the 5 showing no response were moribund at the initial observation. Anemia was a frequent complication, but this was usually due to the disease. The most dramatic improvement occurred in a 66-year-old woman (A.P.) who had had the disease for only six months and had received no previous radiation therapy. She was given relatively small doses of P^{32} , about 1 mc twice a week for a total of 14 mc in the first course. The enlarged lymph nodes regressed, the abdomen which had been distended with masses and ascites became normal, and skin infiltrations disappeared. Recurrences were treated and each time a remission was induced. Four years after initiation of therapy the patient died of a myocardial infarction. Post-mortem examination of the tissues revealed no evidence of lymphosarcomatous disease. Another patient (J.H.) with marked bone-marrow involvement was given a small dose of P^{32} (3 mc) resulting in a long-term remission without other therapy. Immediately after receiving P^{32} he felt much improved.

These instances will emphasize the fact that P^{32} is probably the treatment of choice in selected cases of giant follicle lymphoblastoma, often of course, combined with roentgen irradiation to local areas.

A total of 14 cases of generalized lymphosarcoma were treated with P^{32} . Ten patients were males, 4 females; the ages varied from ten to seventy-two years. When first seen by us, the average duration of the disease in these patients was slightly over one year. Three cases had far advanced disease. Eleven had received previous roentgen therapy; 1 of these cases with skin involvement had also been given Fowler's solution.

P^{32} was administered in total amounts ranging from 5 to 40 mc, the average being about 16 mc per patient. The usual dose was 1 to 2 mc one to three times weekly. The shortest treatment period was two weeks, during which 5 mc was given; the largest amounts of P^{32} were 40 mc administered in a nineteen month period and 30 mc given over a two year period.

The usual clinical criteria such as diminution in size of tumor masses and subjective improvement were used in evaluating response. Definite favorable responses were obtained in 5 patients, 2 other patients showed questionable signs of improvement, and the others showed no evidence of a beneficial response. It may be noted here that the 3 cases showing the greatest degree of improvement were the only patients in our series who had not received previous roentgen therapy. The duration of disease in each

Table I. Giant Follicle Lymphoblastoma

Patient	Sex	Age at onset	History	Initial findings here	P32 dosage	Duration from onset to death	Remarks
A.B.	M	32	1932 Enlargement of cervical nodes; X-ray to 1939 when splenomegaly and generalized lymphadenopathy was observed; biopsy showed giant follicle lymphoblastoma.	1940 Extreme splenomegaly; generalized lymphadenopathy; anemia.	5 mc (9/40)	8 years	No effect from P32. Patient was moribund when first observed by us, dying six days later.
S.B.	F	56	1945-1946 Enlargement of cervical node; 3/46 axillary node also enlarged; X-ray given; 4/46 biopsy showed giant follicle lymphoblastoma.	5/45 Slight enlargement of liver and spleen; enlarged axillary nodes.	5 mc (1/54)	8 years	No effect from P32. The patient had a remission until 1951, when she was treated with X-ray with good response. Patient was moribund when first dose of P32 was given.
F.B.	M	61	1944 Enlargement of nodes in neck, groin, and axilla. Biopsy showed giant follicle lymphoblastoma. Local X-ray given. 1947 abdominal mass observed.	1948 Slight splenomegaly; small, discrete nodes palpable in neck and axilla. Slight anemia. X-ray examination showed fluid in chest.	6 mc (5-6/48)	4 years	No effect from P32. Patient was moribund at first observation here.
R.B.	M	55	1942 Sciatic pain, weight loss; fatigability. Enlarged nodes in axilla & abdominal mass. Biopsy showed giant follicle lymphoblastoma. No treatment.	5/43 Splenomegaly and hepatomegaly; generalized lymphadenopathy.	10.5 mc (5-7/43) 4.2 mc (1945) 2.0 mc (1946) 3.0 mc (1947)	10 years	Good response to P32. Local X-ray given in 1944 and 1947.
R.C.	F	43	1937 Enlargement of supraclavicular node; 2/42 mass in abdomen, enlarged nodes in neck, axillae, and groin. Biopsy showed giant follicle lymphoblastoma. No treatment given.	3/42 Splenomegaly; generalized lymphadenopathy. Abdominal mass.	17 mc (1942)	11+ years	Good response to P32. Later required local X-ray. Patient was alive in 1948 when contact with her was lost.
R.G.	M	47	1944 Enlarged cervical nodes; 5/45 biopsy showed giant follicle lymphoblastoma. Generalized lymphadenopathy later treated by X-ray.	10/49 Hepatomegaly; generalized lymphadenopathy. Anemia lymphocytosis.	4 mc (4-5/50) 2 mc (2/52)	9 years	Good response to P32. Local X-ray given later. Liver became enormous, and hemachromatosis diagnosed from Fe-59 studies, confirmed by autopsy findings. Post-mortem examination showed generalized lymphosarcomatous infiltration.
C.H.	F	—	1940 Enlargement of axillary nodes; splenomegaly and hepatomegaly; 2/43 biopsy showed giant follicle lymphoblastoma. X-ray given to axillae, neck, & spleen.	9/43 Splenomegaly; enlargement of axillary and cervical nodes; lymphocytosis.	6.6 mc (1943)	6½ years	Good initial response; later required X-ray. Developed anemia and leukopenia and required frequent transfusions.

Patient	Sex	Age at onset	History	Initial findings here	P ₃₂ dosage	Duration from onset to death	Remarks
J.H.	M	37	1943 Pain and tenderness over ilium, ribs, and sternum. Later cervical nodes enlarged treated by X-ray. Biopsy in 1945 showed giant follicle lymphoblastoma.	11/45 Splenomegaly; enlarged cervical and axillary nodes.	3.0 mc (12/45-1/46) 4.0 mc	7 years	Improved symptomatically. Continued to have some bone pain throughout course.
E.McG.	F	51	1947 Swelling over clavicle, then cervical and axillary nodes enlarged. Biopsy showed giant follicle lymphoblastoma. Local X-ray given.	12/50 Splenomegaly; enlargement of inguinal nodes; abdominal masses. Anemia.	3.0 mc (12/50-1/51)	4 years	No apparent response to P ₃₂ . Patient moribund when first observed.
A.M.	F	49	1942 Enlargement of node under mandible. Biopsy showed giant follicle lymphoblastoma. X-ray given, followed by severe reaction.	4/43 Splenomegaly; few small nodes palpable in neck.	7.1 mc (1943) 8.6 mc (1944)	4+ years	Questionable response to P ₃₂ . Extremely radiosensitive to both X-ray and P ₃₂ , and patient developed anemia and leukopenia. Patient was living 4 years after onset of disease, when contact with her was lost.
ABM	M	60	1936 Generalized lymphadenopathy; 12/36 biopsy showed giant follicle lymphoblastoma. X-ray given.	2/41 Splenomegaly; generalized lymphadenopathy; fluid in right chest. Anemia.	33.0 mc (2-4/41)	4½ years	No benefit from P ₃₂ . Patient was moribund at first observation. Autopsy showed transformation to reticulum cell sarcoma.
A.P.	F	65	12/40 Enlargement of cervical glands and abdominal mass. 5/41 biopsy showed giant follicle lymphoblastoma. No X-ray.	12/41 Generalized lymphadenopathy. Ascites with abdominal masses. Skin infiltration; edema of legs. Anemia.	14.1 mc (1941) 6.5 mc (1942) 23.7 mc (1943)	4½ years	Excellent response with regression of enlarged nodes; disappearance of ascites and clearing of skin infiltration. Recurrences were treated with P ₃₂ with good response. Patient died of heart attack four years after onset. Post-mortem examination showed no evidence of lymphomatous disease.
H.P.	M	54	1945 Generalized lymphadenopathy. Splenomegaly; 10/46 biopsy showed giant follicle lymphoblastoma. No treatment.	5/47 Splenomegaly; generalized lymphadenopathy. Stern-al marrow infiltrated with lymphocytes.	3.0 mc (5/47) 3.0 mc (4-5/48) 0.5 mc colloidal CrPO ₄	4 years	Improved after P ₃₂ with reduction in spleen size. No effect from terop-terin in 1948. Local X-ray given in 1948.
A.S.	M	37	1942 Fatigability; weight loss; cervical and inguinal nodes enlarged. 7/42 biopsy showed giant follicle lymphoblastoma. No treatment.	9/42 Hepatomegaly; shotty nodes in neck, left axilla, and groin.	9.2 mc (1942) 9.6 mc (1943) 3.0 mc (1944) 3.0 mc (1945) 3.0 mc (1946)	4 years	Questionable benefit. Very radiosensitive. X-ray to lymph nodes benefited, but was followed by anemia and leukopenia.

of these 3 cases was less than six months, and the beneficial response to isotope therapy could have been due to this fact. One of these patients had been given a total of 14 mc of P^{32} in two months. The initial leukemic blood picture had improved markedly, the tumor masses had regressed, and the patient was symptomatically much improved when coronary thrombosis caused his death.

Seven of the 14 patients with lymphosarcoma developed marked anemia and leukopenia while undergoing therapy. In three of these cases radiation undoubtedly contributed to the marrow suppression. The limiting factor in the treatment of these diseases with radioactive phosphorus is the depressing effect on the hematopoietic tissues. However, in an occasional case, the malignant tissue is sufficiently radiosensitive to small doses of P^{32} so that marrow depression is minimal in proportion to the effect on the tumor tissue.

The three cases in our series showing the most favorable responses received the isotopes at low dosage rates over long periods of time. The patients who showed the most marked marrow depression received more intensive radiophosphorus therapy. However, it should be pointed out that most of these patients had been referred to us after roentgen therapy was no longer effective, and only in this refractory group were the higher doses of P^{32} eventually tried.

On the basis of these therapeutic studies, one must conclude that treatment with P^{32} alone is not clearly superior to roentgen therapy, but the treatment of choice in this disease is probably a combination of the two forms of radiation therapy. The occasional good result with P^{32} alone, however, would justify its use in small doses in cases of generalized lymphosarcoma.

LEUKEMIA

At the initial therapeutic attempt in 1936 we realized that radiophosphorus would not provide a specific curative agent for the treatment of chronic leukemia. However, with the demonstration that radiophosphorus in the form of isotonic Na_2HPO_4 would localize to a considerable degree in bone marrow and leukemic tissue,⁴ it appeared clear that we could irradiate the leukemic tissue somewhat more selectively with beta rays with this method than would be possible using whole body spray X-ray. A systematic study was begun at that time in order to evaluate the relative efficacy of this form of radiation therapy, and more than 300 patients have been observed and studied on a continuous basis since that time.^{13,14,15}

The usual clinical and laboratory methods of observation have been employed and sternal marrow punch biopsy used for diagnosis and when necessary to assess the status of the bone marrow in relation to further therapy. The blood volume and rate of red cell production were often determined by isotope

methods as additional precautions against over-irradiation of the bone marrow. In this connection it was interesting to learn that most chronic leukemia patients who are anemic have a normal rate of red cell production or even increased rate, as opposed to that which would be observed if the anemia were on a myelophthitic basis as commonly believed. Further studies of the life span of the red cell provided the predicted explanation for the anemia often associated with leukemia. In the patients studied so far,¹⁶ there is usually a shortening of the life span of the red cell in both chronic myelogenous and lymphatic leukemia, but the patterns observed are different. In chronic myelogenous leukemia the life span is often shortened (usually to 75–90 days, as compared with a normal of 120 days), but the pattern of life duration of the cell is finite, indicating perhaps a defect in the red cell. On the other hand, in chronic lymphatic leukemia, when shortening of the life span did occur, it was usually much shorter than found in myelogenous leukemia and the pattern was one of random destruction of red cells as opposed to finite, indicating some extra-marrow destruction effect on the cell, such as an overactive spleen. Further studies are now under way to clarify these points.¹⁷

In the early days of the work we made a few attempts to give large doses of P^{32} in the hope that the leukemic cells might be inhibited or destroyed selectively and that transfusions and other forms of supportive therapy could compensate for any temporary hypoplasia of the bone marrow. By 1940, however, we concluded that large doses of isotopes could not eradicate the leukemic process and would only produce hypoplasia of the bone marrow that the best we could hope for was palliation and some prolongation of life over that achieved by other forms of radiation, and a small dose schedule has been in use since that time. The average courses of therapy have been 1–2 millicuries of P^{32} once or twice a week for four to six weeks, usually not repeated within six months. The dosage schedule is adjusted to avoid over-irradiation of the bone marrow with resulting depression of red cell and platelet precursors. General supportive care is given as necessary, including transfusions, vitamin and hormone therapy, antibiotics for intercurrent infections, and local roentgen therapy to lymph nodes and/or spleen when they were markedly enlarged and did not respond to safe doses of P^{32} . With reference to hormones, it has been our practice to give patients with chronic lymphatic leukemia 12–18 mg of testosterone orally per day during a good portion of each year. Some benefit in the form of a sense of well being and an improvement in the total red cell volume is observed. It is known that testosterone has some stimulating effect on red cell production¹⁸ and that estrogens are leukemogenic.^{19,20} In some instances we have carried patients on testosterone alone for many months with little progression of the disease.

We have tried to anticipate relapses and begin therapy again when there is evidence of activity of the disease process, since, as Osgood²¹ has emphasized, it seems important to begin re-treatment before there is any marked increase in the number of white cells, enlargement of the spleen or lymph nodes, development of symptoms of relapse, or anemia.

The median survival in our series of 129 chronic lymphatic leukemia patients was 5.4 years, with 31% surviving over 7 years from onset. However, we now have several patients who have already survived 14 years. It cannot be determined at the outset which of the patients will survive for long, but the prognosis for a chronic lymphatic leukemia patient who has an adequate total red cell volume, has no great enlargement of the spleen, liver, and lymph nodes, and is without marked elevation of the white cell count (over 100,000) is good for long survival. The results reported here are considered superior to those achieved by other methods of therapy. Case history F.H.B. illustrates the good results that can be obtained. In this case it was possible to supervise the course of the patient closely.

The median survival in the chronic myelogenous group was 3.2 years, with 20% surviving past the fifth year from onset. These results are equal to but probably not superior to the survival reported with the use of other methods. Unlike chronic lymphatic leukemia, in chronic myelogenous leukemia there is usually an eventual termination of the disease with an acute picture. This is true after all forms of therapy and is not induced by P^{32} , as some workers have thought.

POLYCYTHEMIA VERA

In the past 20 years the author and his associates have observed and treated over 230 patients with polycythemia vera, and follow-up studies have continued on most of these patients over the years.^{22,23,24}

Many patients had a history of thrombosis or hemorrhage. Thrombophlebitis of the leg or arm had occurred in 13%, cerebral thrombosis in 10%, coronary thrombosis in 4%, and other (portal, etc.), 4%. Instances of epistaxis or intractable bleeding following a tooth extraction were frequently reported, and 3% of the patients had gastric hemorrhages, usually associated with peptic ulcers. A history of gout was found in 7%. The most common complaints were fatigability, headache, vertigo, scotomata, precordial and gastric pain, and pruritis. Ruddy cyanosis of the face, nailbeds, or mucous membranes of the mouth and pharynx was observed in 62% of the patients. Some enlargement of the spleen was noted in two thirds of the patients, but the degree was not great in most patients and in only rare instances was enlargement into the pelvis found. The liver was palpable in about one third of the patients. The enlargement was usually slight and was probably on the basis of increased blood volume and distention

of the vascular bed of the liver. About half the patients were hypertensive.

The largest number of patients had red cell counts in the range of seven to eight million, with another large group in the six to seven million range. Hemoglobin was usually between 15 and 20 grams. The white cell counts were over 10,000 in 74% of the patients, but most of these were in the 10,000–25,000 range. In about 70% of the patients there was at least one metamyelocyte or less mature white cell recorded in the peripheral differential count. The majority had platelet counts over 300,000, some ranging from 500,000 to 1,400,000. This emphasizes the often overlooked fact that polycythemia is a condition in which there is total hyperplasia of the bone marrow, not simply erythropoietic tissues. The most conspicuous finding in the sternal marrow differential was the increase in erythroid precursors over the normal and a slight increase in all the neutrophilic series.

There was some increase in the blood viscosity and circulation time, but clotting and bleeding times appeared to be in the normal range. Blood oxygen saturation was in normal range. Unless the patient had received venesections or other therapy shortly before his first visit, the red cell volume was almost invariably elevated, taking 32.2 cm³/kg of body weight as the upper limit of normal (by the P^{32} or radiochromium methods). Some red cell volumes in the 90–100 cm³/kg range were noted. Plasma volume was usually below normal. As measured with Fe^{59} the rate of production of red cells was increased, in some cases to ten times the normal value. Studies of the life span of the red cells in this condition indicate that there are probably two populations of red cells, one with a normal life span and one with a shortened cell life. It is the turnover of the short-lived cells that accounts for the enormous quantities of iron utilized in these patients for red cell production. The short-lived cells probably function normally in the transport of oxygen.

Following diagnosis the patients in relapse are given an initial dose of 3–5 mc of P^{32} . In cases of extremely high red cell volumes and high platelet counts venesections are performed near the time of administration of the radioisotope in order to diminish possibilities of thromboses in the two to three month interval before the full effect of marrow inhibition is seen. In the older patient who tends to be more radiosensitive and also in the very young patients who might need treatment for many years, the initial dose is usually limited to 3 mc. The patient is then observed for three to four months, and if further treatment is needed, a second dose of approximately 4–5 mc is given. In deciding on the need for further therapy, it is often helpful to study the patient with Fe^{59} to learn the rate of red cell production. The effects of the marrow inhibition may not be immediately reflected in a reduced red cell volume although the rate of production of red cells may be normal

or less and will predict an eventual fall in red cell volume. Once active therapy is begun, we try to bring the patient into remission gradually. It may require one to two years before the red count is finally brought down to the 5.5 million level, although some reduction will be seen earlier. When active therapy is begun in patients in relapse, the interval between courses of therapy ranges between six and ten months. Once the patient is brought into remission, less treatment is needed to control the disease and the interval approaches three years. Some patients continued to do well for longer periods without further treatment, as is illustrated by case history S.H.

On the other hand, we also observe a few patients who do not respond to large doses of P^{32} . Patient H.K. is an example. It will be noted that he has developed a slight hypochromia as a result of frequent venesections, but his red cell count has never been held to a value of 5.5 million even with the large doses given.

In general, we withhold P^{32} in cases of myelofibrosis with splenic production of red cells, although small doses may be given in rare instances where there is extreme splenomegaly and polycythemia.

An analysis of the survival in this series of patients shows that the median survival is 13.2 years,²⁴ or approximately twice that observed in series treated by conventional methods.²⁵ There has been no increase in the incidence of leukemic deaths in this series over that reported in other groups treated by conventional methods. Of the 68 deaths, 16 died of leukemia, 10 of coronary thrombosis, and 9 of hemorrhage (8 cerebral and 1 gastrointestinal), the principal causes of death associated with this disease. Thus, there is no increased incidence of leukemia with this form of therapy, although the induction of leukemia in rare instances cannot be ruled out since it is well known that single, large doses increase its incidence and that radiologists have a higher incidence of leukemia than that found in the general population. The major factor in extension of life in this series has been the reduced incidence of hemorrhage and thromboses. In series treated by conventional methods, deaths from hemorrhage and thromboses approximate 50% as compared with 28% in this series.^{25,26}

RADIOCOLLOIDS

A radioelement incorporated into a colloid and injected intravenously is phagocytized by the reticulo-endothelial system cells and constitutes a useful form of internal radiation under some conditions.

The localization of colloidal chromic phosphate in mice has been studied by Jones, *et al.*⁷ and since 1942 we have been using chromic radio phosphate and other radiocolloids in the treatment of leukemia, polycythemia and the lymphomatous disease generally. Suspensions of anhydrous chromic phosphate were prepared from radioactive and non-radioactive

phosphorus. The average particle size was 1 micron. The colloid is inert and insoluble in living tissues, and approximately 90% of the material was found to localize in the liver, with the next highest uptake by spleen and lungs.

The beta-irradiation to the liver and spleen was approximately 100 times that of other tissues, except lung tissue. The lymphoid tissue of the spleen was found to be particularly radiosensitive.

To analyze some of the factors responsible for selective localization in various tissues, a series of colloids were prepared by Gofman and associates²⁸ containing radioactive isotopes of yttrium, zirconium, columbium, and lanthanum. These elements were chosen since it is possible to synthesize a variety of types of colloids incorporating their isotopes and since there is available a large number of radioisotopes of differing nuclear properties, isotopes of short, intermediate, and long half-life, some emitting only beta particles, some only gamma rays, and others emitting both kinds of radiation.

In every case the colloids of large particle size, sedimentable in large part by ordinary centrifuges, showed the common property of localizing in the liver and spleen of mice, rats, and rabbits. These sols were turbid and produced an intense Tyndall effect on illumination. For the colloids which localize in the liver and spleen, there was a rapid rate of disappearance of the colloid from the blood, with a half-time of 30 seconds to 1 minute. Colloids of intermediate particle size were also prepared, of the zirconium-hydroxy-lactate and the yttrium-hydroxycitrate type. These had a less pronounced Tyndall effect and could not be centrifuged by ordinary centrifuges. In contrast with the rapid blood clearance of the large particle colloids, these of intermediate size are cleared from the blood much more slowly in the rabbit, rat, cat, and mouse, showing an exponential decay from the blood stream with half-time from 30–80 minutes. In the rabbit, rat and cat, the slowly disappearing colloids localized primarily in the bone marrow, with the spleen and liver concentrating the next highest quantities of specific activity.

The bulk of both the large and intermediate particle colloids remained at the site of deposition for at least several weeks. By selection of the type of colloid used and the choice of the isotope having an appropriate half-life, controlled irradiation could be given the liver and spleen, or the bone marrow, liver, and spleen.

We have used $CrP^{32}O_4$ and Y^{90} colloids in the treatment of polycythemia vera, chronic leukemia, and other hematopoietic disorders for many years, particularly in those cases in which there was hepatomegaly or splenomegaly. Except for the unusual case, however, the therapeutic results in polycythemia vera and leukemia are not superior to those using P^{32} as sodium phosphate, and colloids do not provide a unique therapeutic tool in this area as they do in

the intracavitary radiocolloid treatment of effusions in carcinomatoses of the pleural and peritoneal surfaces.

DOSIMETRY

A method for estimating the dosage obtained from an intravenous administration of P^{32} has been presented by Low-Beer.²⁷ On the basis of our experimental studies of relative phosphorus uptake and excretion in soft tissue and bone, an initially uniform distribution of P^{32} in the body is assumed. After three days equilibrium is established between one compartment comprising the bone marrow, liver, and spleen and a second compartment comprising the remaining soft tissues, with the ratio of P^{32} concentration in the former to that in the latter being 10:1. From the third day on, bone and soft tissue lose P^{32} through decay and excretion at the rate of 6.1 per cent per day, corresponding to an effective half-life of 11 days. The 12 per cent of the administered dose which decays in the body during the first three days added to the 58 per cent of the administered dose which decays in the body from the third day on gives a total of 70 per cent decaying in the body. By calculation 34 per cent of the administered dose decays in bone and 36 per cent in soft tissue. The total integral doses are 306 gm-r/ μ c for bone and 320 gm-r/ μ c for soft tissue, giving a total body integral dose of 626 gm-r/ μ c.

CASE HISTORIES

Patient R. B.

This 56-year-old man was referred for radiophosphorus therapy one month following diagnosis of giant follicular lymphoblastoma by biopsy. The patient reported a weight loss and fatigability for the preceding six months and had sought care for sciatic pain and discomfort from pressure in the abdomen. On physical examination the spleen was found to extend four fingerbreadths below the costal margin and was firm and nontender. The liver occupied the entire upper abdomen above the umbilicus with an edge in the right flank at the level of the umbilicus. The omentum was reported infiltrated. There were no enlarged cervical nodes, but many discrete hard areas were noted in both groins. The hemogram was within normal range. Radiophosphorus therapy was instituted in small doses once or twice a week for a total of 10.5 mc between May 20 and July 12, 1943. The patient improved remarkably, gained weight, and his spleen decreased in size. A few months later the patient was also given local X-ray to the enlarged nodes, consisting of approximately 200 r each to both lateral cervical regions, both axillae, both inguinal areas, and the mediastinum and abdomen. In January 1944 he developed pain in the sacrum and left lower rib region. Another course of X-ray was given between March 10 and May 17, 1944. The pain disappeared and by August the patient reported feeling better than at any time since onset of his

illness. During 1945 the patient was given two doses of P^{32} (2.07 and 2.13 mc), and his only complaint at this time was pain near the hip joint. Radiological examination indicated osteoblastic metastases in the sacrum and fifth lumbar vertebra. In 1947 the patient was hospitalized twice for pain in the lower spine and was in a terminal status. There was metastatic involvement of several lumbar vertebrae. A dose of 3 mc of P^{32} was given. There was a dramatic improvement in pain symptoms, the patient gained weight, and did well until July 1952, when he reported increased hip and mediastinal pain, dyspnea, and jaundice. X-ray was given, but the patient went into congestive failure, became dyspneic with evidence of acute pulmonary edema and died the following month. Post-mortem examination showed lymphosarcomatous infiltration of the lymph nodes generally, and the liver, gallbladder, bile duct, pancreas, kidneys, lungs, colon, and dura were also involved. The generalized lymphadenopathy was believed to have produced a mechanical obstruction to the external biliary system which was responsible for the terminal icterus observed. The infiltration of the lungs by this process was sufficient to produce a cor pulmonale and was probably responsible for the patient's congestive failure.

Patient F. H. B.

This 40-year-old engineer was referred to us in June 1941, eight months after a tentative diagnosis of chronic lymphatic leukemia had been made. A white cell count of 228,000 with 99.5% lymphocytes, and some lymphadenopathy of the inguinal, cervical, and axillary nodes had been discovered when the patient was hospitalized for treatment of an eye infection. X-irradiation was given the spleen at that time.

The patient had no serious complaints at the time of his first visit. His history showed that he had had an attack of lobar pneumonia, treated with sulfathiazole, a few months earlier, following which his white cell count had dropped sharply. He had a similar attack of pneumonia at the age of 30. With the exception of the enlarged lymph nodes, the physical findings were essentially negative. His blood count at that time was: RBC, 5.55 million; hemoglobin, 14.5 grams; WBC, 15,500; segmented cells, 11%; band cells, 10%; eosinophils, 0.5%; lymphocytes, 69.5%; lymphoblasts, 3%; and prolymphocytes, 6%.

Radiophosphorus therapy was instituted and between June and December, 1941, a course of 12 mc was given. The patient showed a good response and was controlled with doses of approximately 2-6 mc each year. In 1944 he received one course of spray X-ray, and was given testosterone, vitamins, iron, and so forth when he showed a tendency toward an anemia. The patient was hospitalized for pneumonia in 1949 and has occasionally suffered from upper respiratory tract infections, but in general he had a

remarkably smooth course. In 1946 a chest film disclosed a rounded opacity superimposed on the left third anterior interspace measuring approximately 1.5 cm in diameter. This appeared to lie in the central lung field as seen in lateral view. The nodule was checked regularly, but it did not change in size, density, or configuration, and it is believed that this represented a small tuberculoma or other benign nodule rather than a neoplasm.

The white cell count largely remained below the 35,000 level until 1948 when a trend toward an increase was noted. The count stabilized between 50,000 and 75,000 until December 1953 when the count rose above 100,000 and remained high until the terminal illness, when it fell to the 20,000 level.

In April 1955, after fifteen years of an active and successful life as an engineer, the patient was hospitalized for a kidney infection, with fever, chills, frequency, dysuria, and back pain. The patient was given antibiotics and sulfa drugs, but continued a downhill course and expired in May 1955, 15 years after onset of the disease. This is an example of the good result that can be obtained in some of these patients with an opportunity for close supervision of the course of the disease so that treatment can be carefully adjusted to progression of the disease process. This man had a relatively long useful and comfortable life and worked hard at his profession.

Patient S. H.

This 61-year-old white male was first seen here in 1945, when the following history was obtained: Chief complaints were dizzy spells, aches and drawing feelings in legs and arms, swelling of the ankles, and a full feeling in the head which was not associated with true headaches, all of approximately 18 months' duration. In 1944 severe cramplike pains occurred in the back, and in December 1944, he first experienced dizzy spells. The symptom of chronic fatigue was severe enough at that time to make work impossible. On physical examination the patient had a ruddy cyanosis, congestion of the conjunctivae of the eyes and of the mucous membranes of the mouth. His blood pressure was 145/100, the spleen was enlarged approximately 6 cm below the costal margin, and the liver was felt approximately 4 cm below the costal margin. There was pitting edema of the left ankle and foot. In 1945 the patient had venesections totaling 1500 cm³ and approximately 9 mc of P³². This therapy produced a satisfactory reduction in red cells, hemoglobin, white cells, and platelets, and the patient remained in remission for nearly ten years. In September 1954, it was noted that his red count had risen to 6.72 million (from 4.5 million a year before), hemoglobin to 19.6 grams, white count to 18,600, and platelet count to 432,000. He was treated with phlebotomy and 3½ mc of P³². This is an example of a patient who had a very satisfactory and long remission following one course of 9 mc of P³².

Patient H. K.

This 64-year-old white male was first seen by us in March 1947. In 1936 his wife noticed that his eyes were very red. An examination by his physician showed a reddened tongue and throat and a high red cell count. The patient was then treated with phenylhydrazine but had an unsatisfactory response in that there was too great a decrease in the red cell count. He was then treated by phlebotomy every eight weeks for the next six to seven years, but the red cell count was never kept below the 7.0 million level during this interval.

The initial physical examination revealed an athletic and well-nourished man with a very markedly red complexion and cyanosis over the tip of the nose. The spleen and liver were palpable. Roentgen examination of the chest showed no abnormalities. The initial blood picture was RBC, 10.2 million; hemoglobin, 19 gm; platelets, 220,000; and WBC, 18,350. There were no abnormal myeloid cells in the peripheral blood. The patient was treated with eight venesections of approximately 500 cm³ each, followed by 3 mc of P³². Subsequent to this therapy the red cell count fell to slightly over 6 million but soon started to rise again and further therapy by repeated venesections and P³² was given. The spleen decreased in size so that it was no longer palpable. From 1947 until early 1949, the patient had repeated venesections and received a total of 7 mc of P³² and 15.8 mc of Y⁹⁰ colloid, which in its effect is roughly equivalent, millicurie for millicurie, to P³². Following this the red cell count started to rise and he was again treated with venesection and P³². However, the doses of P³² were much smaller than those given previously because the white cell count, which had been in the 15,000–20,000 range, was now in the 5000–10,000 range. The platelet count was also in the normal or low normal range. When patients relapse, the white cell count and platelet count often do not rise to the level seen previously. Although the patient has continued to receive P³² and has had venesections up to the present time, his red cell count continues to be approximately 6 million. As a result of the repeated venesections, the hemoglobin stays in the normal or slightly below normal range, so that he is slightly hypochromic. The white cell and platelet counts remain in the normal range. With the exception of a persistent pruritis and an episode of gout which responded to colchicine, this patient has been asymptomatic.

This is a case illustrating a very difficult therapeutic problem, since the patient has required very large doses of P³² and repeated phlebotomies to control the red cell count. It should be pointed out that less than 5 per cent of the patients so far treated fall into the group that is extremely difficult to bring into a prolonged hematologic remission, and this case history should be contrasted with that of patient S.H., who has responded to treatment with much

smaller doses at longer intervals. He has been one of our most difficult patients to control satisfactorily, yet from his own standpoint he has done very well.

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Accurate Method for the Treatment of Hyperthyroidism with I^{131}

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SUMMARY

We may observe at the present moment a new trend for the treatment of hyperthyroidism with I^{131} . After a period when a single dose was given, the therapists have followed the opposite criterion, giving small and repeated doses to avoid overdosage. Although the difficulty in assessing the different factors intervening in the evaluation of the optimum individual dose is recognised, some authors believe that the bad therapeutical results depend only on individual biological variations of radiosensitivity.

However, we believe that the correct assessment of the physical factors will lead to a more efficient therapy. Among the physical factors the evaluation of the gland volume is one of the most difficult to perform.

To accomplish this evaluation we have used for more than two years, a new method by which a radiographic visualisation of the gland is made. To obtain this visualisation we use a gaseous contrast, by insufflation of 150–400 cm³ of oxygen above the sternal manubrium.

Although avoiding the blood vessels, we establish, repeatedly before injecting, a negative pressure, noticing if there is blood in a visor before the needle. In each case, frontal, lateral radiographs as well as tomographs are taken, and based on these, calculation is made.

The anatomic comparison has shown that the error is less than 10%. This method was employed in 46 patients and proved to be innocuous and painless. The evaluation of other factors (effective half-life and uptake curve) was made in the ordinary manner.

In some cases the uptake curve with the therapeutic doses was somewhat different from the diagnostic one. Due to this fact, in these cases the prescribed dose was not that actually received.

The beta dose initially prescribed was 8000 rep which subsequently we increased to 10,000 rep and later on to 12,000 rep.

The therapeutic results were as follows: 33 cures after the 1st dose, 72%; 2 doubtful after the 1st dose, 4%; and 10 recurrences after the 1st dose, 21%;

one case of post-therapeutical hypothyroidism was observed.

In the 10 recurrent cases are included 4 nodular goiters and 3 other initial cases where the mass evaluation was wrong.

The average beta dose actually received in the cases with recurrences was 6900 rep, in the remissions, one was 11,300 rep, which confirms our working hypothesis.

In another series of 14 patients, with similar clinical cases, the method was not used and the evaluation of the gland volume was done by the palpation method.

In these, the results were: 7 remissions after the 1st doses, 50%; 5 recurrences after the 1st doses, 36%; 1 hypothyroidism after the 1st doses, 7%; 1 doubtful, 7%.

INTRODUCTION

Nowadays we are witness to radical changes in radioiodine methods for the treatment of hyperthyroidism.

After an initial period where a definite remission with a single dose was aimed at, the general trend is the administration of small and repeated doses in order to avoid post-therapeutic hypothyroidism.

The above factors include both physical and biological data. In our opinion we can only assess the importance of the biological factors if the physical ones are accurately known.

Unless the prescribed and effectively administered dose is accurately known we cannot relate a therapeutic result with the individual biological factors.

As is well known for such an accurate dosage the physical factors involved are: the mean beta-disintegration energy of radioiodine, the uptake curve and the thyroid gland mass.

The least accurately known of all these factors is the thyroid gland mass. Although there were suggested several radioactive scanning methods, generally the mass is calculated by palpation of the thyroid region and the errors of this procedure may rise to 100%.

We are going to describe the method we have been using for the resolution of this problem which, we think, offers some advantages over the methods previously proposed.

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EVALUATION OF THE THYROID MASS

The thyroid gland capsule is almost completely surrounded by a lax cellular tissue and so we thought to radiograph the gland using a gaseous contrast.

By analogy with the visualization techniques of retroperitoneal organs, we may call this procedure "pneumo-thyroid".

The patient lies on the left side because the experimental work of Durand, Long and Oppenheimer¹ and a clinical accident reported by Musgrove and MacQuigg² has shown that in this position gaseous embolism is much less liable to occur.

We anesthetize the skin near the median line of the infra-hyoid region with novocaine although the injection in this point is almost painless.

Afterwards we introduce a needle of 0.7-mm diameter until we encounter resistance and cross the investing deep fascia. If the gaseous insufflation is made between the two fascia the pneumothyroid can also be carried out but a radiograph contrast of the muscles of the region appeared and we got a subcutaneous emphysema.

The patient's swallowing can show us if we attain the right point. Insufflation is made with the device of Fig. 1. It consists of two pneumothorax flasks, one of them with oxygen and the other with water connected in series with an insufflation bulb. The oxygen flows through a rubber tube with a manometer and an empty syringe in parallel. The rubber tube is connected to the needle through a glass visor.

Before and during the operation, if there is any vascular accidental puncture, blood will come to the visor by aspiration with the syringe.

Insufflation pressures go up to 7 cm Hg and the oxygen volume injected varies from 300 to 400 cm³.

Radiographs and tomographs are taken after the insufflation on two perpendicular planes.

The method is practically innocuous; in all cases tolerance was perfect although it was applied to hyperexcitable patients, some of them having auricular fibrillation.

In fact there has not been, until now, any embolism, accident or clinical signs of mediastinic emphy-

sema. Some patients feel a slight local pain during absorption of the gas which lasts for a few hours.

We believe that perhaps the one situation where this technique is not to be recommended, is in patients with evident signs of cardiac insufficiency.

In order to simplify the calculations of the thyroid gland masses we assume that each of the thyroid's lobes is comparable to a scalen ellipsoid. The volume is then $V = \frac{4}{3} \times \pi \times a \times b \times c$ where a , b and c are the semi-axis which are measured on the radiographs and tomographs. In six cases the anatomical comparisons have shown that the error made in this evaluation is less than 10%.

It must be noticed that in some patients we cannot obtain useful pictures; in three out of 46 cases we could not calculate the thyroid mass; two of them corresponded to post-operative hyperthyroidism recurrences.

In three other cases the mass calculation was clearly wrong. These cases (Nos. 2, 6 and 18 of Table I) corresponded to earlier pneumothyroids when we still used a superficial insufflation and there was no experience in picture interpretation.

Figures 2 to 7 show some of the radiographs and tomographs taken with this method.

UPTAKE CURVES AND DOSIMETRY

In all the patients to be treated by I^{131} a pre-therapeutic uptake curve with carrier free I^{131} was taken and followed during several days in order to calculate the effective half-life. The procedure is described in another report.³

After the calculation of the thyroid mass by the method described above, the activity to be given in order to get a certain dose was calculated on the basis of the previous uptake curves.

After the oral administration of the dose, measurements were carried out as show in Fig. 8.

The patient is in front and at a distance of about 3 meters from a scintillation detector (thallium activated sodium iodine 1.2-cm diameter and 12-cm high). The pulses from the photomultiplier EMI 5659 went through a cathode follower to an amplifier

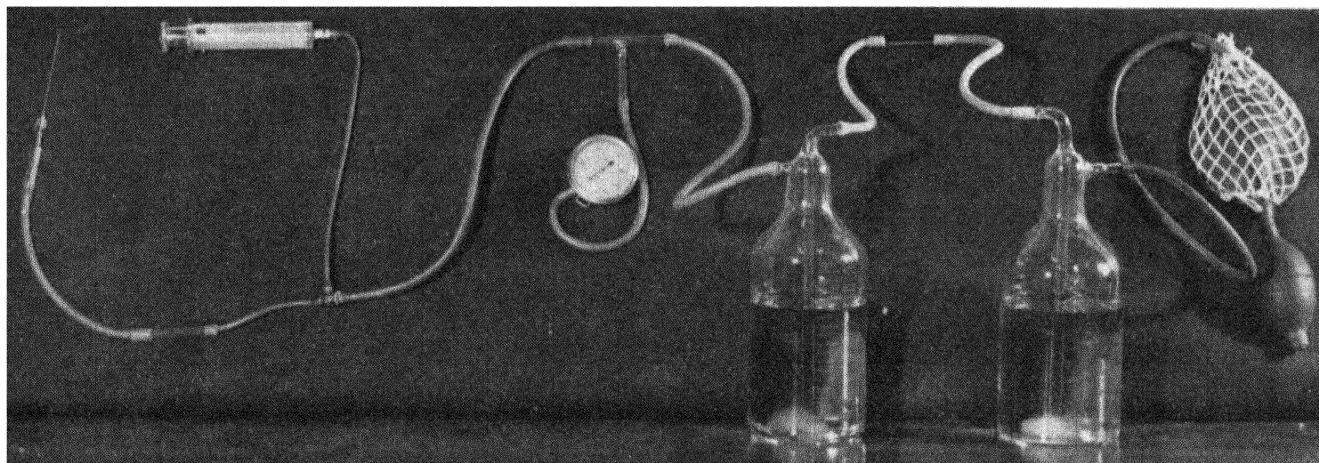


Figure 1. Insufflator device to perform the "pneumothyroid"

(gain 450) and were counted with a ratemeter (Dynatron N 522) with a built-in discriminator.

At this distance of the patient from the scintillation head, the geometrical response of the detector was the same to all parts of the body.



Figure 2. Radiograph and tomograph of the same patient. Although the radiograph only visualizes the right lobe, the tomograph shows clearly all the gland



Figure 3. Postero-anterior and lateral radiograph of a goiter with considerable size. Right lobe = 249 gm, left lobe = 168 gm. In the lateral radiograph superposition of the two lobes gives a double shadow. Afterwards, thyroidectomy was performed

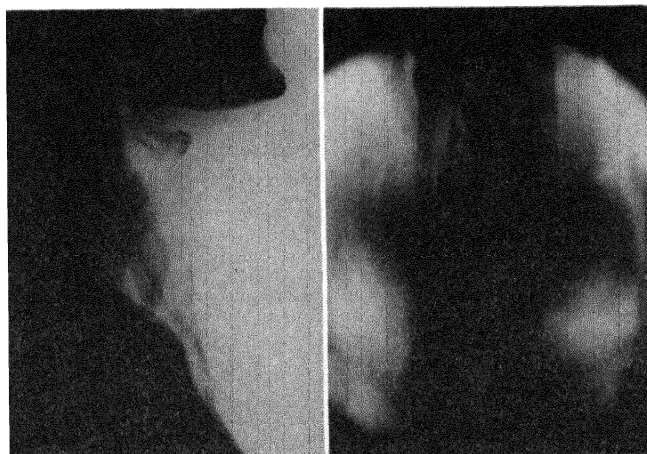


Figure 4. Left. Lateral radiograph shows a clear visualization of the thyroid gland. Mass = 35 gm. Figure 5. Right. The tomograph shows a large goiter with nodular masses well individualized, some of them with calcifications. The trachea is strongly deformed and displaced towards the right side.

Previous to the administration, a bottle containing the activity to be administered was put into a paraffin model of the head, neck and chest, at the same distance of the scintillation head. We could calculate then the transmission coefficient of a lead shield, 1 cm thick, with dimensions just sufficient to cover the neck.

The activity in the gland was measured at different times after the activity administration simply by making two measurements, one without the lead shield and the other with the lead in position (Fig. 8). Taking as unity the reading immediately after the activity administration we could calculate the percentage which remains in the thyroid, in the rest of the body and the activity excreted by the urine, as well as the corresponding doses.⁴

With the amplifier gain of 450 and an interdynode voltage of 136, a discrimination of 15 volts is sufficient, in the operating conditions, to avoid the Compton scattering effects as we verified in a series of measurements.

The uptake curves may be classified within two general types. One where, after the maximum uptake p_{max} , the activity decays with a half-life of T days; the other where after only a few days the curve

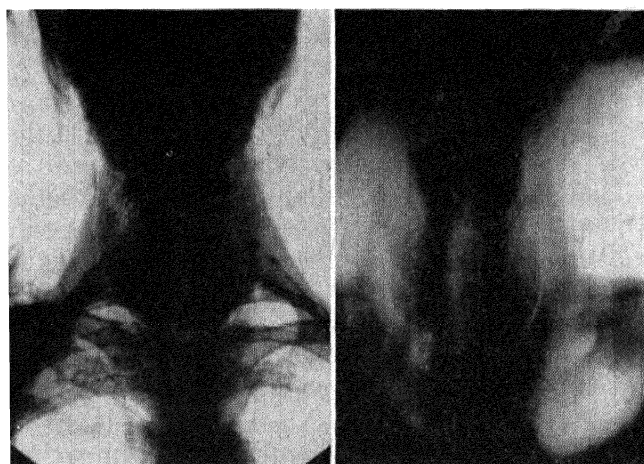


Figure 6. Postero-anterior radiograph and tomograph, plane II, after a superficial insufflation. Mass = 42 gm. Notice the sub-cutaneous emphysema and the muscular dissociation of the region on the radiograph



Figure 7. Postero-anterior and lateral radiograph and tomograph, plane II, of a patient with a diffuse goiter. Mass = 55 gm. Notice in lateral radiograph clear calcifications in the tracheal rings and in tomograph tracheal compression with calcification there only

Table I. Hyperthyroidism Treatment with ¹³¹I

Obs.	No.	Pat.	Age	Diagn.	Uptake Ther.		Post.	Ef. half- life	Mass Q	Dose		Date	Result	
					Prether.					pres.	mc			
93599	1	EC	46	Nod.	26-46-51	* 21-31-44	18-33-30	8	8	8000	6950	9.1	14/ 8/53	Recurrence
92403	2	EF	54	Ex. Dif. Disc.	62-87-81	51-73-68	34-45-69	8	7	22,100	15,000	1.64	28/ 9/53	Recurrence
69395	3	LCS	43	Hypert.	14-30-44	10-17-38		8	8	10,000	8810	5.2	6/ 9/53	Remission
99877	4	CJG	79	Hypert.	62-55-55	54-61-67		7	5	12,000	11,700	13.66	6/11/53	Remission
107724	5	LC	40	Post. op. rec.	34-39-44	15-47-52	19-26-20	6	5	15,000	15,000	2.90	18/12/53	Remission
103025	6	MH	58	Dif.	...-54	64-67-75	42-65-66	7	8	10,000	16,700	2.50	21/ 1/54	Recurrence
99374	7	MIA	43	Hypert.	57-97-88	35-40-48	18-28-39	6	8	8000	5800	1.30	19/ 2/54	Remission
82605	8	MSB	53	Hypert.	51-87-92	30-50-71	23-32-43	8	8	10,000	8800	1.80	21/ 1/54	Remission
102373	9	MJR	41	Hypert.	38-59-70	53-58-68	36-57-71	8	8	8000	7700	6.70	19/ 1/54	Remission
98158	10	CS	63	Hypert. Ex.	57-82-75	72...-60	23-26-26	4	5	10,000	7400	5.10	29/12/53	Remission
109460	11	IMA	44	Post. op. rec. dif.	58-76-72	...-66	...-...	5	5	10,000	6400	7.30	15/ 2/54	Remission
110154	12	AC	43	Hypert.	59-85-85	30-55-69		7	8	10,000	9300	3.69	24/ 3/54	Remission
64921	13	ARR	59	Hypert.	52-73-89	53-61-72	11-15-28	7	7	22,100	9200	1.98	22/ 3/54	Remission
60624	14	EC	35	Post. op. rec.	43-84-81	41	21-45-52	4	2.5	8000		4.58	5/ 4/54	Remission
108742	15	CMB	56	Nod. Ex.	29-47-56	40-51	10-21-18	8	8	10,000	9100	16.96	15/ 5/54	Remission
110847	16	ELSG	45	Nod.	82-83-62	96-85	67-94-67	4	5	10,000	13,300	17.90	30/ 4/54	Remission
101485	17	AC	60	Nod.	37-58-37	69-61	15-21-25	5	5	10,000	8600	13.30	30/ 4/54	Recurrence
40129	18	AAMM	49	Dif.	6-23-40	33-54	27-37-53	6	8	10,000	16,100	6.43	6/ 4/54	Recurrence
106631	19	JC	36	Dif. (d. mitr.)	68-83-73	82-83	40-45-48	8	8	10,000	11,000	8.98	3/ 5/54	Recurrence
102943	20	MAS	37	Dif.	72-94-78	48-62	24-33-40	8	8	10,000	6500	8.66	20/ 5/54	Recurrence
70713	21	MPB	39	Dif. Disc.	19-36-51	34-60	5- 2- 1	7	7	10,000	11,500	5.00	4/ 5/54	Remission
101236	22	EA	45	Nod.	65-80-74	88-86	30-52-65	8	8	10,000	8700	6.15	15/ 5/55	Recurrence
100831	23	MRO	70	Nod.	68-91-97	32-46-59	25-62-54	7	6	12,000	7500	8.50	6/ 7/54	Recurrence
93412	24	RSB	55	Hypert.	20-25-30	20-39-51	24-12- 7	7	6	12,000	16,000	6.10	6/ 7/54	Remission
84991	25	SFPA	45	Hypert.	55-68-82	60-74	15-32-39	5	6	12,000	12,700	4.20	16/ 7/54	Remission
90492	26	MJMB	26	Dif.	63-64-67	85	34-55-60	5	3	10,000	8100	18.30	16/ 7/54	Doubtful
106305	27	AM	47	Dif.	22-60-63	23-28	16-18-18	5	8	12,000	8400	22.80	30/ 7/54	Remission
104442	28	RCFM	37	Hypert.	42-60-65	65-70	11-24-23	6	7	12,000	16,900	2.55	18/ 8/54	Remission
101427	29	MMC	36	Hypert.	33-45-57	24-47-59	33-35-33	7	6	10,000	9900	4.20	23/ 9/54	Remission
101334	29	MSM	44	Hypert.	31-45-59	39-60	14-19-17	5	6	12,000	14,200	4.60	2/10/54	Remission
115096	30	FES	51	Dif.	42-66-66	31-59-72	7- 7- 4	7	7.5	12,000	13,600	6.90	2/10/54	Hypothyroidism
115432	31	LP	48	Nod. disc. Ex.	47-70-55	80-86-68	13-15-11	3	3	12,000	17,100	8.20	20/10/54	Remission
15916	32	ADA	47	Hypert.	81-90-87	72-88-72	17-23-24	4	5	12,000	15,000	6.80	10/11/54	Remission
104280	33	ESS	40	Hypert. Ex.	20-27-53	19-20-46	10-26-13	6	6	12,000	9700	4.95	10/11/54	Remission
113572	34	AM	49	Hypert. Diff. g.	60-84-83	70-91-82		6	6	12,000	13,500	17.90	17/12/54	Remission
29326	35	RRT	52	Sl. Hypert.	44-61-68	47-61-74		6	6	12,000	14,000	4.30	17/12/54	Doubtful
92919	36	BCL	42	Hypert.	24-36-50	21-33-50		8	7	12,000	11,000	3.70	17/12/54	Remission
1114751	37	MCF	52	Hypert. Diff. goit.	15-45-55	27-44-57		6	6	12,000	10,300	15.10	20/12/54	Remission
79060	38	MJG	47	Hypert.	32-83-88	48-57-59	68-82-78	8	8	10,000	7200	4.84	2/10/53	Recurrence
	39	AJHC	41	Hypert.	35-45-51	54-67	6-16-23	8	8	12,000	14,000	4.86	6/ 8/54	Remission
Men														
11682	40	JAF	52	Hypert. Diff. goiter	70-91-83	54...-63	21-37-46	7	7	10,000	6900	16.90	14/ 6/54	Remission
1113477	41	JMSD		Nod.	85-93-89	91-84	13-11- 8	5	5	12,000	11,200	16.00	16/ 7/54	Remission
99196	42	JB	35	Dif. Hypert.		40-57-65	26-26-41		5.5	3000	5000	4.00	10/ 3/53	Remission

Table II. Hyperthyroidism Treatment with ^{131}I

Obs.	No.	Pat.	Age	Diagn.	Uptake		Post.	Ef. half-life	Mass Q	Dose		Date	Result	
					Prether.	Ther.				pres.	adm. mc			
92846	1	ECT	68	Hypert.	82-70-68	19-34-54			30	8000	9700	4.00	28/11/52	Recurrence
100889	2	MFF	49	Post.op.dif.goit.rec.	94-.-78				70		10,300	10.00	4/11/52	Recurrence
98161	3	DGM	41	Post.op.rec.	17-26-34	14-19-30		6	15		12,500	4.00	20/ 3/53	Remission
107682	4	MJSC	55	Dif.Ex.	74-76-76	73-78-84		5	50	10,000	10,400	7.80	25/ 1/54	Remission
97336	5	SLC	54	Dif.Disc.	24-40-82	20-.-78		7	40	10,000	12,900	4.10	19/ 3/54	Remission
98106	6	HAV	49	Dif.	23-47-53	12-35-23		6	80	10,000	8400	14.00	24/ 3/54	Remission
57825	7	ECP	49	Hypert.	23-44-64	50-71	22-34-46	6	40	10,000	13,200	5.80	15/ 5/54	Remission
1112551	8	MJRT	46	Post.op.dif.goit.rec.	48-84-88	84-86	14-30-35	8	60	10,000	9400	4.40	21/ 5/54	Remission
1116138	9	JCM	49	Dif.disc.ex.	61-84-83	6 -79-81	40-57-52	6	30	12,000	11,700	3.80	13/10/54	Recurrence
117190	10	MCCLL	32	Post.op.dif.ex. goit.rec.	63-77-64	85-99-72		4	50	10,000	15,000	9.40	6/10/54	Hypothyroidism
117017	11	MRFC	68	Dif.Disc.	35-62-75	26-45-52		7	30	12,000	7500	3.45	23/ 9/54	Doubtful
101361	12	EC	30	Post.op.rec.	30-79-75	40-60-79	10-26-35	55	40	9000	8400	3.85	22/ 7/54	Remission
Men														
108285	13	DC	57	Post.op.dif.goit.rec.	31-63-82	50-.-67	27-42-62	4	55	10,000	12,100	8.40	28/ 1/54	Recurrence
99982	14	FPC	43	Hypert.ex.	42-82-66	12-31-41		6.5	30		17,800	6.00	23/ 5/53	Remission

Men

decays exponentially with an effective half-life of T days.

Of course in both cases the administered dose and the prescribed one can be calculated simply.

In the first case if we represent by A_0 the activity administered in millicuries, by $\bar{E}$ in Mev the mean beta-energy disintegration of ^{131}I , M , in grams, the thyroid gland mass, T the effective half-life in days and by p_{max} the maximum percentage of the administered activity in the gland, the beta dose D in rads is given by

$$D = 7.39 \times 10^4 \times \frac{p_{max} A_0}{M} \times \bar{E} \times T \text{ rad} \quad (1)$$

With $\bar{E} = 0.197$ Mev and if we suppose the dose contribution of the gamma radiation as 10% of the beta dose we have

$$D_t = 1.60 \times 10^4 \times \frac{p_{max} A_0}{M} \times T \text{ rad} \quad (2)$$

In the second type of curve if the uptake curve only after t_n days decays with an effective half-life of T days we have for the beta doses

$$D = 5.12 \times 10^6 \frac{\bar{E} A_0}{M} \int_0^{t_n} p dt \text{ rad} \quad (3)$$

and for $\bar{E} = 0.197$ Mev and the 10% contribution from gamma rays

$$D_t = 1.11 \times 10^4 \frac{A_0}{M} \int_0^{t_n} p dt \text{ rad} \quad (4)$$

The integral may be calculated by the area between the uptake curve and the abscissa axis or more simply

$$\int_0^{t_n} p dt = \sum_{p_1}^{p_n} p_1 + p_2 + \dots + p_n$$

where p_1 , p_2 and p_n are the mean of the ordinates corresponding to the first day, the second and the n th day.

Of course we must add to D_t from Equation 4 the value we obtain from Equation 2 if we put now $p_{max} = p_n$ (percentage uptake after n days).

RESULTS

Until the end of 1954, 60 hyperthyroid patients were treated in our laboratory.

This technique has been employed since the summer of 1953 and we first prescribed a beta dose of 8000 rep and six months later, since no case of hypothyroidism appeared and there were recurrences, the dose was increased up to 10,000 rep and later on to 12,000 rep.

Table I represents a set of 46 cases where the thyroid mass was calculated by the "pneumothyrroid" method (all the doses are beta-ray doses). Table II represents 14 cases where the patients either were treated before this technique was available or for

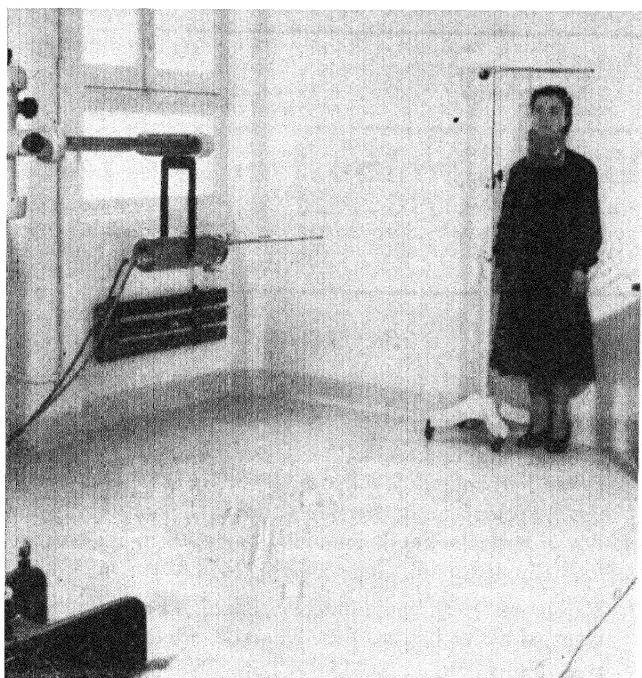


Figure 8 Uptake measurements with therapeutic doses. At a distance of 2 to 3 m response of the detector is the same for all parts of the body

other reasons the thyroid mass was evaluated by palpation.

As the clinical situation is similar in both groups we can compare the results.

Table II deserves a further commentary since this table does not give a clear picture of the results we can expect from this method.

Three out of the 10 recurrence cases corresponded to the cases mentioned above (under-evaluation of the thyroid mass) and four others correspond to nodular goiters.

These seven cases were possible because, in the beginning of this technique, in order not to be subjectively influenced, we ignored on purpose the morphological aspects of the gland.

It is accepted by many authors that the nodular goiters need higher doses in order to get a remission. However we, for the sake of having some personal experience, prescribed the same dose for all the patients independently of the type of goiter, the seriousness of the disease and the existence of complications.

Finally, in the last three recurrence cases, one corresponds to a patient with a cardiac insufficiency and the other two have received 7600 rep and 7900 rep (beta plus gamma).

We got a post-therapeutic hypothyroidism case with a dose of 15,000 rep (beta plus gamma).

CONCLUSIONS

From Table I, including the patients where the thyroid mass gland was evaluated by the pneumothyroid method, we can conclude:

1. The mean dose received by the patients where we got remission of the total was 12,500 rep (beta plus gamma).

2. For the two recurrence cases (5.2% of the total) the mean was 7700 rep (beta plus gamma).

3. The post-therapeutic hypothyroidism received 13,000 rep (beta plus gamma). These results seem to point out that there exists some correlation between the administered doses and the clinical response.

However we may notice that in certain cases, although the dose was very high, remission was observed without post-therapeutic hypothyroidism.

Table II, where the thyroid gland mass was estimated by palpation, shows that:

1. The mean dose presumably received by the remission patients (42% of the total) was 12,200 rep (beta plus gamma).

2. In the recurrence cases (50% of the total) the mean dose was 11,500 rep (beta plus gamma).

3. The post-therapeutic hypothyroidism received 14,200 rep (beta plus gamma).

Although the number of cases we present in Table II is very small the results are comparable with others from some authors.

The results reported above by using this new technique of mass evaluation seem to point out that we can get better results if the dose can be accurately determined. Of course, the studied number of cases is not significant enough to get a definite conclusion about the problem.

It seems that the biological factors indeed intervene in the therapeutic results:

1. Because in certain cases doses of the same magnitude gave different therapeutic results and different doses gave the same clinical results.

2. It seems that the iodine uptake varies with time, due to other biological factors difficult to assess, and so modify the maximum uptake and the effective half-life of I^{131} in the gland (Fig. 9).

If in a more representative set of cases we continue to obtain results similar to the reported ones, we believe that the use of this technique may help to get a better understanding of the biological factors involved and, from the clinical point of view, may also offer some advantages since in most of the cases it avoids the use of repeated doses. Moreover, we may add that, from the clinical point of view, it is hoped to get better results if we take into consideration the individual cases and accordingly the prescription of a more suitable dose; for instance, in

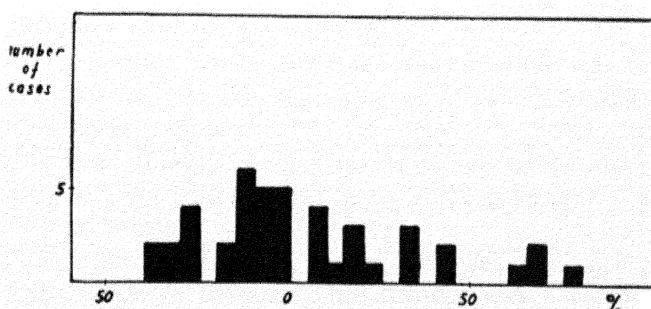


Figure 9. Percentage deviation between the prescribed and the administered doses

Table III. Hyperthyroidism Treatment Results

	<i>Gland volume evaluation by</i>				<i>Pneumothyrroid (corrected statistics)</i>	
	<i>Palpation</i>		<i>Pneumothyrroid</i>			
Defin. remission	7	50%	33	71%	33	85%
Recurrences	5	36%	10	21%	3	8%
Doubtful	1	7%	2	4%	2	5%
Hypothyroidism	1	7%	1	2.1%	1	2.5%
Total	14		46		39	

cardiotoxicosis, nodular goiter and severe hyperthyroidism we must prescribe higher doses. In the cases reported above the policy was different since the aim was to get first-hand experience about the importance of the different factors on the clinical response.

Summing up, we may say that the data reported, mainly the small percentage of post-therapeutic hypothyroidisms, lead us to believe that I^{131} is the best treatment for Graves' diseases in patients over 40 years old and for younger patients in cases of post-therapeutic recurrences. Of course we must exclude the hyperthyroidism with nodular goiter which must be treated as any other tumor.

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Notes on the Treatment of 400 Cases of Toxic Goiter by I^{131}

By L. Brull,* Belgium

Since our laboratory pioneered the use of radioisotopes for biological purposes in this country, we had the pleasure of making the first therapeutic uses of these agents on this continent, in May, 1948.

In addition, we have a reserve of goiter cases, which numbered over 4000 at the time, a figure which, since then, has been more than doubled. Finally, we had experience of long standing in the radiotherapy of goiter, which provided us with an excellent start.^{1,2}

At the beginning, two problems held our attention: that of dosage, and that of measuring the rate of fixation of iodine by the thyroid gland. On the first point, there was a certain amount of confusion. Following the practice of other authors, we tried single doses which varied between 8 and 18 millicuries and finally settled, in most cases, on a 15 millicurie dose, which was repeated, after 2 to 6 months, only in a small minority of the cases.

This paper is concerned with therapy, so that we will not dwell on diagnostics: the reader is referred to our monograph on goiter.³

May it be stated, in passing, that we entertained some hope regarding the use of radioiodine in tracer doses. It was our wish to substitute, for the conventional methods used to measure basal metabolism (the results of which are unfortunately made inaccurate by the agitation of the patients), a technique which would not be influenced by the said patients' nervousness. Along with others, with J. Govaerts in particular, we have measured and summed the radioactivity of the urine following a dose of I^{131} .⁴ This technique, interesting and objective though it may be, particularly on hospitalized patients, requires too long a time to be used as more than a check on other tests.

We devoted considerable time (with J. Govaerts and R. Busset), on the other hand, endeavouring to record thyroid radioactivity in order to find out whether it is possible to obtain any figures which reflect the toxic condition of the gland. Beyond a doubt, there are various methods which give a fairly satisfactory idea of the type of radiation emitted by the gland: however, let the physicists not entertain too many illusions. The results achieved in such a manner are so different from a proper integration

of the clinical signs based on extensive experience that I should like to sum up the situation by stating that, for the time being, we should leave the physicists to their Geiger counters, and the clinicians to their responsibilities.

The anatomical nature of goiter is a delicate matter. Our guiding principle has been, from the very beginning, as well as for X-ray therapy, to leave the nodular goiters which compress cervical organs to the surgeon, along with non-toxic goiters, where an operation was justified. However, we have not ruled out colloid or parenchymatous goiter containing one or two not too hard or recent nodules. Finally, our principle has been that, when the goiter to be operated on was toxic, this toxicity should first be reduced by radioactive iodine therapy, regardless of its anatomical description, in order to present the surgeon with a purely anatomical problem.

After a couple of years of trial and error, our conclusions read as follows (L. Brull and L. Lefèvre): "Our results, on the whole, parallel those of the American authors, who currently have treated by far the largest number of patients. There is only one discrepancy: we have not observed any difference in the response obtained according to the type of goiter, but our series is too small. Treatment by radioiodine seems to be harmless. In most instances, it is very effective."

Two years later⁴ we had treated 40 cases. We could conclude, at that time: "Radioiodine has given us complete cures, without any recurrences, in $\frac{2}{3}$ of the cases; an improvement but no complete cure, in the remaining $\frac{1}{3}$, and it has been completely inactive in one patient. As regards complications we were then noting:⁵ two cases of myxedema following the administration of 12.7 and 9 mc of I^{131} respectively, five cases of arthralgia (a phenomena observed with all treatments); and one case of anemia with a transient leucopenia (following 10 mc).

Thereafter, the number of cases treated was on the increase. At the beginning of 1954⁶ we reached 250 cases, approximately as many as Soley and Foreman (1949) could glean from the world literature in 1949, and our first sixty cases gave us a 2 to 5 year perspective.

We noted one death, that of a 65-year-old patient with Graves's disease. This subject was a cardiac suffering from angina pectoris. A dose of 8 mc brought about toxic symptoms and fatal heart failure.

Original language: French.

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We concluded, at that time: "The administration of radioactive iodine is a therapy which is exceptionally simple and, may we add, exceptionally elegant.

"We can indeed say there is nothing else comparable to it in the field of therapeutics.

"We do not know, as yet, what are the proper doses to be administered; these vary a great deal from one author to the next. In our opinion, this is due to the fact that, as happens with X-rays, individual sensitivity varies enormously and unpredictably." Then, further along: . . . "The differences in thyroid volume do not justify the differences in the doses. At first, we used to vary the dosage according to the volume of the goiter or the degree of toxicity. We then were disappointed by unpredictable differences in the responses. For this reason, we standardized our dosage in the vicinity of 14 to 15 mc. The results are neither better, nor worse, than those of surgery, antithyroid preparations or X-rays. Radioactive iodine solves the problems raised by toxicity without any hospitalization and without surgery. It even solves the anatomical problem in most cases of colloid or parenchymatous goiter. In fact, it is able to cause nodules to disappear where they are neither long-standing nor sclerotic."

Since then, the number of the cases we treated went to over 400, and we had no other accident.

It would not be apposite here to discuss the failures of the various therapeutic methods used in Graves's disease. In our opinion, they are due to the fact that, in most cases, Graves's disease is, above all, a neurosis complicated by a very variable degree of hyperthyroidism. It is understood that the therapies which merely address themselves to the thyroid have very little direct action on the nervous system.

Finally, it is our belief that radioiodine is indicated prior to the ablation of toxic goiters which, at the same time, cause mechanical trouble. It is thus possible to avoid surgery in a case of thyrotoxicosis. One often has the surprise of witnessing such an anatomical regression that the indication for an operation no longer stands.

Let us add, finally, that thyrotoxicosis is neither for the physicist nor for the surgeon, but rather for the experienced specialist in internal medicine.

Summing up, we have treated 400 cases of thyrotoxicosis with radioactive iodine. The method is as effective as X-rays or thyroidectomy. It is of simple application for the patient. It can bring about hypothyroidism and even—although on rare occasions—hyperthyroidism which may prove fatal, as those caused by other agents.

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The Role of Radioactive Iodine in the Treatment of Thyrotoxicosis and Carcinoma of the Thyroid

By D. E. Clark and J. H. Rule,* USA

HYPERTHYROIDISM

In 1942, Hertz and Roberts¹ and Hamilton and Lawrence² described the response of the first patients with thyrotoxicosis who were treated with radioactive iodine. Since then, successful remissions have been obtained using this isotope in many patients with hyperthyroidism. Although the result of treatment has established a definite place for radioactive iodine (I^{131}) in the treatment of selected cases, there is some difference of opinion among groups of investigators using the isotope in the treatment of thyrotoxicosis concerning the criteria for selection of patients. Miller³ and his group treat all cases of hyperthyroidism with radioiodine regardless of age or type of gland except those in which the condition is associated with pregnancy or lactation. They emphasize, however, that the wholesale treatment of children for hyperthyroidism should not be carried out and they are only administering the isotope to children as part of a long-term study. Chapman, Maloof, Maisterrena, and Martin⁴ have concluded that I^{131} should not be used in persons with either a single or multiple toxic nodular goiter; their reasons being the high incidence of carcinoma in clinically solitary nodules and that even though a multinodular goiter may diminish in size after radioiodine, it may still harbor a carcinoma.

During the past 8 years, we have had the opportunity to institute therapy in over 700 patients with primary or secondary hyperthyroidism, and in this interval we have established fairly definite criteria for the therapeutic application of radioactive iodine. These indications, with a few minor variations, agree with those of many other investigators who are using I^{131} in the treatment of hyperthyroidism.

Criteria for Radioiodine Therapy

Almost all of the patients that we have treated have met one or more of the following criteria: (1) Uncomplicated hyperthyroidism in patients over 40 years of age; (2) Recurrent or persistent hyperthyroidism after thyroidectomy; (3) Hyperthyroidism with severe cardiovascular disease or some other concurrent disease; (4) Failure to respond properly to antithyroid drugs; (5) Refusal to accept sur-

gical or other therapy; (6) Presence of severe exophthalmos.

The possibility that the radiation from radioiodine may induce carcinoma of the thyroid has been a deterrent to its use in younger patients with uncomplicated thyrotoxicosis. To date, there has been no report of any adverse radiation effects in any of the patients who have received therapeutic doses of radioactive iodine. Since it will take many years to evaluate fully any possible long-range harmful effects of internal beta radiation on the thyroid or other tissues, we advocate surgery as the treatment of choice in patients under 40 years of age with uncomplicated primary or secondary hyperthyroidism. Older individuals with a large toxic nodular goiter also are better treated with surgery than with radioiodine, providing surgical risk is not excessive.

Contraindications to Radioiodine Therapy

1. Pregnancy and lactation.
2. Clinically solitary nodule associated with hyperthyroidism.
3. Nontoxic nodular goiter.
4. Nontoxic diffuse or simple goiter.

We believe that the coexistence of hyperthyroidism and pregnancy is an absolute contraindication for radioactive iodide therapy. It has been shown that radioiodine readily crosses the placental barrier and that the fetal thyroid is able to concentrate iodine after the third month. Radioiodine has also been found in the milk of lactating women.

Because of the strong possibility of carcinoma, patients with hyperthyroidism and a clinically solitary nodule should have surgery with or without radioiodine unless the nodule can be proved to be a hyperfunctioning adenoma by suitable techniques.

It should be emphasized that I^{131} will not appreciably alter the size of a nontoxic nodular goiter.

Method of Treatment

The diagnosis of thyrotoxicosis is made on the basis of clinical judgment in addition to various laboratory procedures: namely, radioiodine diagnostic studies (conversion ratio and/or uptake), serum protein-bound iodine determination, and one or more determinations of basal metabolic rates. Laboratory evaluation is sometimes foregone in obvious cases of classical hyperthyroidism.

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One of the chief problems involved in the treatment of hyperthyroidism with radioactive iodine is the determination of a suitable dose. Many methods have been reported for estimating the minimum effective dose, but to date, none has been entirely reliable. It is extremely difficult to predict accurately the amount of radiation that any given area of thyroid tissue will receive, nor can anyone ascertain how uniformly the thyroid will take up I^{131} . Since most of the methods that have been used to determine dosage do not seem to increase greatly the accuracy of establishing the minimum effective dose and since many of them are quite time-consuming, we have continued to base our dosage on the estimated weight of gland, type of the gland, and age of the patient.

It has been estimated that a radiation dose of approximately 10,000 to 12,000 equivalent roentgens per g of thyroid tissue is required to bring about a reduction in thyroid activity. Therefore, a large gland will require much more I^{131} than a small gland. We have also found that individuals with toxic nodular thyroids require about twice as much I^{131} per estimated g of gland, to effect a remission, as individuals with a diffuse toxic goiter. Furthermore, older people seem to require more radioiodine to control thyroid activity than younger individuals.

At the present time, a dose of 150 μ c per estimated g of thyroid tissue is given as an initial dose to patients under 40 years of age with a diffusely enlarged gland, and approximately 250 μ c in patients over 40 years of age. From 300 to 350 μ c per estimated gram of thyroid tissue are given as an initial dose to patients with nodular glands.

Within this dosage range, we attempt to obtain a satisfactory remission with one dose. Some individuals prefer to give repeated small doses over a period of time in an attempt to avoid too high an incidence of hypothyroidism.

In hyperthyroid patients who have been pre-treated with iodine and/or antithyroid drugs and who are mildly hyperthyroid or euthyroid, therapy is usually deferred for four weeks following discontinuance of iodine and 48 hours following discontinuance of the antithyroid drugs.

Almost all of the patients have been treated on an ambulatory basis and have been followed at approximately eight-week intervals until a euthyroid condition developed or the need for further treatment was apparent.

Observations

Therapy has been completed in 628 patients, including 384 that have been reported previously,⁵ since the summer of 1947. A few patients who had complete remissions discontinued their clinical visits and are not included in the series. A small group of patients offered no opportunity for any follow-up after therapy either because they failed to return or the referring physician was reluctant to forward such information.

One of the big problems with this form of therapy is that many physicians, who do not have a great deal of experience in the care of patients treated with I^{131} , believe that one dose is all that is required to assure a complete remission. If remission is not accomplished by a single treatment, some will condemn the therapy or will allow the patient to continue inadequately treated.

Twenty-five in the group have expired. In all of these patients, a complete remission of the thyrotoxicosis had been accomplished and the cause of death appeared to be unrelated to the isotope therapy.

Table I. Gland Type and Therapeutic Result

Gland type	Therapeutic result			Total %
	Euthyroid	Hypothyroid	Post I^{131} recurrent	
Diffuse	214	48	(2)	262 (41.7%)
Nodular	201	28	(1)	229 (36.5%)
Post surgical recurrent	105	32	0	137 (21.8%)
Total (%)	520 (82.8%)	108 (17.2%)	(3)	628 (100.0%)

* These cases have all been treated again with I^{131} and are included in the euthyroid groups.

Of the 628 cases followed carefully, the ratio of females to males was 3.5 to 1. Table I shows the therapeutic results in this group. Of the 262 patients with a toxic diffuse goiter, 214 became euthyroid and 48 hypothyroid. Two of the cases had a recurrence 2 and 4 years after a euthyroid state had been established. Two hundred and twenty nine cases of toxic nodular goiter were treated, 201 became euthyroid and 28 hypothyroid. A recurrence developed in one case 5 years after it had been treated successfully with I^{131} . Post-surgical recurrent thyrotoxicosis was seen in 137 cases, 105 had an excellent response, and 32 became hypothyroid following I^{131} therapy.

In all, 520 or 82.8 per cent of the patients became euthyroid. A total of 108 or 17.2 per cent developed varying degrees of hypometabolism, and the recurrence rate was approximately 0.5 of 1 per cent. The incidence of hypometabolism may seem high, but in many of these cases the condition is extremely mild; perhaps some might not be considered to be hypothyroid by less critical standards. Furthermore, some of the patients showed a transitory hypothyroidism that lasted from a few months to a year or more. It is possible that, in time, many of the milder cases of hypometabolism will not require any substitution therapy. A fair number of the patients were purposely rendered hypometabolic in an attempt to improve their cardiac status; if one excludes the latter group and the extremely mild cases, the incidence of hypometabolism drops to 11 per cent.

Table II shows the length of follow-up period and the therapeutic results. None of the patients treated in the latter part of 1954 are included. The highest

Table II. Length of Follow-up and Therapeutic Result

<i>Year treated</i>	<i>Euthyroid</i>	<i>Hypo-thyroid</i>	<i>Total</i>	<i>% Hypo-thyroid</i>	<i>Re-currence</i>
1947	3	3	6	50.0	
1948	34	3	37	8.1	
1949	75	14	89	15.7	2
1950	105	25	130	19.2	
1951	105	27	132	20.4	1
1952	88	18	106	16.9	
1953	72	14	86	16.3	
1954	38	4	42	9.5	
Total	520	108	628		3
(%)	(82.8)	(17.2)	(100)		(0.5)

incidence of hypometabolism occurred among the patients treated during 1950 and 1951. This may be explained by the fact that recently we have observed a gradual development of hypometabolism in several patients who were treated during 1950-51. These patients had been euthyroid for several years, which suggests that, in some patients, the biological effect is a gradual one that acts over a period of years.

Table III. Number of Doses of I^{131} Required for Remission

<i>Number doses</i>	<i>Gland type</i>			<i>Total patients</i>	<i>%</i>
	<i>Diffuse</i>	<i>Nodular</i>	<i>Recurrent</i>		
1	164	102	86	352	56.1
2	52	75	33	160	25.5
3 or more	46	52	18	116	18.4
Total	262	229	137	628	100.0

The number of doses required to effect a remission is shown in Table III. Only one dose was necessary for 352 or 56.1 per cent of the patients; two doses were required for 160 patients or 25.5 per cent; and three or more doses for the remainder. There was complete remission with one or two doses in 81.6 per cent of the 628 patients.

Patients with toxic diffuse goiter received doses ranging from 1.8 to 60 mc, with an average of 11.3 mc. The patients with toxic nodular glands required an average of 20.5 mc to effect a remission, with a range of 3.0 to 140 mc.

The course of exophthalmos following I^{131} therapy is shown in Table IV. There were 189 patients with exophthalmas of varying degree. An increase in the

Table IV. Course of Exophthalmos Following I^{131} Therapy

<i>Exophthalmos</i>	<i>No. of patients</i>	<i>%</i>
Complete regression	49	30.8
Improved	88	55.4
No change	16	10.1
Worse	1	0.6
Developed after therapeutic response	5	3.1
Total evaluated	159	100.0
Inadequately evaluated	30	
Total cases of exophthalmos	189	(30.1% of total pts.)

exophthalmos was observed in only one patient following therapy. We were unable to evaluate adequately 30 patients. These were being followed elsewhere, and we were unable to determine whether the exophthalmos had improved or remained unchanged. Of the 159 patients who were evaluated satisfactorily, 49 or 30.8 per cent had complete regression; 88 or 55.4 per cent showed varying degrees of improvement; and in 16 or 10.0 per cent it was thought that the degree of exophthalmos remained the same. In five cases, post-therapeutic exophthalmos developed 6 weeks to 17 months after administration of the radioiodine.

It has been our impression that the improvement in exophthalmos with radioiodine therapy is somewhat better than with surgery.

Untoward Effects

1. Tenderness of the thyroid gland. A moderate number of patients have noted some tenderness in the region of the thyroid gland following radioiodine therapy. This usually appeared 1 to 3 days after the administration of I^{131} and continued for several days to a week. Furthermore, some patients complained of a sense of constriction in the neck beginning 5 to 6 weeks after therapy and continuing for a considerable period of time.

2. Transient gastritis. Some patients complained for a few days following therapy of symptoms suggestive of gastritis.

3. Localized pretibial myxoedema. Three patients developed localized pretibial myxoedema after they had become euthyroid. They had no other manifestations of hypometabolism.

4. Hypothyroidism.

5. Post-therapeutic exophthalmos. There has been no radiation sickness and no evidence of bone marrow depression or injury to the parathyroids or kidneys. Menstrual abnormalities have not been observed. Some of the patients subsequently became pregnant and delivered healthy children.

Summary

Radioiodine is an excellent means for treating selected cases of thyrotoxicosis and offers many advantages. There is no mortality, no parathyroid destruction, and no recurrent nerve damage. Its cost is relatively inexpensive compared with that of surgery. No hospitalization is required, virtually no time is lost from work, and the administration is painless and produces an excellent cosmetic result. Furthermore, the recurrence rate is negligible, the incidence of hypometabolism is comparable to that observed following surgery, and the isotope should be effective in all cases of true hyperthyroidism if given in adequate amounts.

Once the fear of the possibility of its carcinogenic action in the thyroid has been alleviated, it should largely replace surgical thyroidectomy for Graves' disease unless a better and nondestructive method of therapy is forthcoming.

CARCINOMA

At one time radioiodine was heralded by the lay press as a possible panacea for carcinoma of the thyroid that was not amenable to surgery. Now we know that it has a limited but, we believe, a definite role in the management of such lesions.

In order to obtain a beneficial response from I^{131} , the cancer cell must have an affinity for the isotope. In other words, the cell must have the function of concentrating iodine similar to that of a normal thyroid cell.

In the early days of our program, which is now going into its 8th year, all cases of thyroid carcinoma were treated which could not be satisfactorily cared for by radical surgery. Since not all carcinomas of the thyroid have an avidity for I^{131} , various procedures such as ablation of thyroid by surgery and/or I^{131} , administration of thiourea compounds and thyrotropic hormone, were employed in an attempt to stimulate some of the tumors to pick up the isotope. As our work progressed, we concluded that certain types of tumors would probably not respond to radioactive iodine therapy, and its use in such cases was probably not warranted. We also thought that we could ascertain fairly well which types of tumor might be affected by careful study of the morphological picture. At the present time, all of our cases are screened on the basis of the pathological type. If a definite decision cannot be made from careful morphological examination, then studies to determine whether the tumor has any avidity for iodine are performed or attempts are made to stimulate the tumor cells to take up the isotope. If no uptake is found, such cases are not treated with I^{131} at this time. In the past, some of these patients were given 35 mc of I^{131} every 2 weeks for a period of 6 months to learn whether some benefit might be observed. The results were very discouraging.

The classification of thyroid cancer that we use is a relatively simple one. There are four main groups:

- A. Papillary carcinoma: (1) true, (2) mixed
- B. Alveolar and follicular adenocarcinoma
- C. Hürthle cell carcinoma
- D. Undifferentiated carcinoma: (1) small cell, (2) large cell, (3) giant cell, (4) epidermoid.

Pathology As It Relates to the Use of I^{131}

A true papillary carcinoma from a patient who received 0.5 mc of I^{131} 48 hours prior to surgery is shown in Fig. 1. Survey, radioautographs of tissue removed, and direct counting of a known amount of digested tumor tissue failed to reveal any localization. Unfortunately, none of the cases of this type has shown an avidity for I^{131} , nor have we been able to stimulate such a type to take up radioiodine. It is doubtful whether I^{131} is warranted in such cases.

Others have also failed to find evidence of I^{131} concentration in cases with predominantly papillary tumors by radioautographic technique and direct counting of the tissue.

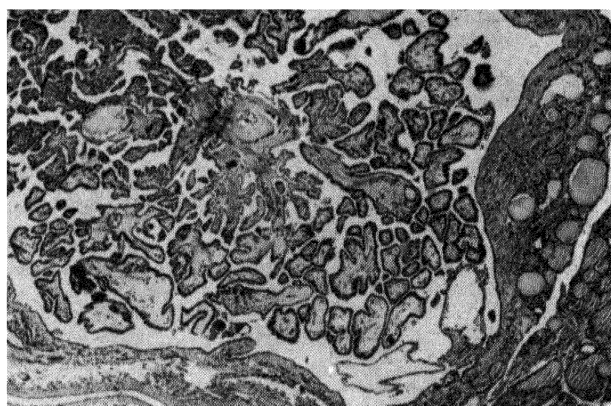


Figure 1. A true papillary carcinoma of the thyroid composed almost entirely of papillary elements. There is no acinar or colloid production. Such a tumor is not functioning

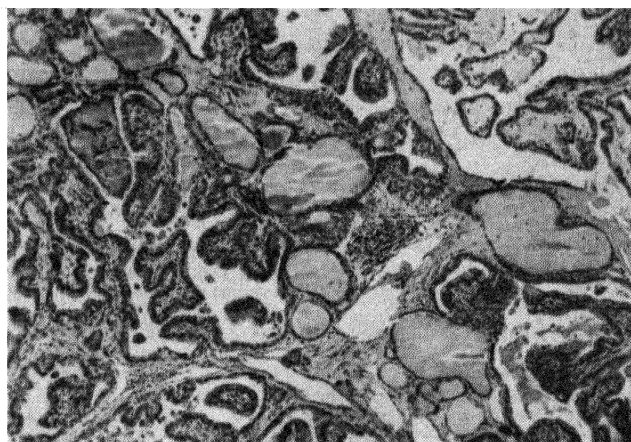


Figure 2. A mixed papillary carcinoma of the thyroid. Twenty-five per cent or more of this tumor is composed of acini with colloid formation

Figure 2 shows a mixed papillary carcinoma. Twenty-five per cent or more of the tumor was made up of acini with colloid formation. This tumor is one that should be functioning and taking up I^{131} . The specimen was taken from a male, age 41, who had a left radical neck resection in October 1947. Thirty months later (April, 1950), a recurrence was noted in the lower neck. Forty-eight hours after a tracer dose, the nodule was removed. Direct counting of the tissue and examination of radioautographs revealed a good localization of I^{131} in the tumors. Even though there was no clinical evidence of any residual carcinoma, he was given 35 mc of radioiodine every 2 weeks for a period of 6 months, receiving a total of approximately 450 mc. A fair number of cases with this type of tumor have been treated prophylactically with a similar dosage schedule. He has remained clinically free of any further recurrence since the radioiodine course, a period of approximately five years.

The radioautograph in Fig. 3 shows that the localization of radioiodine is spotty and corresponds to areas of maximum colloid formation; there is no localization in the papillary areas. When one carefully examines the microscopic sections and corre-

sponding radioautographs, it is evident that some of the glandular areas do not concentrate iodine. This is one of the principal reasons why we treat carcinomas of the thyroid with multiple moderate doses at regular intervals of time. It is believed that in the tumors that concentrate iodine, the cells have a "phasic function" similar to that of the thyroid. Therefore, by the administration of repeated doses, the cells functioning at the time of one dose would be affected and subsequent doses would affect other cells or follicles as they began to function.

We realize fully that in such tumors there is little chance of affecting the papillary elements of the tumor, but individuals with this type of tumor should probably be given the benefit of I^{131} not only as a therapeutic measure, but as a prophylactic one also.

An example of an alveolar type of carcinoma of the thyroid is shown in Fig. 4. It is a biopsy specimen taken from a 42-year-old woman, 48 hours after she had received a tracer dose of radioiodine. The tumor is well differentiated; there are acini and colloid formation. This type of tumor always has an avidity for I^{131} .

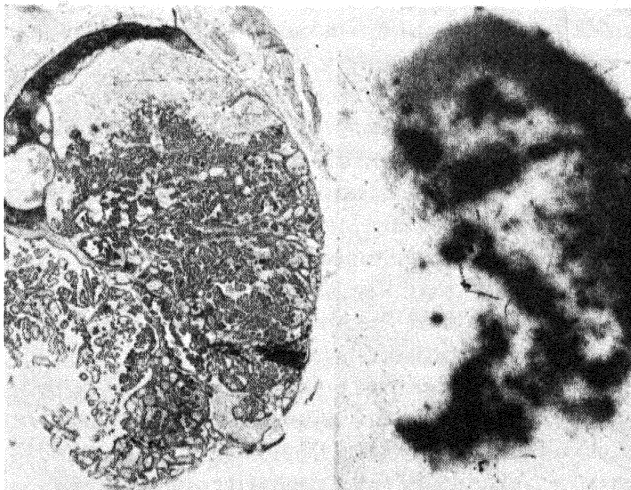


Figure 3. A photomicrograph and corresponding radioautograph of mixed papillary carcinoma of the thyroid. Localization is spotty and corresponds to areas of maximum colloid formation

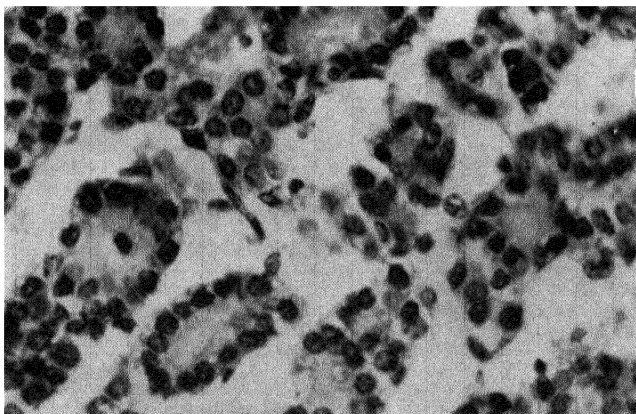


Figure 4. An alveolar carcinoma of the thyroid. The morphological appearance is similar to that of normal thyroid. The acini are well formed and there is an abundance of colloid

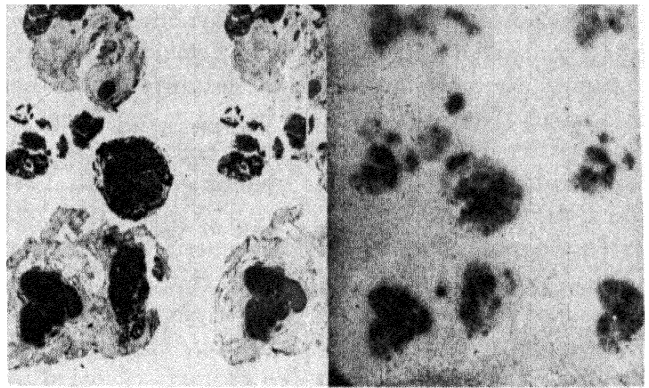


Figure 5. A low power photomicrograph and a radioautograph of the alveolar carcinoma. There is definite localization of radioiodine

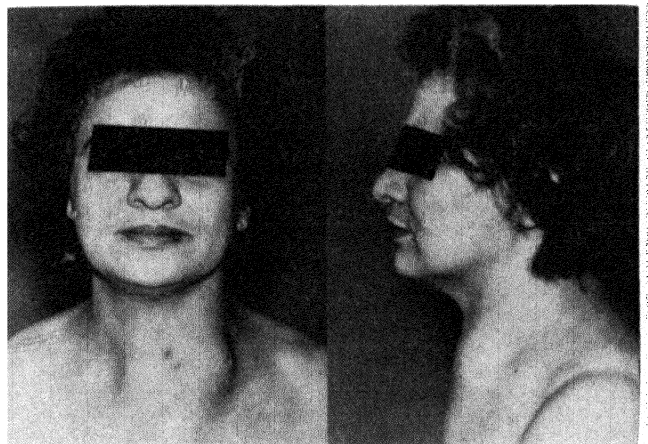


Figure 6. Patient prior to radioiodine therapy. Note large mass in lower anterior neck and over manubrium of sternum

Figure 5 shows a radioautograph made from the tumor. Direct counts of the tissue also revealed considerable concentration of I^{131} , and an *in vivo* external survey showed marked localization over the tumor mass in the neck and over the mediastinum. This patient had two thyroidectomies, one at the age of 13 and the second at the age of 40. The second thyroidectomy in January, 1948, revealed carcinoma. We first saw her in December, 1948, at which time she had a large recurrent mass, which was growing rapidly, in the lower neck, and a mass involving the manubrium of the sternum (Fig. 6). A roentgenograph of the chest (Fig. 7) revealed large mediastinal masses that were thought to represent metastatic lesions since there was marked localization of the tracer dose over the mediastinum. She was started on I^{131} therapy in January, 1949, receiving approximately 35 mc of I^{131} every 2 weeks for a period of about 2 years. She was given a total of approximately 1500 mc; her therapy was discontinued about 4½ years ago. At the conclusion of her therapy the masses in her neck and over the manubrium had completely disappeared to external palpation (Fig. 8); the lesion in the manubrium of the sternum was much smaller and showed marked sclerosis; and the mediastinal masses had completely disappeared as far as could be ascertained by roent-

genographs (Fig. 9). At the present time she is enjoying excellent health and her hypometabolism is being controlled with 2 grains of thyroid every day.

Figure 10 shows a photomicrograph from another case of an alveolar carcinoma from a young man, 22 years of age, who had had several operations including a radical neck resection sometime between 1944 and 1947 (Fig. 11).

He was first seen at the University of Chicago Clinics in December, 1947, at which time he complained of headaches, back pain, diplopia, anorexia, nausea, and vomiting. He had lost 11 pounds in weight. Examination of his eye grounds revealed bilateral papilloedema of 3 to 4 diopters. There were no localizing neurological signs. A diagnosis was made of an unlocalized, expanding, intracranial lesion, probably metastatic from the thyroid. He was given a tracer dose of I^{131} , and on survey 24 and 48 hours later, an area of localization was found in the left occipital region (Fig. 12).

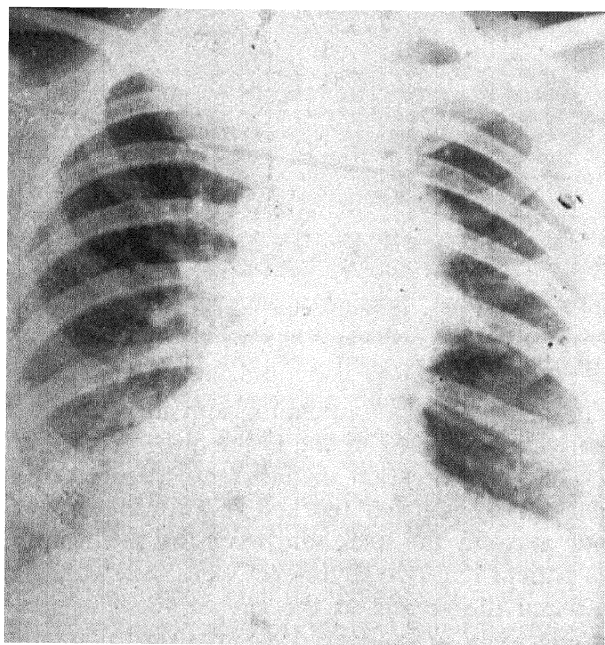


Figure 7. Roentgenograph of chest. Note large mediastinal masses. A large mass is also present in left superior mediastinum deviating the trachea to the right.

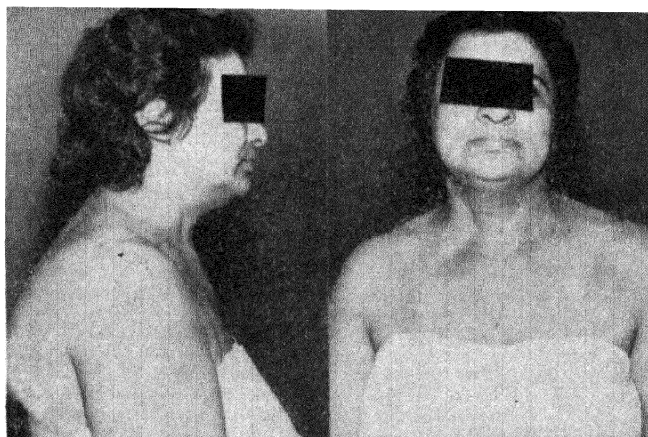


Figure 8. Patient at conclusion of radioiodine treatment. Masses have completely disappeared.

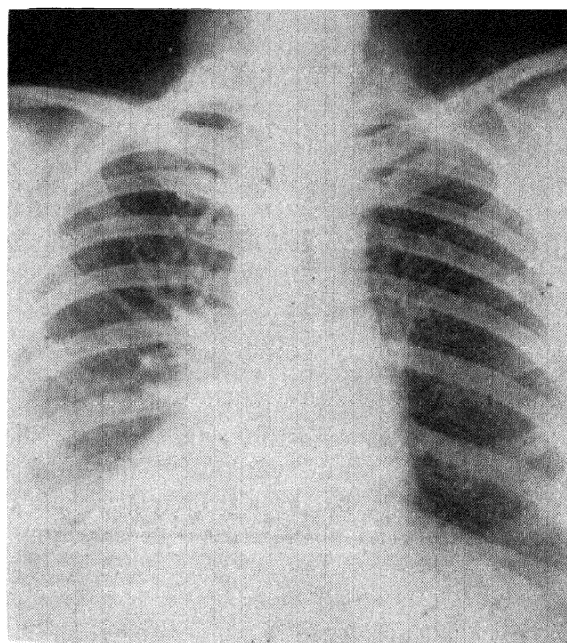


Figure 9. Roentgenograph of chest. Mediastinal masses have disappeared. Trachea is almost in midline.

He was treated with multiple doses of I^{131} , receiving a total of 237 mc. His condition improved under therapy and by March, 1948, he felt well. An *in vivo* survey following a tracer dose of I^{131} at that time failed to reveal any localization. He has remained well for over 7 years and is without any clinical evidence of recurrence at this time.

All of our cases of alveolar and follicular carcinomas that have been treated therapeutically have benefited from the isotope therapy and are still living. One case has recently shown a progression of a metastatic lesion in the spine, and radioiodine treatment was reinstituted.

Since this tumor has a predilection to metastasize to the skeletal system, we feel that all patients with this type should receive I^{131} not only therapeutically but prophylactically following surgery.

An example of a Hürthle cell carcinoma is shown in Fig. 13. These cells have no resemblance to the cells of the thyroid. No acini or colloid formations

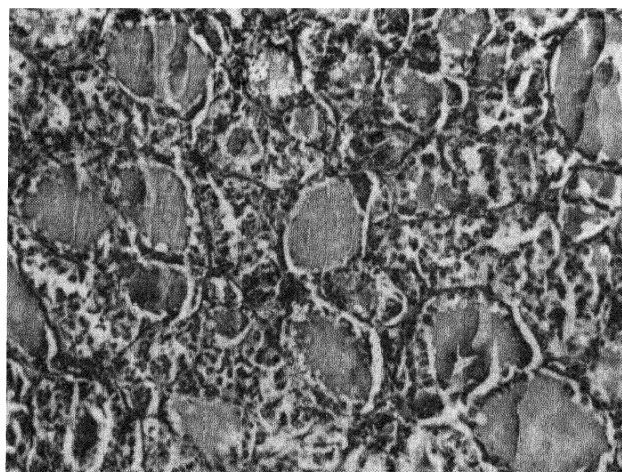


Figure 10. An alveolar carcinoma. Note acini and colloid formation.

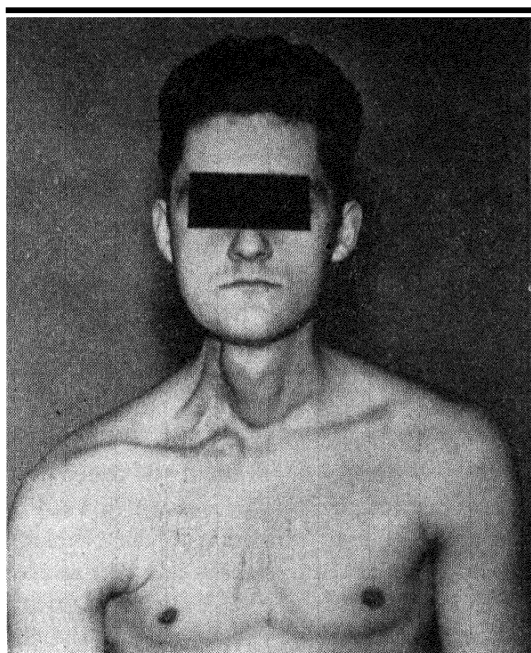


Figure 11. Photograph of patient. Note incision from radical neck resection

are present. This type of tumor does not function, nor have we been able to stimulate such a tumor to concentrate I^{131} . This has been the general experience of other groups. It is doubtful whether these tumors will benefit from I^{131} therapy.

Figure 14 shows a photomicrograph of an undifferentiated carcinoma of the thyroid. No beneficial effect from I^{131} has been observed in individuals with this type of tumor, nor has the clinical course been altered. Such a tumor is not functioning nor have we been able to stimulate such tumors to take up I^{131} . All of the patients who have been treated therapeutically are dead except two, and, at the present time, such cases are not being given radioiodine.

On the basis of our experience, many patients with mixed papillary carcinoma and alveolar or follicular carcinomas should benefit from I^{131} therapy where surgery has failed. Some instances require the employment of techniques to enhance the avidity. It is also possible that the incidence of recurrence in these types of carcinoma may be materially reduced by the administration of a prophylactic course of radioiodine immediately following adequate surgery.

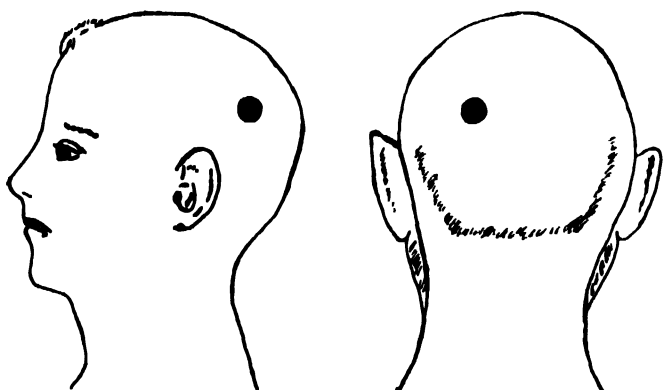


Figure 12. Area of localization of radioiodine in left occipital region

Treatment

There is considerable variation in the methods of treating carcinoma of the thyroid. Some groups ablate the normal thyroid either surgically or with I^{131} , wait for a period of time, then give a course of thyrotropic hormone (25 to 30 mg I.M. every day for a period of 5 to 6 days) in an attempt to further enhance the uptake of I^{131} , and then administer a large dose of radioiodine. Other groups, after ablation of the thyroid, give thiouracil compounds, such as thiouracil (1 to 1.5 gm every day) for a long period of time and give massive doses of I^{131} only after it is found by *in vivo* studies or urinary excretion studies of a tracer dose that a large per cent of the dose is retained. Another group gives moderately large doses at irregular intervals of time. The method we employ is to give moderate doses at regular intervals over a long period of time. Crile and his associates at the Cleveland Clinic are using a similar method.

For an individual with a carcinoma of the thyroid that is thought not to be amenable to surgery and for whom it is decided to consider I^{131} treatment, the

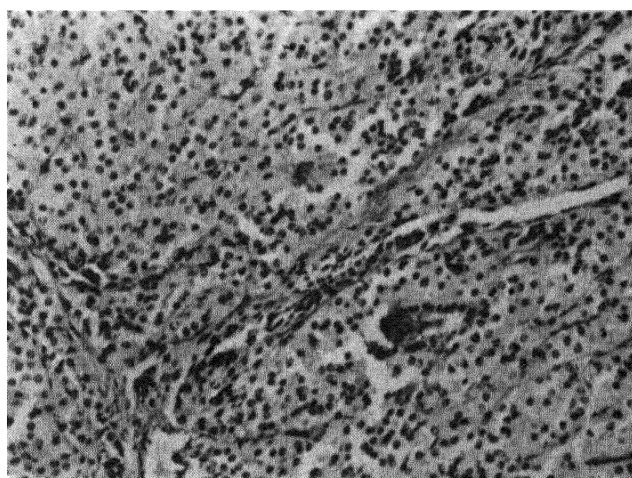


Figure 13. Photomicrograph of a Hürthle cell carcinoma. There is no acinar or colloid formation

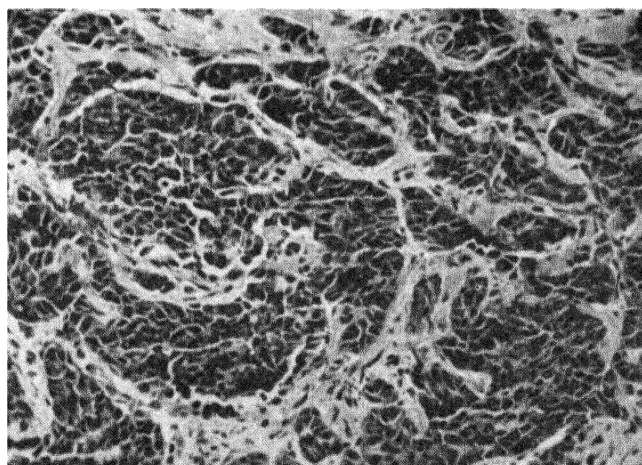


Figure 14. Photomicrograph of an undifferentiated carcinoma of the thyroid. This type of tumor has no avidity for I^{131}

following program is usually carried out. Careful blood studies are made. Necessary roentgenographs are made. The patient is then given a tracer dose of I^{131} orally. In most cases this is usually 0.5 to 1 mc. In 48 hours, the patient may be surveyed to determine whether there has been any localization in the metastatic lesions. Also, at this time one of the metastatic lesions, if accessible, or a portion of the primary, if still present, is removed under local or general anesthesia. The specimen that has been removed is divided into three portions. One portion is weighed and either slowly digested with sodium hydroxide and then counted, or homogenized, and an aliquot of the homogenate is counted in a Texas-well counter. A frozen section is made of the second portion, and the tissue slice is counted and then applied to a nuclear track plate for radioautography. The third portion is used to make permanent microscopic sections. If the tumor is a true papillary carcinoma, Hürthle cell type, or an undifferentiated type and there is no concentration of I^{131} , the patient is not selected for therapy. If the tumor is a mixed papillary type or an alveolar type and there is a concentration of I^{131} , the patient is then given 35 mc every 2 weeks. The therapy is continued until there is no longer any localization of I^{131} that can be detected by external survey or until it is thought that the patient has obtained maximum clinical benefit. The duration of the therapy is variable—in some, it has been continued for as long as 2 years. The course of the patient during therapy is followed by roentgenographs, tracer studies, and when feasible, biopsies for histopathologic and radioautographic studies. Peripheral blood studies are performed at regular intervals in order to evaluate possible radiation effects on hematopoietic function. In many cases, repeated sternal marrow biopsies are performed. Urinalyses are also made at intervals.

Except when very ill, these patients are ambulatory. At the termination of therapy, thyroid extract is administered to control hypothyroidism, and the patients are then seen at periodic intervals for follow-up studies. Thyroid extract is withheld during the course of treatment with the hope that a hypometabolic state will increase the production of thyroid-stimulating hormone by the pituitary, which will enhance the uptake of I^{131} by the tumor. Occasionally, the patients complain so bitterly of the symptoms produced by the hypometabolism that small doses of thyroid extract are given during the latter part of the therapeutic period.

In some patients with an inoperable mixed papillary carcinoma or an alveolar carcinoma, the tumor will *not* concentrate I^{131} . In such cases, the tumor cells usually can be stimulated to concentrate I^{131} by ablation of the thyroid. At the present time, we are using I^{131} rather than surgery to eliminate the normal thyroid. Thus, such cases are treated with 35 mc of I^{131} every 2 weeks and their clinical course is evaluated in the way described previously. The

Table V. Present Status of Patients Treated with Radioiodine. Therapeutic

Type	Total no. cases	Number benefited	No. living not benefited	Number Expired
Papillary:				
True	5	0	1	4
Mixed	17	15	0	2
Alveolar	11	11	0	0
Hürthle	4	0	1	3
Undifferentiated	16	0	2	14

period of treatment lasts for at least 6 months. If, at that time, there is no clinical evidence that the patient is benefiting from the isotope therapy or we have not been able to demonstrate that ablation of the thyroid by the radioiodine has stimulated the tumor to concentrate iodine, therapy is discontinued. If the patient seems to be responding to the therapy or if there is definite evidence of localization of I^{131} in the tumor, therapy is continued until it is thought that a maximum benefit has been obtained.

Thiourea compounds and thyrotropic hormone may be employed to further enhance the uptake of I^{131} after ablation of the normal thyroid gland and are being used quite extensively by others. We feel, at the present time, the most important aspect in stimulating such tumors to take up I^{131} is ablation of the normal thyroid and we are not using any of these agents.

The rationale for eliminating the normal thyroid is: First, the normal thyroid has a greater affinity for iodine than malignant tissue, and its elimination affords the tumor a greater opportunity to concentrate the isotope; second, athyroidism may cause the tumor to function and thus concentrate iodine.

Patients who have had recent adequate surgery for a localized tumor of a mixed papillary or an alveolar type are given radioiodine prophylactically. These patients have no clinical evidence of recurrence or distant metastases. They receive 35 mc of I^{131} every 2 weeks for a period of 6 months. It is realized that this study will be very difficult to evaluate, but it is hoped that, over a long period of time, we will be able to determine whether the incidence of recurrence in such cases has been reduced materially by the prophylactic course of radioiodine.

The best way to treat carcinoma of the thyroid with radioactive iodine is not known as yet. It is gratifying that various methods are being studied thoroughly by different groups. The overall period of observation is still relatively short, but perhaps in the future, some unanimity will evolve as to the best course of therapy.

Results and Comments

The present status of patients treated therapeutically with radioiodine is shown in Table V. Five cases with true papillary carcinoma have been

Table VI. Present Status of Patients Treated with Radioiodine. Prophylactic

Type	Total no. cases	No. living without recurrence	No. living with recurrence	Number Expired
Papillary:				
True	5	2	3	0
Mixed	14	14	0	0
Alveolar	13	13	0	0
Hurthle	1	0	1	0
Undifferentiated	2	1	0	1

treated. Four have expired and one is living but did not obtain any benefit from the isotope. Seventeen cases with mixed papillary carcinoma have been treated. Of this group, fifteen were thought to have been benefited, and two have expired. One of the patients who expired also had a carcinoma of the breast, and her death was due to the breast carcinoma. Eleven cases with alveolar carcinoma have been treated and all gave an excellent therapeutic response. None of the four cases with Hürthle cell type were benefited, and three have expired—one from a cause other than the tumor. Fourteen of the sixteen cases with an undifferentiated carcinoma have expired. Two who were treated more recently are living, but were not benefited by a long course of I^{131} .

Table VI shows the present status of patients treated prophylactically with radioiodine. Five cases with true papillary carcinoma have received such treatment; three are living with recurrence, two without. Fourteen cases of mixed papillary carcinoma have been treated prophylactically. So far as we know, none has developed a recurrence since the I^{131} therapy. A similar course has been observed in the 13 cases with alveolar carcinoma. One of the two patients with an undifferentiated carcinoma is living without recurrence. This case had a localized lesion, and it is possible that surgery completely eradicated the tumor.

The time followed since the onset of I^{131} therapy in the therapeutic group is shown in Table VII. Fifteen cases of mixed papillary carcinoma were thought to be benefited by the treatment and are still living; one has been followed for 1 year, three for 2 years, five for 3 years, two for 4 years, two for 5 years, and two for 6 years. Of the 11 cases treated for alveolar carcinoma, one has been followed for 5 years, three for 6 years, five for 7 years, and two for 8 years.

Table VII. Time Followed Since Onset of I^{131} Therapy. Therapeutic

Type	No. cases	Years							
		1	2	3	4	5	6	7	8
Mixed papillary	15	1	3	5	2	2	2	0	0
Alveolar	11	0	0	0	0	1	3	5	2

Many more years of observation will be required before it can be established how many of these have been "cured." Perhaps none has been cured, but most of them have been benefited and have had added years of comfortable living. Some still have residual tumor, others have no clinical evidence of any remaining tumor.

Table VIII shows the time followed since the onset of I^{131} therapy in the prophylactic group. Of the 14 with mixed papillary carcinoma, one has been followed 1 year, one for 3 years, two for 4 years, six for 5 years, and four for 6 years without clinical evidence of recurrence. Of the alveolar group, three have been followed 1 year, two for 2 years, two for 3 years, one for 4 years, three for 5 years, and two for 6 years without clinical evidence of recurrence.

The patients in the prophylactic group have all received between 400 and 450 mc over a period of 6 months.

In the therapeutic group, the individuals with alveolar carcinoma have received from 180 to over 1500 mc. Five have received between 1000 and 1500 mc and five over 1500 mc. The individuals with mixed papillary carcinomas have received 230 to 2218 mc.

Although a fair number of our cases have been women in the premenopausal period, only one case of amenorrhea developed during therapy. This was a woman, age 43, in whom the amenorrhea developed after 450 mc had been given. It is possible the I^{131} was not the cause of the amenorrhea. One girl, 18 years of age, has received over 2000 mc without any disturbance of the menstrual cycle.

Three of the cases have had pregnancies; two post-therapy. One became pregnant while she was undergoing treatment. She had received over 350 mc of I^{131} prior to conception; therapy was immediately discontinued. All have borne normal children.

There has not been any clinical evidence of toxic effects on the pituitary, parathyroid, kidney, bladder, or liver in any of the cases. Nor have clinical signs and symptoms of hyperthyroidism appeared during I^{131} therapy. Most of our cases of alveolar carcinoma in the therapeutic group have had very large amounts of I^{131} before signs and symptoms of hypometabolism developed. In one case, hypometabolism did not develop until after the patient had received 1400 millicuries of I^{131} . This evidence suggests that the tumor continues to function and produce thyroxine until it is completely or almost completely destroyed. In individuals with no demonstrable functioning tumor, a hypometabolic state may occur after 2 or 3 doses.

Table VIII. Time Followed Since Onset I^{131} Therapy. Prophylactic

Type	No. cases	Years							
		1	2	3	4	5	6	7	8
Mixed papillary	14	1	0	1	2	6	4	0	0
Alveolar	13	3	2	2	1	3	2	0	0

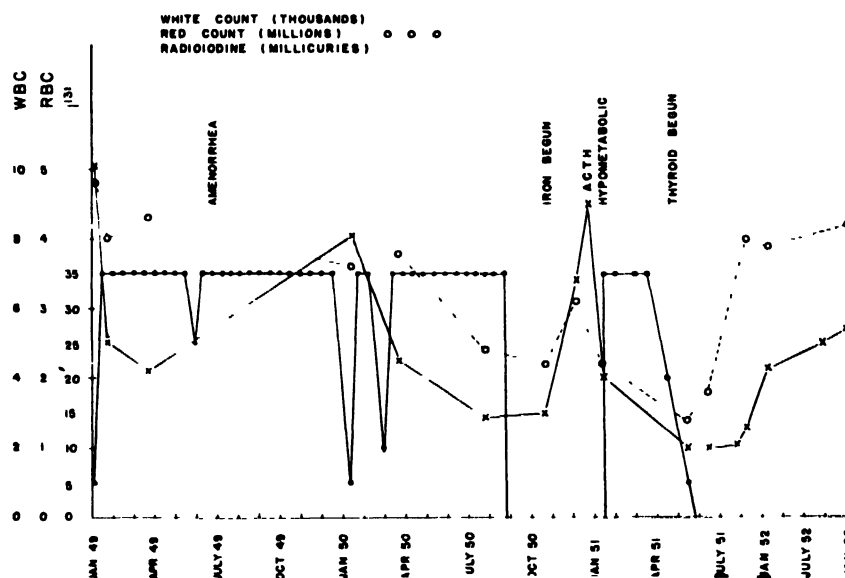


Figure 15. Changes in the red blood and the white blood cell counts in a patient who received over 1500 mc I^{131}

A fair number of our cases have shown moderate to severe depression of the peripheral blood elements. Almost all of these have occurred in patients who have received over 500 mc. All have returned to normal after the cessation of I^{131} therapy except one who has a persistent leukopenia. Some of our cases have received over a 1000 mc without any

demonstrable change in the blood elements. Studies of the bone marrow have shown a marked aplasia in some of the patients who have received the larger cumulative doses. No serious complications have developed in any of our patients that could be ascribed to the effects of irradiation on the hematopoietic system.

Figure 15 shows the changes in the red and white blood cell counts in a patient who received over 1500 mc of I^{131} . There were no demonstrable changes until she had received approximately 1000 mc. ACTH had no effect on the count. Cell counts gradually returned to normal levels after I^{131} was discontinued. The bone marrow changes are shown in Figs. 16 and 17. In this patient, the bone marrow became quite aplastic after 1000 mc. Most of the marrows that have been studied showed remarkable regeneration after therapy was discontinued.

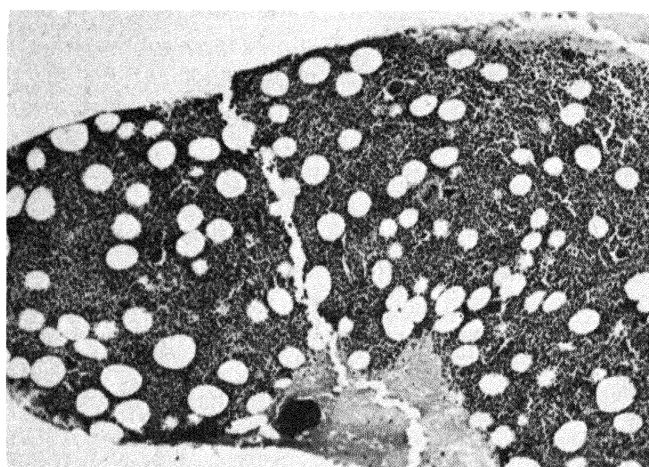


Figure 16. Bone marrow of the patient prior to I^{131} therapy. Note that its appearance is normal

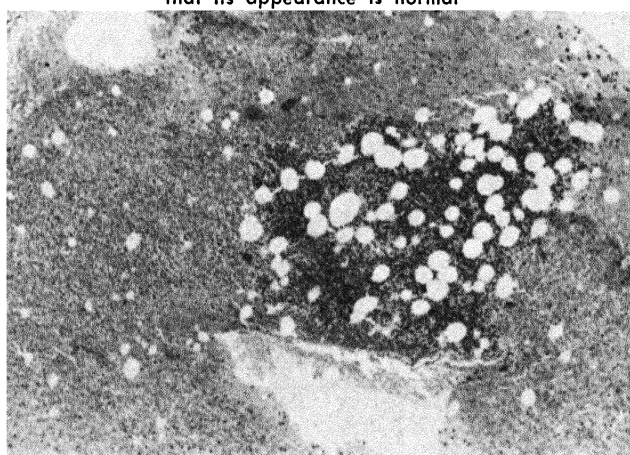


Figure 17. Aplastic bone marrow after 1000 mc of I^{131}

CONCLUSIONS

Carcinoma of the thyroid is still a surgically-treated disease, but we believe that radioactive iodine has a limited but definite value for the treatment of certain cases.

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Hypothyroidism Induced by I^{131} in the Treatment of Euthyroid Patients with Intractable Angina Pectoris and Congestive Failure

By H. L. Blumgart, A. S. Freedberg and G. S. Kurland,* USA

The induction of hypothyroidism by I^{131} , to lessen the work of the heart, has been studied by us since 1947 in the treatment of certain patients with intractable angina pectoris and congestive failure.¹ Our present method of treatment was suggested by the fact that the work of the heart is lessened in myxedema and that alleviation of congestive failure and angina pectoris has long been witnessed in thyrotoxic patients when the metabolic rate is lowered from hyperthyroid to normal levels.^{2,3} The rationale of the treatment is, in brief, that lowering the total metabolism of the body so reduces systemic circulatory requirements as to place them within the limit of cardiac reserve.

We believe that the radioiodine treatment of angina pectoris and congestive failure is necessary only for that small group of patients who remain disabled and in great discomfort despite all standard measures of therapy. For most patients with angina pectoris and congestive failure, medical treatment successfully alleviates discomfort and provides increased capacity for a satisfactory program of life. The 87 patients who have been treated by us since 1947 had been incapacitated for many months or years prior to treatment. Each patient was euthyroid as attested by clinical criteria, basal metabolic rate, serum cholesterol thyroid uptake or tracer doses of I^{131} , and, in some instances, by serum protein bound iodine determinations.

The clinical course of our patients had been stationary or only slightly worse during the months immediately prior to treatment; consequently, any striking improvement could be confidently attributed to I^{131} treatment. For each patient, the pretreatment level of disability may be considered as a comparative control for that case. The methods we have employed in the course of our studies have been described in previous communications.^{4,5,6}

We felt that ultimate evaluation of this therapeutic measure would be furthered by the results of independent studies in various clinics. A questionnaire has been distributed, therefore, to all clinics in the United States using radioactive iodine. The results

of I^{131} treatment of euthyroid patients with advanced cardiac disease have been accumulated and analyzed. The entire group consists of 1298 patients, 87 of our own series and 1211 patients reported through the cooperation of 49 other clinics.

The results of treatment were evaluated as excellent, worthwhile and not worthwhile.

An *excellent* result denotes that the patient is markedly improved over pretreatment status, with either no recurrence of symptoms or a marked decrease in the frequency and severity of angina pectoris or congestive failure, despite markedly increased activity; in many instances rehabilitation and return to gainful employment occurred.

A *good* or *worthwhile* result denotes definite improvement, with a decrease in frequency and severity of attacks of angina pectoris or congestive failure on the same amount of activity as before treatment. In the remainder of the patients, there was no worthwhile improvement.

ANGINA PECTORIS

The results in the 835 patients with angina pectoris, approximately 200 of whom also had evidences of congestive heart failure, are shown in Fig. 1. Seventy-eight per cent of the patients treated elsewhere showed worthwhile improvement; 39% showed excellent results, and 39% showed good results. Twenty-two per cent showed no worthwhile result. Our own experience, shown in the right hand columns of Fig. 1, is similar.

CONGESTIVE FAILURE

The results in the treatment of 463 patients with congestive failure are shown in Fig. 2. Sixty-eight per cent of the patients treated elsewhere showed worthwhile improvement; 27% showed excellent results, and 41% showed good results. The remainder, 32%, failed to show improvement. Our own experience, denoted in the right hand columns of Fig. 2, is quite similar.

It should be noted that the over-all results of treatment are not as gratifying in congestive failure as in angina pectoris; however, more than half of the patients showed worthwhile improvement, and 27%

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experienced an excellent result. The duration of follow-up after hypothyroidism in our own series was from 2 to 84 months, with an average of 30 months. Approximately half of the patients treated elsewhere have been observed for more than 18 months.

CRITERIA FOR SELECTION OF PATIENTS

Patients with angina pectoris who may be expected to gain the greatest benefits from treatment with I^{131} are those in whom the disease, regardless of its severity, has been relatively stationary or only slightly progressive over a period of one year or more. Patients with congestive failure should show evidence of some cardiac reserve, such as improvement in signs of congestive failure and other symptoms on bed rest or with the use of diuretics and digitalis. A basal metabolic rate of more than -10% is desirable. It is important that the patient be alert, cooperative, and emotionally stable. It has been our experience that hyperactive, tense patients are particularly favorable candidates.

The very recent onset of angina pectoris or of status anginosus, or other evidence of rapidly progressive cardiovascular disease, is considered by us a contraindication to I^{131} therapy. In this connection it should be emphasized that we have no reason to believe that hypothyroidism retards the progression of the underlying pathological process. The treatment of heart disease with I^{131} is not to be considered as curative; it is a palliative procedure that is to be regarded as an adjunct to recognized medical measures. It is unwise to treat patients who are in terminal phases of their disease, particularly since two to six months intervene before the hypometabolic state is attained. Some clinics, in contrast to our own earlier experience, have not regarded rapidly increasing angina pectoris or congestive failure, intermittent claudication, or low metabolic rate as contraindications to treatment; further detailed studies are required to settle these points.

Several clinics reported the use of single doses of I^{131} as high as 50 mc. Reports from those clinics

indicate, as might be anticipated on the basis of earlier experience, that some of their patients suffered temporary aggravation of their angina pectoris or congestive failure; there were a few deaths. In prior reports, we and others have observed that, with unduly large doses, radiation thyroiditis may be associated with release of relatively large amounts of thyroid hormone into the blood stream, with temporary hyperthyroidism.⁷ This is manifested by clinical evidences, by lowering of the cholesterol level, by rise in basal metabolic rate, and by increased serum protein bound iodine. That such seriously ill persons cannot tolerate this temporarily increased burden is not surprising.

Our practice, therefore, has been to give patients with I^{131} uptakes in the euthyroid range, such as 30%, an initial dose no larger than 20 mc and, in the instance of those with higher uptakes or those who suffer attacks of angina pectoris while at rest in bed or during the night, to utilize smaller doses of 10 mc. These doses are usually given at weekly intervals for three doses. The period during which the complicating temporary hypermetabolism may occur is usually the second or third week of treatment; it is, therefore, our practice to insist that all patients with angina pectoris decubitus restrict their activities during the three weeks after the initiation of therapy. At one to two month intervals additional single doses are given until hypothyroidism becomes manifest. Since adoption of the above program, thyroiditis has not been a serious problem. Mild, transitory thyroiditis occurs in approximately one-third of the patients. In one clinic, one of three patients given I^{131} therapy while taking bishydroxycoumarin (Dicumarol) suffered hemorrhage into the thyroid and died.

The use of single large doses has evidently been motivated by a desire to shorten the period of treatment and to secure earlier onset of hypothyroidism. Similarly, pretreatment with the thiourea derivatives or with thyroid-stimulating hormone has been used. Our experience and that of others seem to indicate

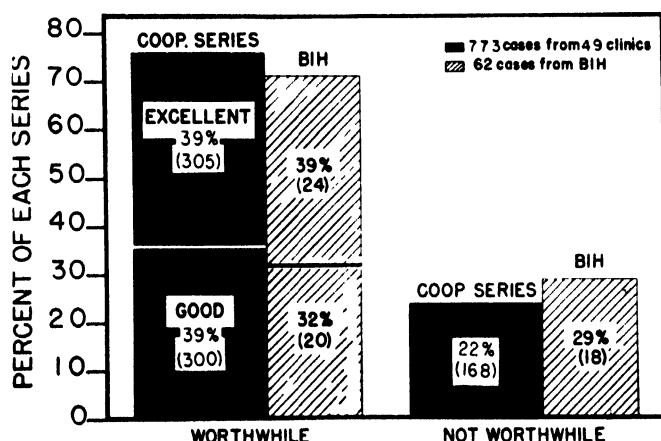


Figure 1. Results of radioactive iodine treatment of 835 euthyroid patients with angina pectoris in a cooperative series and in a group of cases studied at Beth Israel Hospital

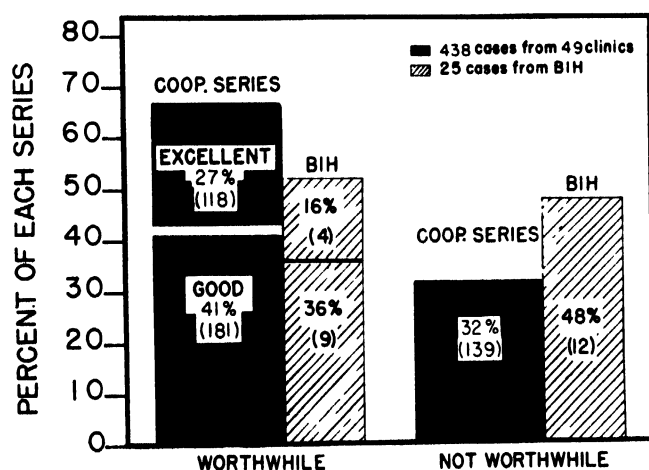


Figure 2. Results of radioactive iodine treatment of 463 euthyroid patients with congestive heart failure in a cooperative series and in a group of cases studied at Beth Israel Hospital

that the use of these measures to produce more widespread and sudden destruction of the thyroid may be hazardous.

In patients whose uptake is unduly low because of prior ingestion of iodine or who have received iodine for purposes of intravenous pyelography or for cholecystography, the deiodination of the thyroid by means of thiourea derivatives may be employed. Tracer studies should then be instituted and the dosage of I^{131} calculated in accordance with the result of such studies. No toxic effects of radiation have been observed. Extensive studies of the blood and urine have revealed no abnormal findings. No clinical or laboratory evidence of parathyroid insufficiency has appeared. Pathological studies of the radiation effects⁷ on the thyroid have been reported. The parathyroids, evidently protected successfully by their capsules, showed no morphological changes.

The onset of improvement was coincident with the occurrence of hypothyroidism and was usually two to six months after the initiation of therapy. The irregular interval of eight weeks to six months after the first treatment with I^{131} before hypothyroidism becomes evident is an additional safeguard against the possible effect of suggestion; the time at which hypometabolism and clinical improvement will occur cannot be foretold by the patient or the physician. The clinical improvement, first noted by the patient and later found to be coincident with the inception of hypometabolism, when the results of the serum-cholesterol and basal-metabolic determinations are made available, is consequently the more impressive.

The first clinical intimations of incipient hypothyroidism may consist of one or more of the following: slight fullness or puffiness of the face; increased sensitivity to cold; or slight stiffness of the joints, arthralgia, or paresthesias. Concomitantly, the patient usually reports that the attacks of angina are milder or less frequent or that dyspnea and orthopnea are diminished. A daily dose of desiccated thyroid, 6 or 12 mg, should then be administered. In each patient, thyroid dosage must be adjusted to maintain him at the lowest metabolic level at which he experiences the maximum relief from his cardiac disease and the minimum discomfort from myxedema. Occasionally this may not be possible, the patient showing little or no improvement over his pretreatment status when sufficient thyroid is administered to obviate the discomfort. Most patients are maintained with little discomfort at a level approximately -20 to -25% on a daily dose of 6 to 30 mg of thyroid. Occasionally, patients may suffer a recurrence of their symptoms because residual thyroid tissue has regenerated and caused return of the metabolic rate to pretreatment levels. In such instances, thyroid is withdrawn, and, after tracer doses show adequate uptake of I^{131} , an additional therapeutic dose is administered. Besides these considerations, the same careful supervision and therapeutic program indicated for all patients with heart disease are essential.

HYPERCHOLESTEREMIA, MYXEDEMA, AND ARTERIOSCLEROSIS

The question naturally arises whether the high cholesterol values and myxedema dispose the patient to an increased progression of arteriosclerosis. The situation is not strictly analogous to untreated complete myxedema, since these patients receive small doses of thyroid. In this connection we have reviewed the clinical course and postmortem findings of patients who survived from 1 to 11 years after surgical total thyroidectomy and in whom hypometabolism with elevated cholesterol values was present. We have been particularly interested in the younger patients with rheumatic heart disease. In them, only slight or minimal coronary arteriosclerosis would ordinarily be anticipated at death. If decided arteriosclerotic lesions were observed post mortem in such patients, the lesions might well be attributed to the myxedematous state. In a study reported elsewhere,⁸ the clinical and postmortem findings are reported of eight such patients with rheumatic heart disease or cor pulmonale in whom hypothyroidism or myxedema was present and who survived from 1 to 13 years (average, 7.4 years) following surgical total thyroidectomy. None of the eight cases showed complete occlusion of any of the coronary arteries; five of the eight showed no narrowing of any of the main stems or branches of the coronary arteries; only three of the eight showed slight narrowing of one of the main stems. In the other arteries, damage from atheromatosis and atherosclerosis varied greatly, but conditions were similar to those usually observed in similar euthyroid patients.

Our results do not, of course, disprove a role of cholesterol in the production of atherosclerosis. These results do demonstrate, however, that over the observed period of time the hypothyroid state, controlled with small daily doses of desiccated thyroid, is not necessarily, in itself, sufficient cause for the production of coronary arteriosclerosis.

Studies of the cardiovascular system in spontaneous myxedema have demonstrated an increased size of the cardiac silhouette, diminished force of cardiac contraction, decreased cardiac output and velocity of blood flow, diminished plasma and total blood volumes, and lowered voltage of the electrocardiogram. There is considerable difference of opinion regarding the clinical significance of these changes; in most cases, cardiac function remains adequate. The "myxedema heart," in the sense of a condition precipitating congestive failure, as described by Fahr,⁹ occurs rarely. Extensive studies in our patients¹⁰ indicate that the "myxedema heart," in the sense of a condition precipitating angina pectoris or congestive failure or accompanied by marked changes demonstrable on electrocardiographic tracings or roentgenograms, does not occur in patients with prolonged, controlled hypothyroidism induced by administration of radioactive iodine.

The question naturally arises whether I^{131} treatment of heart disease prolongs the duration of life. Statistical analysis of our data does not yield a decisive answer to this question. It appears that duration of life of these severely ill patients has not been decreased and may have been prolonged;¹¹ future studies may well clarify this point.

CONCLUSIONS

1. Hypothyroidism can be regularly induced in euthyroid patients by one or more appropriate doses of radioactive iodine.

2. After such treatment in 50 clinics of 1298 patients with intractable angina pectoris or congestive failure, approximately 75% of the 835 patients with angina pectoris showed worthwhile improvement (half of these showed marked improvement and half a good result).

3. Sixty-eight per cent of the 463 patients with congestive failure showed worthwhile improvement (approximately 27% of these showed marked improvement and 41% moderate improvement).

4. In the clinical management of these patients, small daily doses of thyroid are usually administered to maintain the lowest metabolic rate consistent with comfort.

5. In intractable cardiac cripples who are usually considered for cardiac surgery hypothyroidism induced by radioactive iodine gives improvement through medical means without risk and complications of surgery.

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Physiological and Biochemical Studies of Astatine-211 (Eka-Iodine)

By J. G. Hamilton, P. W. Durbin, C. W. Asling and M. E. Johnston,* USA

The use of the cyclotron for the production of heretofore unknown elements resulted in 1940 in the positive identification of element 85, among others, the heaviest of the halogens. The instability of its isotopes, all radioactive, with half-lives of from a few seconds to 8.3 hours, earned it the name "astatine" (from the Greek *astatos*, unstable). For the past eight years part of the program of the Crocker Laboratory 60-inch Cyclotron has been the production of At^{211} , the alpha-particle-emitting 7.3-hour isotope,† in quantities sufficient to support a sustained inquiry into its chemical and biological properties. (The decay of the 8.3-hour At^{210} to a long-lived polonium radioisotope makes it unsuitable for biological experimentation.) Because of the juxtaposition of astatine and iodine in the periodic table, and because of the relationship of iodine to thyroid physiology, the investigations in this laboratory have been focused upon the selective accumulation of astatine by the thyroid gland.

The discovery of At^{211} by Corson, MacKenzie, and Segrè² was closely followed by studies of its thyroidal accumulation by Hamilton and Soley.³ The chemistry of astatine has been discussed by Johnson *et al.*⁴ and by Garrison *et al.*⁵ At^{211} is selectively accumulated by the thyroid glands of rats, guinea pigs, monkeys, and man, but to a lesser degree than I^{131} .^{3,6,7,8} The metabolism and acute toxicity of At^{211} were initially reported by Hamilton *et al.*⁶ It was demonstrated that certain of the nuclear characteristics of At^{211} (70-micron tissue range and 7.3-hour half-life) in some respects gave it advantages over I^{131} (2000-micron tissue range and 8.1-day half-life) in the capacity to deliver an intensive irradiation in a restricted region.^{6,10,11} (An attempt has been made to utilize these properties in the treatment of an epithelial cyst in the anterior chamber of the eye.⁹)

Further comparative studies of At^{211} and I^{131} have shown that the thyroidal accumulation of both may be increased by pituitary thyrotrophin (TSH)³ and by a low-iodine diet,⁶ or may be decreased by exo-

genous thyroxine,¹² by high iodine intake,⁶ and by thiocyanate.¹² An interesting exception to these similarities is the enhancement of astatine retention (rather than the lowering of the iodine retention) following the administration of goitrogens of thiouracil type.^{8,13}

It is the purpose of this paper to indicate some of the potentialities of At^{211} as a tool in biological research by the presentation of two specific experiments: (a) the chemical state of At^{211} in the thyroid gland; and (b) radiodestruction of the thyroid gland by At^{211} as a function of dose and time.

The At^{211} used in these experiments was separated from the bismuth target by a modification of the method of Garrison *et al.*⁵ It was injected intravenously as At^- in physiologically normal saline. The valence state was stabilized by the addition of sodium sulfite. The rats used were females of the Sprague-Dawley strain, injected when 55 days of age (160 to 180 grams body weight). All animals were maintained for at least two weeks prior to use on tap water and either Purina Lab Chow or "Diet 14" of the University of California Institute of Experimental Biology.‡ All radioassay procedures used were standard and are described by Hamilton *et al.*⁶ Histological specimens were prepared by Bouin or neutral 10% formalin fixation, dioxane dehydration and clearing, paraffin embedding, and hematoxylin and eosin staining.

THE CHEMICAL STATE OF At^{211} IN THE THYROID GLAND

A Preliminary Report

Investigation of the possible organic binding of At^{211} by the thyroid gland was stimulated by three observations: (a) the concentration of At^{211} in the thyroid gland of the rat 18 to 24 hours after its intravenous administration is several hundred times that in the plasma;^{6,8} (b) the thyroidal accumulation of At^{211} as a function of time closely resembles that of I^{131} under similar conditions;⁶ and (c) some preliminary experiments suggested that the At^{211} was closely associated with the thyroid protein.¹⁴ The experiments described are studies of the thyroidal accumulation of At^{211} in the presence of the anti-

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† At^{211} is produced by the bombardment of bismuth by 29-Mev alpha particles: $\text{Bi}^{209}(d,2n)\text{At}^{211}$. Sixty per cent of the At^{211} nuclei decay by K-capture to form Po^{211} with a 0.3-second half-life, and 40% decay by the emission of 5.9-Mev alpha particles to form Bi^{207} .¹

‡ Purina Lab Chow and "Diet 14" contain 1.2 ppm and 2.9 ppm of iodine respectively.

Table I. The Leaching of At^{211} from the Thyroid Glands of Normal and PTU-treated Rats in Histological Fixatives. Values Are Expressed As Per Cent of Total Thyroid At^{211}

Fixative	Per cent leached	
	Normal thyroid	PTU thyroid
80% ethanol, pH 6-7	59.2	—
10% neutral formalin, pH 5	7.9	11.5
10% formalin in 95% ethanol, pH 6-7	57.5	85.9
Tellyeniczky's fluid (5% formalin and 5% glacial acetic acid in 70% ethanol), pH 5-6	17.1	16.5
Bouin's fluid (25% formalin and 5% glacial acetic acid in saturated picric acid), pH 2	3.3	2.9

thyroid compounds, the propyl thiouracil (PTU) and thiocyanate ion (KSCN), the partition of At^{211} between the protein and nonprotein fractions of thyroid homogenates, and the extent to which At^{211} is leached by various histological fixatives.

Procedures

Groups of 10 to 20 rats were prepared as follows: (A) controls: no pretreatment; (B) KSCN: all animals received a single subcutaneous injection of 0.2 ml of a 10% solution of KSCN 1.5 hours before sacrifice; (C) PTU: tap water was replaced by a 0.1% solution of PTU for 11 days prior to the injection of At^{211} and I^{131} ; and (D) PTU and KSCN: animals on the PTU water were given a single subcutaneous injection of KSCN as in Group (B). All the animals received $10\ \mu\text{C}$ of At^{211} , and the control and PTU-treated groups also received $2\ \mu\text{C}$ of I^{131} . After 18 hours, thyroids were removed and homogenized in normal saline; cellular debris was discarded. For At^{211} and (or) I^{131} determinations, an aliquot was taken from each thyroid homogenate. The remainder of each homogenate was treated with

an equal volume of either 20% trichloro-acetic acid (TCA) or a saturated solution of ammonium sulfate. Protein precipitates thus obtained were washed once with their precipitating reagent and assayed.⁶

For histological preparations, thyroids were removed from two rats of each group seven hours after At^{211} administration and placed in the fixatives listed in Table I. After eight hours of fixation, the At^{211} activity of the thyroid glands of their fixing and dehydrating agents was determined by X-ray counting.

Results

Table II again demonstrates that PTU increased the thyroidal accumulation and retention of At^{211} . When KSCN was given 1.5 hours before sacrifice, about 30% of the At^{211} was discharged from the normal thyroid gland and nearly 70% from the PTU-treated gland.

The per cent of soluble radiohalogen associated with thyroid protein of both normal and PTU-treated rats is shown in Table III. The results obtained for the I^{131} in both normal and PTU-treated thyroids with the two reagents tested agreed with the findings of Chaikoff and Taurog,¹⁵ so that despite the small number of samples, the data presented appear to be reliable. The amount of I^{131} associated with the protein depended upon the extent of the organic binding of I^{131} , and not on the protein precipitant employed. The behavior of At^{211} was generally similar to that of I^{131} in the normal thyroid gland (controls), although the per cent of At^{211} associated with the thyroglobulin (ammonium sulfate precipitable) was lower than the per cent of I^{131} . In the PTU-treated glands, however, the behavior of At^{211} from that of I^{131} was quite different. Trichloro-acetic acid precipitated the same proportion of At^{211} from PTU-treated glands as from the controls. The amount of At^{211} precipitated from these glands by ammonium sulfate was about 60% of the control value. In con-

Table II. The Effects of Pretreatment with PTU and KSCN on the Thyroidal Accumulation of At^{211} and I^{131} Rats. Values Are Expressed as Per Cent of Administered Dose and Are Presented with the Standard Error of the Mean*

Group and no. of rats	At^{211}		I^{131}	
	%/organ	%/gram	%/organ	%/gram
1-Control (20)	0.78 ± 0.03	50.6 ± 2.4	12.6 ± 0.69	811 ± 52
2-KSCN (8)	0.56 ± 0.04	34.8 ± 1.6	—	—
3-PTU (21)	1.53 ± 0.06	47.0 ± 2.1	0.42 ± 0.03	13.3 ± 1.0
4-PTU + KSCN (10)	0.59 ± 0.04	14.7 ± 0.9	—	—

* Standard error of the mean, S.E. = $\pm \sqrt{d^2/n(n-1)}$.

Table III. The Extent of Association of At^{211} with the Protein of the Thyroid Gland Compared to I^{131}

Group and no. of rats	Per cent saline soluble halide in thyroid gland precipitated by			
	10% TCA		Half-saturated $(\text{NH}_4)_2\text{SO}_4$	
	At^{211}	At^{211}	I^{131}	I^{131}
1-Control (7)	90.0 ± 5.7	93.0 ± 5.1	69.9 ± 4.3	97.5 ± 2.6
3-PTU (7)	90.0 ± 5.7	21.8 ± 1.8	44.3 ± 5.7	24.9 ± 6.7
4-PTU + KSCN (4)	72.4 ± 3.4	—	71.9 ± 1.1	—

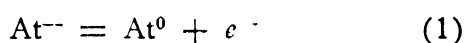
trast, the I^{131} precipitated by both the TCA and ammonium sulfate was about 25%.

The leaching of At^{211} from normal and PTU-treated thyroid glands by five histological fixatives is shown in Table I. The astatine was more readily leached from PTU-treated than from normal thyroid glands. This halogen appeared to be more firmly "fixed" by solutions of pH less than 2. Alcohol-based fixatives apparently increased the leaching of At^{211} . It was shown previously that following Bouin fixation, up to 20% of the At^{211} but none of the I^{131} was removed when alcohol was used as a dehydrating agent.^{6,16} Leaching of either astatine or iodine was negligible during dioxane dehydration, irrespective of the fixative employed.

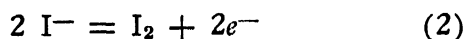
Discussion

It has been clearly established that the same factors influence the accumulation of At^{211} and I^{131} , and to a similar degree.^{6,8,12} In the case of I^{131} iodide is presumably oxidized to iodine, which is then organically bound.¹⁵ It is more difficult to understand what happens to astatide after it is collected by the thyroid gland. The large thyroid-to-plasma ratios for At^{211} 8 to 48 hours after administration^{6,8} indicate that the At^{211} in the normal thyroid gland is to a great extent combined in some manner with the thyroid protein.

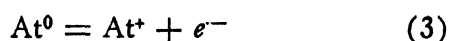
The data presented show that in the normal thyroid gland about two-thirds of the At^{211} is rather firmly bound or "stable," and about one-third is quite labile except in the presence of an organic acid, in which case At^{211} is apparently "fixed" firmly to the denatured protein. The position of astatine in the periodic table, and the present knowledge of its chemistry,⁴ make it seem likely that the oxidation potential of the reaction



is less negative than for the reaction



Therefore, it is fairly certain that the normal thyroid gland is capable of oxidizing the At^{211} injected as At^- to At^0 . Elemental astatine possesses certain metallic properties² and, as a metal, could chemically adsorb onto the protoplasm of the thyroidal epithelial cells. (The decay of the 8.3-hour At^{210} to a long-lived polonium radioisotope makes it unsuitable for biological experimentation.) If the normal thyroid gland is capable of further oxidizing astatine from the elemental to a positive valence state,



then there is the additional possibility of the formation of fairly stable complexes of At^+ with the intracellular protein or with the colloid. Autoradiographs presented by Hamilton *et al.*⁶ and the extent of the association of At^{211} with the thyroglobulin fraction suggest that the "stable" At^{211} in the normal thyroid gland is bound in some manner to this protein. The

labile fraction may be At^0 chemically adsorbed onto the cellular protein, or may even be astatide ion; iodide ion has been shown to exist in the thyroid gland to some extent.¹⁷

Hamilton *et al.*⁶ and Shellabarger and Godwin,⁸ have shown that the administration of PTU or thiouracil for subacute periods enhances the thyroidal uptake of astatine. Shellabarger¹⁸ has further shown that the thyroid glands of rats given thiouracil for 18 hours accumulated less At^{211} than did control rats. This would rule out direct action of the thiouracils on the accumulation of astatine by the thyroid gland. The goitrogen, PTU, prevents synthesis of thyroid hormone,¹⁹ but does not interfere with the ability of the thyroid gland to collect iodine.¹⁷ The ensuing hypothyroid condition results in hypersecretion of TSH. This, in turn, results in hypertrophy and hyperplasia of the thyroid gland and activation of the thyroid iodide-concentrating mechanism.^{17,20} Potentiation by PTU of the effect of TSH on the iodine-trapping mechanism, as postulated by Halimi *et al.*,²¹ could account for the enhanced astatine uptake observed in subacute thiouracil treatment.

The Vanderlaans have shown that the administration of thiocyanate after the organic binding of I^{131} discharges little or no iodine.¹⁷ In this experiment, KSCN discharged approximately 70% of the astatine from the PTU-treated thyroid glands, indicating that most of the astatine initially present was in a labile form. This lability was further emphasized by the increased leaching of astatine from the PTU-treated thyroid glands by certain histological fixatives. The major portion of the astatine in these glands may be in one of the forms previously suggested for the "labile" fraction of astatine in the normal thyroid, as astatide ion by analogy to the above mentioned findings for iodide¹⁷ or as a chemically adsorbed At^0 . The prevention of thyroid hormone production by the thiouracils appears to be due to inhibition of oxidative enzymes involved in the iodination of tyrosine.¹⁰ The oxidation of astatide to astatine, however, may still proceed to the point where some of the At^0 is formed, provided that the energy necessary to drive Reaction (1) is sufficiently small. *In vitro* experiments support the possibility that the administration of KSCN could reverse Reaction (1), releasing At^{211} from the thyroid gland as astatide. The energy necessary for further oxidation of At^0 to At^+ makes it unlikely that astatine exists in these glands as a true metal-protein complex.

The stability of the At -protein combinations at low pH's is very likely the result of the exposure of new and perhaps more stable astatine-binding sites in the denaturation of the protein.

RADIODESTRUCTION OF THE THYROID GLAND BY At^{211} AS A FUNCTION OF DOSE AND TIME

The objective of this study was to establish the acute and chronic manifestations of the varying degrees of damage to the thyroid gland that would

be produced by increasing dosages of At^{211} . Most experimental groups consisted of five to ten rats. The degree of damage was estimated in each case (a) functionally, by measuring the ability of the remaining thyroid tissue to take up a tracer dose of I^{131} ; and (b) structurally, by weight and histological study of the tissue.

Results

Table IV shows the per cent of the administered I^{131} found 24 hours after intramuscular injection of 5 to 10 μc tracer dose. It will be seen that the iodine uptake diminished as the dosage of At^{211} increased, reflecting the functional damage to the gland. The maximal effect on iodine uptake of functional damage developed within six days, was reached at the 1.2 μc dose level.[§] This level is close to the MID_{50} .¹¹ Over a longer period of time, however, lower doses were effective in bringing about equivalent impairment of function, as shown by the low iodine levels of the 0.8 μc At^{211} groups after 40 days and 1 year. This suggests that the earliest phenomena of damage following At^{211} irradiation of the thyroid are related to impaired cellular physiology, while the subsequent processes reflect cell death and glandular atrophy. To explore this concept, analysis of the degree of structural damage at the different doses and time intervals was necessary.

The weights of the glands removed six days after astatine administration underwent only slight reduction from control values, even at the highest dose level. These weights do not fully represent the gland, however, since edema and extravasation of blood were common and resulted in a misleadingly high weight. The weights a year after astatine administration are more representative of the actual tissue; a marked reduction below control value was achieved even at the lowest dosage group (0.4 μc).

§ It will be noted that it was possible to check the completeness of thyroid destruction by use of a tracer I^{131} dose six days after At^{211} administration. This indicates a possible advantage of At^{211} over I^{131} as "thyroidectomizing" agent in endocrine experiments. When thyroid destruction is achieved by I^{131} weeks must pass before such checking, owing to the residual radioactivity in the neck region from the long-lived I^{131} .

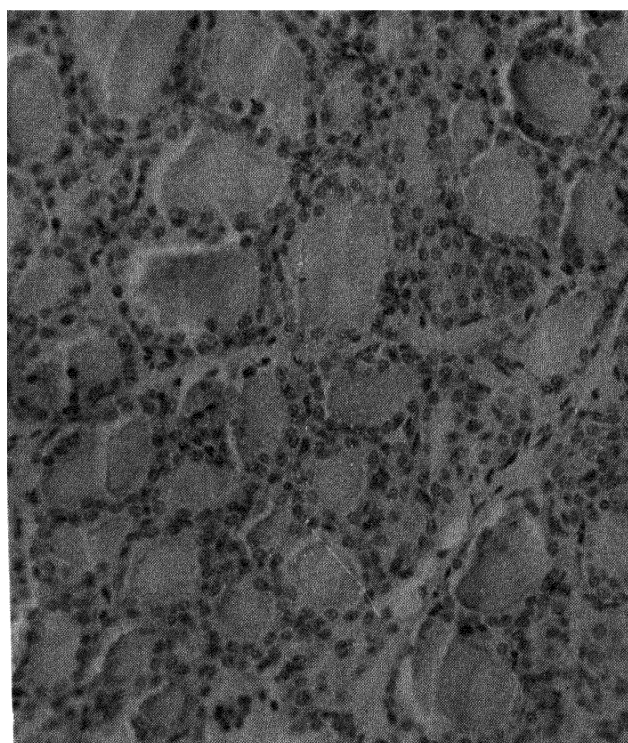


Figure 1. The thyroid of a normal 55-day old rat illustrates the condition in the controls at the time of At^{211} injection ($\times 267$)

Histologically, the thyroids taken six days after administration of the lower dose levels showed particularly interesting structure (Fig. 2, to be compared with a normal thyroid, Fig. 1). The epithelium of the follicles was unusually high. The abundant vacuolation of the margins of the colloid (especially in the larger peripheral follicles) suggested very active resorption. The abundant capillary vasculature was dilated. Evidences of cell destruction were quite scanty. This structure characterized not only the glands from the 0.8- μc dose level, but also some from an experiment (not tabulated) in which only 0.5 μc of At^{211} per gram of body weight was administered. The impression was that of a very active gland. It is conceivable that functionally these glands were not secreting adequate amounts of normal thyroid hormone, and that the pituitary was, in consequence, putting out excessive TSH, to which the cells could still respond structurally.

Table IV. Mean Weight and 24-hour Iodine Uptake of Residual Thyroid Tissue of Rats Following At^{211} Administration

Dosage At^{211} $\mu\text{c/gm}$ body wt	No. of rats	6 days		Post-injection interval 10 days		1 year	
		Thyroid weight, mg	% I^{131} Uptake	No. of rats	% I^{131} Uptake	Thyroid weight, mg	% I^{131} Uptake
Controls	6	14.3 $\pm$ 0.5	9.0 $\pm$ 0.7	10	16.7 $\pm$ 0.9	9	26.0 $\pm$ 1.4
0.4	—	—	—	3	4.8 $\pm$ 2.1	7	10.6 $\pm$ 1.0
0.6	—	—	—	—	—	5	11.6 $\pm$ 1.7
0.8	6	12.7 $\pm$ 0.4	4.1 $\pm$ 0.7	3	0.8 $\pm$ 0.3	6	9.3 $\pm$ 0.9
1.2	6	11.3 $\pm$ 0.3	1.3 $\pm$ 0.1	—	—	1	—
1.5	5	13.2 $\pm$ 0.4	0.3 $\pm$ 0.1	—	—	—	—
1.8	5	11.3 $\pm$ 0.6	1.3 $\pm$ 0.2	—	—	—	—

* Data for 40 day group taken from that published by Hamilton *et al.*,⁶ thyroid weights unavailable.

† No thyroid tissue was found at autopsy. The I^{131} uptake was measured on neck tissues *en bloc*.

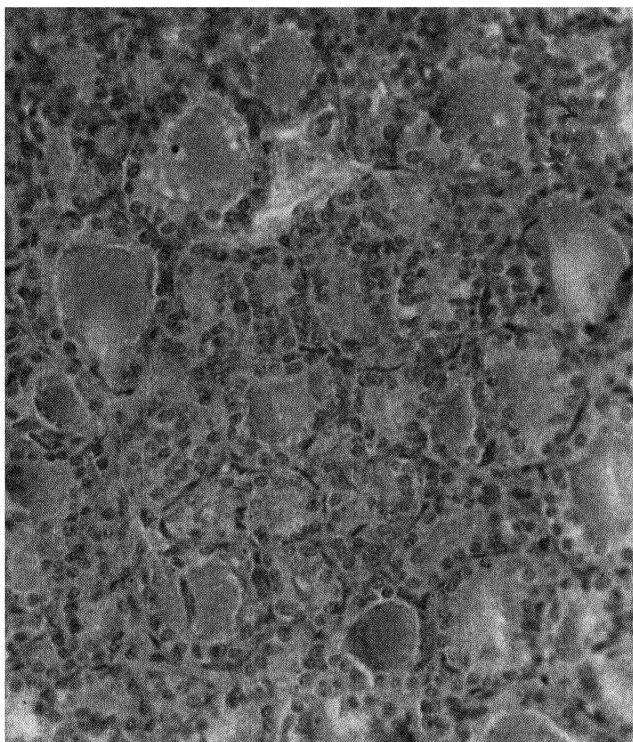


Figure 2. Six days after administration of $0.8 \mu\text{c At}^{211}$ /gm body weight, the thyroid shows heightened epithelium, colloid resorption, and an abundant supply of capillaries ($\times 267$)

At the $1.2\text{-}\mu\text{c}$ dose level (six day interval) the thyroid tissue was divided between follicles like those above, and other follicles in which the cells were arranged as dense sheets or cords. Some proliferation was evident; on the other hand, areas of cell death were also found.

At the $1.5\text{-}\mu\text{c}$ dose level, unqualified evidence of tissue destruction was found (Fig. 3). The greater part of the epithelium was disintegrating, and in patches only amorphous substance appeared at the former site of follicles. There could still be found a variable but small number of structurally normal follicles. Some even showed heightened or proliferating epithelium. The stroma was markedly altered, showing edema, fibrinous exudation, extravasation of blood, and an abundance of macrophages.

At the $1.8\text{-}\mu\text{c}$ dose level, some specimens showed structure like that of the $1.5\text{-}\mu\text{c}$ group. Other specimens, however, showed more extensive epithelial destruction, and the stroma was more profoundly affected.

In the chronic studies (one year after At^{211}) appreciable glandular damage was evident even at the lowest dose level. Substantial areas of fibrosis were seen in which macrophages containing golden-brown pigment were often found. Many well-vascularized follicles could also be found, however, containing intact epithelium (medium to high cuboidal cells) and colloid. These follicles were usually small, whereas in the controls they were mostly medium to large. The impression was that of a gland in which much of the thyroid tissue had disappeared;

the residual tissue was possibly under increased stimulation by TSH.

The next two levels ($0.6 \mu\text{c}$ and $0.8 \mu\text{c}$) showed greater damage. There was a progressive increase in the proportion of fibrotic tissue, and the epithelial elements tended more to be dense cell clusters than follicles.

Histologic studies were also made from 11 "thyroids" obtained from rats one year after administration of At^{211} at dosages of from 1.1 to $1.6 \mu\text{c}$ per gram of body weight. (Only one of these levels, $1.2 \mu\text{c}$, is represented in Table IV). In only two sections did the sparse epithelial remnants bear any resemblance to thyroid follicles; in the remainder they consisted of dense small clusters or isolated cells in fibrotic tissue (Fig. 4). These cells were sometimes vacuolated; some contained intracellular colloid droplets, suggesting that they were viable and might be under TSH stimulation. Occasionally evidence of epithelial proliferation was found. Vascularity was poor. The remainder of the tissue was fibrous. Often an abundance of pigment-containing macrophages gave evidence of the extravasation of blood that had occurred earlier (see previous descriptions). Developing fat tissue was often seen in these sections. The parathyroids consistently showed structural normality.

Discussion

This experiment indicates that functional tests (^{131}I uptake) and structural tests (weight and histologic change) of thyroid damage after At^{211} admin-

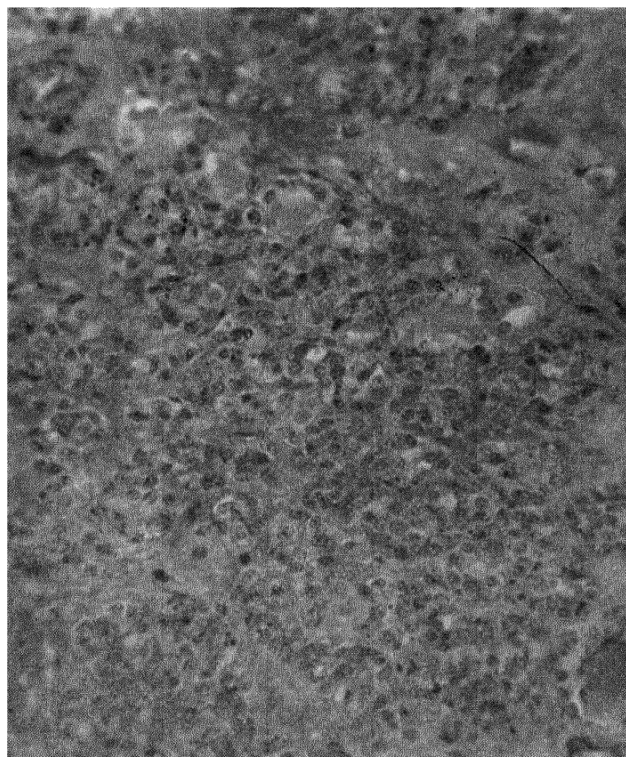


Figure 3. Six days after administration of $1.5 \mu\text{c At}^{211}$ /gm body weight, the thyroid shows masses of epithelial cells, many of them degenerating. The stroma between these cell masses is edematous and hemorrhagic ($\times 267$)

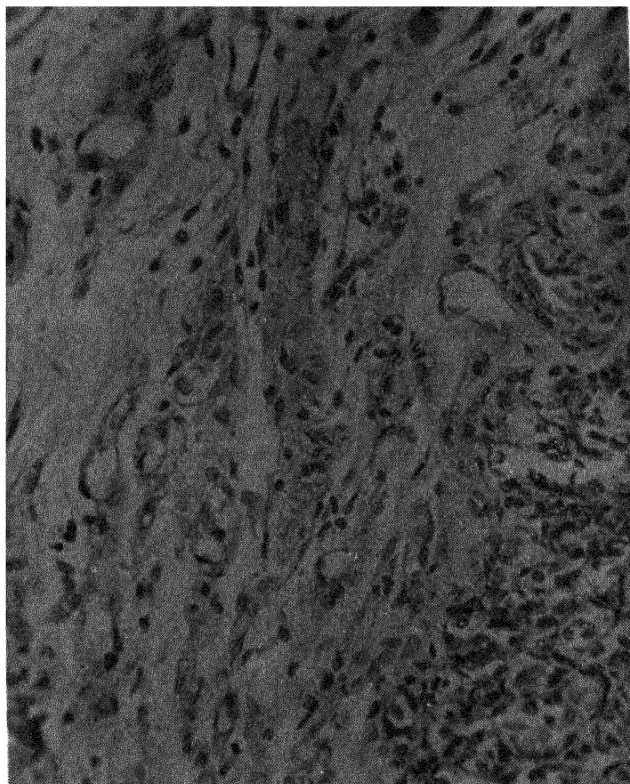


Figure 4. One year after administration of $1.1 \mu\text{C At}^{211}$ /gm body weight, no recognizable thyroid tissue is found, although some epithelial remnants are seen. The parathyroid gland (lower right) appears intact ($\times 267$)

istration are complementary. The I^{131} test seems more sensitive; reduction of uptake could be demonstrated after moderate At^{211} dosages in glands that appeared normal or even hyperactive structurally. Furthermore, the finding of even considerable epithelial remnants in the paratracheal region long after a thyroidectomizing dose of At^{211} is not necessarily associated with any substantial evidence of thyroid function. It appears that at the lower levels of At^{211} dosage, cells retain their capacity to respond to TSH stimulus by hypertrophy and hyperplasia. Obviously, the first consequences of the At^{211} radiation must be sought in impaired biochemical processes within the cells. This reinforces the demonstration for the need of further biochemical studies such as are described in the first section of this report.

SUMMARY

Astatine-211 is an alpha-particle-emitting halogen which is selectively accumulated by the thyroid gland. It has been possible, by employing standard protein chemistry procedures (trichloro-acetic acid or ammonium sulphate precipitation), to draw some conclusions as to its chemical state in the thyroid. Such biochemical studies have been combined with experimental regimens using antithyroid drugs (propyl thiouracil, potassium thiocyanate), and deductions have been made as to some of the physiological mechanisms which affect the transformations of astatine within the thyroid.

Advantage has been taken of some of the special nuclear properties of At^{211} (short half-life, rapid energy release, restricted tissue range) to achieve graded damage to the thyroid gland. Combining functional tests (I^{131} uptake) and structural tests (weight and histology) for thyroid damage has made it possible to assess the effective thyroid-destroying dose over varying time periods. Altered glandular physiology following lower doses has also been demonstrated.

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Use of the Nuclear Reactor for Neutron Capture Therapy of Cancer

By L. E. Farr, J. S. Robertson and E. Stickley,* USA

Neutron capture therapy is an experimental procedure for achieving selective irradiation of cancer.^{1,2,3} The selective irradiation is accomplished by utilizing nuclear reactions to generate short-range energetic heavy particles within and throughout the malignancy. The energetic heavy particles are produced by the immediate disintegration of unstable nuclear complexes which are formed when slow neutrons are captured by suitable target nuclei.

The procedure for neutron capture therapy begins with the intravenous injection of a suitable target or neutron capturing material and is arranged to provide, at least transiently, a concentration of this target material much higher in the cancer than in the surrounding normal tissues. While this favorable concentration difference exists, the area is exposed to slow neutrons, resulting in irradiation of the area by energetic heavy particles. Localization of the radiation depends on the short range of such particles and on the relative concentration of the target isotope in the disease tissue.

This radiotherapeutic modality is in distinct contrast with other uses of high energy machines or large radioactive sources for radiation therapy. Other methods in use are properly described as teletherapy, in that the radiation originates at a target or source external to the subject being irradiated. Neutron capture therapy uses the external source only to supply the slow neutrons, relatively ineffective in their own right, while the therapeutic radiation actually originates throughout the tumor tissues being treated. In this sense, the modality is one of inter-jacently originating radiation and the methods for determining dosage from internally administered radioactive isotopes are applicable. This modality is also in contrast to those methods depending primarily upon radiation sensitivity to achieve differential effects.

The accepted applications of radiation therapy in wide use for the treatment of malignant disease rely chiefly on penetrating electromagnetic radiation, that is, X-rays and gamma rays. In addition, there are instances in which irradiation with light particles such as beta rays or electron beams is used. Except for very recent developments which employ narrow beams of protons or deuterons of extreme energy,

the application of heavier particles has been restricted to wholly superficial sites, since the penetration of alpha and other such particles is quite limited at energies hitherto generally available. Neutron capture therapy involves an important physical difference in the approach to radiation treatment of disease in that the malignancy is treated in depth and presumably in a uniform manner with heavy particle radiation. The kinetic energy of these particles is transferred to the tissues within a very short range from their point of origin. This range is of the order of 10 microns for the nuclear reaction used in our work. Such intensive transfer of energy along a short path in contrast to the lesser linear energy transfer along the path of an X-ray or gamma ray might be expected to produce qualitative as well as quantitative differences in the results observed.

Nuclides desired for this work must have large capture cross sections for slow neutrons and must provide an instantaneous disintegration into massive particles with high energy. Consideration of the neutron capture cross sections and the heavy particle production characteristics narrows the choice of target elements to three: lithium-6, boron-10, and uranium-235. For our work, boron-10 was selected because of its availability and because certain specific data had been obtained by Sweet⁴ indicating a satisfactory physiological behavior of boron.

From the physiological standpoint, the problem is to achieve, at least transiently, a significantly greater concentration of the capture element within cancer tissue than in the surrounding normal structures, with an absolute concentration great enough to provide tumoricidal radiation in a short interval. Infiltration or injection by mechanical means cannot provide the uniform distribution of target element which is required. The use of boron-containing dyes known to localize to a degree in tumors is limited by toxicity problems and unavailability. There is no known compound for which tumors have a significantly preferential uptake to the degree exemplified by the iodine uptake behavior of thyroid tissue. We have, therefore, developed an alternative approach to this problem which we have termed "selective kinetics". This is based on the phenomena of transient differences in concentration of the target-carrying substance which result from differing rates

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of transfer from blood to the several tissues of immediate interest. In working with glioblastoma multiforme, a rapidly infiltrating tumor, we are able to use to advantage the so-called blood-brain-barrier characteristic to retard entrance of the target element into the normal brain structures while rapid permeation of the tumor occurs, since no barrier exists here.

The first practical opportunity to apply the slow neutron capture principle to therapy of malignant disease in patients was provided by the high flux of slow neutrons available from the Brookhaven research reactor. A treatment facility was constructed in the top shielding of this reactor from which a flux of about 2×10^8 neutrons/cm²/sec was delivered to a presenting skin surface area of 5 by 10 centimeters in the first series of experiments. The patient was prepared by intravenous injection of borax, with maximum differences in concentration of boron between tumor and normal brain being assumed at about 10 minutes postinjection. The advantageous difference gradually decreases, disappearing at some time after an hour. For best effect, these conditions indicated that the procedure follow a schedule of neutron irradiation of roughly from the 10th to the 50th minutes.

Measurements of the neutron exposure were carried out by using the slow neutron activation of gold foils on the treatment port and on the patient's skin. In some instances, intracranial measurements of neutron flux were made by activation of gold wires which were inserted through the treatment area. Direct measurement of the heavy particle radiation which provides the therapeutic effect was not possible. Potential radiation doses delivered were computed from the experimentally determined values of neutron flux and selected values of boron concentration in the tissues.

Following the conclusion of the first experimental series of ten patients, the reactor medical facility was redesigned to provide several needed advantages. Most important was the increase of slow neutron flux to the presenting skin surface. This can now be as high as 3×10^9 neutrons/cm²/sec and is now presented through a treatment of port 10 by 10 centimeters. A second major advantage of the new facility is the incorporation of a shutter mechanism sufficient to protect the patient and attending personnel during the preparatory stages without requiring that the reactor be shut down. In constructing the new facility, the bismuth gamma-ray shield was relocated to provide better neutron economy and the customary aluminum coating was removed from the top layer of bismuth to eliminate unnecessary exposure of the patient to the induced radioactivity of the aluminum.

The shielding pieces which cover the treatment facility when not in use were simplified and fitted with access doors, so that auxiliary experiments on small animals, tissue sections, or bacterial cultures can be put into position and irradiated at any time without disturbing other experimental work at neigh-

boring locations. This unique facility (Fig. 1) has accelerated many phases of the contributory research program of the Medical Department and has been used to advantage in the work of other Brookhaven Departments as well. It is used extensively to investigate experimental approaches to the problem of dosimetry by means of special ionization chambers made of entirely tissue-equivalent materials and by the development of dosimeters based on the biological response of selected systems.

The first clinical study of neutron capture therapy was begun in February, 1951, and was completed in February, 1953. Ten patients with glioblastoma multiforme were given one to four capture therapy irradiations. Details have been reported in the literature.¹ Survival in this series ranged from 43 days postirradiation to 184 days postirradiation. The two patients in this group who received four irradiations survived longer than the others. The average survival time was 107 days. In this group, the skin surface neutron flux was approximately 2×10^8 neutrons/cm²/sec. A second clinical study began in April, 1954, with the enhanced neutron flux of 3×10^9 neutrons/cm²/sec. To date, nine patients have been treated in this facility. Three patients have received two treatments and six a single treatment. Five patients have died, but average survival time postirradiation of these was 169 days with a range of 93 to 337 days. Four patients are surviving whose survival postirradiation as of June 1, 1955, ranged from 42 to 279 days. (See Table I, which extends these data to August 1, 1955.) At the time of writing, no prediction can be made of the expected survival of this group. It appears, however, that the increase in boron dose and slow neutron flux was accompanied by a significant lengthening of survival span. On the basis of calculations and experience, until a slow neutron flux of 10^{11} neutrons/cm²/sec at the skin

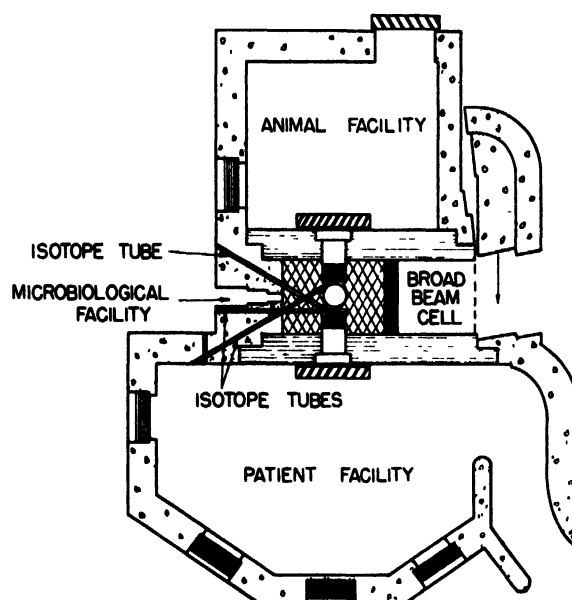


Figure 1. Treatment facility

surface can be obtained, adequate penetration to optimum depth cannot be achieved.

New problems arose when treatment with the higher intensity neutron beam was begun. It should be noted that at this same time we had discovered means allowing the safe injection of greater quantities of boron. Thereby, the potential amount of radiation delivered to the tumor was increased considerably. One problem of first importance which has developed following use of the higher neutron flux is a severe skin reaction which requires detailed care so that the patients had to remain in the hospital much longer than their clinical condition otherwise indicated. This skin effect was due at least in part to the more intense neutron beam which, along with the high energy gamma rays, was able to release energetic protons, electrons, and perhaps other massive particles from the shielding materials and intervening air. An additional thin filter of material of low atomic weight (3 mm plastic) eliminated part of this difficulty. The area of adverse reaction of the skin was also limited to a degree by the use of strong neutron absorbers to define the treatment area more closely. Both of these changes were made at a sacrifice of a fraction of the neutron intensity but with unquestionably desirable results.

Many other aspects of the program require further study. Among those of considerable immediacy are the pharmacology involved in delivering adequate boron target element concentrations to the tumor

site, the physiology of selective kinetics which provides the differences of concentration necessary for localization of the irradiation, the toxicology of the target element and its vehicle compound, and the physics of neutron energy selection and control for optimum penetration at the tumor depth. Following any new development in any of these directions, a careful clinical assessment of effect of the modification is absolutely essential and, on the clinical side, it is also necessary to improve the means of analysis employed in establishing the state of the disease and its changes.

From our experience with the neutron capture therapy experiment and with other biological investigations utilizing the nuclear reactor, specifications have been detailed for a proposed reactor to be built exclusively for our medical applications. This reactor should be capable of providing external thermal neutron beams at intensities of 10^{11} neutrons/cm²/sec at the presenting skin surface. This intensity is about thirty times the flux we are now using. Fast neutron and gamma-ray contaminations must be maintained well below tolerable limits. To continue and extend our investigation, the medical research reactor should provide a patient irradiation facility with a widely adaptable treatment port incorporating both gamma-ray and neutron shutters and with an auxiliary preparation room. Means should be provided for modifying the character of the neutron flux, in order to explore the enhanced penetration of epithermal neutrons. A similar exposure facility should be provided for animal irradiations. In addition, a large cell directly exposed to a bare face of the reactor should be available. A collimated neutron-beam hole should be included for irradiation of microbiological specimens and for study of radiation effects or neutron depth dosimetry in tissue or equivalent media. Our work also includes the application of radioactive materials of very short half-life, for which an irradiation tube inside the reactor should be arranged with direct delivery into the treatment rooms or laboratories. A reactor so designed will permit adequate exploration of the field which has been touched upon in the studies mentioned in this report.

SUMMARY

A brief resume of clinical experience to date with neutron capture therapy has been presented. On the basis of this experience and the collateral studies carried out, it is possible to establish operating performance characteristics for a nuclear reactor for medical purposes which will provide an adequate thermal neutron flux at depth in human tissues. This reactor must provide a variable energy neutron spectrum through the epithermal range for satisfactory depth effects. Other facilities desirable in such a reactor have been mentioned. Physiological theory and practice to provide suitable environmental characteristics for neutron capture therapy have been presented in outline.

Table I

<i>First series (1951-1953)</i>			
<i>Patient</i>	<i>Sex</i>	<i>Number of irradiations</i>	<i>Days lived after first irradiation</i>
4045	♂	1	43
5227	♀	3	66
3977	♀	1	67
4055	♂	1	93
4709	♂	3	97
4202	♂	1	100
4227	♂	1	126
4753	♂	2	136
5144	♀	4	152
4653	♂	4	185
Average survival first series			106
<i>Second series (1954-1955)</i>			
<i>Patient</i>	<i>Sex</i>	<i>Number of irradiations</i>	<i>Days lived after first irradiation</i>
6408	♀	1	93
5972	♂	1	121
6546	♂	1	137
6480	♀	1	147
5984	♂	2	173
6024	♀	1	244
6012	♀	2	337
Living as of August 1, 1955			
6629	♂	1	102
6351	♀	1	311
Average survival in patients who died before August 1			179

The present statistics of survival time of patients with glioblastoma multiforme show a significant lengthening following the increase in boron dosage and in thermal neutron flux provided by our latest facility. This is objective evidence supporting the basic tenets advanced in explanation of neutron capture therapy.

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The Distribution and Excretion of Hexavalent Uranium in Man

By E. G. Struxness,* A. J. Luessenhop,† S. R. Bernard* and J. C. Gallimore,* USA

This study was undertaken, first to determine the intravenous tolerance dose of fissionable uranium in man for its possible application to neutron capture therapy of brain tumors; and secondly, to obtain human data with which to describe the excretion of uranium in urine and its relation to distribution in the body. This information is needed to permit a more realistic evaluation of occupational exposures in enriched uranium processing and fabrication plants. The study was a cooperative undertaking by the Health Physics Division, Oak Ridge National Laboratory, and the Department of Neurosurgery, Massachusetts General Hospital.

Previous investigations by W. H. Sweet, and others pointed out the use of the neutron capturing isotope, B¹⁰, in the radiation therapy of intracranial tumors.^{1,2,3} Their results, suggestive of temporary retardation of tumor growth in eight out of twenty-one patients, indicated that further exploration of neutron capture therapy was warranted. After careful theoretical evaluation of the clinical use of fissionable uranium, they concluded that its possibilities in capture therapy were worthy of investigation.

Some of the questions that arise out of consideration of this possibility are: (1) Will a high differential concentration of uranium in the tumor be achieved following intravenous administration? (2) Will a high differential concentration in the tumor persist long enough to permit effective exposure to thermal neutrons? (3) What is the absolute concentration of uranium in neoplastic brain tissue and in other tissues? (4) What is the highest possible dose of fissionable uranium intravenously administrable without seriously affecting the patient?

Uranium is known to be nephrotoxic, but the dose required to produce toxic manifestations in man is not known. Small animal data show that a striking species variation exists, e.g., the mouse tolerates 100 to 200 times more uranium than the rabbit. The initial objective of this study was to investigate the toxicity of uranium in man with a view to determining the highest intravenous injection dose.

This investigation provided a unique opportunity to obtain uranium distribution and excretion data on

humans for application to a mathematical method of evaluating industrial exposures. A specific problem of great interest to industrial workers is the possible radiological effect of chronic exposure to the enriched uranium-contaminated dusts, smokes and fumes ever present in production plants. Heretofore, these exposures have been characterized by the arbitrary and limited monitoring techniques presently available to industrial health groups. Such methods are incapable of detecting evidence of biological significance in the case of continuous, long-term, low-level exposure to insoluble radioactive aerosols. For example, the body burden cannot be determined, with any degree of confidence, on the basis of airborne-uranium measurements; neither is it possible to estimate concentration in critical organs on the basis of excretion measurements until a definite relationship between distribution and excretion is established. Here was a chance to obtain information on which to construct and test part of the evaluation method, i.e., the relationship between distribution and excretion of uranium as it leaves the circulation.

Although the primary justification for these experimental injections of uranium in man grew out of its theoretical value in neutron capture therapy, only the distribution and excretion portion of the study will be included here. The toxicological findings will be published by other authors at a later date.

METHODS

Selection and Care of Patients

The six patients selected for this study were in the terminal phase of severe irreversible central nervous system disease. Virtually all had brain tumors of a most malignant type. The ages of the patients were 26, 34, 39, 47, 60 and 63 years, and, aside from the central nervous system disease, they were in generally good physical condition without definite evidence of other pathological processes.

At the time of injection all patients were in coma and receiving the usual hospital care consisting of frequent turning, skin care, gastric tube feedings, catheter drainage and frequent tracheal suction. Three of the patients had tracheotomies.

The patients who did not terminate during the two to three week period following injection were transferred to a nursing home where they could still be closely observed.

Including work by C. S. Banks,* B. L. Harless,* J. R. Muir,* J. Robinson† and W. H. Sweet†.

* Oak Ridge National Laboratory.

† Massachusetts General Hospital.

Administration of Uranium

Preparation of Injection Solution

Pure uranium oxide (U_3O_8) was converted to nitrate ($UO_2(NO_3)_2$) by dissolving the oxide in an excess of nitric acid and evaporating to dryness. The resulting nitrate crystals were dissolved in distilled water and twice evaporated to dryness to eliminate final traces of nitric acid. The crystals were then dissolved and diluted to volume with distilled water. The solution was assayed at this point colorimetrically and by alpha count. The desired quantity of nitrate was then removed, placed in a rubber sealed container and autoclaved for sterility. A 0.4M sodium acetate solution was prepared and autoclaved. Equal volumes of each were combined shortly before the beginning of each study and the desired quantity removed for injection. All administrations were given at a pH of from 5.5 to 6.0. Except in the case of Patient I, all injection solutions were similarly prepared. In this case the uranium nitrate was placed in physiological saline and adjusted to the proper pH with sodium hydroxide and hydrochloric acid.

Following the injection, the syringe employed in the administration was used to deliver a replicate volume of the injection solution to a flask for quantitative analysis. This procedure accounted for any volume errors as a result of inaccurate markings on the syringe.

Injection Procedure

The uranium was injected intravenously in all the patients. The procedure consisted of first starting an intravenous of normal saline in an antecubital vein. After careful inspection to preclude any possibility of extravasation, the uranium solution was injected over a period of 10 to 15 seconds through the rubber intravenous tubing. In the first patient this was done through a metal 3-way stopcock. However, a small amount of the solution was lost because of leakage from the stopcock. In the second patient a glass 3-way stopcock was employed, but during the injection the glass side arm broke resulting in the loss of a small, but significant, amount of the solution. Thereafter, the injections were made by inserting the syringe needle into the rubber I.V. tubing. In all cases the syringe was irrigated 4 to 5 times with saline from the I.V. bottle prior to its removal from the tubing.

Collection of Specimens

Blood specimens of 1 to 3 millimeters were taken by phlebotomy in the arm not used for the uranium injection. During the first 24 hours, hourly specimens were taken; then at 12 hour intervals for several weeks, then at 24 hour intervals and, following transfer from the hospital, at 1 to 3 week intervals until expiration.

For the determination of initial bone uptake several bone biopsies were taken from the anterior tibia

employing a $\frac{1}{2}$ -inch trephine through a small skin incision.

The urine samples were collected from indwelling catheters. During the first 24 hours they were collected at hourly intervals, or more frequently if the output was great; thereafter at 12-hour intervals for 2 to 4 weeks and finally 12-hour samples at 1- to 4-week intervals.

All feces specimens were collected during the time the patient remained under close observation in the hospital.

During the period of collection of samples for uranium determination numerous blood and urine specimens were taken for measurement pertinent to indices of chemical toxicity.

At the time of writing, 5 of the 6 patients have expired and permission for autopsy has been obtained on 4 of these. Samples of all body tissues were taken for uranium determination.

Preparation and Analysis of Specimens

Urine

Three 20 millimeter aliquots were removed from each specimen, when possible, and 20 millimeters of concentrated nitric acid were added to each aliquot. These solutions were reduced to dryness on a steam bath and shipped to the Oak Ridge National Laboratory for analysis.

Upon arrival at the Laboratory, 20 milliliters of a hydrochloric-nitric acid solution (1:3 proportions) were added to each specimen bottle and allowed to stand until all the residue was in solution. The resulting solution was carefully rinsed with 0.1N nitric acid into a 100 milliliter beaker and evaporated to dryness. This acid digestion was repeated five or more times until a white residue resulted at dryness. A final digestion with 20 ml of nitric acid for chloride destruction was carried out.

Following evaporation, the residue was dissolved in 0.1N nitric acid and diluted to volume. Triplicate aliquots were removed from each volumetric dilution for electrodeposition⁴ of the uranium and subsequent alpha counting.

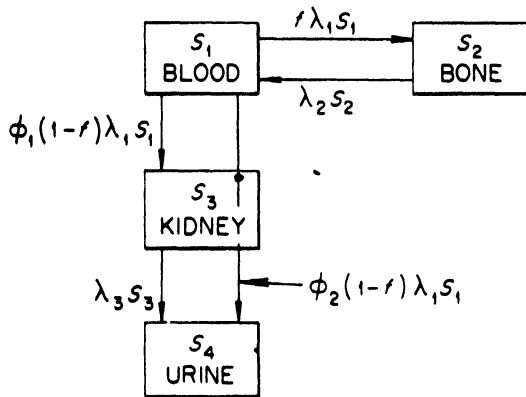
Blood

The blood specimens were prepared in the same manner as the urine with the exception of the final nitric acid digestion. At near dryness, the sample was removed from the steam bath. The small quantity of acid and residue remaining was dissolved in 20 milliliters of distilled water and triplicate aliquots were removed for electrodeposition and subsequent alpha counting.

Soft Tissue Specimens of Less than 2 Grams Wet Weight

Soft tissue specimens (biopsy or autopsy) were weighed and muffled in platinum crucibles at 600°C for 24 hours. The ash was dissolved in 0.1N nitric acid and the entire volume analyzed by electrodeposition and alpha counting.

THE MODEL



THE DIFFERENTIAL EQUATIONS:

BLOOD

$$\frac{dS_1}{dt} = \lambda_2 S_2 - \lambda_1 S_1$$

BONES

$$\frac{dS_2}{dt} = f\lambda_1 S_1 - \lambda_2 S_2$$

KIDNEY

$$\frac{dS_3}{dt} = \phi_1(1-f)\lambda_1 S_1 - \lambda_3 S_3$$

URINE

$$\frac{dS_4}{dt} = \phi_2(1-f)\lambda_1 S_1 + \lambda_3 S_3$$

THE INTEGRATED EQUATIONS:

BLOOD

$$S_1(t) = \frac{I}{\mu_2 - \mu_1} \left[(\mu_2 - \lambda_1) e^{-\mu_1 t} + (\lambda_1 - \mu_1) e^{-\mu_2 t} \right]$$

BONES

$$S_2(t) = \frac{f\lambda_1 I}{\mu_2 - \mu_1} \left[e^{-\mu_1 t} - e^{-\mu_2 t} \right]$$

KIDNEY

$$S_3(t) = \frac{\phi_1(1-f)\lambda_1 I}{\mu_2 - \mu_1} \left[\frac{\mu_2 - \lambda_1}{\lambda_3 - \mu_1} e^{-\mu_1 t} + \frac{\lambda_1 - \mu_1}{\lambda_3 - \mu_2} e^{-\mu_2 t} + \frac{(\lambda_2 - \lambda_3)(\mu_2 - \mu_1)}{(\lambda_3 - \mu_1)(\lambda_3 - \mu_2)} e^{-\lambda_3 t} \right]$$

URINE

$$S_4(t) = I - \frac{(1-f)\lambda_1 I}{\mu_2 - \mu_1} \left[\frac{(\mu_2 - \lambda_1)(\lambda_3 - \phi_2 \mu_1)}{\mu_1(\lambda_3 - \mu_1)} e^{-\mu_1 t} + \frac{(\lambda_1 - \mu_1)(\lambda_3 - \phi_2 \mu_2)}{\mu_2(\lambda_3 - \mu_2)} e^{-\mu_2 t} + \frac{\phi_1(\mu_2 - \mu_1)(\lambda_2 - \lambda_3)}{(\lambda_3 - \mu_1)(\lambda_3 - \mu_2)} e^{-\lambda_3 t} \right]$$

APPLICATION OF MODEL

GIVEN AN EXCRETION EQUATION

$$\text{URINE} = I - \sum_1^3 a_i e^{-a_i t} \quad \text{WHERE} \quad \sum_1^3 a_i = I$$

THE ROOTS OF

$$x^2 - \sum_1^3 \left(\frac{1 - \frac{a_i}{I}}{a_i} \right) x + \frac{\sum_1^3 a_i \frac{a_i}{I}}{\pi a_i} = 0$$

ARE $1/\lambda_2$ AND ϕ_2/λ_3 , WITH $\mu_1 < \lambda_3 < \mu_2$

$$\lambda_1 = \mu_1 + \mu_2 - \lambda_2$$

$$(1-f) = \frac{\mu_1 \mu_2}{\lambda_1 \lambda_2}$$

Figure 1. A model for the distribution and excretion of hexavalent uranium

Feces, Bone, and Tissue Specimens Greater than 2 Grams Wet Weight

All specimens were wet weighed and muffled in platinum crucibles at 600°C for approximately 24 hours. Additional time was required for several specimens of bone to insure complete organic destruction. The resulting ash was weighed and analyzed for uranium using the aluminum nitrate-diethyl ether extraction procedure with subsequent evaporation in a stainless steel planchet for alpha counting.

Mathematical Interpretation of Data

A mathematical model, similar in structure to that designed by Teorell⁵ for studying the tissue distribution of intravenously administered drugs, was designed to represent the distribution and excretion of uranium following intravenous administration.⁶ Its design, shown in Fig. 1, was based on a simple diagram constructed by W. F. Neuman.⁷ The values of f , ϕ and λ were then taken from available research data on animals.⁸ Only a few terms are used to represent the complicated metabolic processes in the four major compartments, the major premise being that these physiological processes are simple first order reactions.

The test of this model involves only the measured excretion and deposition values obtained in the experiment. After determining the excretion equation and the values of each parameter, it is possible to calculate the concentrations in each compartment for a comparison with the observed deposition values.

RESULTS

Blood Disappearance

Hexavalent uranium leaves the blood stream very rapidly. The blood values obtained in this experiment at varying time intervals after administration are presented in Fig. 2. On the average, 99% of the injected dose is removed from the circulation in a matter of 20 hours, assuming uniform distribution at injection (time zero) and a blood volume of 7.14% of body weight. When they are once reduced, the blood values remain relatively constant until time of expiration.

Urinary Excretion

Concurrent with the fall of the blood-uranium level large quantities of uranium appear in the urine. Most of the excretion takes place in the first twenty-four hours after administration; thereafter, ever decreasing quantities are excreted. Essentially all uranium is excreted in the urine, since less than one per cent of the injected dose is found in the feces. Figure 3 shows the urine excretion data obtained in this study. These data are presented as log-log plots for the purpose of comparing the excretion rates on an identical time scale. It will be seen in all cases that the excretion rate is non-linear over the total observation period.

Distribution in Tissue

The per cent of injected dose per gram of tissue found in tibia biopsies during the first 40 hours after administration is shown in Table I. These data indicate that the accumulation of the injected uranium ranges from 2×10^{-4} to 23×10^{-4} per cent of injected dose per gram, the average for the first 24 hours being 10.1×10^{-4} per cent. This corresponds to an initial deposition of 6.0 per cent of the injected dose in bone assuming an average patient skeletal weight of 6000 grams.

In contrast to this value of initial deposition in the skeleton, are the autopsy values shown in Fig. 4. Here the average skeletal content, in terms of per cent of injected dose per gram, is plotted as a function of time. The data show that the initial deposition in bone, estimated by extrapolation, is 23.9×10^{-4} per cent of injected dose per gram, or 14 per cent of the injected dose. This is suggestive of a skeletal build-up which peaks somewhere between 24 and 60 hours. However it must be remembered that biopsy samples may not be representative of skeletal distribution since they are very small and essentially compact bone, while the autopsy specimens are larger and include portions of all bone structures.

The kidney findings at autopsy are shown in Fig. 5. Extrapolating these data to time zero indicates an initial deposition of 16 per cent of the injected dose. The uranium is mobilized from the kidney with a 16 day half-life accompanied by a leveling off at approximately 1 per cent.

A typical gross autoradiogram of the kidney, Fig. 6, shows uranium non-uniformly distributed and concentrated primarily in the cortical structures. Figure 7, a microscopic autoradiogram of a kidney section from Patient VI, illustrates in detail, a typical site of deposition. Here the uranium is observed to be deposited within or upon epithelial cells of a proximal convoluted tubule.

The autopsy values of all tissues analyzed for uranium are listed in Table II. They are tabulated in terms of per cent of injected dose per gram, because it was impossible to obtain weights on all the organs and tissues sampled. Thus, there is no other basis upon which to adequately compare these values. However, if one assumes the weight values of standard man⁹ to convert these values to per cent of in-

Table I. Uranium Content of Bone Biopsy Samples in Per Cent of Injected Dose per Gram $\times 10^{-4}$

Patient no.	I	II	III	IV	V	VI
0-24 hr	8.7	11.2	12.9	10.8	8.0	8.8
	7.9	6.7	3.3		6.3	9.3
	23.4	4.4	2.0		5.9	
	16.2	15.9				
	8.2					
	13.4					
24-48 hr	17.9					
	7.0				2.2	
	15.1					

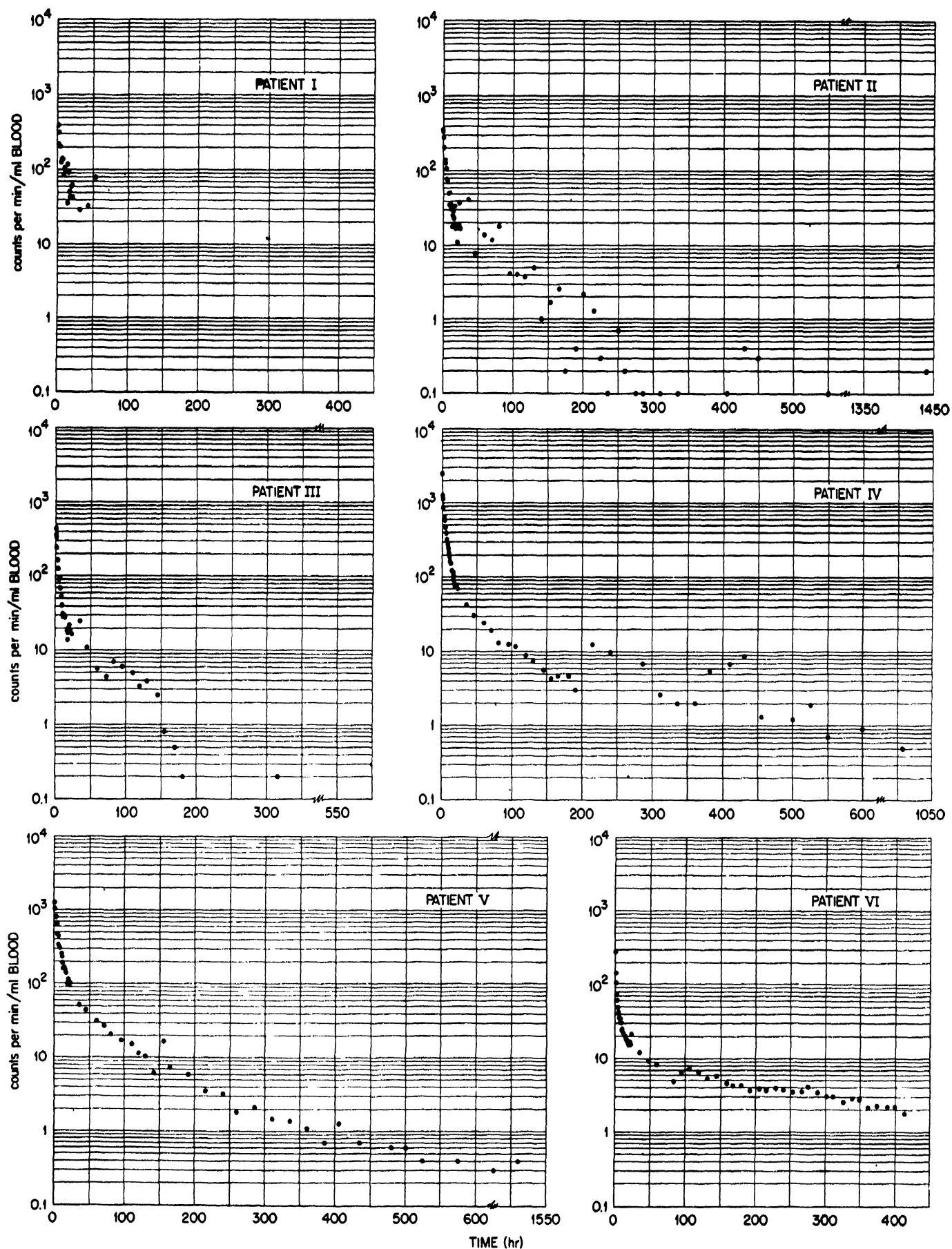
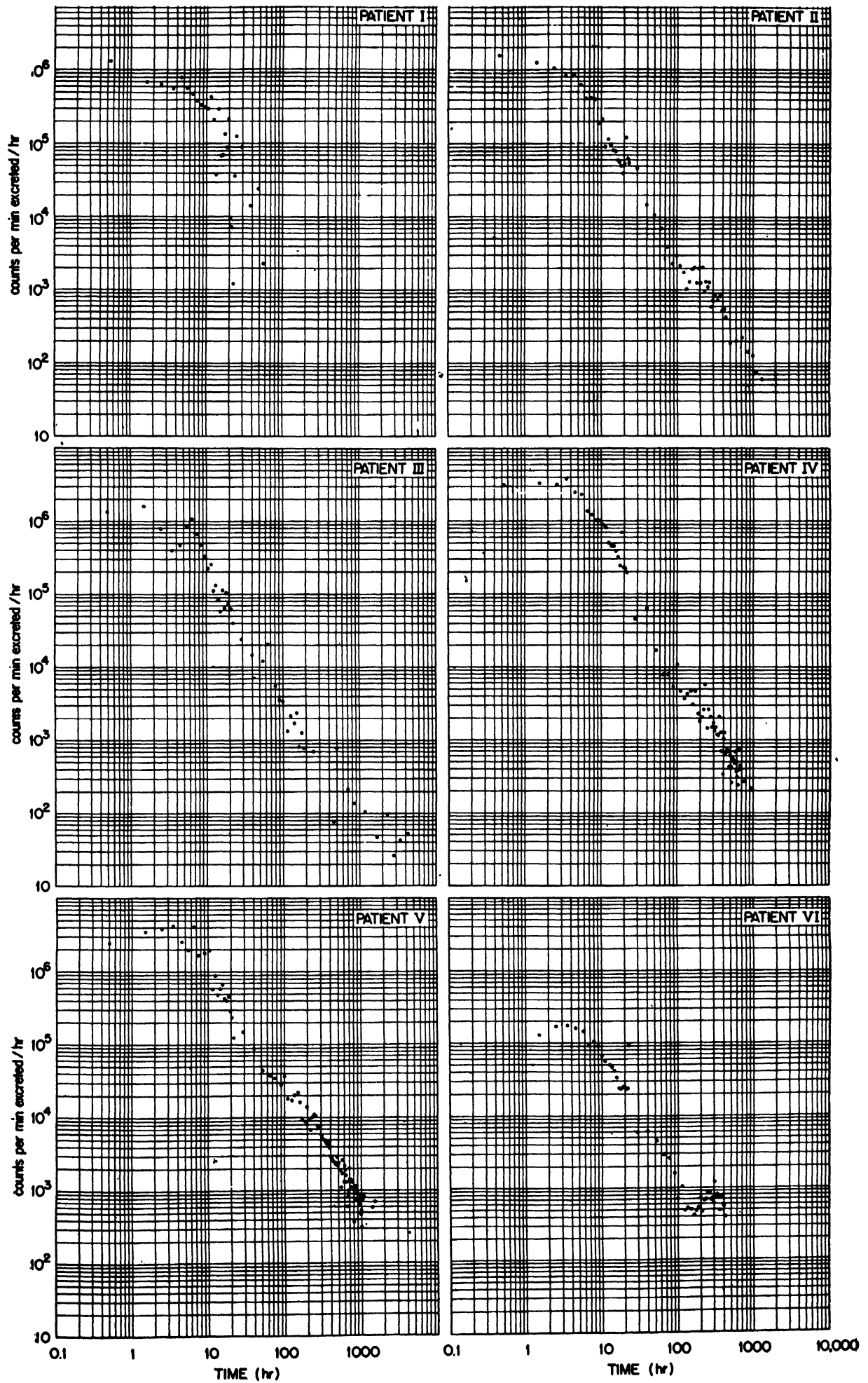


Figure 2. Uranium disappearance from blood



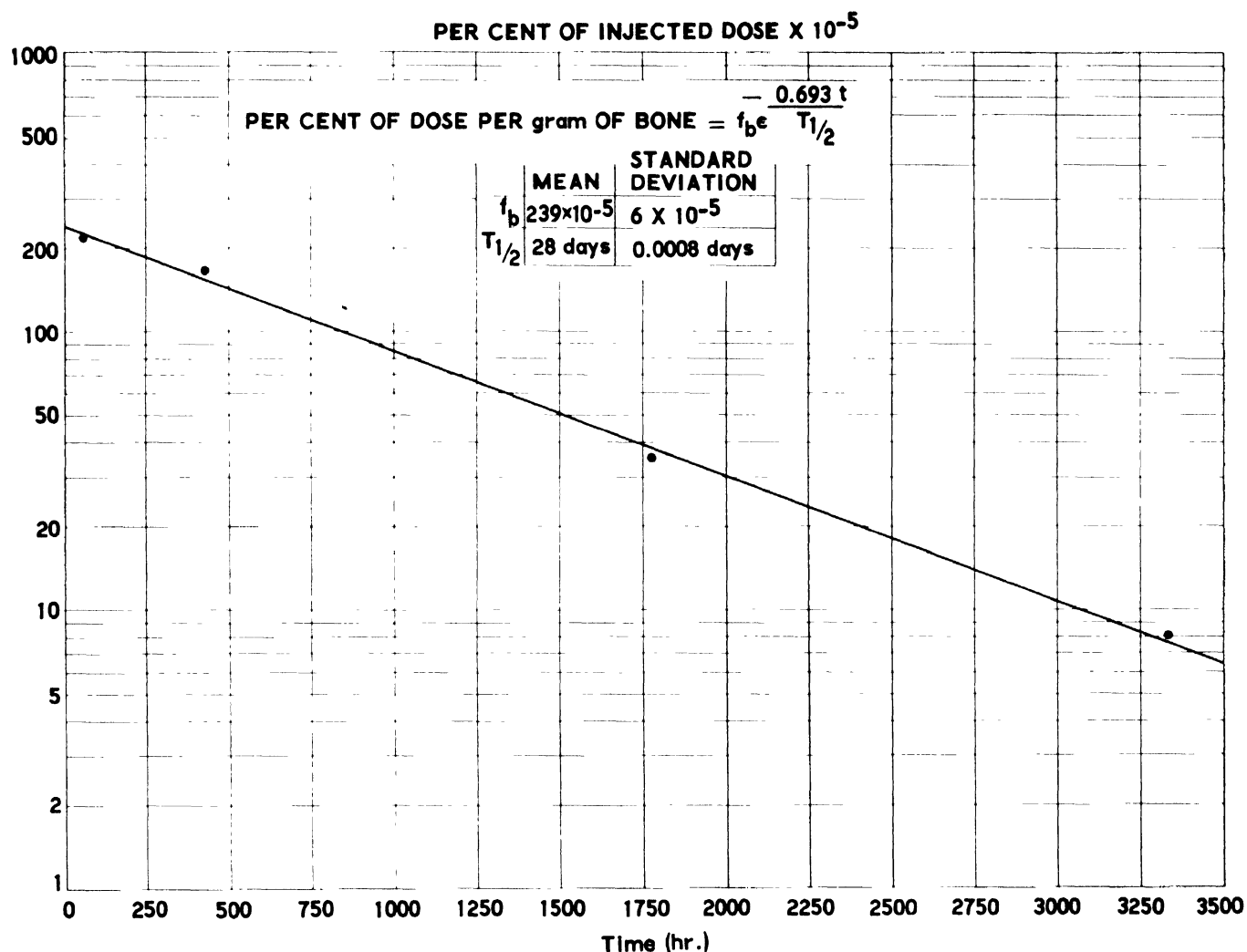


Figure 4. Per cent of injected dose per gram of bone (wet weight) at autopsy

jected dose per organ or per tissue, it is evident that all soft tissues, excluding kidney, contain approximately 20 per cent at 60 hours after injection and less than 1 per cent at 1500 hours. This is unlike the results obtained in small animal experiments, where the soft tissue content fell 1 to 2 per cent of the injected dose concurrent with the fall in blood uranium.¹⁰ This difference is markedly evident when the soft tissue concentration is related to the blood disappearance curve, as will be shown later.

DISCUSSION

Estimating the Injection Dose for Patients I and II

As described previously, injection solution replicates were collected following administration. These replicates were analyzed in a manner identical with the analysis of urine and blood specimens.

In the case of Patients I and II, injection incidents prevented administration of the predetermined dose. It was necessary, therefore, to estimate the injected dose. This was accomplished in the following manner: the excretion curves of Patients III and IV were plotted; the first part of each curve, being linear, was fitted with an exponential term; this single component, when integrated, represented 0.54348 and 0.53476 of the measured dose, respectively. The same

procedure was followed with the excretion curves of Patients I and II. Dividing the area under their curves by the average of the values from Patients III

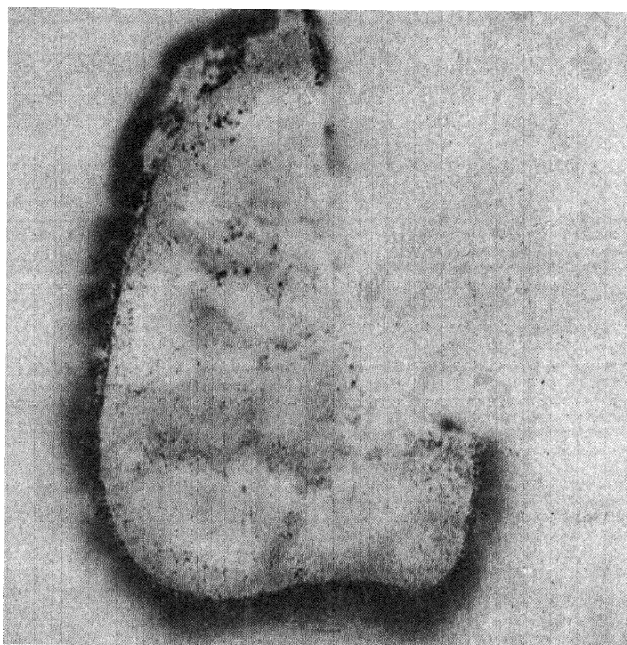


Figure 6. Gross autoradiogram of kidney showing distribution of uranium

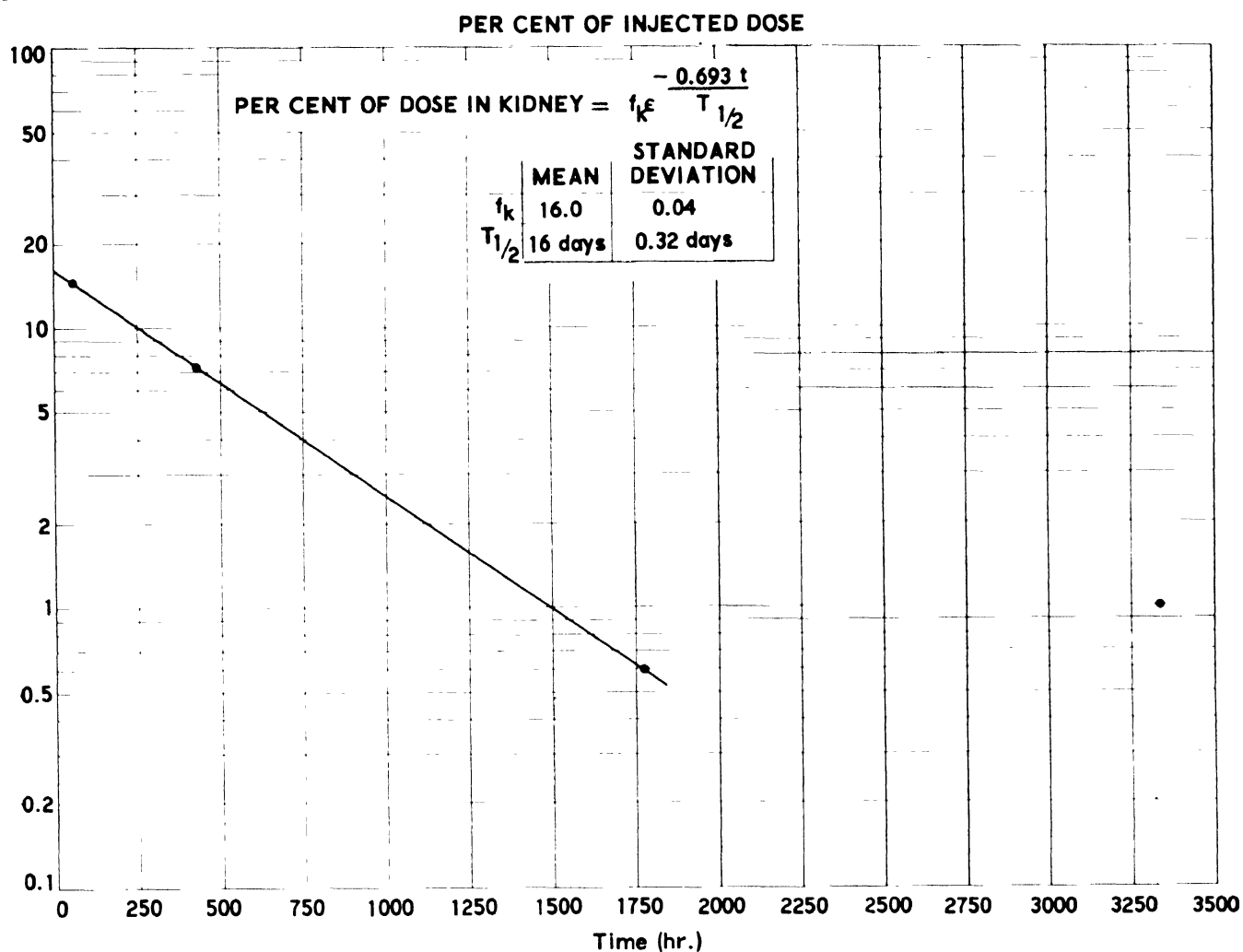


Figure 5. Per cent of injected dose in kidney at autopsy

and IV, 0.53908, it was possible to estimate their injected dose.

Comparison with Research Animal Data

The fractions of injected dose initially deposited in soft and hard tissue obtained in this study are in good agreement with those obtained in animal experiments. For example, Neuman states that from 5–20% of the injected dose in rats concentrates in

bone.¹¹ Two independent estimates of these fractions in per cent of injected dose determined by the analyses of biopsy and autopsy bone samples in this study gave values of 6% ($\pm 3\%$, one standard deviation) and $14 \pm 0.04\%$, respectively. The half-life in the bone, however, differs by a factor of 4, i.e., 28 days for man, while that for rats is reported to be 100 days.¹² The bone half-life was determined from a plot of the autopsy results shown in Fig. 4.

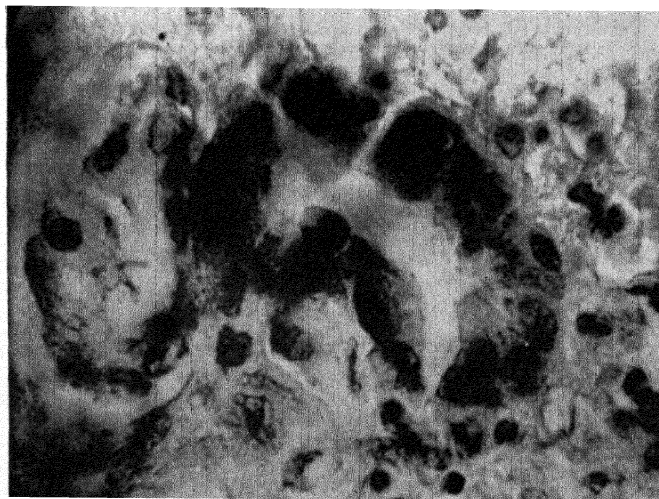
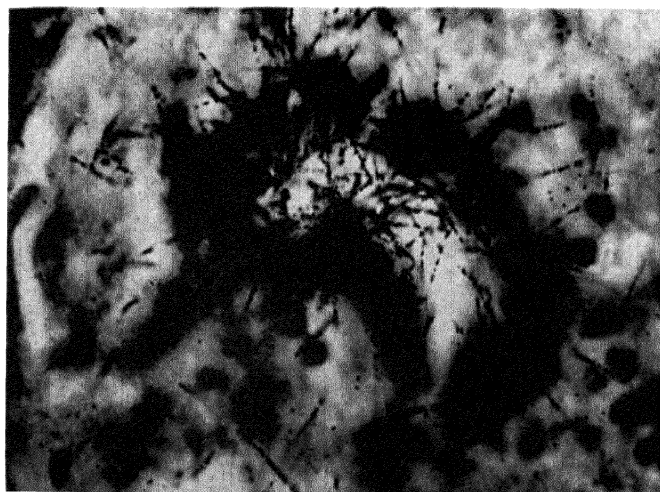


Figure 7. Microscopic autoradiogram of kidney showing uranium in or upon epithelial cells of a proximal convoluted tubule

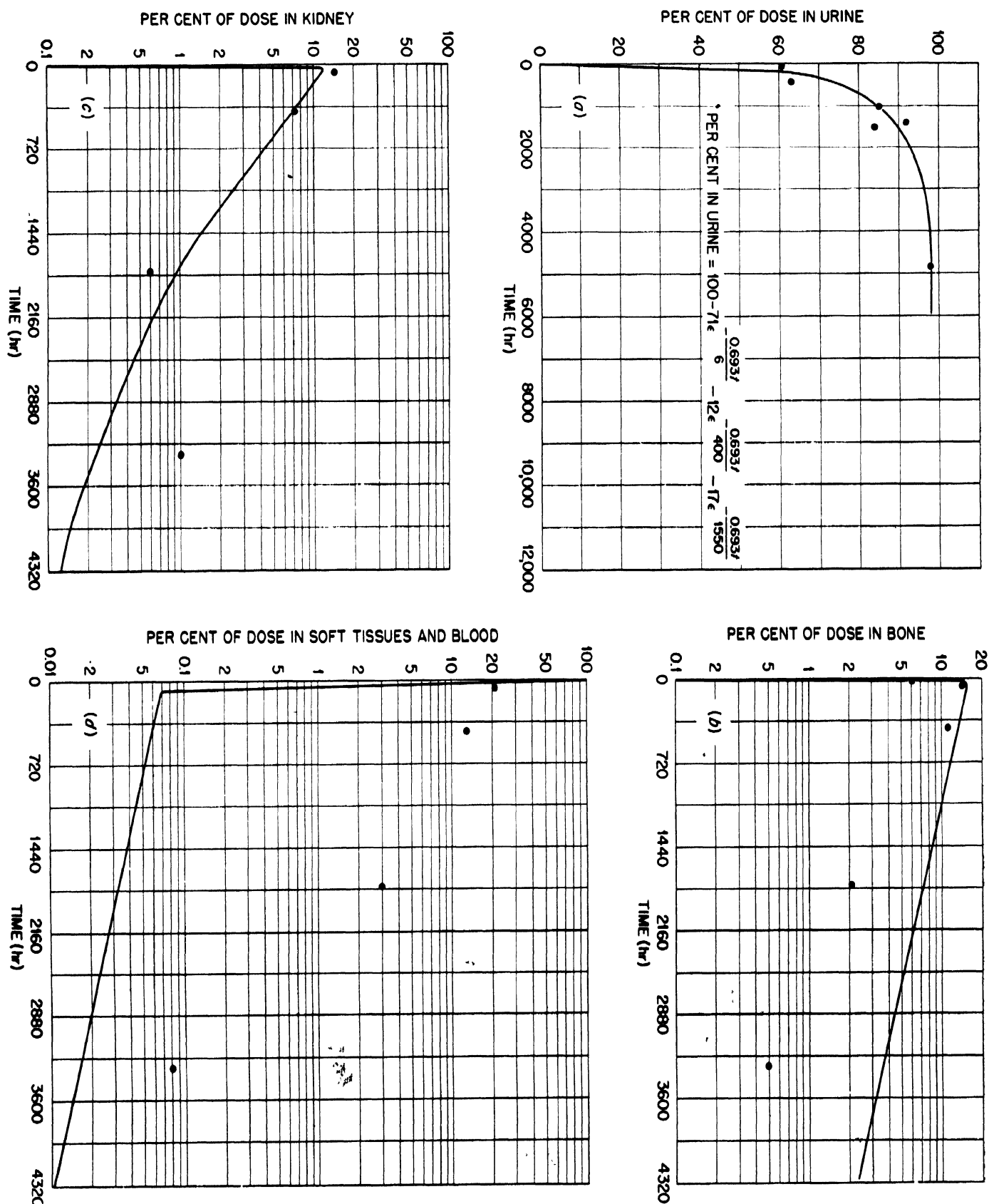


Figure 8. Comparison of observed and estimated organ contents

Rat kidney initially contains $\frac{1}{5}$ of the dose and has a half-life of 4–6 days.¹³ A plot of the per cent of injected dose found in the kidneys of these patients at autopsy gives the fraction initially deposited as $16 \pm 0.04\%$ and a half-life of 16 ± 0.32 days. This graph is shown in Fig. 5.

These patients excreted from 49 to 84 per cent of the injected dose in the urine during the first 24 hours as shown in Table III. Neuman cites excretion values covering the same time interval of 50 per cent in mice, 60 per cent in rats, 70 per cent in dogs, and 73 per cent in humans. In this study, which covered periods of observation ranging from 60 hours to 7 months, 61 to 98 per cent of the injected uranium was recovered in the urine.

Previous animal work has demonstrated considerable variations in bone deposition with age, activity and diet. Also, deposition of uranium in the kidney has been shown to be markedly affected by the acid-base balance. It is apparent that the metabolic state

Table II. Autopsy Results in Per Cent of Injected Dose per Gram $\times 10^{-4}$

Patient no. Post injection time	I 60 hr	II 1775 hr	IV 3335 hr	VI 430 hr
Adrenal gland	11.1	1.6	0.6	6.0
Aorta	7.1		0.01	7.1
Bladder	2.2	0.1	0.09	
Brain			0.05	13.8
Brain (frontal lobe)	270.8	0.2		
Brain (parietal lobe)	28.2			
Brain (temporal lobe)		0.1		
Cartilage			0.1	
Cartilage (rib)		0.9		
Cartilage (tracheal)				3.5
Fat (subcutaneous)	0.6			0.6
Fat (from skin)				8.7
Femur (II & V bone from shaft)				
(I & VI bone from distal end)	5.8	0.5	0.6	4.4
Gall bladder	4.8		0.2	13.7
Heart	2.0	0.1	0.2	0.7
Intestine (small)	2.1	0.3	0.1	1.8
Kidney	554.0	22.1	39.3	239.9
Liver	10.4	1.4	0.9	6.5
Lung	4.7	0.3	0.2	3.6
Lung (necrotic)	23.9			
Marrow (bone)		0.1	0.2	
Muscle	0.4	0.3	0.09	1.0
Muscle (psoas)				0.3
Pancreas	96.0	1.2	0.08	1.1
Prostate		0.2	0.2	1.6
Rib	19.7	2.6	0.9	42.1
Skin	3.0	0.2	0.1	1.7
Skull	7.9	11.9	1.9	
Spleen	18.8	3.6	0.5	7.7
Sternum			0.5	
Stomach	3.2	0.1	0.05	0.8
Testis		2.1	0.5	2.9
Thyroid		0.1	0.07	1.5
Tumor (tissue from edge)	24.8	0.6	0.4	
Tumor (viable)		0.6		
Vertebrae (lumbar)		1.8		
Vertebrae (thoracic)	53.8			

Table III. Per Cent of Dose Excreted in Urine

Patient no.	24 hr	48 hr	Last sample	Time of last sample, hr
I	59	61	61	57
II	78	84	92	1437
III	84	88	98*	4823
IV	77	81	85	1008
V	67	72	84	1511
VI	49	53	63	425

* Last sample analyzed; patient has not expired

of these patients would differ from that of healthy subjects. None of the patients showed the salt loss or retention occasionally noted following brain damage, but their bed-ridden condition indicated a state of negative calcium and nitrogen metabolism. No doubt these factors, and perhaps others, played an important role in some of the variations noted in these patients. For example, it might be inferred that the effect of a negative calcium balance would hasten the removal of uranium from the skeleton.

In general, considering factors of 2 to 4 as being small, the data obtained in this study are in good agreement with those obtained in small animal research.

Preliminary Application of the Model

The ultimate purpose of the model is to evaluate individual excretion curves in terms of distribution. By fitting a plot of excretion data with a sum of three exponential terms and equating this to the theoretical excretion equation, it is possible to estimate the amounts of uranium in principal sites of deposition. This requires analytical curve fitting methods to obtain the best fit of the excretion data.

An attempt has been made to obtain the best fitting excretion equation for each of the patients involved in this study. Two analytical methods^{14,15} have been tried without success and other methods are being investigated at this time.

However, in order to illustrate the application of the model, a tentative schema employing the distribution and excretion measurements of all patients can be used. First, the data in Table III are plotted. Then a free hand curve is drawn and the equation of the curve is determined graphically. This curve and its equation are shown in Fig. 8a. The unknown parameters of distribution can then be determined in the manner shown in Fig. 1. When these parameters are substituted into the integrated equations for blood, bone and kidney, the uranium content in these sites can be calculated. The resulting curves, together with related biopsy and autopsy values, are shown in Figs. 8b, 8c and 8d.

While the calculated amounts of uranium in bone and kidney are in good agreement with the observed values, the difference between blood and soft tissue evident in Fig. 8d is striking. This suggests a significant fixation of uranium in soft tissues (other than kidney) not found in small animals.

It must be pointed out, however, that final conclusions cannot be drawn because this application does not represent the final test of the model.

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Record of Proceedings of Session 8C

THURSDAY AFTERNOON, 11 AUGUST 1955

Chairman: Mr. F. Leborgne (Uruguay)

Vice-Chairman: Mr. M. Gokmen (Turkey)

Scientific Secretaries: Messrs. L. F. Lamerton, C. Polvani and I. D. Rojanski

PROGRAMME

P/685	Medical application of some radioactive isotopes	A. V. Kozlova
P/446	Some problems of radiotherapeutics	J. S. Mitchell
	DISCUSSION	
P/179	Teletherapy devices with radioactive isotopes	M. Brucer
	DISCUSSION	
P/925	Internal radioactive isotope therapy of neoplastic diseases by means of radioactive suspensions	J. H. Muller
P/367	The use of colloidal chromium radiophosphate in the treatment of malignant tumors	A. Chevallier and C. Burg
	DISCUSSION	
P/183	Radioactive isotopes in hematologic therapy	J. H. Lawrence
P/970	Accurate method for the treatment of hyperthyroidism with I^{131}	V. H. Franco <i>et al.</i>
P/177	Use of the nuclear reactor for neutron capture therapy of cancer	L. E. Farr <i>et al.</i>
	DISCUSSION	

The CHAIRMAN: Ionizing radiations represent an important method of therapy, but their effectiveness is limited by biological and topographical conditions. The papers to be submitted at today's session report on experience with new radiotherapeutic agents and cover the following subjects:

1. The use of various methods and media of delivery—the blood, intracavity and interstitial injection and selective metabolic localization—which provide better access to the site of the lesion.

2. New types of teletherapy machines for percutaneous treatment with different and more powerful radioactive sources.

3. Radioactive sources recently used in place of radium for internal and external focal therapy and their technical and economic features.

4. Lastly, therapy with heavy particles produced by neutron capture at the site of the lesion, a subject on which clinical experience is limited.

These papers will serve to increase our basic clinical knowledge in regard to dosage, radiation quality and geometry.

Mrs. M. N. FATEYEVA (USSR) presented paper P/685.

Mr. J. S. MITCHELL (UK) presented paper P/446.

DISCUSSION OF P/685 and P/446

Mr. M. H. BRUCER (USA): The excellent results presented by Mrs. Fateyeva are a little difficult for me to interpret because of some of the words used. In order to get over this semantic difficulty, I would ask what is the precise meaning of the word "recovery" which is used by the author of the paper, and, as concomitant to that question, were all these cases histologically examined?

Mrs. FATEYEVA (USSR): A diagnosis of clinical recovery is made on the basis of observation of patients' subsequent progress. If there has been no recurrence of the disease five years after the cessation of treatment, we usually consider that for all practical purposes recovery has taken place. Biopsies were made in all the cases referred to in our paper; they are in fact made in all cases of cancer treated in our clinics, and the diagnosis of cancer is normally confirmed by histological examination in every case.

Mr. AEBERSOLD (USA): I should like to ask if those slides illustrating therapy with cobalt were with teletherapy or with cobalt-60 needles or with other gamma-ray needles. I should also like to know how many years' experience institutions in the USSR have had with cobalt teletherapy.

Mrs. FATEYEVA (USSR): Depending on the special features of each case and on the clinical picture, ordinary teletherapy or, as in the case of treatment of cancer of the bladder, teletherapy combined with intra-tissue therapy was prescribed. In the cases of cancer of the skin, lips and tongue, both external and intra-tissue irradiation were employed.

As regards the Soviet Union's experience of teletherapy, 166 oncological institutions are supplied with necessary equipment, and the results obtained are normally analyzed in central organizations.

Mr. C. WU (China): I wish to ask Mrs. Fateyeva about the last case that she presented to us. Is that a case only of diagnosis by X-ray or is it proved by other pathological examination? May I ask another question? Mrs. Fateyeva, have you ever had any cases treated by radioactive isotopes for cancer of the prostate and if so, with what results?

Mrs. FATEYEVA (USSR): The data available to me, or more correctly, to the author of the paper I have just presented, did not include any information about cancer of the prostate. The type of treatment mentioned is, however, given in the Soviet Union.

Mr. A. SOMERWIL (The Netherlands): I was very interested to hear that the possible use of germanium for dosimetry has been investigated. As we in our institute have tried to use germanium diodes for dosimetry and have not found them very useful, we should like to ask if you at your institute were successful with their use and we should like to hear what the results were.

Mrs. FATEYEVA (USSR): We are not at present using germanium therapeutically on a sufficiently large scale to form any definite conclusions. I am not in a position to make a full statement on the basis of the information at present in our possession. Experimental work is being carried out in individual clinics and laboratories.

Mr. M. H. BRUCER (USA) presented paper P/179.

DISCUSSION OF P/179

Mrs. LEBIDINSKY (USSR): Radiocobalt-60 machines in the hectocurie range are being extensively used in the Soviet Union. I am glad to hear that such machines are also being used in the United States at the present time. I should like to hear Mr. Brucer's views on the prospects of the further use of radiocobalt machines in the 1000 curie range.

Mr. BRUCER (USA): There is in the United States a large number of therapists, each of whom has his own idea of what is the best curiage, the best design of machine and the correct output for any particular machine.

About 60 to 100 machines (I am not sure how many) have been developed so far. Of those with which I have personal acquaintance most of the individuals who now have sources in the 200, 300 and 400 curie range would very much like to have a

higher curiage for the same design machine, usually in the 600 to 700 curie range. Fifty per cent of those who now have machines in the 1000 curie range would be perfectly willing for their next machines to go down to the 600 curie range, and 50 per cent would be willing to go up to the 1500 and 1800 curie range. I think the question is one of personal interpretation, and it could be answered by various people in various ways. Therefore, our program is to furnish a whole range of machines rather than to standardize on any one particular source size.

Mr. K. C. BORA (India): On what basis has the assumption been made that an output of less than 5 rhm (roentgens per hour at a meter) is inadequate for teletherapy?

Mr. BRUCER (USA): That assumption was made only for the purpose of the study of these various machines. There are two people in the United States who would much prefer to have machines with outputs of less than 5 rhm. However, they are the only two people in the United States who would accept less than that. Most of the people in the large meeting of therapists agreed to accept arbitrarily, as it were, a figure of 5 r per hour as a minimum output for any particular machine. It is admittedly an arbitrary figure.

Mr. V. R. KHANOLKAR (India): What is the treatment policy on fractionation and total dose?

Mr. BRUCER (USA): In most cases the treatment policy that has been followed is very similar, especially with the fractionation machine, to the treatment policy followed with the 250-kv X-ray generators. In the case of some of the very large machines, many of the radiotherapists have increased the total dose. The usual fractionation policies are followed, almost the same as they have been for any other form of supervoltage therapy.

Mr. J. H. MULLER (Switzerland) presented paper P/925.

Mr. A. CHEVALLIER (France) presented paper P/367 as follows:

The work we are doing leads us to believe that of the radioisotopes at present in industrial production phosphorus-32 is probably one of the most suitable for effecting local irradiation *in situ* for the treatment of malignant tumours.

The radioactive element must enter an absolutely insoluble molecule and the colloidal suspension obtained must have relatively fine particles and a high specific activity. For this purpose, we prepare chromium radioactive phosphate, which has particles with an average diameter of 100–150 Å and has a specific activity of over 10 millicuries per milligram.

In our view, this preparation has three advantages:

1. It has a high-energy beta radiation (1.7 Mev in the case of phosphorus-32) with no emission of gamma radiation. Thus, irradiation carried out at the tissue level remains completely localized. Its action extends over a radius of roughly 5 millimetres

from the injected element but not beyond. There are thus no secondary reactions in other organs or tissues.

2. The half-life of phosphorus-32—approximately 15 days—is long enough to permit a regenerative reaction by the tissues and stroma and hence to expose differences in radiosensitivity at their maximum. Consequently, large quantities of chromium phosphate can be placed in the immediate vicinity of large vessels without any risk of hemorrhage, provided that the vessel walls are healthy and normal. A gradual thickening of the walls in fact takes place, which eliminates the risk of necrosis.

3. An important fact is noted—that the lymphatic system of the area into which the chromium phosphate is introduced becomes activated. This activation is due to the carrying of radioactive particles by macrophages. The resultant radioactivity of the lymph nodes may be sufficient to cause the destruction of the node and any cancerous cells that may be present. This secondary specific action is connected with the colloidal nature of the radioactive preparation and, in particular, with the size of its particles and its specific activity.

The document P/367 gives quantitative data on this point.

In view of these facts, we have applied the following general procedure to various cases of malignant tumours (different cancers). It requires adaptation to each individual case, according to the character, the degree of localization and size of the tumour.

First, the colloidal suspension of chromium phosphate is injected into the tumour site. This is done at points one centimetre apart, 1 cm³ of liquid being injected at each. The radioactivity injected is in the region of 200 microcuries per cubic centimetre at each point. Then, a few days later, the tumour is excised surgically and chromium phosphate is placed in the operative field in the form either of implants of hardened gelatine activated with chromium phosphate or of a suspension.

The implants activated with chromium phosphate are manufactured by preparing an absolutely sterile viscous solution of gelatine, in which the colloidal suspension of chromium phosphate is incorporated: the solution is poured on to sheets of glass and the liquid is allowed to evaporate. Sheets of hardened gelatine are thus obtained which can be cut as and when needed. These sheets, whose radioactivity is proportional to their surface, can be implanted in the tissues it is desired to treat.

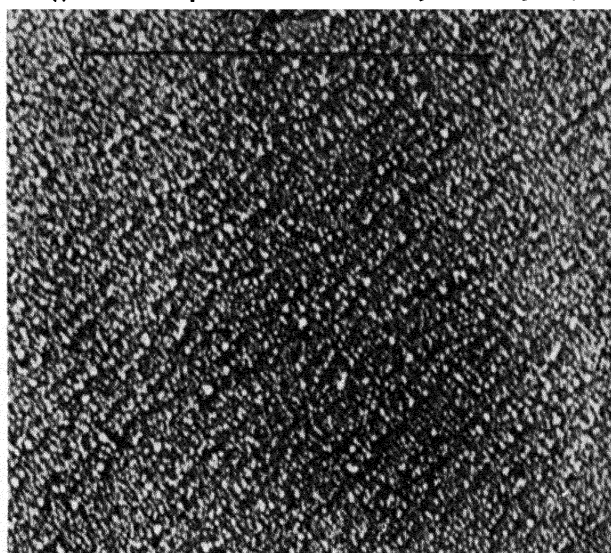
The gelatine is very rapidly absorbed and digested in the tissues within a few hours and the chromium phosphate particles therefore remain exactly where implanted.

We have treated by this method, adapted as necessary, cancers of the bladder, prostate, rectum, lung, breast and thyroid and inoperable metastases adhering to the large vessels. In all cases, it was used in conjunction with surgery, which had not previously been considered possible.

Slide 1 shows the chromium phosphate suspension we prepare under the electron microscope. The size of the particles is approximately 150 Å.

Slide 2 shows a gelatine implant in the thigh of a rat. It is a radioautograph with an X-ray photograph superimposed. You will see here that the radioactivity is completely localized in the muscle in which the chromium phosphate-impregnated gelatine was implanted.

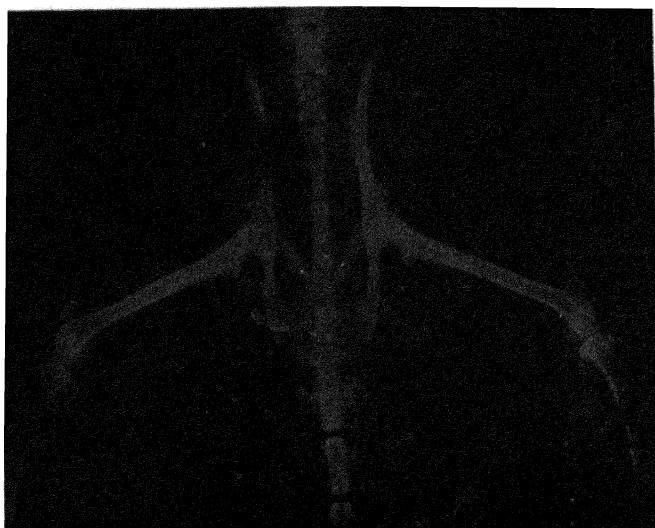
Slide 3 is also a radioautograph with an X-ray photograph superimposed. The subject is a rat. A quantity of chromium phosphate was injected in the upper right leg. It is only necessary to compare the darkness of the photograph here and in the other leg. It will be seen that radioactivity is localized in the right lumbar ganglion corresponding to the leg in which the injection was made. This is due to the migration of particles carried by macrophages.



Slide 1



Slide 2



Slide 3

The degree of radioactivity that can be obtained by this method is considerable. It depends on the size of the particles and, as I have already said, on the specific activity. When applied therapeutically, the radioactivity obtained in the lymphatic system is several tens of thousands of roentgens per glandular volume.

Slide 4 shows a specimen taken from a patient with a cancer of the rectum treated by chromium radiophosphate.

The tumour site, into which chromium phosphate was injected a few days before surgical intervention, was partly covered by a lead plate on account of excessive radioactivity and photographic diffusion.

The injection was confined to this area. It will be noted that there is a very high degree of radioactivity, which is very widely dispersed and disseminated, in all the lymph nodes in the part of the intestine next to the rectum, an indication of the extent and importance of dissemination by the lymphatic system.

Slide 5 is also a radioautograph with an X-ray photograph superimposed which was obtained in the

course of a large number of experiments we made on dogs before we attacked the problem of the treatment of cancer of the lung.

Before treating cancer of the lung, it was necessary to make sure that, in view of the proximity of large vessels, gelatine implants would be tolerated and would not involve any risk.

The implantation was made. This represents a dog's lung with one mediastinum. The implantation was made in two places. The rest of the slide shows the disseminated lymphatic activity spreading from these two points of implantation.

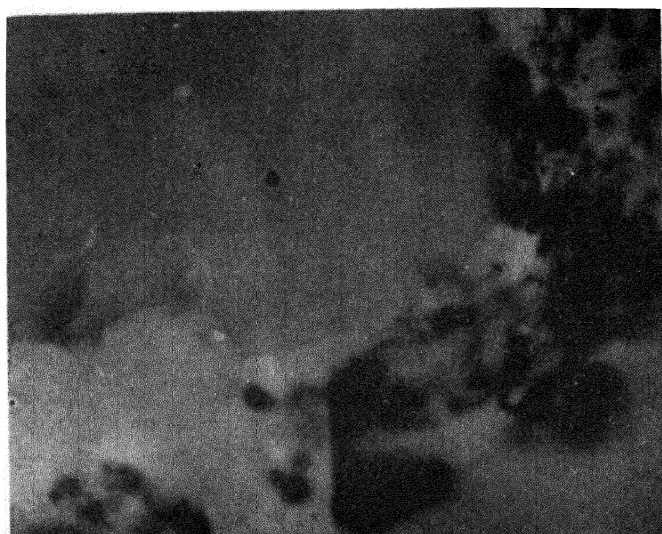
We found in these cases that the implant was extremely well tolerated, and we still have dogs in perfect health which were operated upon two years ago.

Slide 6 shows a dog's brain. We have in fact used the method in some cases of brain tumours. Before doing so, we carried out a large number of experiments on dogs and this slide shows sections made in a dog's brain from the point, here, at which an implantation with gelatine was made. The sections were approximately 2 millimetres in thickness. The slide therefore shows the distribution of radioactivity in depth. It will be seen that the activity is completely localized and does not affect more remote sections of the brain.

Slide 7 shows the brain of a dog on which an autopsy was performed two months after a gelatine implant had been made in one lobe. Here there is a clearly defined area of necrosis and histological examination showed that the cerebral cells bordering on this area were perfectly healthy.

With this method, therefore, the surface affected by irradiation is strictly limited not only as regards the destruction of cells but also as regards reactions and particularly sclerotic reactions due to irradiation.

It is obviously much too early to evaluate this method, the purpose of which is less to destroy the tumour directly than to facilitate its surgical excision and to protect the patient against local secondary recurrences in the form of glandular metastases. The results so far obtained have been encouraging.



Slide 4



Slide 5

DISCUSSION OF P/925 AND P/367

Mr. MITCHELL (UK): Could Mr. Muller discuss the problem of dosage in injection implants? To obtain useful therapeutic effects one often requires to deliver mean doses of 50,000 rep and sometimes more. In many cases the gamma-ray dose will not explain the effects; nevertheless in some otherwise untreatable cases useful palliative results are obtained.

Mr. MULLER (Switzerland): The question is difficult to answer. It is true that in various interstitial applications we have great difficulties in assessing exactly what we do about dosage, and there is also the question of the danger of having too many very hot spots. It depends, of course, on the general volume. I would say that with very small volumes we can go to 1 millicurie of gold to 1 cm³ of tissue, but we have to go very much down if the volume increases. Apart from special situations where you can see what you are doing, when you blindly inject the region, you have to use in my experience rather large volumes of liquids in order to disrupt the cellular spaces, but if you use this technique I do not think that there is any danger.

Mr. SIWABESSY (Indonesia): I have two questions for Mr. Muller. First, what is the influence of intra-abdominal radioactive gold on the blood and spleen? Secondly, have you had any experience with carcinoma of the liver treated with radioactive gold?

Mr. MULLER (Switzerland): I would answer the first question by saying that the application of radioactive gold does not involve particularly high danger for blood formation and for spleen function. It is a little astonishing to see that patients after a large course of X-ray treatment will still stand, say, one application of 100 to 150 millicuries of radioactive gold quite well. It may be that due to the resorption within the lymphatic there is some stimulation of the system which affords an explanation.

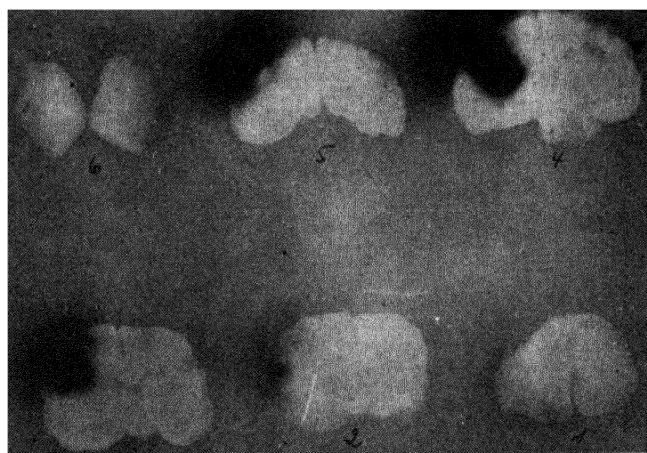
The second question concerned carcinoma of the liver. I have had rather a limited experience, and I would say that treatment with radioactive gold intravenously is only a palliative method of treatment, but it is reasonable in certain cases of radio-sensitive tumours. I remember one case of a huge tumour which responded extremely well to radioactive gold given combined intravenously and intraperitoneally. An interesting palliative effect was also obtained in a case which I have already shown, although the patient lived only a few months.

Dr. Ross (USA): I should like to address this question to Mr. Muller and Mr. Chevallier. When a metastatic disease is present in lymph nodes, is it not usual to find that the drainage of the radioactive colloid to the involved node is blocked, and therefore that the radioactive colloid method is not an effective method of treatment?

I have a second question. Will Mr. Muller explain his preference for radioactive colloidal gold as against colloidal phosphate, because we might expect the gamma-emission of gold to be a hazard.

Mr. MULLER (Switzerland): In reply to Mr. Ross, it is clear that in the cases which have only a huge, bulky metastasis within the lymph nodes there is no chance to reach them with this procedure. However, as I have shown, it seems that when the process of metastasis is only beginning, the flux of the particles is even increased in comparison with nodes which have not yet received any cancer deposits. I would say, therefore, that the use of this method is sensible in those cases in which the process is only beginning, or where it is not far advanced; but as soon as we have huge masses which are fixed it is no good at all. Cases where large masses are concerned have to be treated classically by some form of deep radiotherapy or surgery if possible. Even in cases where there are big masses, however, it might be reasonable to remove surgically the very large masses and then infiltrate immediately the surroundings with some colloidal material, gold or phosphate. We have done that in a few cases, in recurrent cancer of the mammary glands, removing the large masses and infiltrating the surroundings.

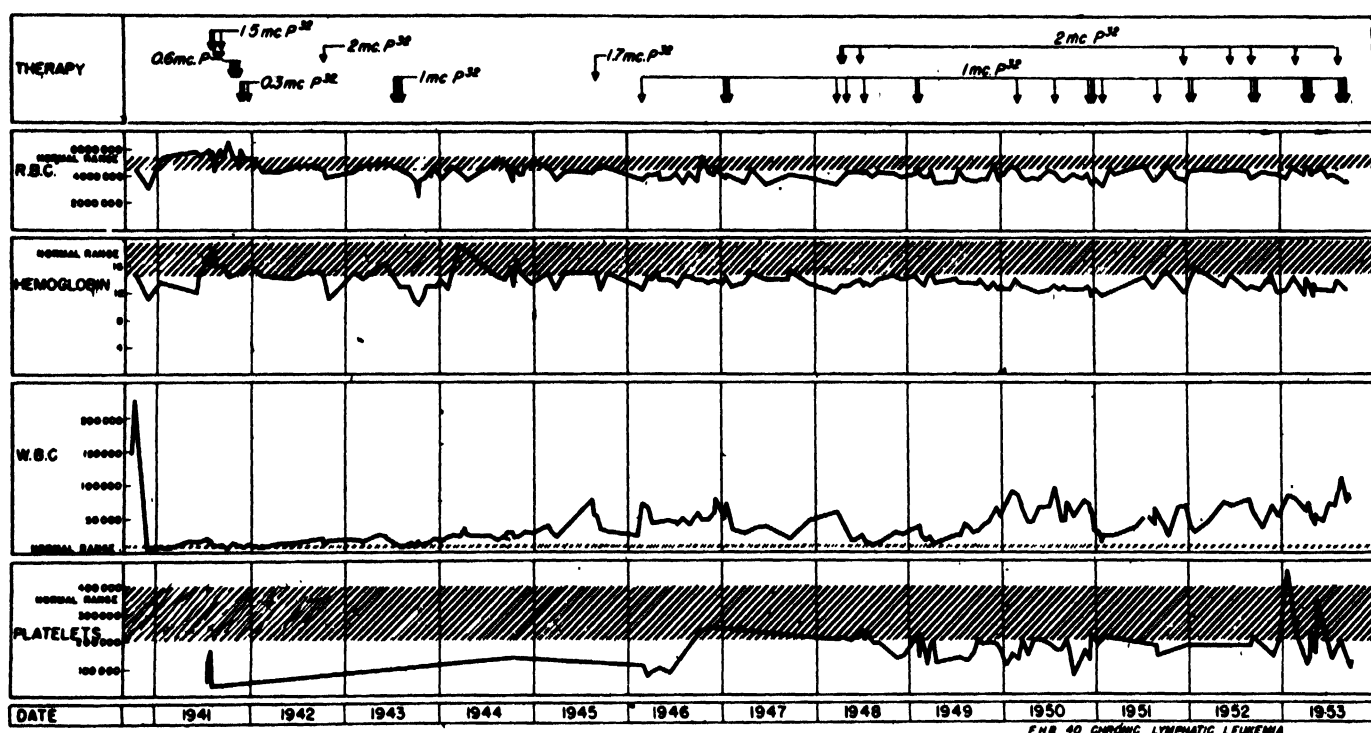
The second question concerned my preference for gold. Gold in my opinion has an advantage, which can also be a disadvantage, in giving gamma radiation. The gamma radiation, having an energy of order of magnitude about 400 kev, procures substantial homogenization of the radiation effect, be-



Slide 6



Slide 7



Slide 8

cause all the beta effects are rather spotty and never quite homogeneous. On the other hand, of course, there may be situations in which too much gamma radiation may have certain dangers. Personally, I think that this whole question should be discussed more thoroughly and systematically, and there might be certain advantages in discussing whether there are cases in which we should use, say, a mixture of different colloidal materials in order to have some gamma radiation. Take the abdominal structures. If you use radioactive gold, the range of the beta particles reaches only the outer part. Using yttrium, with the higher energy you may reach deeper into the bowel. There are many factors which should be considered in each case.

Mr. W. H. SWEET (USA): I should like to ask Mr. Chevallier whether he has had any experience, with his beautiful technique, with the treatment of craniopharyngiomatic tumours by instillation therein of his colloidal radiophosphate. This would seem a peculiarly favourable sort of tumour for this treatment.

Mr. CHEVALLIER (France): I should like to associate myself with what Mr. Muller said just now in reply to the previous questioner. It would be useful to discuss the respective advantages and drawbacks of gold and chromium phosphate in connection with the problem of glandular metastases.

I should like to mention an important fact in this connection. In cases of breast cancers with axillary metastases, we first make an injection in the primary tumour, as I explained just now. Then we look for radioactivity in the axillary area with a beta counter.

The problem of course does not arise if large metastatic masses are palpable in the axillary area. In that case the lymph nodes are of course blocked and the radioactive colloid consequently does not

penetrate into the large metastatic masses. But where these masses are fairly small or barely perceptible, tests with the counter enable us to determine the degree, though not the amount of penetration. If a high degree of radioactivity is detected in the axillary area, the infiltration around the tumour is likely to be sufficient to provide a solution of the problem. If not, treatment of the axillary area as if it itself were a tumour is indicated. In short, the metastasis would then be considered on the same footing as the primary tumour and treated accordingly.

As regards the second question on tumours of the pharynx, I am unable to reply as I have not had occasion to study the matter.

The CHAIRMAN: I wish to thank the speakers who have spoken so far and to invite Mr. Gokmen to take over the Chair until the end of the session.

Mr. Leborgne then vacated the Chair, which was taken by the Vice Chairman, Mr. M. Gokmen (Turkey).

Mr. J. H. LAWRENCE (USA) presented paper P/183, as follows:

It is just twenty years ago this summer that we first had available artificially produced radioisotopes from the 37-in. cyclotron in Berkeley, and at that time began using them in tracer medical studies on patients. In the short time available, I can only discuss the end results in the therapy of two blood diseases representing a summary of twenty years' experience with P^{32} , and present some tracer investigations on the nature of these conditions, which have been helpful in therapy.

Early experiments with radioactive phosphorus showed considerable localization of P^{32} in leukemic and neoplastic tissue, in addition to localization in

bone and bone marrow and so it seemed obvious to us that this isotope would be of value in the therapy of chronic leukemia and polycythemia vera. The now wide use of P^{32} throughout the world in the treatment of these conditions makes it unnecessary for me to go into detail here today. Many patients with chronic leukemia survive long and comfortable lives under P^{32} therapy.

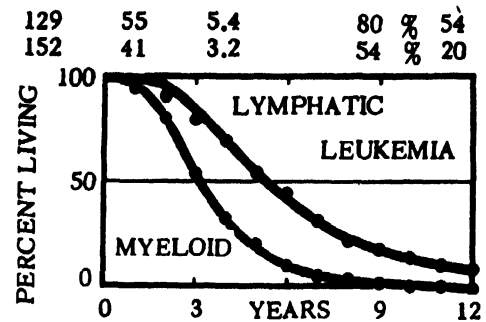
As an example, Slide 8 presents the hematologic course of a forty-one-year old engineer, treated over a period of fifteen years with the infrequent administration of P^{32} , with the red, white and platelet counts being maintained at near normal levels, and the patient leading an active and comfortable life.

Slide 9 shows the end results of the 20-year study. Fifty-four per cent of patients with the lymphatic variety survive over five years and the median survival time is five and one-half years. Later I shall present some tracer studies on the nature of this condition which at present is not well understood or well treated by any known method.

In the case of polycythemia vera, the results are much better, giving the patient a near normal life expectancy. After one or two doses of P^{32} long remissions are possible, as shown in Slide 10. This fifty-two year old man received two single doses totalling 9.5 millicuries, producing a remission lasting ten years.

The end results are depicted in Slide 11, showing that 91½% survive over five years, 70% over ten years and the median survival time is thirteen and two-tenths years, like that in diabetes treated with insulin and pernicious anemia treated with liver extract. This represents a successful therapeutic application of the atom to medicine. Thus the first disease treated with an artificially produced radioisotope was chronic leukemia, but the first disease successfully treated with a radioisotope artificially made is polycythemia vera.

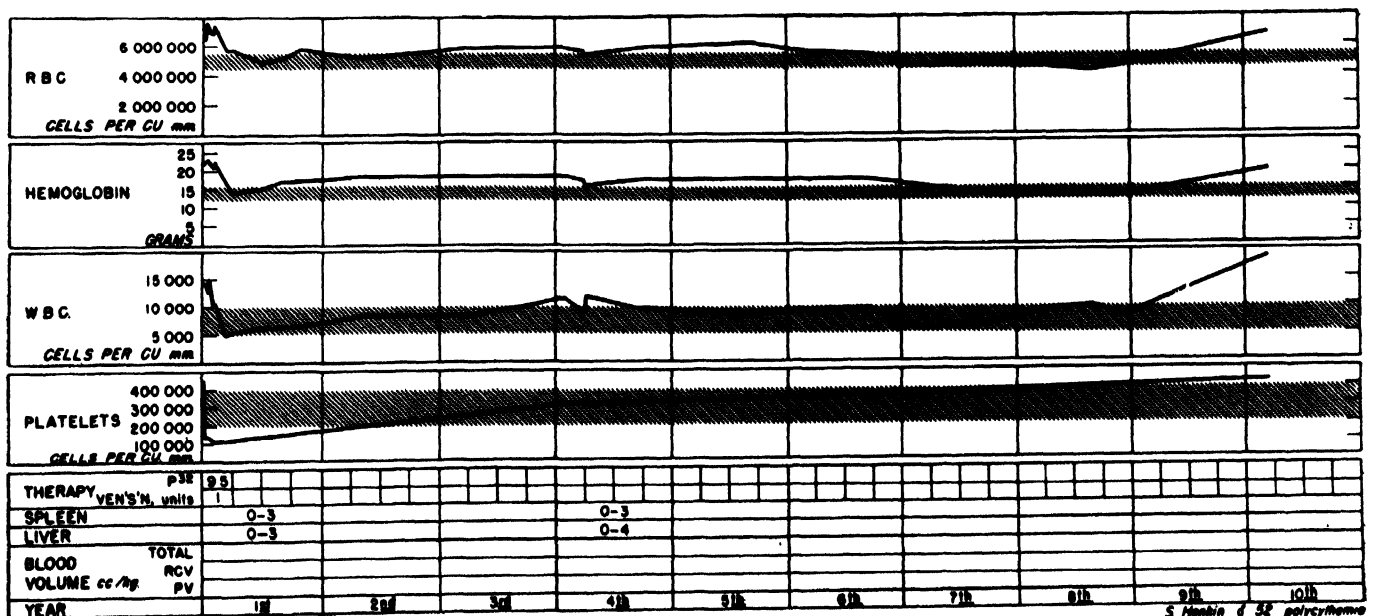
Cases Av. Age Median-Survival-3 Year' 5



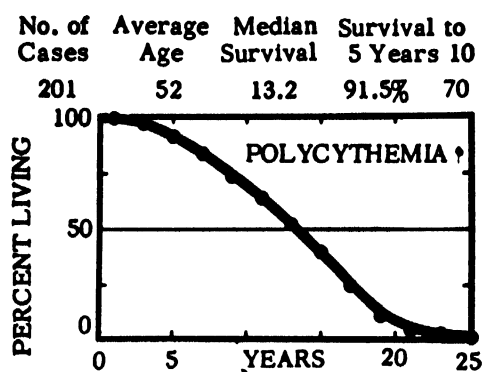
Slide 9

Many isotopes are helping us treat and understand the nature of these diseases, especially Fe^{59} and C^{14} . The rate and site of production and destruction and the life duration of the red cell can be measured with these isotopes. The radioactive iron disappearance rates from the plasma can be measured after intravenous injection of a few microcuries of Fe^{59} . The normal disappearance rate gives a half-time of two hours. Early we were surprised to find that in chronic leukemia this half-time was usually normal, indicating that these patients did not have a myelophthisic anemia, since their red cell production was normal. It was concluded that the life duration of their red cells must be shortened. This was demonstrated using C^{14} glycine which is a building block for hemoglobin.

Slide 12 shows the C^{14} specific hemoglobin activity plotted against days in a normal person, and in a patient with myelogenous leukemia, showing a finite shortening of the red cell life to approximately ninety days, indicating a defect in the red cell. In a patient with lymphatic leukemia a random shortening of the life span is shown to less than thirty days, indicating some extrinsic factor causing premature destruction of the cells.



Slide 10

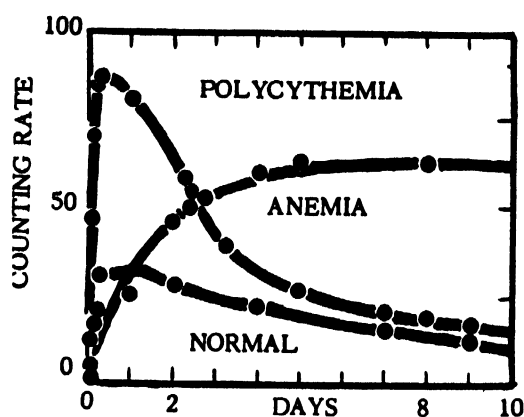


Slide 11

Using a scintillation counter over the spleen of the patient who has received radioiron, it is possible to get information on the site of red cell production and destruction, as shown in Slide 13, showing extra medullary red cell production in the spleen in a patient with polycythemia vera and abnormal destruction of red cells in a patient with anemia and leukemia.

In the therapy of these patients their anemia may be often corrected by either splenectomy or the administration of ACTH or cortisone. Slide 14 shows the red cell life duration in this patient to be short, with random destruction, but after splenectomy the life duration has returned to normal and the anemia disappears. A similar change from a shortened life to a normal one is observed after ACTH administration.

Recently, with the aid of isotopes, we have been looking for the stimulus for red cell production, hoping to throw light on the abnormality causing polycythemia vera. Using isotope techniques we found red cell production to rise rapidly on ascent from sea level to altitude. Slide 15 shows radioactive iron disappearance rates from the plasma in four subjects at Lima, Peru, and after ascent in the Andes to 15,000 feet, a marked increase at altitude. This occurs after one hour's exposure to 15,000 feet. From the plasma of patients with polycythemia vera and secondary polycythemia a humoral factor can be ex-



Slide 13

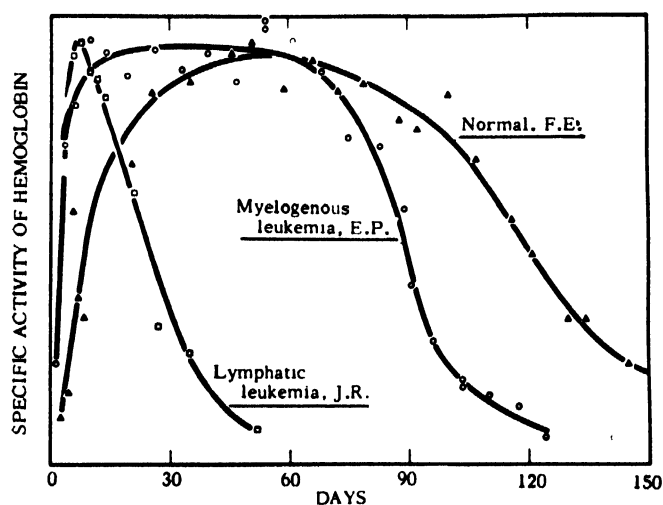
tracted which stimulates red cell production in rats. Recent investigations by my colleagues Contopoulos and Simpson *et al.* in the Institute of Experimental Biology, have revealed a similar erythropoietic factor in the pituitary gland which may be at the basis of polycythemia vera. We have shown that with daily injections of this pituitary extract a polycythemia can be produced in the normal hypophysectomized rat. This pituitary factor is apparently not mediated through the adrenals since it is free of ACTH.

The possibility of pituitary hyperfunction in polycythemia vera brings me to a brief discussion of some recent investigations with the synchrocyclotron, which produces a somewhat unique form of high energy irradiation—340 million volt protons or 190 Mev deuterons. This beam can be delivered to tissues in a well-delineated pencil of particles and small areas of tissue inhibited or destroyed.

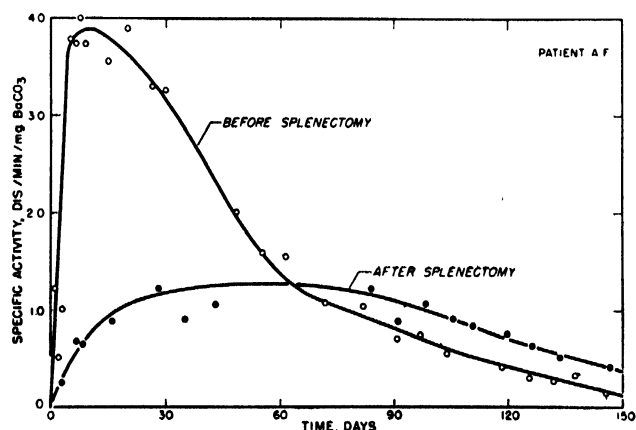
Slide 16 shows the unique depth-dose characteristics of the beam compared to 200 kv X-rays or 16.4 Mev electrons. The Bragg curve here shows the depth dose at 14 cm in tissue to be over four times the surface dose, unlike X-rays or electrons.

Slide 17 shows the sella turcica housing the pituitary gland, and to the right the beam passing through the sella and blackening the X-ray film in a sharply delineated area.

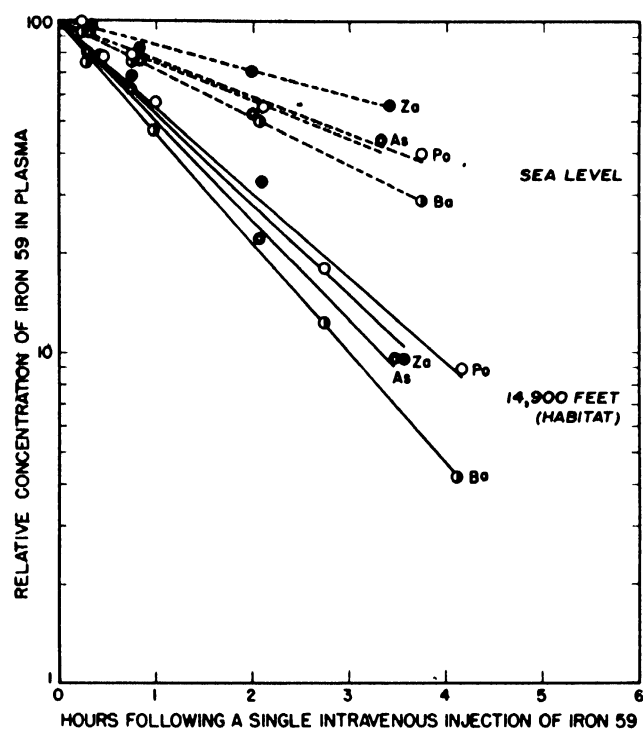
Slide 18 shows diagrammatically the set up developed by my physicist colleague, Cornelius Tobias, for animal or human exposure, the beam emerging



Slide 12



Slide 14



Slide 15

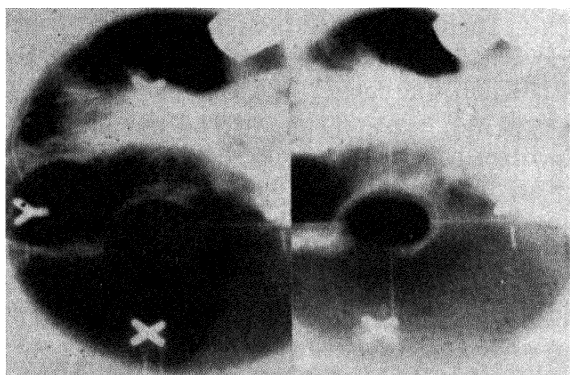
from the cyclotron into the physics room and passing into the next room, where animal or human studies are carried out.

That hypophysectomy can be done in this way is shown by Tobias in Slide 19. These are four rats of the same age. The dwarfed black rat had a surgical hypophysectomy, the other dwarf a radiation hypophysectomy. The one on the left is normal and the one on the right has obesity from selective hypothalamic irradiation.

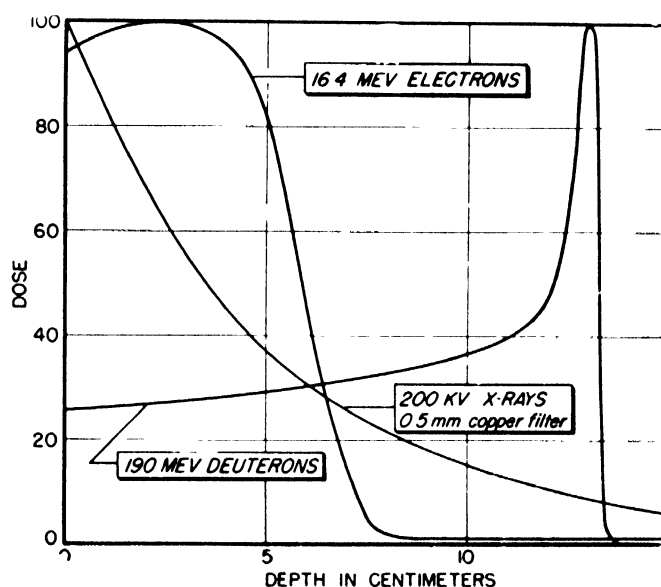
Slide 20 shows the patient set up for treatment with the deuteron beam and with two X-ray diagnostic units for alignment of the pituitary.

Slide 21 shows that with this beam the effects of hypophysectomy can be simulated, as shown by the disappearance of pituitary gonadotropins from the urine after 13,850 roentgens to the pituitary. There is also cessation of thyroid function as measured by uptake of radioactive iodine by the thyroid gland.

In several countries, in recent years, surgical hypophysectomy is being carried out for several different serious medical conditions. If hypophysectomy



Slide 17



Slide 16

or inhibition of pituitary function becomes necessary in the future, here is another method of carrying it out, without surgery.

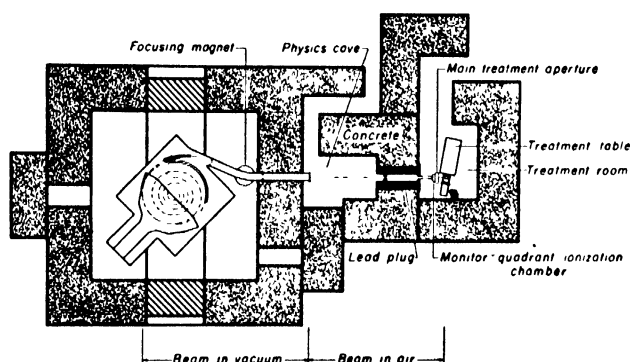
Mr. V. H. FRANCO (Portugal) presented paper P/970.

Mr. L. E. FARR (USA) presented paper P/177.

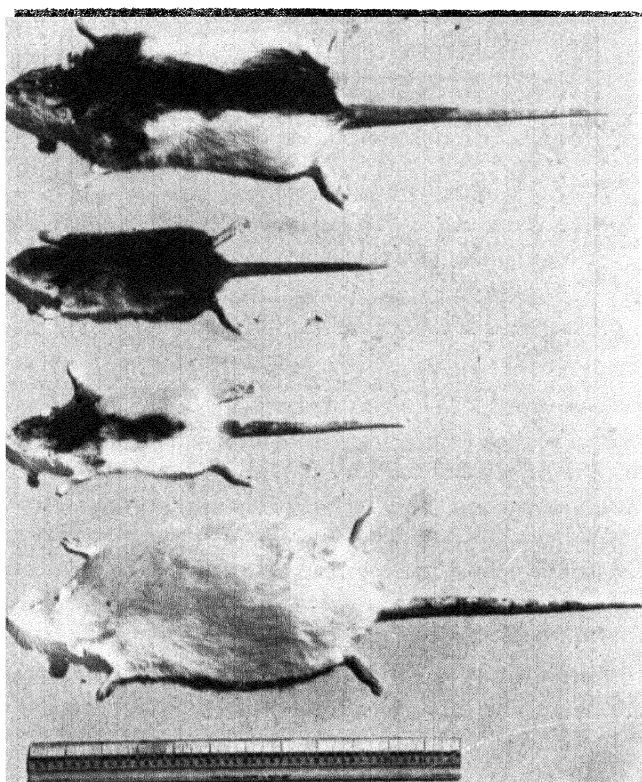
DISCUSSION OF P/970 AND P/177

Mr. J. F. Ross (USA): I should like to compliment Mr. Franco on his excellent presentation.

I should also like to ask him several questions in regard to the therapy of hyperthyroidism with radioiodine. I completely agree with him as to the importance of attempting to quantitate accurately the dose of ionizing radiation delivered to the hyperthyroid gland by radioiodine. He very appropriately emphasized its importance. Perhaps of equal importance in determining the clinical response of hyperthyroidism to radioiodine therapy is the severity of the disease of the patient. Those patients with severe hyperthyroidism appear to require a larger dose of radiation to induce a remission than those patients with milder disease. Does Mr. Franco's experience agree with this? Does he attempt to control the radioiodine dosage on the basis of the severity of the disease?



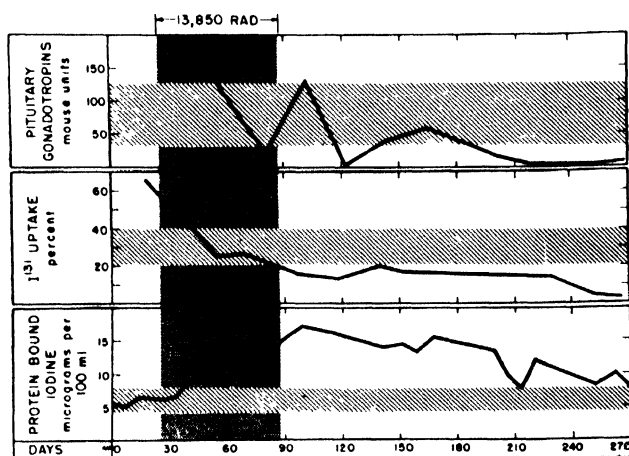
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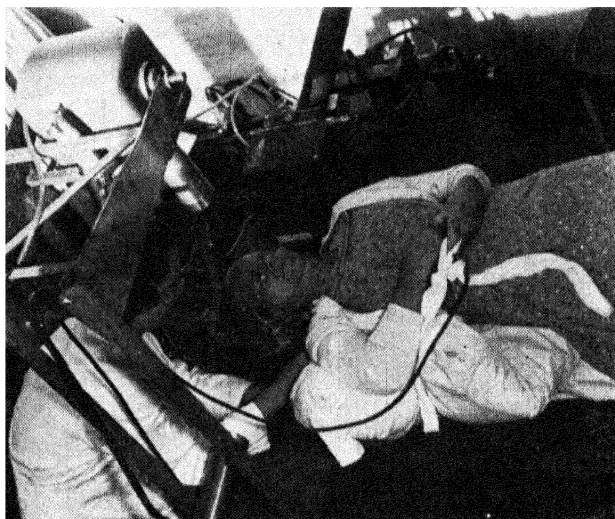
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Secondly, I should like to comment on the interstitial injection of air. In the United States similar techniques have been employed to visualize the adrenal gland in an attempt to demonstrate the presence of a tumour. In this procedure with the adrenal gland there have been several quite serious complications and many clinics have discontinued this particular procedure. I should like to have Mr. Franco's comments on the possible hazards of the procedure. I recognize, as he pointed out, that he had had no ill event from this procedure.

Thirdly, I should like to ask how the estimation of the size of the thyroid gland by Mr. Franco's method compares in accuracy with estimates of the thyroid gland size as indicated by scintillation crystal scanning methods and with the clinical estimation of thyroid gland size by palpation.



Slide 21



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Mr. FRANCO (Portugal) · I am afraid I shall not have time to reply to the first question. It is a question of the clinical state of the patient. I naturally agree with Mr. Farr that some clinical states call for the administration of different dosages; nodular goitres, for example, require larger doses than other goitres. But this was purely experimental work and in this case we had intended that all patients should receive the same dose. Unfortunately, as I said, they did not all do so.

We treated eight cases of nodular goitre solely with a view to reducing thyroid activity, as the patients were later treated surgically, and found that four had recurrences.

As I have said, we are making an experiment and for that reason wish to administer an equal dose to all patients in order to evaluate the biological factors involved.

I now pass on to the second question regarding the interstitial injection of air.

I do not know the exact number of these injections, but we have certainly made over fifty and have had no accident so far. We have admittedly taken certain precautions against them. Using the syringe I have just shown you, we pause before and during the injection, establish a negative pressure in the circuit and note whether any blood appears in the visor. On the rare occasions on which this has occurred, we made the injection elsewhere.

The patient lies on the left side, as the American work I have already mentioned has shown that in this position the danger of an air embolism is much reduced. We have not in fact had any cases of this.

In regard to the third question, we have made no comparisons. We have followed only the method I have described. I would point out that, with the transverse axial tomographic machine, we could readily determine the anterior-posterior diameter of the lobes. The lateral projection of the thyroid lobes in the scintigram is always unsatisfactory because the counter receives the radiation emitted by the two lobes of the gland simultaneously.

Section 9C

RADIOACTIVE ISOTOPES AND NUCLEAR RADIATIONS IN MEDICINE (*continued*) — DIAGNOSIS AND STUDIES OF DISEASE

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The Use of Radioactive Isotopes in Diagnostic Procedures

By W. V. Mayneord,* UK

It is now well recognised that radioactive isotopes may be used in clinical medicine both therapeutically and diagnostically. In both we make use of the energy radiated from the materials; in the diagnostic applications to detect the presence of the radioactive isotope and study of its metabolism. It is not therefore surprising that many diagnostic uses lie on the borderlands of clinical medicine with physiology and biophysics, or that the most useful methods are often those in which the radioisotopes are selectively taken up and concentrated in organs such as the thyroid, or carried in important tissue constituents around the body as, for example, the iron of haemoglobin.

RADIOACTIVE IODINE

Radioactive iodine-131 (as well as 128 and 132) has been used extensively in the investigation of thyroid disorders, and many tests have been developed, most of them based on two aspects of thyroid activity, namely (*a*) the removal of iodine from the plasma by the thyroid and (*b*) the subsequent synthesis and secretion of thyroxine. Phase (*a*) may be studied by measurements of the total amount and speed with which iodine is concentrated in the thyroid and phase (*b*) by a measurement of the fraction of the radioiodine in the blood plasma which is protein bound. The measurement by an external counter of the fraction of the iodine in the thyroid, say 24 hours after oral administration of perhaps 20 microcuries of iodine-131 in the form of sodium iodine, still forms the basis of most procedures and is conveniently carried out with roughly collimated scintillation counters (Fig. 1). Values greater than about 50% uptake indicate hyperthyroidism; normals show from 10% to 45%, and hypothyroids less than 10% to 15%, but great care and experience is required in the interpretation of these numbers.

The urinary output of radioactivity is often as characteristic as the uptake by the thyroid and forms a valuable check. (Fig. 2). Following intravenous injection uptake is very rapid and, again, may be used to delineate thyroid function. These tests measure primarily the ability of the thyroid to store iodine, but the production of thyroxine is even more directly related to the metabolic processes. Thyroxine is firmly bound to a specific globulin and we may, therefore, by precipitation, measure the activity associated with

plasma protein and hence study the activity of the thyroid. Some workers maintain that the ability of the salivary glands to concentrate iodine is also of diagnostic significance and may be of use particularly in the diagnosis of hypothyroidism.

It has been suggested that the rate at which radioiodine is secreted from thyroid carcinomata is, in general, faster than that for euthyroids and this rapid turnover in thyroid carcinoma may have diagnostic value, but there are many uncertainties. For example, the uptake in carcinomata of the thyroid is frequently hardly detectable at all until normal thyroid has been destroyed or removed.

RADIOACTIVE IRON

Another promising field of investigation depends upon the use of tracer doses of radioactive iron, particularly iron-59. This technique is of great interest in the study of erythropoiesis both in health and in diseases affecting the bone marrow. Roughly some 65% of the haemoglobin is in the circulating erythrocytes and by injecting intravenous radioiron in a similar chemical form to that found in the plasma, this iron will be used for red cell formation in preference to the ferritin iron. It is interesting that studies with iron seem to be approximating in type to those with iodine. We may, for example, study plasma clearance rates, that is the rate at which iron leaves the blood plasma to be utilised in red cell formation (Fig. 3). This quantity may be estimated by measuring the activity of samples of blood taken

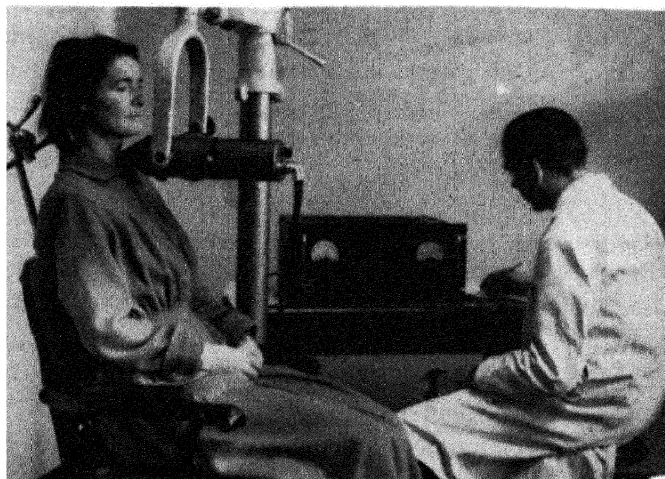


Figure 1. Measurement of uptake of radioactive iodine in the thyroid with scintillation counter. [Mayneord, W. V., *Proc. Roy. Soc. Med.*, 46: 225 (1953)]

* Department of Physics, Institute of Cancer Research, Royal Cancer Hospital, London.

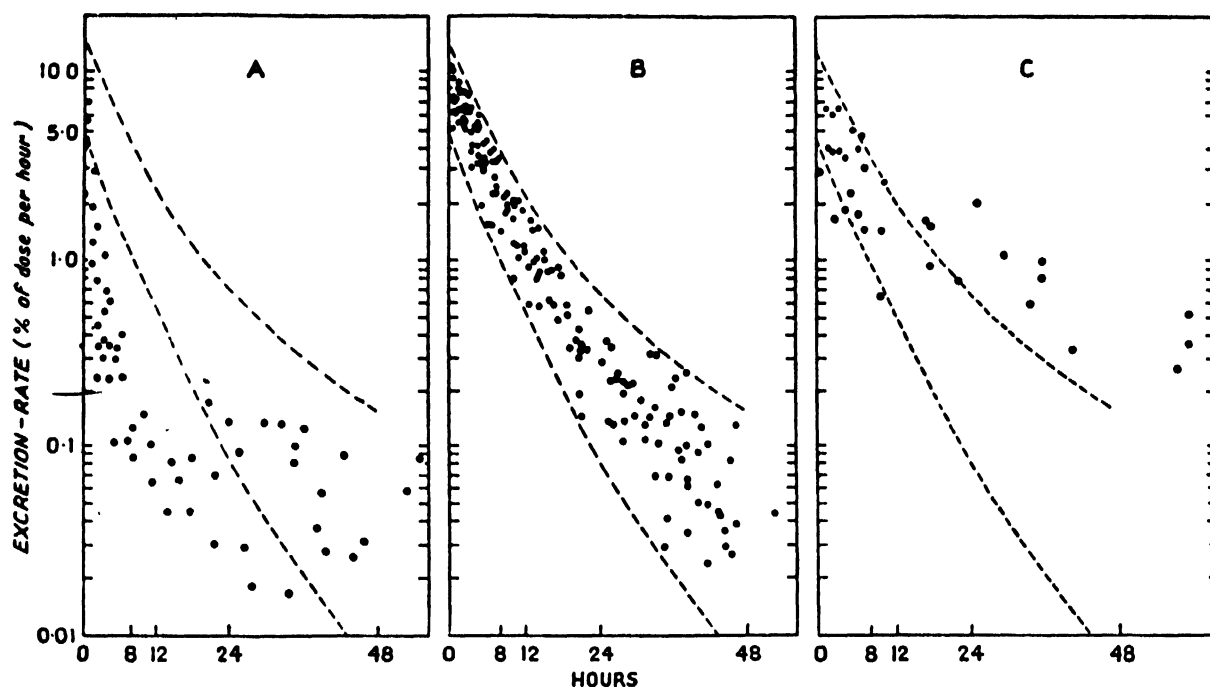


Figure 2. Urinary excretion of radioactive iodine. [Arnott, D. G., Emery, E. W., Russell Fraser, Hobson, Q. J. G. *The Lancet*, Sept. 10, p.460 (1949)] Results of the radioactive-iodine tests in the three groups studied: (A) thyrotoxic patients; (B) normal men; (C) hypo-

thyroid patients. The results of the test on each person were obtained as for Fig. 1, and each single observation of the rate of excretion has been plotted as a dot. The area enclosing the normal observations, (B), has also been outlined in (A) and (C)

at intervals after the injection of iron and gives a sensitive measure of the activity of erythropoietic tissue. During the increased marrow activity of polycythaemia the clearance rate is increased but if the marrow is hypoplastic, plasma clearance is reduced. It is also of interest to use these techniques to study the comparative uptake in various organs as, for example, by counting over the heart to obtain a measure of total blood activity. Measurements may also be made of activity in bone marrow by counting over the sacrum. Similarly we may make observations over the liver or the spleen (Fig. 4). The method may be used to study the effects of radiation treatment as, for example, the results of X-ray treatment of Hodgkin's disease.

BLOOD VOLUME AND CIRCULATION

The use of radioactive isotopes in physiological studies of treatment results is already very important. A group of clinical tests using radioactive materials is now available for the investigation of the circulatory system. For example, we may measure blood and plasma volumes, or the rates of flow in various parts of the circulatory system both in normal conditions or after pathological or surgical interference. The blood and plasma volume determinations normally depend upon the simple dilution principle, that is, injecting a known volume of a standard slowly eliminated substance and measuring its dilution when mixed uniformly with the total circulating blood. By the use of radioactive iodine attached to serum albumen, or iron-59 tagged red cells, accurate estimates can be made. The latter is inconvenient, for to provide iron-59 in cells they must be grown

in a donor maintained on a diet containing iron-59, but fortunately an alternative exists in the use of sodium chromate which enters red cells quite rapidly and is then apparently transformed into a non-diffusible form providing a permanent label. The

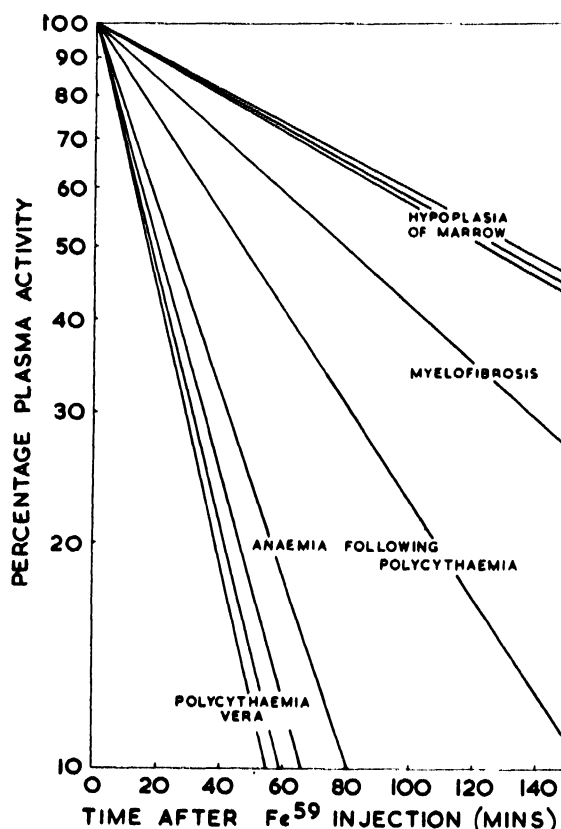


Figure 3. Plasma clearance rates. [Ledlie, E. M., Baxter, C., *Radio-isotope Conference, 1954*, 1: 97. Butterworths Scientific Publications, London (1954)]

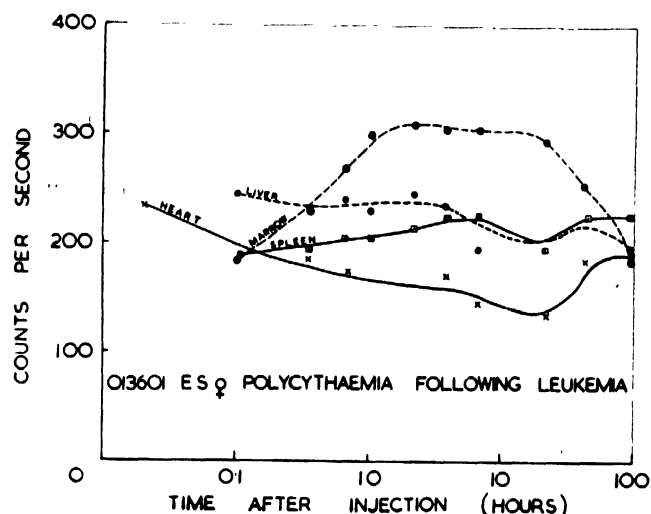


Figure 4. [Ledlie, E. M., Baxter, C., *Radioisotope Conference, 1954*, 1: 97. Butterworths Scientific Publications, London (1954)]

precise mechanism of this action is not understood but the subject of considerable enquiry. Chromium-51 is the most suitable isotope to use inasmuch as it has a convenient half-life of 26 days and is easily detectable by its 10% of transitions giving rise to 0.32 Mev gamma rays. We have found this chromium labelling of blood cells a very convenient and reliable method of measuring blood volumes. Such techniques also permit studies of red cell survival times in normal and pathological conditions, and these again appear to promise much clinical information. Attempts have been made to use phosphorus-32 in the study of malignant processes but so far with little clinically valuable results. There is no difficulty in observing the increased uptake of phosphorus-32 in rapidly growing cells but the interpretation is often difficult and the results very variable.

One of the most important features of the use of radioactive isotopes lies in the possibilities of the study of dynamic action inside the body. This is perhaps best exemplified by studies of the circulatory system. If a small quantity of labelled serum albumen labelled with iodine-131 is injected into an arm vein, then it is possible by making observations of the passage of the material through the heart and integrating the curve of activity to estimate the cardiac

output, and this particular method has already found use in the study of pathological conditions (Fig. 5). It is of course also well known that one may in this way study blood circulation in limbs. The early experiments using radioactive sodium are of interest in this connection. Since the viability of an injured limb or extremity depends upon its blood supply it is evident that any information on this subject is useful in the diagnosis, prognosis and evaluation of the therapy of vascular diseases (Fig. 6). We have developed a double counting rate system (Fig. 7) consisting of two scintillation counters coupled by simple light guides to the same electron multiplier which when placed upon a vein enables the mean speed of flow of material within the vein to be determined. This system has been used to study portal flow. By a closer analysis of such results a good deal might be learnt about the variation of velocity of flow along different axes of blood vessels, but such studies demand detailed physical and mathematical analysis and have so far not been carried very far. It does, however, seem probable that they will one day be possible and useful.

ACTIVATION TECHNIQUES

So far, we have spoken of the use of radioactive materials which are ingested or injected into the body but other techniques are possible as, for example, the so-called "activation" techniques. If small portions of tissues are irradiated at large neutron fluxes in a pile, then high activity may be induced in them and small specimens yield counting rates which are quite usable. The main activity produced is often sodium-24 but fortunately this has a short half-life and soon dies away, so that the presence of other elements such as arsenic or gold having high capture cross sections for low energy neutrons may be detected. The method can indeed compete with spectroscopic determinations of the amounts of material in the tissues. In conjunction with A.E.R.E., Harwell, we have made use of such techniques in, for example, the study of the gold content of the liver of a patient who had been given colloidal gold therapy. Amounts of the order of microgrammes per gramme of liver may be identified and measured with a scin-

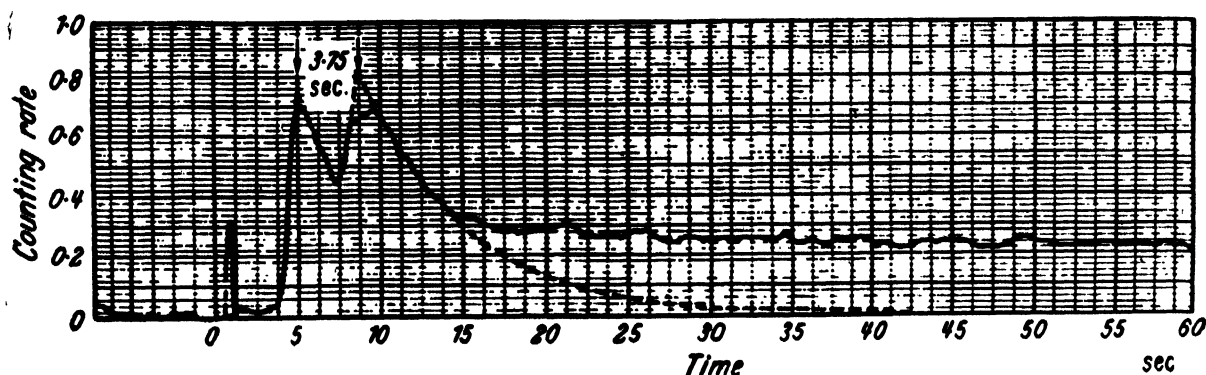


Figure 5. Measurement of cardiac output. Trace obtained from injection of ^{131}I labelled human serum albumin. [Veall, N., Pearson, J. D., Hanley, T., Lowe, A. E., *Radioisotope Conference, 1954*, 1: 183. Butterworths Scientific Publications, London (1954)]

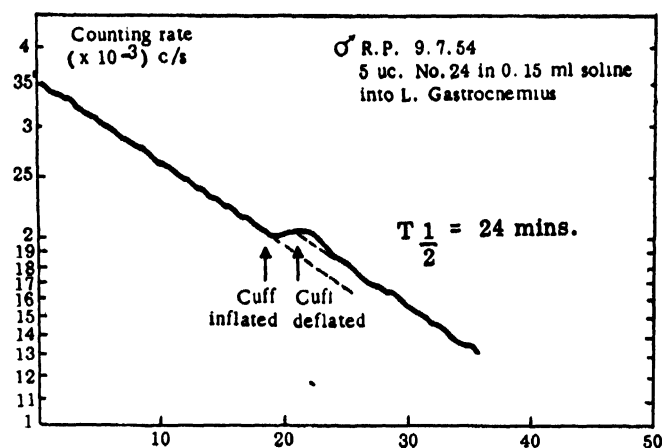


Figure 6. Local rate of clearance of sodium-24 in vascular disease



Figure 7. Double scintillation detector

tillation spectrometer (Fig. 8). We have also made a study of the arsenic content of skin of patients suffering from tumours of arsenical origin. It seems very probable that this method could be extended in a variety of ways. We showed some years ago that under the action of very high energy X-rays (22 Mev) that a number of materials in the tissues become radioactive, in particular by production of oxygen-15 and carbon-11 (Fig. 9), as well as radioactive isotopes of nitrogen, phosphorus, iodine and potassium. We are interested at the moment in the development of proportional counting techniques since, coupled with anti-coincidence counting methods, they promise the possibility of the measurement of extremely low activities in tissues and the detection of small quantities of materials made active by proton, neutron or high energy gamma activation.

VISUAL IMAGES OF ISOTOPE DISTRIBUTIONS

So far, we have spoken of uses of radioactive isotopes in which we have been content with the detection of the material as it moves from place to place, and thought little about the size and shape of the tissues infiltrated by it. We now turn to a group of techniques in which we aim rather higher, namely at the formation of a picture of the shape and size of the radioactive mass of material. As an obvious

example, it is frequently of great importance to know the size and shape of the thyroid gland both in disease and in health. It has been found possible to develop scanning equipment which can form a visual picture of such radioactive masses.

In the earliest experiments a collimated counter was moved in front of the gland and lines drawn on a diagram joining places having the same counting rate, so-called "equinumerals" (Fig. 10). The method, analogous to the use of isodose curves of radiotherapy, is lengthy and not very satisfactory. Using well-collimated scintillation counters and feeding the pulses to a recording device which moves in space simultaneously with the counter, it is possible to draw a picture of the area from which the radioactive radiations come. The apparatus (Fig. 11) may make use of a relatively simple electronic device such as a pen recorder, or use a "storage tube" and more complex electronic gear incorporating television display systems. In either event it is possible to obtain reasonable visual pictures of the outline of the radioactive tissues, provided that there are sufficient quanta reaching the instrument to form the image. If the amount of radioactive material is large (of the order of millicuries in a few millilitres) then there is no difficulty, but as the amount of radioactive material decreases, first of all any contrasts in the picture disappear, and eventually one is left with only a rather indefinite outline (Fig. 12). As the amount of radioactivity declines still further the random distribution of pulses of the background predominates and we are merely able to say whether or not radioactive material is present without being able to define its position at all precisely. Such techniques have

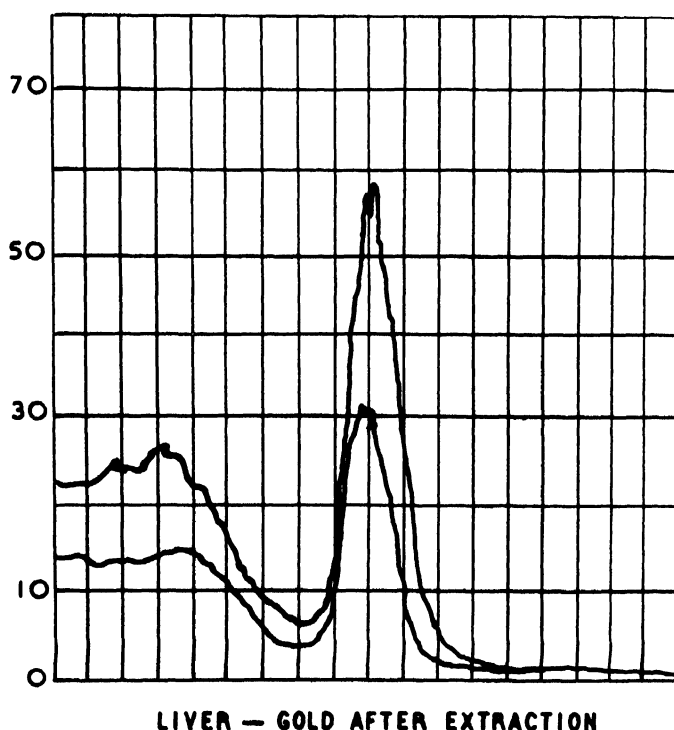


Figure 8. Record of scintillation spectrometer. Upper curve, specimen of liver; lower curve, standard solution containing colloidal gold (by courtesy of AERE, Harwell)

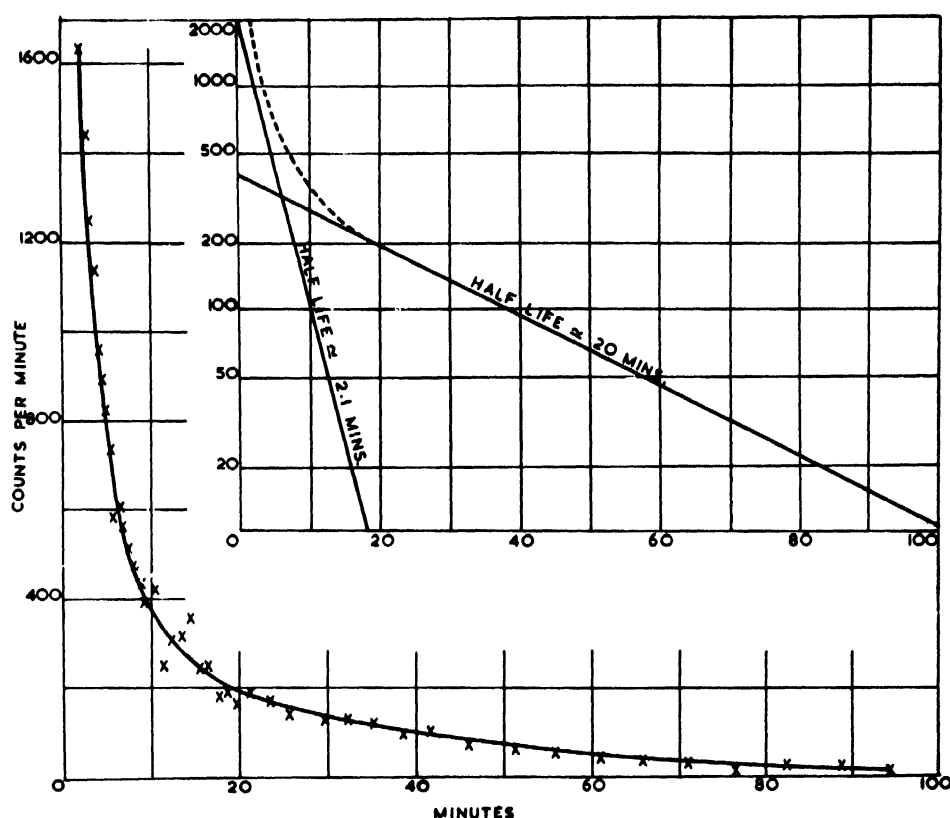


Figure 9. Decay of activity of tissues irradiated at 22 Mev. [Mayneord, W., Martin, J., Layne, D., *Nature*, 164: 728 (1949)]

been used considerably in the study of the thyroid and it is possible to watch the effects of radiation therapy in changing the size and shape of the gland either by external irradiation or by radiation from the radioactive iodine in the thyroid itself. We have been able to find by this technique secondary tumours not revealed by normal radiographic methods (Fig. 13).

Similar techniques may be applied to the study of brain tumours. It is well known that certain dye-stuffs may be used as vital stains in neurosurgery and it was therefore natural to use materials such as diiodo sodium fluorescein incorporating radioactive iodine, to see if the position and size of cerebral tumours could be determined. A number of groups of workers have tackled this problem with varying success. Our own experience obtained with some 500 patients is that the detecting gear must be of very high sensitivity (Fig. 14), but that even then precise localisation is difficult to obtain. Other radioactive materials such as potassium-42 have been used by us in extensive clinical trials but, again, with relatively small success. It is possible to correlate the finding of higher radioactive uptake with known positions of the tumours, but only rarely have we been able to add significantly to the information available to the clinician in charge of the case. An ingenious technique which has also been employed makes use of a positron emitter giving rise to annihilation radiation consisting of two quanta emitted at 180 degrees to each other. By the use of coincidence counting techniques it is therefore possible to find

the point of origin of the two quanta. Such a method has great physical elegance but unfortunately suffers also from the limitation that the uptake of tumours of the brain for the materials involved is frequently not much greater than that of the normal tissues,

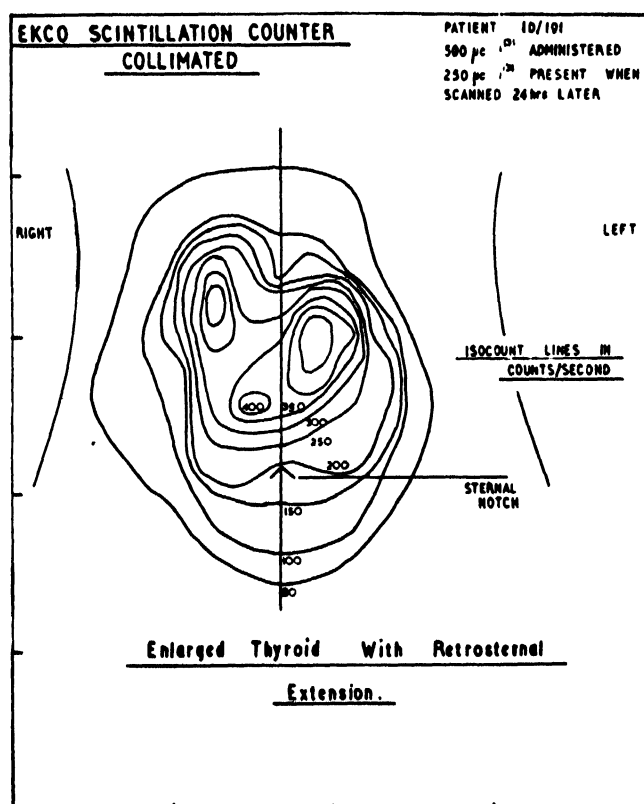


Figure 10. Equinumerals of thyroid

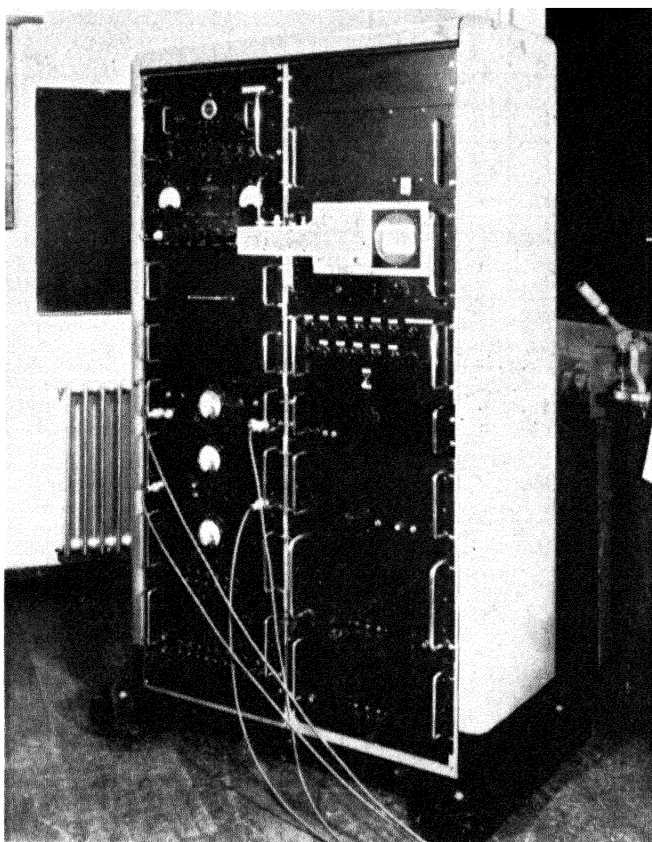


Figure 11a. Scintillation scanner. Electronic equipment and display unit. [Mayneord, W. B., *Brit. J. Appl. Phys.*, 4: 353 (1953)]

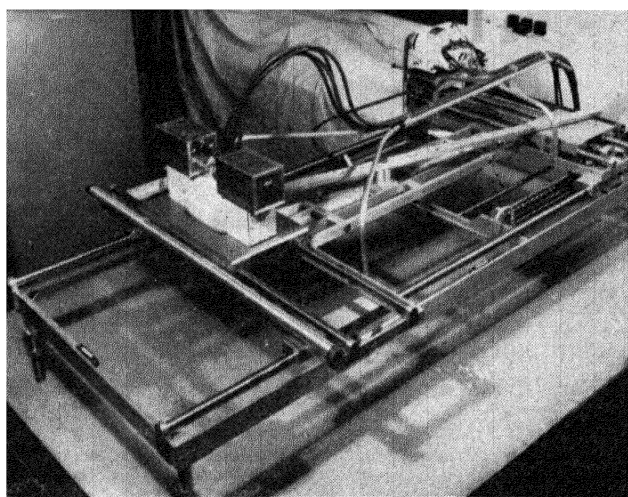


Figure 11b Scintillation scanner. Mechanical pantograph and coincidence scintillation detector

and again the differential determination of the activity is vital and difficult. The uptake of the brain is usually only a small fraction of the total amount of radioactivity injected into the body and the background of radiation from such organs as the liver difficult to avoid. Moreover, there are wide variations in the vascular character of the brain and the other tissues. The selective concentration in tumour tissue has only been observed by us in a few of the specimens removed at craniotomy from normal and neoplastic brain areas of the same patient. There can be no doubt that radioactive isotopes are useful in

the delineation of brain tumours but perhaps particularly after the administration of phosphorus-32 and search by opening the skull and using a Geiger-Muller probing counter. With such an instrument it is certainly possible to define the position and boundaries of a tumour with some precision. We are, of course, most anxious to develop techniques which avoid the necessity of opening the skull.

ISOTOPE RADIOGRAPHY

Finally, we would like to describe work on the radiographic use of radioactive isotopes. It was suggested some years ago that radioactive isotopes might be used in this way but difficulty arises in finding sufficiently strong sources emitting the right kinds of radiation which must have low quantum energy in order to give high contrast in the picture. This limits the materials available. The two materials which we have found most useful so far are thulium-170 and xenon-133. Our original experiments with thulium carried out some years ago showed that high-contrast excellent radiographs could be obtained with dried skulls (Fig. 15) and also of extremities. Thulium-170 is formed by placing normal thulium-169 in the pile and making use of the usual n, γ reaction. Its 85 kv gamma ray is accompanied by the characteristic K radiations (energies 59.4 and 52 kv) of the next element, ytterbium, into which it disintegrates. Spectra of these radiations have been obtained by classical X-ray methods (Fig. 16). One

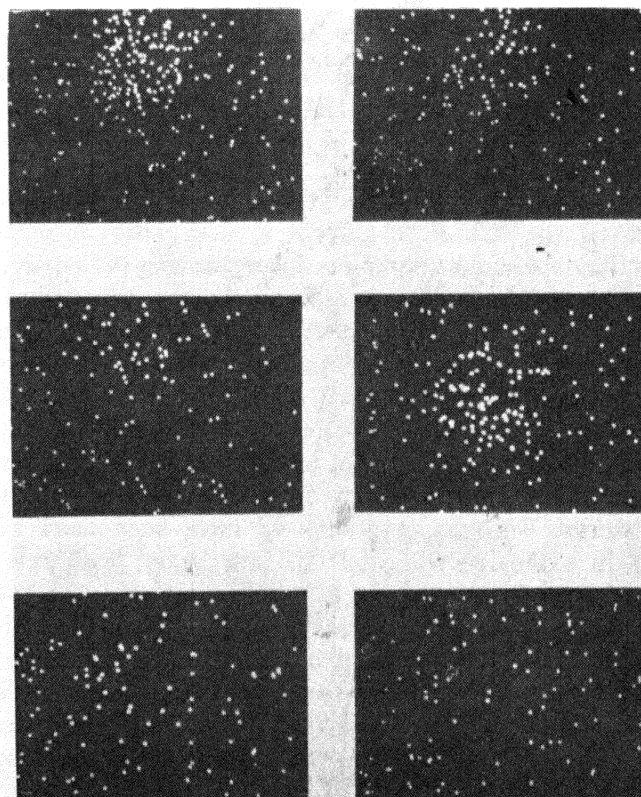


Figure 12. Scintillation records of square sources having very low activities. [Mayneord, W. V., Evans, H. D. Newbery, S. P., *J. Sci. Instr.*, 32, 45 (1955)]

deteriorate, since in the thulium disintegration two approximately 1 Mev beta rays are emitted which give rise to strong bremsstrahlung of high energy. These radiations lower the contrast and also make the problems of protection more difficult. We have therefore turned to xenon-133 which gives primary gamma radiation of approximately the same energy, namely 81 kv, but lower energy characteristic X-rays (35 kv and 31 kv from caesium). Moreover, this material is a fission product and has therefore very high specific activity. Its chief disadvantage is its short half-life of 5.3 days. We have found that satisfactory radiographic sources can be made by taking a small pellet of active carbon (1 mm diameter granule) and cooling to liquid nitrogen temperature in the presence of compressed xenon. The xenon is adsorbed on the carbon which thus becomes an active source of radiation. Quantities of the order of two curies may be adsorbed on the particle of this size. The radiographs obtained with this material are distinctly better quality than those obtained with thulium. It is possible to use a small rod carbon source (Fig. 17) viewed approximately end on, in a way analogous to the line focal spot of the X-ray tube and with such sources we have been able to obtain pictures of extremities of patients (Fig. 18) in times of the order of 10 seconds and in some instances even of shoulders and chests, though here the times are longer of the order of a minute. It must be emphasized that there is no question of competing with X-ray tubes, as simple calculation shows

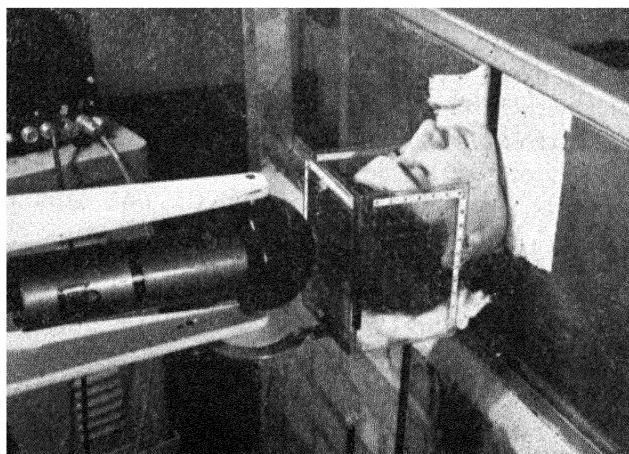


Figure 14. Scintillation equipment for the detection of brain tumours

that the specific activity and the strength of source can never be such as to render possible exposures now possible with X-ray tubes. Nevertheless, the small size and easy protection of these sources together with their freedom from electrical supplies makes them available in circumstances in which X-ray tubes cannot be used. For example, it is possible to take pictures from inside the rectum showing the lower end of the vertebral column and sacrum. Alternatively, in the mouth we may take pictures of teeth having only one side of the skull exposed to the radiation without the overlapping which is so difficult a feature of normal radiographs. It should also be possible to take pictures of, let us say, the

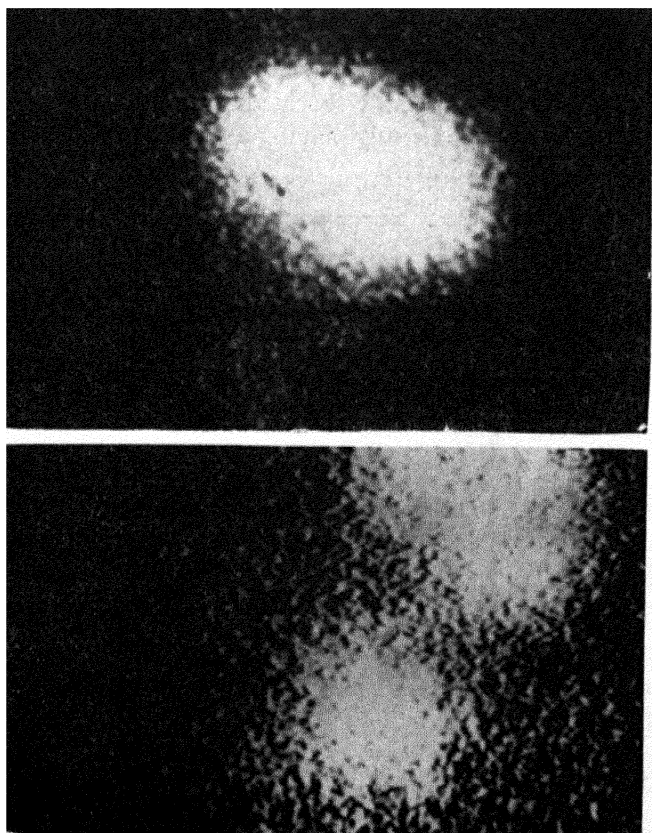


Figure 13. Record of tumour of the pituitary (upper picture). Extension three months later (lower picture)

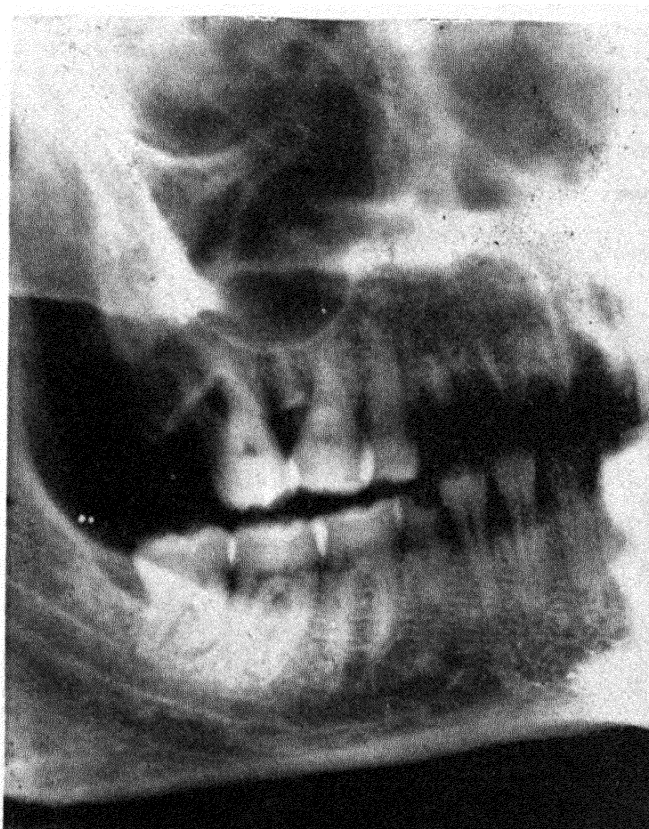
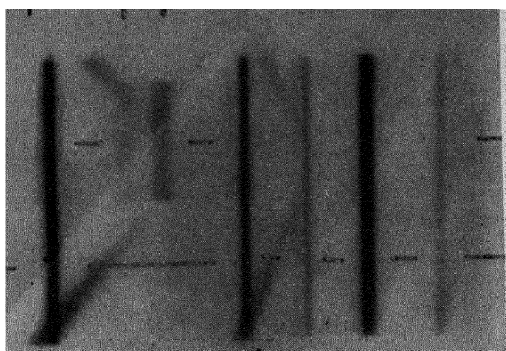


Figure 15. Radiograph of dried skull taken with weak source of thulium-170. [Mayneord, W. V., *Brit. J. Radiol.* 25: 517 (1952)]

atlas, a notably difficult region of the body to radiograph. There are also many other industrial uses as well as in veterinary science, but these cannot be dealt with here.

To sum up, we see that the diagnostic use of radioactive isotopes already covers a very large field. There is a growing tendency on the one hand for



1st order
81 kV. γ ray.
Xenon.

2nd order

3rd order

84 kV. γ ray
Thulium.

K series of
ytterbium

Caesium

Figure 16. Spectra by X-ray methods

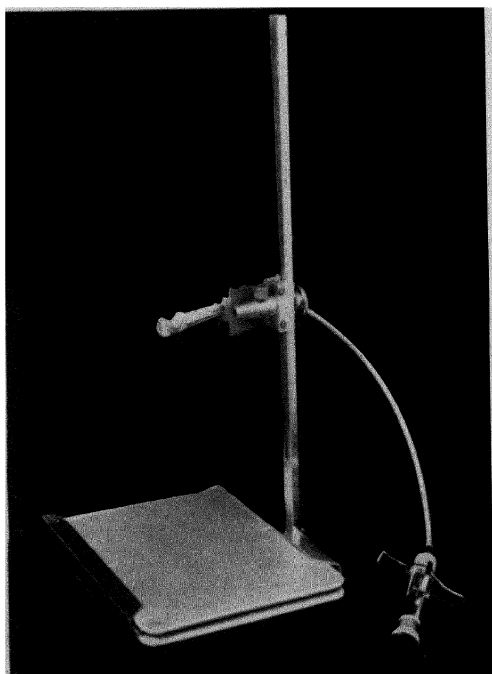


Figure 17. Simple form of radiographic apparatus



Figure 18. Radiograph of ankle taken with xenon-133

the isotopes to be used in physiological investigations and on the other in techniques similar to those of normal radiography. Many physical problems of safety and reliable detection still remain, but there can be no doubt that the lively dynamic picture of the workings of the body revealed by the use of radioactive isotopes is already playing a part in a reshaping of the diagnostic art.

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Experience in Clinical Diagnostic Application of Some Radioactive Isotopes in the USSR

By M. N. Fateyeva, USSR

The basic principles of Soviet health protection can be defined as a wide scope of medical aid to the population, free medical service, high specialization of such aid and a prophylactic trend in medicine. The organization of work in the diagnostical and curative application of radioactive isotopes can serve as a concrete illustration of the system of medical aid in the USSR. Medical establishments are supplied with isotopes free of charge, and through central agencies.

This paper is confined to an outline of some problems connected with the determination of the functional state of the thyroid gland by using radioactive iodine as well as with the study of hemodynamics by means of radioactive sodium and radioactive phosphorus.

Radioactive iodine has been used for a number of years in Soviet clinics to determine the functions of the thyroid gland. Investigations conducted on a great number of people (to cite an example, as many as 2,000 persons were examined at the Institute of Therapy of the USSR Academy of Medical Sciences) have made it possible to determine the average figures of radioactive iodine absorption by the thyroid gland in healthy people and in patients with hyperthyroidism and hypothyroidism.

We have applied the method of placing a gamma-counter on the front part of the neck surface to determine the degree of radioactive iodine absorption by the thyroid gland. The indicated dose of I^{131} did not exceed 2 microcuries.

The investigations were carried out dynamically, the indices of radioactive iodine absorption being determined 2-4 and 24 hours after the injection of isotopes. Such a method of determining the curve of I^{131} absorption has essential advantages over single measurement.

The observations conducted in a number of clinics (Institute of Therapy of the USSR Academy of Medical Sciences, therapeutic clinic of First Medical Institute in Moscow, Institute of Endocrinology) have shown that absorption of radioactive iodine by healthy people two hours following injection amounts to 5-9 per cent of the injected dose; 10 to 30 per cent in 24 hours; in almost 80 per cent of the cases the

absorption indices vary within a narrower range—from 15 to 25 per cent. In case of hyperthyroidism, the absorption indices usually rise reaching by 24 hours 35-75 per cent of the injected dose (Fig. 1). There were cases when the percentage absorption of I^{131} went up as high as 20-25 per cent of the injected dose two hours after the injection of the isotope. The maximum absorption was recorded in 8-12 hours with a consequent rapid decline. The number of such cases is insignificant—they can be detected only in serial examinations.

Absorption of radioactive iodine in hypothyroidism is lower and retarded; it is generally less than 5 per cent in two hours, and it does not reach 10 per cent

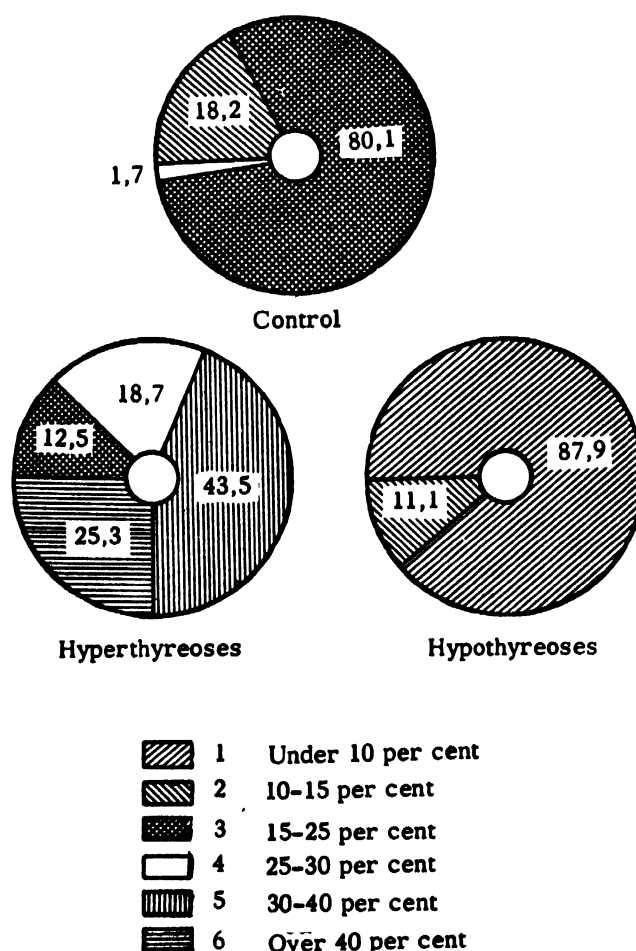


Figure 1. Uptake of I^{131} by thyroid gland in cases of thyroid pathology (percentage of number of cases examined)

of the injected dose in 24 hours. The lowest figures have been recorded in severe forms of cretinism. In cases of myxoedema, as shown by our observations, there occurs no selective absorption of I^{131} .

At present this method of investigation of the functional state of the thyroid gland is widely used in the practical clinical examination of a number of medical establishments (Institute of Therapy of the USSR Academy of Medical Sciences—up to 600 persons annually, one of Sverdlovsk hospitals—up to 1200 persons annually, the goitre dispensary in Stanislav—up to 500 persons annually, etc.).

Mass-scale examinations of the population with the help of I^{131} were carried out in the centres of endemic goitre in the Urals, in the Chernovitsky region, and in the Abakan area. The examinations were conducted by special expeditions. The results of the examinations have made it possible to determine the characteristic features of the functional state of the thyroid gland among the population of the endemic areas and to specify the clinical nature of the endemias.

Another test of the functional state of the thyroid gland, excretion of radioactive iodine with urine, has been used in diagnostically difficult cases, along with the determination of radioactive iodine absorption by the thyroid gland. Investigations of our clinics and the data of foreign researchers have shown that in hyperthyroidism the excretion of radioactive iodine in urine is generally reduced, while in hypothyroidism it rises as compared with indices recorded in healthy people.¹

Most of the work of Soviet scientists in the last few years has been devoted to the study of the function of the thyroid gland in pathogenic conditions when the thyroid gland did not play a leading part, though its participation in the process seemed obvious. In the main these investigations dealt with the study of the functional state of the thyroid gland in cardio-vascular diseases.

A large number of papers (Institute of Therapy of the USSR Academy of Medical Sciences, First Medical Institute in Moscow, the clinic at the health resort in Arsini, etc.) have been devoted to the study of the functional state of the thyroid gland in essential hypertension.

The results of these investigations have led us to the conclusion that the change in the functional state of the gland depends on the stage of the disease. Thus, according to the data of the Institute of Therapy of the USSR Academy of Medical Sciences², an examination of 360 persons has shown that the absorption of radioactive iodine by the thyroid gland either increases or is at the highest margin of the norm in over 50 per cent of cases of hypertensive disease of the I stage. It decreases in the III stage in practically 65 per cent of cases (Figs. 2 and 3). The deviations from normal radioiodine absorption recorded in the first and third stages are not drastic. These deviations do not reach the figures character-

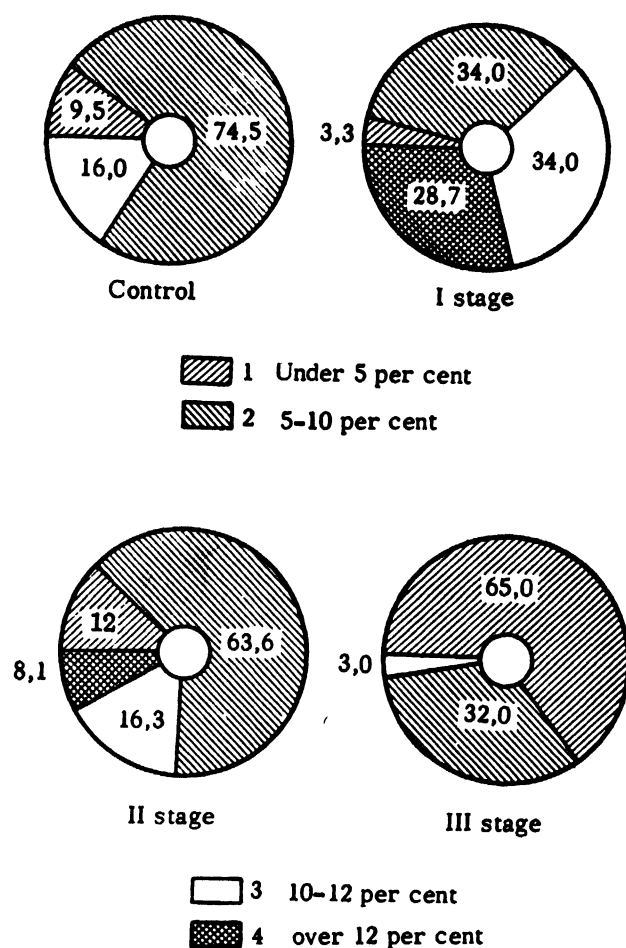


Figure 2. Uptake of I^{131} by the thyroid gland in cases of hypertensive disease, within two hours

istic of hyperthyroidism, however some hyperfunction of the gland in this stage of hypertensive disease undoubtedly exists, which is corroborated by clinical data.

In the third stage of the disease the decrease in iodine absorption is very pronounced. This may be caused by secondary profound disorders in the blood circulation of the body, in the brain tissue, in the hypophysis and in the thyroid gland itself.

Interesting findings have been obtained when investigating the functions of the thyroid gland in patients with rheumatic cardiac lesions. An examination of a group of patients with rheumatic heart diseases (280 cases) has shown that the absorption of radioactive iodine by the thyroid gland in the stage of compensation does not substantially differ from normal. With the development of decompensation phenomena, radioactive iodine absorption by the gland decreases (Fig. 4).

Sometimes a subtotal thyroidectomy is performed in cases of decompensated heart diseases. B. V. Alyoshin³ has found that the histological structure of the removed glands testifies to the depression of the gland function which is in accord with the figures of I^{131} absorption as well. On the basis of these data it may be assumed that thyroidectomy may produce a therapeutic effect only in cases when there is real thyrotoxicosis.

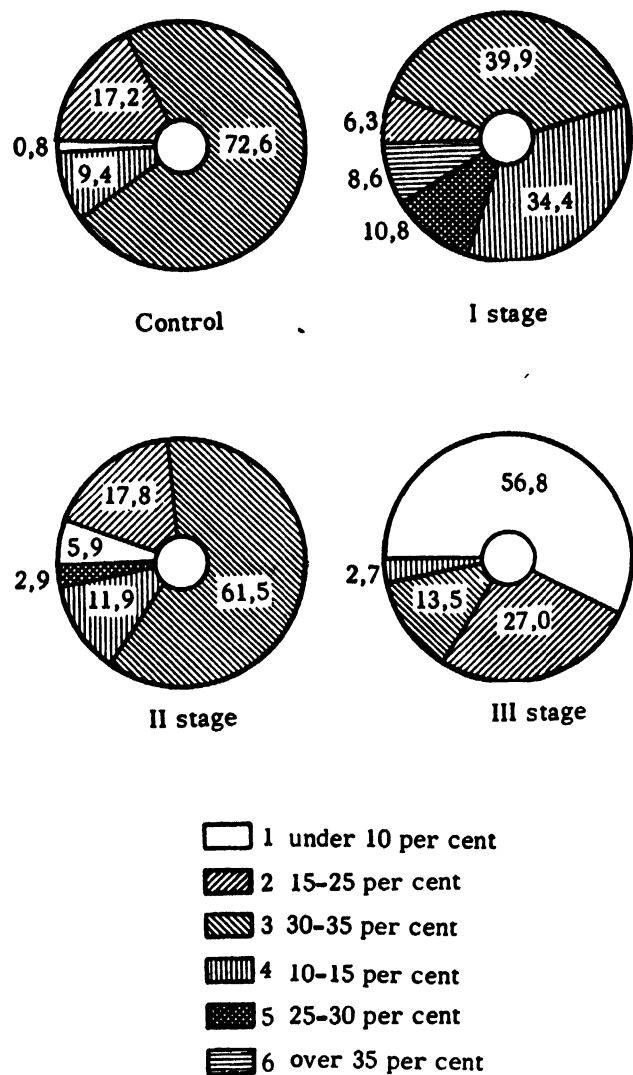


Figure 3. Uptake of ^{131}I by thyroid gland in cases of hypertensive disease, within 24 hours

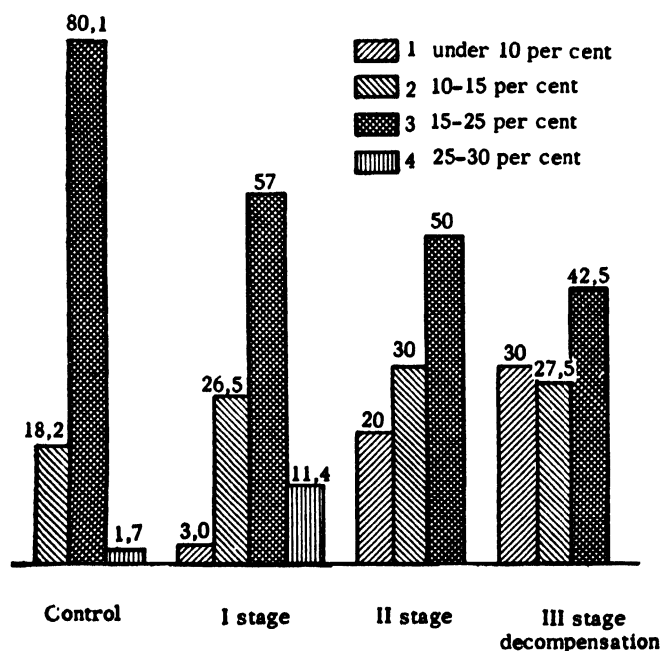


Figure 4. Uptake of ^{131}I by thyroid gland in cases of heart diseases within 24 hours (number of cases examined, per cent)

Examinations of the functional state of the thyroid gland in cases of rheumocarditis, carried out with the help of radioiodine (Institute of Therapy of the USSR Academy of Medical Sciences), have shown that in 50 per cent of cases at the highest point of the disease a slight increase in the function of the gland is recorded with corresponding clinical microsymptoms (Fig. 5, Table 1).

Interesting findings have been obtained in determining the function of the thyroid gland in various endocrine diseases. According to the results of the examinations of the Institute of Endocrinology (Moscow) in patients with acromegaly, an increase, sometimes up to 50 per cent of the injected dose of radioiodine uptake, is often recorded. In the Itsenko-Cushing disease during remission radioiodine absorption is within the norm; with the deterioration of the patient's condition radioiodine absorption decreases. These findings lead to the assumption that in diseases of the diencephalon and hypophysis there occurs a disturbance of the function of the thyroid gland.

In practical clinical work one often encounters cases of vegetative neuroses whose clinical symptoms approximate the symptoms of hyperthyroidism. It becomes particularly difficult to diagnose if a slight enlargement of the thyroid gland is combined with a slight increase in basal metabolism which is based on extrathyroid factors.

Investigations conducted by the Institute of Endocrinology (Moscow) and the Institute of Therapy of the USSR Academy of Medical Sciences have shown that in cases of vegetative neuroses radioiodine

Table 1. Uptake of ^{131}I in Cases of Rheumocarditis in 24 Hours, As Percentage of the Injected Dose

Number of cases examined	Under 10%	10-15%	15-25%	25-30%	Over 30%
75	2	13	21	13	26

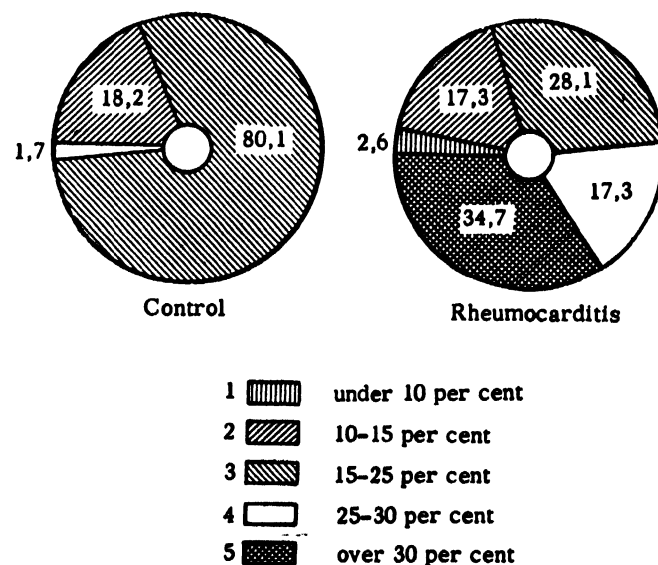


Figure 5. Uptake of ^{131}I by thyroid gland in cases of rheumocarditis, within 24 hours (percentage of cases examined)

uptake by the gland is within the norm, hence the function of the thyroid gland does not increase.

In a number of clinics a study is being made, with the help of the radioiodine test, of the hormonal influence of the thyroid gland on the course of clinical-physiological metabolic processes in the body; the function of the thyroid gland is being investigated in various periods of pregnancy, as well as its influence on the development of arteriosclerosis, etc.

Radioactive sodium and radioactive phosphorus have been used to investigate hemodynamics. Hemodynamics has been examined mostly in patients with cardio-vascular diseases. A group of 550 patients have been examined at the Institute of Therapy of the USSR Academy of Medical Sciences. A study has been made of the rate of blood flow in the systemic and pulmonary circulation, and the local and tissue blood flow in cases of hypertensive disease, rheumocarditis, and cardiac diseases have been determined.

Table 2. Rate of Blood Flow in Hypertensive Disease

Stage of disease	Number of investigated cases	Average rate of blood flow "from hand to hand" (per sec)	Limit of fluctuations (in seconds)
Control	20	13.2	10-18
I stage	38	11.7	8-18
II stage	60	15.3	8-23
III stage	47	18.4	10-30
	165		

In determining the rate of blood flow in the general blood circulation, the method consists in injecting into the ulnar vein a sodium chloride solution containing 60-70 microcuries of radioactive sodium; the patient holds the counter in the other hand. The inflow of the wave of radioactive blood is automatically registered by the recording instrument.

The rate of the blood flow in patients with the first stage of hypertony approximates the normal⁴, while in the second stage the average figures of blood flow rate show a slight retardation. In the third stage the deceleration is more pronounced. This slower rate of the blood flow in the later stages of the disease may be regarded as a sign of a latent cardio-vascular insufficiency. Considerable individual fluctuations have been recorded in all stages. The results of the observations are shown in Table 2 and Fig. 6.

The rate of the blood flow in rheumocarditis in a compensated state does not differ sharply from normal. A pronounced deceleration of the blood flow rate averaging up to 20.7 seconds (the norm being 13.2 seconds) has been recorded in a group of decompensated patients. The blood flow rate slightly decreases in mitral valve lesions in a compensated state. This deceleration is more pronounced in the compensation stage.

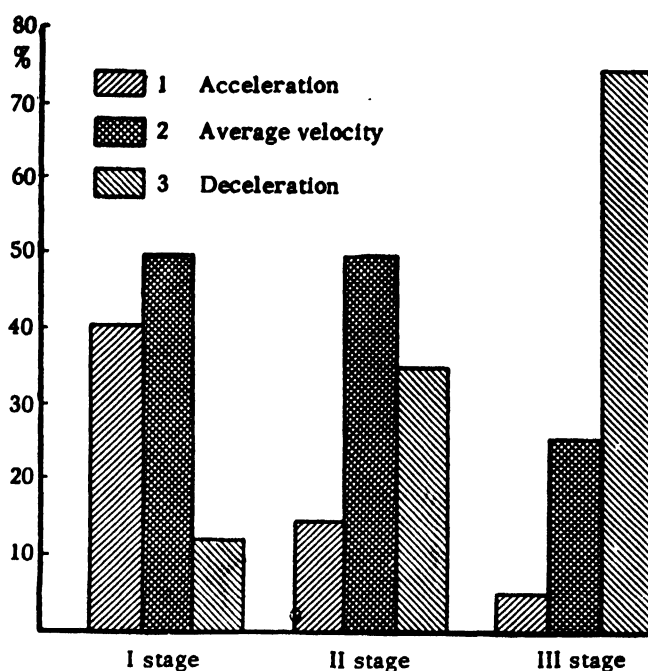


Figure 6. Rate of blood flow in hypertensive disease

This technically feasible method has made it possible to tackle the problem of determining the blood flow rate in the pulmonary circulation. The blood flow rate in the pulmonary circulation of healthy people⁵ varies within a comparatively narrow range—from 5 to 7 seconds. The blood flow rate in the pulmonary arterial bed decreases in the third stage of the hypertonic disease. This decrease is less pronounced in the second stage of the disease (Table 3).

Of interest are the findings on the rate of the blood flow obtained by P. M. Zakharova with the help of this method. It has been found that in epilepsy cases the blood flow rate is more frequently accelerated and averages 7.7 seconds in one and the same part of the pulmonary circle "from hand to hand"; in patients with organic diseases of the nervous system (disseminated sclerosis, encephalitis) a slight decrease in the rate of the blood-flow has been recorded with abrupt individual fluctuations ranging from 9 to 27 seconds; the blood flow rate has been retarded and varied from 12 to 30 seconds, averaging 19 seconds, in schizophrenia cases during a catatonic state.

Table 3. Rate of Blood Flow in the Pulmonary Circulation in Hypertensive Disease

Group of examined cases	Number of patients examined	Average rate of blood flow (in sec)	Range of fluctuations in seconds
Control	20	6.2	5-7
I stage	30	7.2	4-11
II stage	51	7.5	5-17
III stage	12	9.2	7-12
	113		

In 1949 S. Kety⁶ worked out a method of studying the dynamics of sodium exchange between the blood and tissues. S. Kety studied the dynamics of the passage of the easily diffusing sodium from the tissue deposit into the blood stream. The rate of the flow, termed as clearance of the tissue, is in a way an indication of the local tissue blood flow.

Table 4 shows our findings on the determination of the tissue muscular blood flow in healthy people and hypertonic patients. The data in Table 4 show that with the disease progressing, the muscle blood flow tends to grow worse, which is manifested in the slower absorption of radioactive sodium from the tissue deposit. It is worth mentioning that the range of individual fluctuations in all the groups of patients greatly varied.

Similar work has been conducted on studying the intensity of the tissue blood flow in the subcutaneous tissue of patients with rheumocarditis. A comparison of data in groups of compensated and decompensated forms shows that the slower passage of radioactive sodium from the subcutaneous tissue into the blood stream is more pronounced in decompensated than in compensated cases. Most likely, disorders of circulation are the main factor affecting in this case the intensity of the tissue blood flow.

A retarded passage of radioactive sodium from the intratissue deposit into the blood stream was found in well defined heart diseases without rheumocarditis phenomena. The deceleration was less pronounced in compensated heart diseases.

Table 4. Passage of Sodium-24 from the Tissue Deposit to the Blood Stream

Group of examined cases	Number of cases	Time of absorption in minutes		"K"—constant of absorption	Percentage of absorption per minute
		50% Na ²⁴	80% Na ²⁴		
Control	25	13.9	30.2	0.057	2.77
Hypertonic patients:					
I stage	17	12	29.1	0.054	2.80
II stage	58	14.1	34.7	0.049	2.49
III stage	17	16.4	38.5	0.043	2.28
	117				

Table 5. Tissue Blood Flow in Rheumocarditis

Group of examined cases	Number of cases	Time of passage in minutes		"K"—constant of absorption	Percentage of absorption per minute
		50% Na ²⁴	80% Na ²⁴		
Control Rheumocarditis:	22	13.3	29.2	0.066	3.12
(a) Compensated	24	16.0	38.0	0.052	2.73
(b) Decompensated	11	20.7	42	0.041	2.30
	57				

Table 6. Tissue Blood Stream in Heart Diseases

Group of examined cases	Number of cases	Time of passage in minutes		"K"—constant of absorption	Percentage of absorption per minute
		50% Na ²⁴	80% Na ²⁴		
Control	22	13.3	29.2	0.066	3.12
Heart diseases:					
(a) Compensated	9	15.4	41.0	0.043	2.53
(b) Decompensated	11	20.7	40.0	0.038	2.23
	42				

Hevesy⁷ and his associates have elaborated a method of determining the mass of circulating blood by introducing a suspension of erythrocytes labelled with radiophosphorus. B. E. Tartakovskaya and D. N. Strazhesko (1954) conducted experiments on introducing labelled erythrocytes with whole blood, which considerably simplifies the method and makes it more accessible for wide clinical application. A comparison of both variants—the introduction of washed tracer erythrocytes and the introduction of tracer erythrocytes with whole blood—has shown that the latter method is more convenient and safer in clinical conditions.

The above method has been used for determining the mass of circulating blood in some blood diseases. It appeared that the amount of circulating blood decreased in pernicious anaemia. The decrease in the mass of circulating blood was frequently recorded in hemolytic anaemias. In systemic diseases—chronic myelosis, chronic lymphadenosis—an increase in the volume of circulating blood was recorded in most of the cases under observation.

The method has also been used in the examination of a group of cardio-vascular disturbances. In cases of myocarditis and myocardio-dystrophy without circulatory failure, the volume of circulating blood decreased on account of the fall of the total volume of erythrocytes and the amount of plasma. This reduction is possibly a compensatory reaction of the body, aimed at alleviating the heart activity. Most of the patients showed an increase in the volume of circulating blood during a deficiency of blood circulation of the second and third stages, occurring in various forms of heart disease; this increase is mostly due to the increase in the plasma, the volume of erythrocytes being normal or even somewhat lowered. In some cases of heart diseases (*affectio mitralis*), attended with a predominating stenosis of the left venous opening, a normal or decreased amount of circulating blood was recorded; this is evidently due to a considerable deposit of blood, mostly in the liver and the lungs.

The percentage of erythrocytes according to hematocrit and the total volume of erythrocytes were smaller during endocarditis, both in cases of increase or decrease in the volume of circulating blood.

G. Hevesy and others have elaborated a method of studying the permeability of erythrocytes for radioactive phosphorus.⁸ We have used this method in various pathological conditions. Greater permeability of erythrocytes in cases of obliterative endarthritis has been recorded, which appears to be related to the depression of oxidizing-reducing processes. It has been found that erythrocyte permeability increases in various pathological processes: in rheumatic polyarthritis, in generalized and infiltrative forms of tuberculosis, broncho-pneumonia, etc. Low permeability figures have been recorded in chronic nephritis, as well as in hepatic cirrhosis.

Naturally enough, this report does not cover all the problems dealing with the use of radioactive isotopes in clinical examinations. Still the findings cited above, which have won wide recognition in the

medical establishments of the Soviet Union, testify to the fact that the use of radioactive isotopes for diagnosing some human diseases are of value and hold out great possibilities.

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Application of Nuclear Energy in the Solution of Special Problems in Biomedical Research

By Masanori Nakaidzumi,* Japan

DIAGNOSTIC PROCEDURES

Since 1950 radioisotopes have been imported into Japan and used for the diagnosis and therapy of various diseases. Among others, one of the most interesting applications is the so-called scintigram whose purpose is to map the distribution of I^{131} deposited in the thyroid gland, and to estimate its mass for therapy. The apparatus has been experimentally manufactured in Japan, and is, as explained below, a simple and inexpensive one.

Scintigram of Thyroid Gland

When radioisotopes are used for diagnostic and therapeutic purposes, it would be clinically advantageous if we could make a scintigram of particular regions of the human body, by making use of the gamma rays emitted by the isotope deposited there.

A scintigram is particularly important for measuring the distribution of I^{131} deposited in the thyroid, and for measuring the mass of the thyroid when necessary for therapy. At present, no scintigram recording apparatus is on the market in Japan, and the first experimental model was only completed recently. Although we have not reached the stage of clinical use, a fair number of types of clinical applications have been tried. The experimental apparatus is described below.

The apparatus consists of a scintillation counter, a support for the counter and a recording device. The scintillation counter consists of a thallium activated sodium iodide crystal, and photomultiplier, surrounded by a lead cylinder, with small holes in the front face to form a collimator. The pulse is first amplified by a pre-amplifier and then by an amplifier in the recorder.

The recording device consists of a neon lamp in series with the meter circuit. In front of the neon lamp is a slit, and both lamp and slit are attached to the scintillation counter. A dry-plate or film is placed below the slit so that when the neon lights, an image of the slit is recorded. The neon lamp and slit move simultaneously with the detector, while the cassette of the film or plate is fixed. The detector and neon lamp are attached to a frame, which allows

both horizontal and vertical motion, manually controlled by a screw thread.

The neon lamp will light when the detector passes over an active region, and an image of the slit will be printed on the film. Since the higher the gamma intensity, the longer the neon will remain alight, it will be seen that figures of the slit will be wider for higher gamma intensities. In this way, a scintigram can be obtained.

By making a scintigram of the thyroid with the above apparatus, and by measuring the I^{131} uptake by means of a Geiger-Müller counter or scintillation counter, we can determine the I^{131} absorption rate of the thyroid gland.

In most cases of hyperthyroidism, the mass of the thyroid gland, estimated from its scintigram, is greater than the normal thyroid mass. Even in cases where a thyroid tumour is not obvious by inspection or palpation, the scintigram method can easily detect the thyroid enlargement without fail. In cases of thyroid tumour, the scintigram permits a clearer understanding of the relationship between tumour and normal gland. In fact, we can determine whether or not the tumour is of the I^{131} absorption type, and this will be a great help in differential diagnosis to supplement the clinical observations.

Counting Tube for Stomach Cancer Diagnosis

A counter has been constructed which can be introduced into the stomach via the mouth and oesophagus; and whose position can be adjusted from outside. The diameter of the thin glass end-window is 3.5 mm, and the effective length of the counter is 5 mm. The tube is halogen quenched. The clinical applications are now being studied.

THERAPEUTIC PROCEDURES

Co^{60} for Telecurie Irradiation and the Manufacture of Co^{60} Needles

Co^{60} was first used as a gamma-ray source in a telecurie unit in 1953, and is now in use at many universities and national hospitals. Two sizes of bombs have been manufactured for trial purposes, of 50 curies and 100 curies. The trend is now to increase the activity up to about 500 curies.

The main material for the Co^{60} container is lead. It is desirable to limit the leakage dose to 50 mr a

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day; but since using this figure involves too large an amount of lead, it has not been found practical to keep the dose down to 50 mr a day. Instead, operators are instructed not to approach within 1 metre of the radiation centre for more than 4 hours a day, since the leakage dose cannot be kept below 50 mr for 4 hours. Since then, improvements have been made wherever necessary. Depth dose data and isodose curves for these units have been determined using ionization measurements with condenser chambers, or photographic films, and are used as a therapeutic guide.

Various needle containers are made in America and Europe, and some are already imported into and used in Japan. Recently, however, Co^{60} wire was made for trial, and it has proved by various tests to be convenient, safe, and relatively inexpensive. The Co^{60} wire is put into a needle-like container made of stainless steel 1-8-8. The outside diameter is 1.6 mm, and they can be obtained in any length. The needle is made so that each 1.5 cm contains 1 mg of radium (0.6 mc of Co^{60}).

I^{131} for Hyperthyroidism

I^{131} is widely used in Japan for the therapy of hyperthyroidism. Its therapeutic results are very

good, and in most cases, such subjective symptoms as palpitation, dyspnoea and overperspiration become alleviated, pulse rate becomes normal and thyroid tumours contract.

The logical method to determine the amount of I^{131} to be given to a patient can be calculated from the average amount of I^{131} absorbed per gram by the thyroid. This can be estimated from the I^{131} absorption rate and the weight of the thyroid gland. For this purpose, we are anxious to have a home-made thyroid scintigram recording apparatus, employing a scintillation counter.

Miscellaneous

P^{32} , Sr^{89} and Sr^{90} beta-emitters have been used for the external irradiation of angiomas and various kinds of naevi.

The method is to absorb the P^{32} solution into pieces of good quality blotting paper, which are then cut to the same shape as the diseased area. The paper applicator is then covered with cellophane, and stuck over the area to be irradiated. The applicator is usually backed with sheet lead for protection.

When Sr^{90} is used, it is prohibited to contaminate the skin with even the slightest amount of Sr^{90} ; it must, therefore, be handled with extreme caution.

The Use of Artificial Radioisotopes in Diagnostic, Therapeutic and Clinical Research in Argentina

By Constantino Nuñez,* Argentina

When the National Atomic Energy Committee as such began to operate, provision had to be made at once for every aspect of the medical applications of radioisotopes and for their use in biological research. For the National Committee, this meant the establishment of an organization for the study, planning and implementation of such activities. The Department of Medicine and Biology was founded on that basis.

The main problems faced by this organization, from its inception, may be reduced to three. In the first place, it had to investigate the problems connected with the medical applications of certain radioisotopes suitable for diagnostic, therapeutic, and clinical research purposes. In the second place, some biological research techniques meant the use of these radioelements as unique tools in carrying out certain projects. On the other hand, it was of vital importance to complete research projects based on the action of radiation and energetic particles upon the cellular structure with a double end in sight: the extension of our knowledge of the structure and functioning of the normal and pathological cells, and the drawing of conclusions of possible usefulness to the various methods of biological protection against radiations. Finally, it was indispensable to draw up, perfect and check, the standards for the protection of the personnel employed at the generating facilities and "hot" laboratories. One of its Divisions, the Division of Medical Activities, is in charge of all matters having to do with the clinical application of radioisotopes, the supervision of their proper utilization and the dissemination of advice to the centers which make use of them. The Committee has advocated the view that proper use of the radioisotopes is basically determined by the competence of the medical personnel who uses them. For this reason, these elements have not been issued indiscriminately. On the contrary, a limited number of medical centers, in departments of various hospitals, directed by professionals familiar with the handling of radioisotopes have been equipped and supplied. At the present time, there are 16 centers making use of isotopes, in the city of Buenos Aires and in the interior. At the same time, these centers serve the

purpose of training physicians who wish to make use of them. In spite of the short time that has elapsed since the establishment of the first of these centers (about three years) the results obtained are deemed encouraging with respect to some therapy and diagnostic problems. Let it not be forgotten, that, at the present time, almost all the radioisotopes used are imported from abroad, which rules out the short half-life radioisotopes, so that the elements used up to this time for diagnosis and therapy, are reduced to the following three: iodine-131, phosphorus-32 and cobalt-60.

UTILIZATION OF IODINE-131

Iodine-131 has been used in Argentina since 1951. At that time, a project was started for the study of endemic goiter in the Province of Mendoza. This was the idea of Dr. James Howard Means, when he learned of the features of the endemicity. The project was carried out at the Mendoza Goiter Institute with the cooperation of the National University of Cuyo, by the Thyroid Clinic of the Massachusetts General Hospital of Boston, under the sponsorship of the Atomic Energy Committee and with the aid of the Loomis and Rockefeller Foundations. Active part was taken in the work by a group of professionals and technicians, under the direction of Dr. Hector Perinetti, John B. Stanbury, Gordon L. Brownell, Enrique B. del Castillo, Jan E. Itoiz, and several others.

A selection was made of 129 patients, most of whom had greatly enlarged thyroids. Several of them had multinodular glands, especially some under 15 years of age, who had enormous multinodular glands. The weight of the thyroid, for these patients, varied between 40 and 250 gm; in some patients, the glands were heavier still. The study was limited to patients over 35 years of age. The measurements were taken with bismuth cathode Geiger tubes, the geometry being properly adjusted. The standard solution was placed in a 100 cm³ volumetric flask and measured with the same geometry.

Most of the patients examined, who behaved clinically like euthyroid subjects, showed a heavy concentration of I¹³¹ in their thyroid glands. Figure 1 shows patient distribution 48 hours after administration of the radioisotope. It is compared with the

Original language: Spanish.

* Comisión Nacional de la Energía Atómica.

number of patients

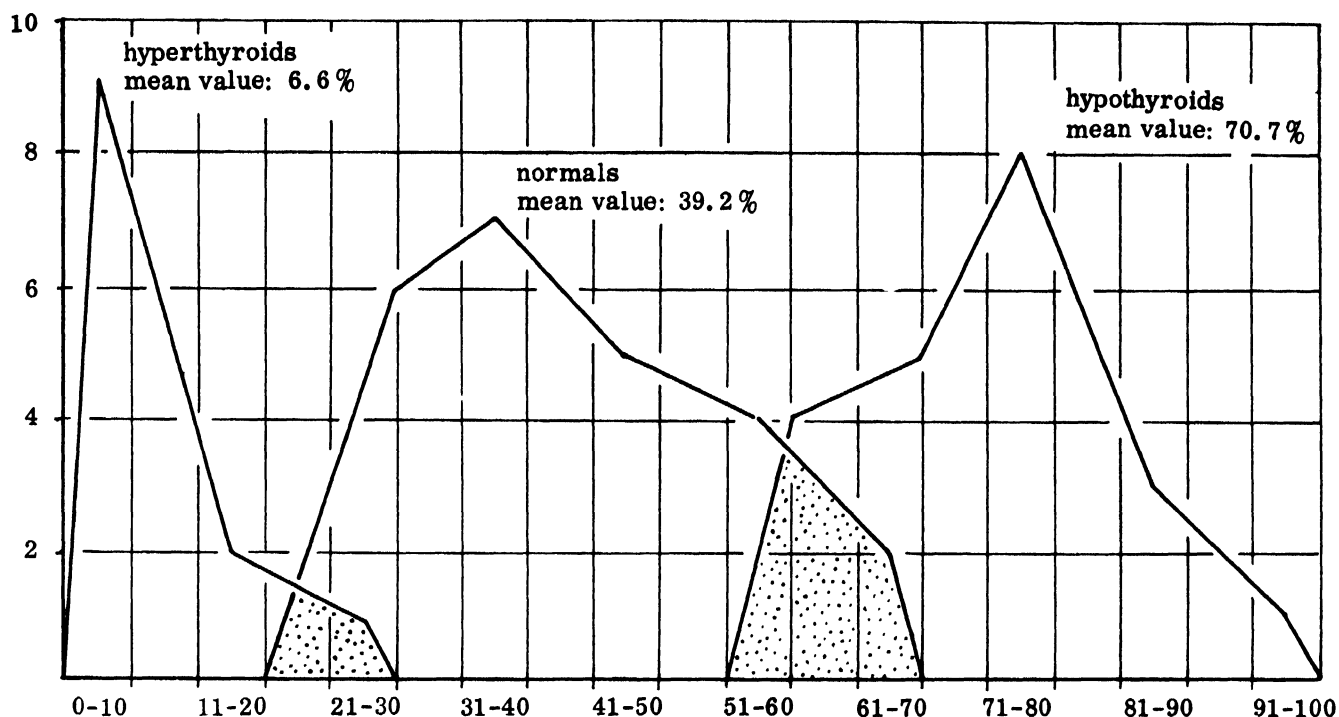


Figure 1. Per cents of accumulation of iodine-131 in the thyroid gland at 48 hours after administration of the radioisotope. The mean values correspond to normal persons and to patients suffering from hypo- and hyperthyroidism. The overlapping of zones corresponds to the doubtful cases

distribution curve for normal subjects in the area of Boston, Massachusetts (USA), (Fig. 2) as drawn by Skanse. It also was shown that the rate of I^{131} accumulation in the thyroid gland of iodine deficient patients was rapid, while the apparent rate of release was slow. Upon administration of a gland blocking drug, a much sharper drop could be noted in the retention curve of the tagged hormone. Upon ad-

ministration of thyrotropic hormone, there was accelerated release of the hormone, since these glands were not subjected to maximal stimulus by the endogenous thyrotropine. In these patients, the administration of dessicated thyroid caused a depression in the I^{131} concentration, even though the administration of stable iodine did little to reduce the rate of I^{131} accumulation.

A description of the work carried out at Mendoza also was published in book form in the United States. We here present excerpts of their most significant parts, which are considered to be of definite importance:

1. The accumulation of radioactive iodine was higher in the Mendoza patients than in those from non-endemic areas. There is an inverse ratio between the incorporation of iodine by the thyroid and its excretion through the urine. In other words, the accumulation depends on the iodine utilized by the patient and having its origin in the diet. The rate of iodine secretion in the thyroid hormone was computed in 98 euthyroid subjects. The average found was 56.7 micrograms per day for subjects taken individually, and 46.5 micrograms per day when the rate was figured by a graphical method. The average concentration of the protein bound iodine of the serum was similar to the average normal value obtained in a region where iodine was plentiful, however, the range of values found was larger.

There was a significant inverse relationship between the serum protein-bound iodine and the accumulation of iodine.

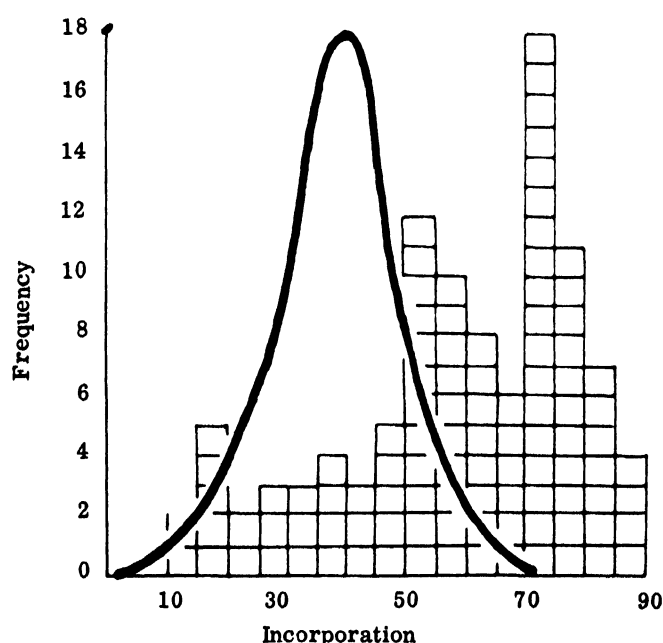


Figure 2. Histogram of the frequency of incorporation of iodine-131 by the thyroid glands of 103 euthyroid persons suffering from endemic goiter (Mendoza). The superimposed curve represents the normal distribution of incorporation in Boston (USA), calculated from the data of Skanse. Each square represents one patient

2. Some experiments were undertaken relative to the administration of complementary doses of iodides to patients with manifest iodine deficiencies. Thirteen patients were taken from three groups; the first group received 150 micrograms of iodides, the second between 500 and 600 micrograms, and the third 1500 micrograms; frequent counts were made of the tagged iodide incorporation and excretion.

3. It was found that there was a slow trend to a net balance between the fixation and excretion of the iodide during the following weeks. The extent to which this balance was achieved was not ruled by the size of the daily supplement. The amount of iodide retained every day varied in direct ratio to iodide fixation. The net positive iodide balance as computed in the new equilibrium condition was greater as larger supplements were given. It should be mentioned that one of the patients who received 1500 micrograms of iodide daily developed a clear case of thyrotoxicosis. Where an iodide was added as a carrier for the tracer dose of tagged iodide, the proportion of carrier accumulated by the thyroid gland was independent of amounts under 1.5 micrograms, but, when larger amounts were given, progressively smaller fractions of the dose were accumulated.

4. The administration of dessicated thyroid to a certain number of patients caused a quick drop in the absorption rate of the iodine deficient thyroid, a very large difference in the response to a specific dose of dessicated thyroid becoming apparent. The drop in the fixation also was incomplete after two weeks, in some instances. The studies undertaken on the iodide balance apparently showed a progression toward a new equilibrium condition in these patients. No increase was observed in the sensitivity of these patients to dessicated thyroid.

5. As regards antithyroid drugs, methylmazol was effective to prevent the incorporation of tagged iodine in the thyroid of individuals suffering from endemic goiter, there being an increase in the release of labeled iodine by the thyroid, and an increase in the urinary excretion rate of labeled iodides. This increase was much larger than that foreseen by bearing in mind that the action of methylmazol is the inhibition of the reutilization of the iodides produced by the degradation of the hormone.

6. The administration of thyrothrin induced an increase in the release of tagged iodine, as well as in the rates of urinary excretion of the iodides and tagged iodides. Some of the values found, tend to vary from one patient to another, suggesting the existence of a residual component of thyroid iodine which is mobilized only with difficulty.

7. The administration of large doses of iodine to 4 patients who had previously received tagged iodine induced the excretion of increased amounts of tagged iodine from the gland, as well as a greater rate of urinary excretion. The iodine released from the gland during the iodide course was about twice that observed during the control period, which strongly sug-

gests that the extra iodine leaves the thyroid in the form of an iodide. These data are different from those observed, under similar circumstances, in the hyperplastic glands of Graves' disease patients. The iodide given to a Graves' disease patient causes the same amount, or even a smaller amount, of tagged iodide release.

The Goiter Institute of Mendoza comes to the conclusion, regarding I^{131} and its clinical use, that it is an excellent diagnostic means for the determination of thyroid function, and perfect for the localization of thyroid cancer metastasis. Furthermore, from a purely therapeutic standpoint, it is felt that here is an element which can cure hyperthyroidism in any of its clinical forms, provided adequate doses be used. The ideal case is that of diffuse hyperthyroid over forty, in which it can cure all the disorders and reduce the thyroid to its normal size. In the nodular goiter, it should be used as a preoperative treatment, since the presence of the nodules always suggests the possibility of cancer, so that the surgical decision should be the main consideration. In the treatment of thyroid cancer, I^{131} comes as a support to surgery, it being possible to destroy malignant metastases in organs which may be inaccessible to surgery. Furthermore, the Institute believes that I^{131} is irreplaceable for the study of the physiology of the thyroid, and particularly of the pharmacology of iodine in the living organism.

In 1954 the personnel of the Mendoza Goiter Institute and of the Atomic Energy Commission undertook a study, at San Carlos de Bariloche (Territory of Rio Negro), of endemic goiter and of the iodine factor as the determining element of the disease, using I^{131} . To this end, a laboratory was set up and equipped, and a total of 1901 persons were examined, of which 1700 were school children. The results obtained confirmed the supposition.

In 1953, at the Endocrinology Department of Policlínico Rivadavia in the city of Buenos Aires, under Dr. Enrique B. del Castillo, an attempt was made at evaluating I^{131} as a means of diagnosis in thyroid function studies, using a technique similar to the one employed by the Mendoza group, measuring the accumulation of iodine in the thyroid with a Geiger tube on 143 persons, 24 and 48 hours after the ingestion of the diagnostic dose. The results indicated that the mean value of I^{131} is 38.3% for normal subjects at the end of 24 hours, and 39.2% at 48 hours. Combining these findings with the results obtained in euthyroid patients (nonthyroid patients and ophthalmopathies without hyperthyroidism) the average, for both groups were 39.4% after 24 hours, and 41.7% after 48 hours. The results obtained agree with those accepted as normal in the United States.

Determinations also were made in hyperthyroidism and hypothyroidism, with an average percentage of fixation of 70.7 for the former, and 6.6 for the latter. The following values show the limits for the three groups:

	Maximum	Minimum	Average
Hyperthyroids	100%	51%	70.7%
Euthyroids	70%	9%	39.2%
Hypothyroids	24%	1%	6.6%

The figures overlap between the various groups, close to the limits. This overlapping is clearly shown in Fig. 1. Among the cases studied, no visible relationship could be found between age, sex and clinical form of the disease of the patients, on the one hand, and the percentage of accumulation on the other, in cases of hyperthyroidism and hypothyroidism. These findings are similar to those of Werner *et al.*, in 1950. An obvious coincidence between the rate of incorporation of I^{131} and the basal metabolism has been proven.

These determinations were subsequently extended to about 600 cases, confirming the values found in 1953. In this form, it has been possible to ascertain that the average rate of iodine fixation, in the city of Buenos Aires, is: euthyroids, 20–60%; hypothyroids, 1–20%; hyperthyroids, 50–100%. As a complement to the determination of I^{131} fixation, autoradiographs were taken of the thyroid tissues by the conventional thyroid biopsy techniques. By this technique, it also proved possible to determine, among other elements needed for the exercise of clinical judgment, the possibility of treating patients suffering from metastases of cancers of the thyroid.

For certain problems of a cardio-vascular nature, clinical studies of the use of I^{131} are under way at the Institute of Semiology, a branch of the Medical Department of Buenos Aires, under the sponsorship of the Atomic Energy Committee, under the direction of Professor Pedro Cossio. The main theme has been the use of this radioelement in cases of angina pectoris due to aortic stenosis attended with many daily attacks, which kept the patient in a state of chronic invalidity. Treatment has already been started for 8 patients. The dosages employed in such cases vary from 20 to 63 millicuries.

The results obtained to date have been excellent. In three patients, therapy has terminated upon achievement of the desired effect; i.e., the total eradication of pain. This has been achieved, in one case, at the cost of a hypothyroidism, and, in the other two, with marked hypothyroidism, but which has been compensated, in part, by the daily administration of 1 mg of levothyroxin, without any recurrence of the pain. In these three cases, complete functional recovery can be claimed: the patients suffer no pain, they can go about their daily work unhindered, the measure of hypothyroidism is slight, and it produces no discomfort. In two cases, curtailment of the thyroid function brought about improvement of the pain symptoms, but treatment has not ended. Three more patients have recently received their initial doses during the first of May.

Considering the previous condition of the patients, who experienced difficulty in the performance of their daily work or had to abandon this work or

employment due to the pain, the eradication of pain and the recovery of the functional capacity already obtained in three patients cause the results to be viewed as most promising.

Independently from this work, the following work is under way at the same Institution with the use of iodine-131: (a) action of the anoxia on the thyroid function as a preliminary to the study of the thyroid in relation to aviation; (b) study of the thyroid function on eunuchoid cases and its modification by treatment with chorionic gonadotrophin and testosterone; (c) effects of the stress of surgery on the thyroid function; (d) furthering of the study of treatment of hyperthyroidism.

In the study of protein-bound iodine in human serum, Doctors Jorge F. Sproviero and Juan Carlos Pérez are making evaluations of the protein-bound iodine of the human serum through the application of the S. B. Barker method,⁶ as modified by E. A. Carr *et al.*⁷ at the Branch Laboratories of the National Atomic Energy Committee, operating under the Endocrinology and Central Laboratory Department of the Policlínico Rivadavia, in the city of Buenos Aires.

The determining conditions for the technique were studied, proving that its sensitivity may reach 0.01 γ I_2 /ml of serum; the catalytic effect of the iodide ion, at 0.0058 γ I_2 /ml of serum was negative cerium-arsenic system. In the tests of recovery of known quantities of iodine quantitative results are obtained up to 0.04 γ I_2 /ml of serum, but when an attempt is made at recovering 0.028 γ I_2 /ml of serum, the results vary. Consequently, the method is not very sensitive when the serum values are under 1 γ I_2 /ml of serum.

Determinations were made in the following groups of patients: hyperthyroids (11 cases), hypothyroids (18 cases), nodular or diffused nontoxic goiters (10 cases), healthy controls (doctors, students and laboratory technicians) (36 cases), and patients with other diseases, the euthyroid (9 cases).

In the *normal individuals*, a mean value of 6.2 ± 0.5 γ I_2 /ml of serum was found, the maximum and minimum values being, respectively, 4.5 and 8 γ I_2 /ml of serum. Values were obtained for the cases of hyperthyroidism, which varied between 8.6 and 17 γ I_2 /ml of serum. In hypothyroidism, the values varied between less than 1 γ I_2 /ml of serum and 4 γ I_2 /ml of serum. For the nodular or diffuse non-toxic goiter, the values fall between 2.5 and 10.2 γ I_2 /ml of serum. These results have demonstrated their clinical value in the functional investigation of the thyroid.

Hartman, Cardeza and Portela⁴ also have studied, in the city of Buenos Aires, the action on thyroid fixation of radioiodine in rats, as well as the goitrogenic effects of certain sulphur compounds. The various products were: 2-mercaptobenzothiazole; 4-mercapto-2-methyl-quinazoline; 1,10-decamethylene-bisithiourea; 1,4-dimercapto-phthalazine; 2,2'-de-

camethylene-bisthiourea; 2-mercapto-5-acetamidothiazole; 4,5-dihydro-2-thiouracil; N-(2-propylidene)-2-mercapto-5-aminothiazole; 5,5'-octamethylene-bis-(2-amino-1,3,4-thiazole); 5-amino-2-mercapto-4-(2,4,4-trimethyl-pentyl-thiazole); 4,6,6-trimethyl-2-mercaptothiazine; 3-mercapto-5-methyl-1,2,4-thiazole; 2,4-dimercapto quinazoline; 5-(2-propylidene)-2-thiohydantoin; 2-thiohydantoin; rhodanin; 1-2cetyl-2-mercapto-5-isopropyl hydantoin; and 6-methyl-2-thiouracil.

As this work went along, it could be observed that the absorption of radiation from external sources (Albert, 1951; Perry, 1951; Wolf, 1951; Myant, 1953) or by submersion (Rawson *et al*, 1944; Searle *et al*, 1950; Searle, 1951) may both be used, since the ratio between them is of the order of 0.45. A mean thyroid weight per 100 gm of body weight of the control rats was found, which varied according to the product used, and will be found on the enclosed graph. It was evident, after 20 days of administration *per os* of the mentioned products, that those possessed of antithyroid action bring about an increase in the size of the gland. Such hypertrophy bears a relation to the histological modifications. The goitrogenic action was produced by 6-methyl-2-thiouracil. It is followed, as regards this action, by 1-acetyl-2-mercapto-5-isopropyl hydantoin, rhodanin, and 5-(2-propylidene)-2-thiohydantoin. It was verified that rhodanin and 2-thiohydantoin are intensely toxic and very often fatal to the animals. The more goitrogenic drugs also had the greater blocking action on the assimilation of I^{131} , there being an inverse ratio between the goitrogenic action and the reduction in iodine fixation.

USE OF PHOSPHORUS-32

Phosphorus has been used as a therapeutic agent in hematological diseases in Argentina for many years, Dr. Alfredo Pawlowsky being the first and only one to use it up to the year 1952, at which date its use was extended to a certain number of the country's Medical Centers, the principal ones being the Hematological Department of the Buenos Aires Naval Hospital under Dr. Alfredo Pawlowsky and the Policlínico Rivadavia under Dr. Miguel Angel Etcheverry. It is also used at the Policlínico Rawson (Model Institute of Medical Clinic), the Institute of Neurosurgery, and the Faculty of Medical Sciences of the University of Cuyo.

The experience of the Buenos Aires Naval Hospital with this radioisotope agrees, as to its main lines, with that of the foreign centers. It can be used as a supportive measure for the conventional treatments, particularly radiotherapy and chemotherapy which, if properly handled, may bring about benefits which, temporary though they might be, can also be really effective. The utmost care in the selection of cases is one of the most important factors contributing to the success of the treatment. Treated cases have been, basically, the following: polycy-

themia, vera and symptomatic, myeloid leukemia, chronic myeloid and lymphoid leukemia, acute leukemias, lymphosarcomas, lymphadenosarcosis, myeloid metaplasias of the spleen, and multiple myelomas. Properly handled, it may give good remissions in the first stages of chronic myeloid leukemia. The same may be the case in chronic lymphoid leukemias. It is absolutely ineffective in the final stage of all acute or chronic leukemias. It is particularly useful in the leukemids (Pawlowsky). In the polycythemias, it continues to be a valuable element, although, in some cases, relatively high doses must be administered.

At the Rivadavia Polyclinic of the city of Buenos Aires, P^{32} is used in blood diseases, under the control of Dr. Miguel Angel Etcheverry. The isotope is given to the patient in the form of acid bisodium phosphate diluted in saline. Work began at this center in July, 1954. Thus, the data available to date are limited, both as to the duration of the period of observation and as to the number of patients treated. It has been possible, nevertheless, to make a series of observations having clinical and experimental value. In respect to the chronic myeloid leukemia cases, although they were under treatment for only a short time, the P^{32} proved its therapeutic value, since all patients improved, both clinically and hematologically. It may also be deemed to be of considerable therapeutic value in those cases without any adenopathies or hepato or splenomegaly, where it is supposed that the basic lesion is located in the bone marrow. In some cases of chronic lymphoid leukemia, some clinical and hematological improvement was achieved. In all cases of polycythemia vera, there was marked improvement almost immediately after the administration of P^{32} , even before there were any appreciable effects on the blood cells. The headaches and vertigo disappeared, as did the feeling of discomfort in the stomach and legs. The splenomegalia dropped appreciably in all cases. The flushing of the face, hands and legs disappeared in all cases treated. Generally speaking, all the patients experienced marked well-being and a healthy feeling. It can be stated that there was a remarkable improvement in all the cases followed, with hematological remissions in all of them. Determinations of the blood volume were also made, as well as determinations of the average life of the red cells, circulatory rate and cell volume.

One of the most interesting diagnostic aspects of the use of P^{32} has to do with the location of brain tumors. A series of experiments were made at the Neurosurgery, Faculty of Medical Sciences of Buenos Aires, under the sponsorship of the Atomic Energy Commission, based on the use of P^{32} as the detecting agent. The equipment used was designed by Robinson and Selverstone. The technique consists, in the main, of the intravenous administration of 2 mc of P^{32} twenty-four hours before the operation. After an exploratory craniectomy in the general area in

which the tumor is revealed by the clinical examination and arteriography or ventriculography, the detection is carried out as needed by means of a Geiger counter through small holes in the dura. The activity is detected in this form in a normal zone of the brain, on the periphery of the tumor. Once this has been localized, incision and exeresis are carried out by the usual techniques. The localization of the tumor in depth is effected with the aid of detector equipment. The counting rate usually increases with the degree of malignancy. This is very useful in cases of infiltrating astrocytomas, whose appearance blends with normal nerve tissue. There is always a risk of failing to detect the small gliomas or metastases due to the short range of beta particles in the tissues. Should resection of the tumor prove impossible, the surgeon could obtain a useful biopsy by suction at the point of maximal activity. These experiments have been undertaken in a series of 15 patients, on some of whom surgery had been performed earlier, without being able, at that time to localize the tumor.

The pathological material extirpated was used for research on the metabolism of P^{32} by malignant nerve cells, by radioautography using Pelc's stripping film method. P^{32} deposition can be observed in the cellular protoplasm, there being no incorporation in the nucleus. Generally speaking, a larger accumulation of the silver grains was observed in the perinuclear zone. The time of metabolism of P^{32} in these cells was particularly taken into account, since in all cases 12 to 14 hours elapsed between the administration of the radioisotope and surgical exeresis and tissue fixation. The Laboratories of the National Atomic Energy Commission continue their autoradiographic studies on this type of material.

USE OF COBALT-60

This radioisotope has been used in Argentina mainly for interstitial therapy, in the form of needles, tubes and pellets. Its applications began in 1952 at 5 medical centers; namely the Institute of Experimental Medicine "Angel H. Roffo", the Central Military Hospital, the British Hospital, the Urology Service of the Ramon Mejía Polyclinic, all of Buenos Aires, and the Institute of Cancer Research, a branch of the University of Cuyo. The most extensive projects conducted at the Institute of Experimental Medicine and at the British Hospital of Buenos Aires.

The work with Co^{60} started in September of 1953 at the Institute of Experimental Medicine, with needles and tubes. The accepted therapeutic criteria for the dose of irradiation were practically the same as those used on radium and radon therapy, the relevant corrections being made according to the radiation characteristics of these elements. The patients were not selected; treatment was given to those responding to curative or palliative radium therapy. Most of the treatments were applied by the interstitial techniques, except for the penis, where a cylindrical molding was employed, and the cavum and ear

(postoperative therapy) where intercavitary technique, with lineal sources, was used. Up to mid 1954, a total of 216 patients were treated, broken down as follows:

<i>Location</i>	<i>No. of cases</i>
Upper lip	3
Lower lip and commissures	91
Face	41
Tongue	31
Eyelids	10
Eyes: inner angle	11
Eyes: outer angle	3
Upper maxillary	1
Scrotum	1
Penis	8
Floor of the mouth	5
Cheek	3
Palate	4
Pharynx	1
Cavum	1
Ear (postoperative lodge)	1
Ano-genital region	1
Total	216

Dosage computation and distribution of the material were made following the Paterson and Parker system (Manchester School). The radium therapy dosage tables, corrected by Quinby for Co^{60} , proved of value. The above mentioned Institute was able to prove that, with the usual doses and similar physical conditions, the immediate local reactions produced by cobalt are similar to those of radium, although evidently less intense.

The postoperative course, in most cases, is as follows: the local reactions appear on the second week in the form of erythema, congestion and tumescence of the treated area. At the end of the second week, there is a pseudomembranous exudative epithelitis with an increase in the subjective symptomatology, which recedes during the third week with the shedding of the pseudomembrane and a decrease in the size of the tumor, already palpably in lysis. Towards the fourth week, the congestion, burning and epithelial erosion are in complete regression; during the fifth week the epithelization increases concentrically, with a tendency towards the disappearance of the remanent infiltration and edema. Alopecia is always in evidence, according to the location. There seems to be a lesser reactive response to implantations in the oral cavity, as compared with radiotherapy, particularly in so far as the immediate reactions are concerned, such as congestion, erosive exudative pseudomembranous cobalto-epithelitis and the first degree tegumentary necrosis, named "first degree cobalto-necrosis" by Taboada, meaning by it the tegumentary degeneration of the foci created by necrosis producing doses of radiation, characterized by their sensitivity, their atony and small tendency to re-epithelization. Due to the short time elapsed, it is not possible to come to any conclusions about the slow cobalto-necrosis of the bone as compared with the osteonecrosis seen in radium therapy, but there is reason to believe that there will be no osteonecrosis.

judging by the aspect of very extensive processes of the tongue treated for palliative purposes, notwithstanding the fact that the doses applied reached the maxillaris which bring about osteonecrosis with intensities equal to or greater than those when radium is used. In so far as the implanting methods per se are concerned, there is greater flexibility and ease of handling of the active material than of radium, implantation being possible with much less trauma. Its lighter weight facilitates attachment with finer thread, with slippage from implantation position less likely. Similarly, implantation in delicate or small areas, such as the eyelids, is easier.

Use has been made of cobalt-60 in the treatment of tumors of the bladder. At the British Hospital of Buenos Aires, and with the help of the National Atomic Energy Commission, Dr. Samuel Stuart Pennington, with the collaboration of Drs. Flores, Pelaunde and Rocchi, encouraged by the results obtained by Darjet in France and Cahill in North America with curietherapy, as contrasted with the discouraging results of surgery at even such great institutions as Karolinska and Royal Mardse, began using Co⁶⁰ in the treatment of tumors of the bladder. The selection of cobalt was based on the fact that, physically speaking, it produces a greater beta density per cubic centimeter of tumor than does radium, because, furthermore, its radiation is more penetrating and, particularly, because there were available long enough needles for proper implantation of the tumor. Essentially, treatment consists of adequate surgical exposure of the vesical tumor through cystostomy, its reduction by electrosurgery, total implantation with radioactive cobalt needles according to the technique of Quimby and, whenever possible, to that of Patterson and Parker. The needles are taken out on the sixth to ninth day, as warranted by the dosage computed. According to the degree of parietal infiltration of the tumor and to the possibility extravascular permeabilization, implantation is made in a single plane or two planes. If, after "flattening" by electrosurgery the tumor remains of considerable size, the intraparietal or intramuscular plane is complemented by another artificial extravascular plane, consisting of a set of needles in "spongostan". Tolerance of the pelvis to the latter is very good and, since the material can be absorbed, needle extraction never entails any difficulties, in most instances through the abdominal incision. Palpation of the relevant lymph node areas is always made before the bladder is incised, from the obturator to above the crest of the ilium. This makes true classification of the pathological condition of the tumor possible by checking not only the degree of infiltration, but also possible adenopathies. When these are present, exeresis is undertaken, at least when there is no very close adhesion to the larger vessels. If this is not possible, direct implantation will be made, taking certain precautions relative to the dose. This is generally computed to the highest limit compatible with

the non-production of vascular necrosis, permitting that the external radiotherapy gradually reach the high level desired for effective control of this adenopathy. It is always essential following pelvic exploration, to insure perfect vesical drainage before going on to implantation. A bladder that does not of itself drain completely and normally is not satisfactorily conditioned for irradiation. Thus, one should insure that there is no obstacle whatsoever to the outflow from the kidneys and that there is no obstruction in the ureters. In the first case, when a ureter is embedded in the tumor or very close to it, reimplantation should be made, in a zone of the tumor where neither the tumor nor the postradiation fibrosis interfere with ureter function. Should this not be practicable, thought should be given to sigmoid or cutaneous implantation. Initially, the needles were secured to the bladder by means of thread or Deknatel clips, a second cystostomy being carried out several days later for their removal. The surgical incision was slow in healing, a fistula persisting up to 4 months. Now, after perfecting the general technique, the clips are no longer required, making unnecessary the second cystostomy: vesical healing has been much accelerated by this progress. The postoperative management of this tumor varies somewhat, depending on the intensity of the pain referred to the bladder, and whether the tumor was pediculated or consisted of an ulcerative and infiltrative neoplasm. It will be understood that, in the first case, there only remained a small surface to implant, made up of the base or pedicle of the tumor. In the second case there is a large volume to implant, even after electrosurgery, requiring a much greater number of needles for the correct implantation with cobalt.

It has been proven that the vesical mucosa is the most highly sensitive mucosa of all the system to the action of radiation. It reacts by pain, in direct ratio with the amount of cobalt used and the depth of its implantation. It reacts by producing edema; developed by two different mechanisms, one to be expected in any irradiated mucosa and the other due to blocking of the lymphatics blocking by the tumorous or post irradiation fibrosis. The time factor is the only point which can be distinguished. It has been proven that the reaction of the mucosa persists for up to several months (6-9), as established by Darjet. The possibility exists that, in one of the cases treated to date, surgical closure of the vesical fistula may be necessary, since the tumor in question was large, with total infiltration of the muscular layer. The fact that, on the same patient and in the same surgical procedure, prostatectomy was carried out and a wedge of the vesical cervix extirpated to insure perfect drainage, brought about no clinically perceptible change in the subsequent course of the irradiated tumors ascribable to that exeresis. Insufficient time has elapsed for an evaluation, in exact terms, of the usefulness of the treatment, but the

prevailing opinion is that it promises to do more for the patient than surgery alone. The cases treated to date number 19, none of which has been selected, all means and all available tools having been used to benefit those subjects. Thus it is that surgery has been closely integrated with irradiation, this meaning, at times, the association of radioactive cobalt with roentgentherapy.

In the treatment of lingual carcinoma Dr. Stuart Pennington of the British Hospital of Buenos Aires obtained immediate and promising results with Co⁶⁰. Since this cancer extends rapidly along the muscular fasciculi, this author thought of median and posterior blocking of the lesion. A fact that should be borne in mind, is that neither the anatomico-clinical form (surface, ulcerative, papillary, infiltrative, etc.) nor the degree of histological differentiation (from the most anaplastic to the most adult, most differentiated, as is the keratinized with horny globules) will modify, either the type of implantation, or the subsequent dosage. If the lesion is very exophytic, it will be flattened, as a preliminary step, by partial resection by electrosurgery; if it is very ulcerative, particularly if extending to the floor of the mouth, it will be coagulated in preparation for the implantation. If it is very hemorrhagic, the external carotid or the lingual is to be ligated; if there is a danger of edema of the larynx by tumorous infiltration, the implantation is complemented by a tracheotomy.

From the implantation technique point of view per se, Dr. Pennington classifies the following types, from the anatomical and therapeutic standpoints: *tip and base, dorsal, marginal and basal*.

Tip and Base Type

Two varieties can be distinguished at once, according to the macroscopic extent of the infiltration, one of the *first and simple type*, in which the lesion is restricted to the tip itself or lower surface of the tongue, whether in the center or on the side; the *second type* when the tumor, as it develops is directed downward and infiltrates the floor of the mouth, to a greater or lesser degree. In the first case, implantation will be of the single plane type; in the second, the single lingual implantation will be complemented by suturing the tongue to the gingival edge with a submental plane of needles, preferably done under visual control. This approach offers the advantages of making it possible simultaneously to carry out the exeresis of the submaxillary, the ligation of the lingual and facial arteries, and prophylactic evacuation of the lymph nodes. Early invasion of the submental ganglia through the mylohyoideus and possible bilateralizing to the submaxillaries or directly to the omohyoid bundle should be borne in mind.

Dorsal Type

Implantation is made basically in the plane form on the dorsal region, but with two variations; namely, one posterior blocking 2 cm from the lesion in order

to avoid interfascicular propagation; and, two, vertical implantation along the central line to act upon the vessels and small lymphatics between the genio-glossi, which could be a cause for relapse. The lymphatics which serve this region discharge in the nodes of the submaxillary group, the jugular-digastric group and up to the group of the carotid fork.

Marginal Type

(a) Those extending toward the back of the tongue progress more deeply, and transpose the septum linguae before the ulcerative aspect evidences the transformation, producing bilateral submaxillary metastases or contra lateral cervical node. This justifies the median vertical blocking used.

(b) Those extending mostly towards the floor of the mouth are complicated by the invasion "en bloc" of the sublingual salivary glands by the primary lesion, on the one hand, and by the lymphatic nodular metastases in direct contact, extending as a mass through the floor of the mouth, and rapidly reaching the bony plane of the mandible and invading the periosteum. In our department, we try to avoid such developments using submaxillary surgical implantation, with the extirpation of the submaxillary gland and often the separation of the periosteum of the mandibular branches.

(c) When developing predominantly toward the fauces, extending over the glosso-palatine arch, the process easily reaches the tonsillar fossa and soft palate, infiltrating, at the same time in the direction of the internal pterygoid muscle, blocking the mandibular joint, and creating trismus, with all its consequences. When this extension is suspected, and it is easy to recognize it by palpation of the glosso-tonsillar furrow, it is related to dorsal implantation, implantation of the furrow and of the tonsillar fossa with needles through both fauces and tonsils to the pterygoid apophysis, thus achieving the implantation of the pterygo-lingual type, (Manchester). If there already is marked trismus, which is completely reduced by general anesthesia, operation on the mandibular branch should be considered, severing the internal pterygoid.

(d) It may further progress laterally, surrounding the two back molars from behind, crossing the mandible and reaching the cheek in the region of the masseter.

Basal Type

In this situation, there are two distinct varieties; first, when the lesion, being of medium size, turns glosso-laryngeal, infiltrating the medium fold and later the epiglottis, invading and drying, at the same time, the pre-epiglottic cavity, to appear in the suprahyoid region; the other is, when being somewhat lateral, it invades the pharyngeal wall on one side, and progresses downward towards the piriform sinus through the lateropharyngeal furrow. The first may be attacked, either by direct basal implantation

of the tongue, or by the suprahyoid approach. A large dose is achieved in that fashion. The operation may be complemented by bilateral node extirpation when the nodes are small and can be moved, when, on the contrary, they are fixed, it is better, according to Stanford Cade and Berven, to attack them with external radiotherapy or radium from a distance. When the lesion is lateral, that is to say, already glosso-pharyngeal, the radiotherapy or teleradium is only really possible.

The described activities are some of those currently under way in Argentina on the medical and biological planes. Our experience of these problems is relatively new. The minimum time required for evaluation has not elapsed as yet to allow useful appreciation of the real efficiency of some therapeutic methods, and, on the other hand, the cases treated today are not numerous enough as a base for definite evaluations. The only aim pursued, in this brief exposé of the work under way is to evidence the interest of the Argentine medical profession in work of this nature, with the hope of being able to contribute to the general effort with an effective contribution in the fight against disease.

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The Medical Use of Radioactive Isotopes at the Radium and Tumor Institute, Hadassah University Hospital, Jerusalem

By A. Hochman, J. Stein and Y. Feige, Israel

For medical diagnosis, therapy and research, the following isotopes are in common use at this Department: I^{131} for thyroid function tests, examination of various thyroid tumors, and the treatment of thyrotoxicosis and thyroid metastases; Au^{198} in colloidal suspension for the treatment of carcinomatous effusions into pleural and peritoneal cavities; P^{32} for the treatment of genuine polycythemia, for external use on vascular cutaneous lesions, and occasionally for blood volume determinations.

IODINE-131

The metabolic affinity of iodine to thyroid tissues makes this γ -ray emitting isotope particularly suitable for diagnostic tracer work. Since January 1952, about 900 cases have been investigated, as to the state of their thyroid gland. These studies have been carried out for the following purposes: (a) corroboration of the clinical diagnosis of hypothyroidism or hyperthyroidism; (b) determination of the necessary therapeutic dose of I^{131} in such cases of established hyperthyroidism, in which medical considerations preclude an operation; (c) investigation of the relative I^{131} uptake of nodules and various tumors within the thyroid region and of inoperable, metastatic thyroid cancers so as to select cases suitable for radioiodine treatment.

After application of 50 microcuries of I^{131} in water, the percentage of I^{131} uptake in the thyroid is measured during the 48 hours following ingestion, with a special gamma-sensitive Geiger counter (containing a bismuth-coated cathode). Determination of the radioactivity excreted in the urine during this interval serves to check the uptake measurements.

In the rare cases which fail to permit a clear diagnosis by the above method, the amount of protein-bound I^{131} per liter of blood is determined 24 hours after administration of the radioactive material. This test supplies information as to the conversion rate of inorganic iodine into thyroxine in the thyroid gland. New methods are being developed for a rapid and precise determination of the exceedingly small quantities of I^{131} present in the blood samples available.

In the treatment of thyrotoxicosis, the dose of radioiodine applied varies with the thyroid uptake and the estimated weight of the gland. 85 cases have been treated so far with 4 to 15 millicuries of I^{131} .

The treatment of metastatic cancerous thyroid

lesions with I^{131} has been disappointing, as the affinity of malignant thyroid tissue to iodine is generally much inferior to that of normal tissue. Nevertheless, I^{131} has been given in the few cases in which the metastases show a sufficient uptake to assure a reasonable damage to the malignant cells. These cases are selected after a preliminary assay of the uptake of a tracer dose, using a small lead-shielded directional Geiger counter. Doses of 40–80 millicuries of I^{131} have been administered in these cases once or successively. Insufficient time has elapsed to permit final conclusions as to treatment efficacy.

GOLD-198

Since July 1953, radioactive colloidal gold (Au^{198}) has been administered to 24 cases of carcinomatous effusions within serous body cavities; in these cases, this particular condition constituted the chief distressing ailment. The patients treated were in a relatively good general condition, and their life expectancy was at least six months. This treatment proved superior to other radiological methods owing to the intense radiation effect which can be delivered towards a given site, without causing injurious total body irradiation. Of primary importance for the therapeutic action is that the Au^{198} is colloidal, which prevents its rapid removal from the body cavity.

The average individual dose employed is about 100 millicuries per body cavity. Whilst the β -radiation emitted by the radiogold is absorbed within the first millimeters of the irradiated tissue layer, about 5% of the accompanying γ -rays are absorbed into the body. This effect limits the permissible dose.

Whilst the mode of action of the gold suspension is not yet fully understood, the therapeutic results have been gratifying.

PHOSPHORUS-32

Radioactive phosphorus (P^{32}) has proven a highly advantageous agent in the treatment of genuine polycythemia. Since February 1953, 10 cases have been treated with doses ranging from 4 to 8 millicuries. In some cases, these doses had to be given repeatedly, until a lasting improvement was achieved.

It has become apparent that P^{32} when applied topically to *naevus flammeus*, might be superior to the conventional treatment. Our own experience with about 15 cases, mostly infants, tends to confirm this.

Tritium Measurements of Lung Ventilation Volumes and Kinetics (Radioisotope Use in Medical Diagnosis)

By Rex L. Huff and Daniel Parrish,* USA

Tritium-labeled air has several decided advantages in the evaluation of lung ventilation volumes and kinetics. The foremost is the fact that the tritium is present in trace amounts so that the normal gaseous content of the lungs is not changed while the measurements are being made; on the other hand the influence of changes in gaseous content can be evaluated if desired. The time constants of the ionization chamber and electrometer used for assay of H^3 are short enough (2–2.5 seconds), so that the early rapid changes of the “washout” phase of the tagged gas can be observed. This is in contradistinction to the use of helium, in which long time constants of the katharometer prevent the accurate analysis of the early rapid changes. Nitrogen analysis by recent techniques allows examination of the early rapid changes in concentration but the continued elimination of nitrogen from the blood into the lungs makes interpretation of the data difficult and impractical. Tritium is markedly insoluble in body fluids which allows for adequate equilibration in the lungs with insignificant absorption.

The data here reported were obtained using an open system, the source air being labeled with about 2–3 microcuries of H^3 per liter. The individual breathed the air until equilibration was achieved as shown by the tracing on the recorder. Then the source was changed to room air and the data of the “washout” phase recorded, that is, the time concentration of tritium in the exhaled air. During the “washout” phase the exhaled air was collected in a Douglas bag so that functional residual capacity could be calculated by the classical open circuit technique. The data were graphically analyzed, and by means of an analogue computer solutions for compartment sizes and rate constants were obtained.

Figure 1 is a schematic diagram of the apparatus and their relationships. A small amount of tritium is delivered from a calibrated gas burette to the source Douglas bag, which is then filled with room air. The subject breathes from this source with the valve to the gas meter closed. A large portion of the air adjacent to the mouthpiece is drawn through the ionization chamber at 170 cm³/sec and returned to the main circuit distally. The chamber volume is

about 200 cm³. The exhaled air during this build-up phase is discharged to the outside atmosphere. The current generated by ionizing particles in the ionization chamber is amplified by the vibrating reed electrometer and recorded on the strip chart recorder. During the second phase of the study unlabeled air from the room is inhaled via the gas meter. The exhaled air is collected in the Douglas bag, and the respiration rate is recorded. The concentration of tritium in the collection bag is analyzed after the individual has completed the “washout” phase. From this concentration, the equilibrium value and the volume of air ventilated during the “washout” phase, the functional residual capacity is calculated.

DATA OF NORMAL SUBJECTS

Figure 2 is a plot of the graphic analysis from a representative normal subject. All of the 17 normal subjects examined, except one, show the two component exponentials. The exception exhibited a single exponential. Table I lists the values of the coefficients and time constants of the components. The half-time of the fast component ranged from 8.0 to 20.4 seconds, while that of the slow component ranged from 19.0 to 56.5 seconds. The functional residual capacity ranged from 2.13 to 4.54 liters. Since the tritium concentration was not precisely the same for each subject, comparison of the amplitudes (coefficients) of the two components can be made easily only by normalizing the values. This is automatically done in the computer analysis.

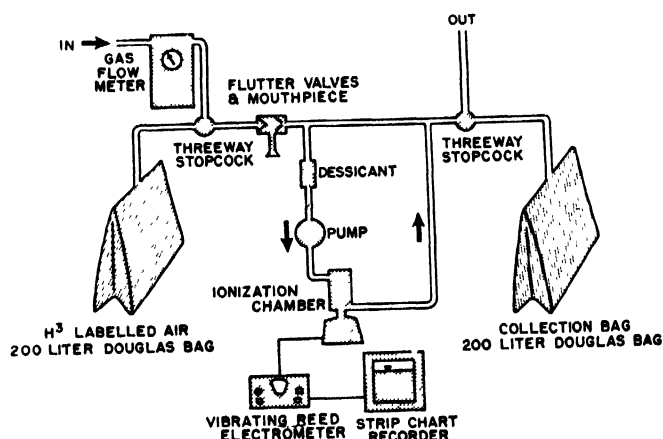


Figure 1. Schematic diagram of equipment for tritium ventilation studies

* Radioisotope Unit (R. L. Huff, Director), Seattle Veterans Administration Hospital, and University of Washington School of Medicine, Seattle, Washington.

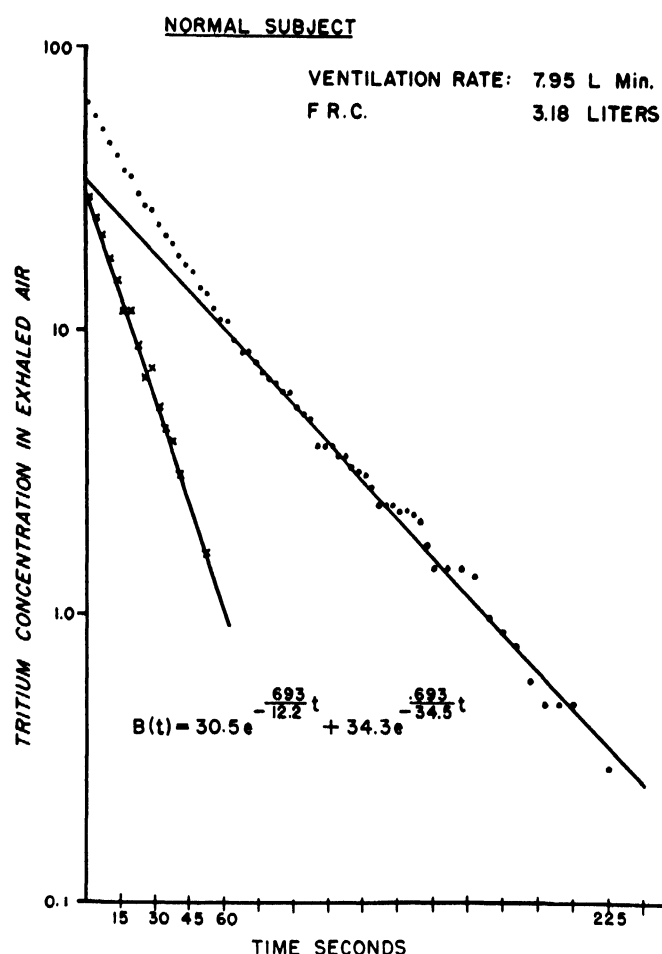
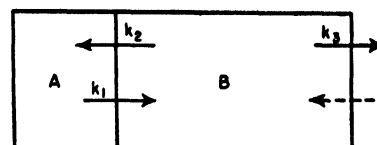


Figure 2. Typical data and graphic analysis. The dots represent concentration of tritium in the exhaled air as a function of time. The sum of the values at each point along the two lines is equal to the data point, $B(t)$

DATA OF PATIENTS

These differ from the normal in that at times there are three exponentials rather than two. In other instances there are two components but these may

$$B(t) = 30.5e^{-\frac{693}{12.2}t} + 34.3e^{-\frac{693}{34.5}t}$$



EQUATIONS FOR ANALOGUE

$$\frac{dB}{dt} = k_1 A - (k_2 + k_3) B$$

$$\frac{dA}{dt} = k_2 B - k_1 A$$

STEADY STATE

UNKNOWN

V_B = Volume of B
 V_A = " of A
 $k_1 = k_{AB}$
 $k_2 = k_{BA}$
 $k_3 = k_{BO}$

KNOWN

$K_3 B$ = Ventilation rate
 $V_A + V_B$ = Functional residual capacity

Figure 3. Model for normal lung H^3 kinetics

have quite different amplitudes (coefficients) and time constants than those of normals.

USE OF ANALOGUE COMPUTER

The double exponential character of the "washout" curves of the normal subjects is compatible with a model as shown in Fig. 3. The equations for this system are also shown in Fig. 2. The available data, the concentration of H^3 in the exhaled air, $B(t)$, and the functional residual capacity are sufficient to give a solution for the rate constants and volumes by ordinary algebraic procedures for the two compartment case. In the three compartment case, the analogue computer is used to obtain values for the rate constants and volumes. Solutions may be obtained by using the measured ventilation rate or the measured functional residual capacity. When the

Table I. Data and Derived Parameters for Normal Subjects

Subject	Ventilation rate, liters /min	Open circuit functional residual capacity, liters	Graphic analysis							Functional residual capacity, liters	k_1	k_2	k_3
			Intercept A	Half-time A, sec	Intercept B	Half-time B, sec	V_A , liters	V_B , liters					
BK	8.65	4.54	57.0	12.7	27.2	56.5	2.06	3.43	5.49	0.0166	0.0100	0.0420	
EF	8.65	2.55	34.0	8.0	41.0	29.5	1.25	2.77	4.02	0.0390	0.0175	0.0520	
CN	9.61	3.30	32.0	10.4	37.0	31.9	1.44	3.44	4.88	0.0372	0.0155	0.0465	
EW	11.58	3.35	42.5	10.0	41.0	32.5	1.03	4.23	5.26	0.0315	0.0110	0.0455	
EN	5.65	3.63	39.5	12.7	31.5	47.7	1.23	2.48	3.71	0.0222	0.0110	0.0380	
PL	7.95	3.18	30.5	12.2	34.3	34.5	1.02	3.40	4.42	0.0313	0.0094	0.0390	
CB	6.66		51.2	13.2	34.4	25.3	0.26	2.56	2.81	0.0340	0.0034	0.0434	
HF	7.50		55.5	14.2	39.0	42.1	1.15	3.68	4.83	0.0239	0.0075	0.0340	
LG	9.12		71.0	11.6	11.5	44.5	0.66	2.82	3.47	0.0175	0.0043	0.0560	
JB	9.20		60.0	10.2	78.0	34.5	1.41	3.78	5.19	0.0333	0.0125	0.0405	
DF 1	6.70	4.04	52.0	17.7	30.3	36.0	0.65	3.25	3.90	0.0550	0.0100	0.0345	
DF 2	5.82		57.0	17.2	61.0	40.5	0.79	3.23	4.02	0.0280	0.0068	0.0300	
WL	8.38	2.13	17.0	12.5	45.5	19.0	0.41	2.96	3.37	0.0980	0.0140	0.0470	
GJ	8.20	4.08	26.0	20.4	31.3	41.7	1.46	4.13	5.59	0.0850	0.0300	0.0330	
EP	7.92	2.29	42.0	9.3	34.0	30.8	0.92	2.72	3.64	0.0300	0.0100	0.0486	
JR	8.32	3.36	22.5	15.0	40.0	35.0	1.50	3.74	4.24	0.0950	0.0380	0.0370	
GH	5.87	2.40	31.0	8.0	47.9	36.0	0.83	2.04	2.87	0.0334	0.0200	0.0450	
PH	12.00	3.49	81.0	12.5									

Table II. Patient Data

Graphic Analysis								
Patient	Ventilation rate, liters/min	Open circuit functional residual capacity, liters	Intercept A	Half-time A, sec	Intercept B	Half-time B, sec	Intercept C	Half-time C, sec
LW	15.0	1.99	22.5	4.8	35.5	18.0		
JF	20.5	2.53	40.0	6.5	49.0	14.7		
CV	11.2		61.0	6.0	22.5	36.3		
BT	7.69	2.47	66.0	10.0	8.0	39.0		
ER	8.10		65.0	8.0	19.0	30.5	10.1	92.5
1 CW	9.79		28.0	3.5	13.9	15.5	11.0	71.3
2 CW	9.79		28.0	3.5	13.9	15.5	11.0	71.3
1 ED	9.91		30.0	6.5	50.0	14.5	12.5	53.7
2 ED	9.91		30.0	6.5	50.0	14.5	12.5	53.7

Computer Analysis									
Patient	V_A , liters	V_B , liters	V_C , liters	Functional residual capacity, liters	k_1	k_2	k_3	k_4	k_5
LW	1.04	3.57		4.61	0.0510	0.0150	0.0700		
JF	0.47	4.73		5.38	0.0600	0.0060	0.0700		
CV	1.71	2.00		3.71	0.0260	0.0223	0.0932		
BT	0.38	2.00		2.38	0.0267	0.0005	0.0640		
ER	0.33	1.56	2.08	3.97	0.0166	0.0035	0.0150	0.0200	0.0650
1 CW	1.86	1.63	1.42	4.91	0.0100	0.0114	0.0275	0.0240	0.1000
2 CW	1.36	1.87	1.63	4.85	0.0220	0.0160	0.0400	0.0350	0.1000
1 ED	0.42	2.73	1.14	4.28	0.0072	0.0011	0.0288	0.0120	0.0620
2 ED	0.42	1.21	2.73	4.35	0.0127	0.0044	0.0160	0.0362	0.0620

Diagnosis				
LW	Congenital heart disease		ER	Senile, emphysema
JF	Emphysema, pulmonary fibrosis		CW	Emphysema
CV	Asthma, emphysema		ED	Congenital heart disease
BT	Partial collapse			

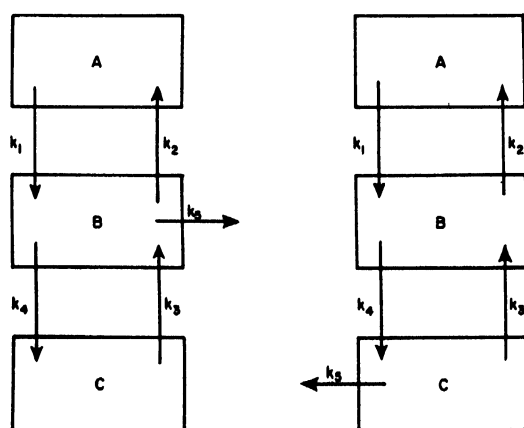
former is used a volume is obtained which is greater than the measured functional residual capacity by about 25%. This apparently is because the tidal volume is not mixed entirely in the first pool. Instead, only the tidal volume minus the dead space is the portion determining the rate constant between the environment and the first space. In this manner, "dead space" can be calculated. Insufficient data has

not allowed precise comparison of this "dead space" with the conventionally determined "dead space."

In Table I are shown the rate constants k_1 , k_2 and k_3 , and the volumes V_B and V_A . Apparently the sum of the two spaces V_B and V_A is analogous to functional residual capacity; while V_B is comparable to *maximum expiratory reserve* and V_A to *residual air*. More data of this type, as well as data obtained by conventional spirometry are required to establish this contention.

In Table II are listed the values for the patients with respiratory disease. In some instances there are two additional rate constants corresponding to the presence of a third exponential term in the data. In two cases these constants appear twice and are presented because satisfactory solutions on the analogue can be obtained for two different models, Fig. 4, and there is now no experimental proof for the correctness of either.

The data obtained by this method are unique in that the entire curve of "washout" is observed, that the gaseous state is normal, and that values obtained for compartment sizes are analogous to conventionally named lung spaces. Additional information pertaining to the transfer coefficients between these spaces is obtained.



$$B(t) = C_1 e^{-m_1 t} + C_2 e^{-m_2 t} + C_3 e^{-m_3 t}$$

$$C(t) = C_1 e^{-m_1 t} + C_2 e^{-m_2 t} + C_3 e^{-m_3 t}$$

Figure 4. Model postulates for three-compartment data. Either of these models yield satisfactory solutions for the data exhibiting three exponential components

Integration of a Radioactive Isotope Program Into Medical School Research and Teaching

By A. K. Solomon,* USA

There are two ways to approach the problem of the introduction of a radioactive isotope program into medical school research and teaching. Either a large central facility can be set up, well equipped to carry out such a program, or individual developments of a smaller kind can be started in many of the diverse areas of the school. The advantages of a central facility are numerous. Since the equipment required is complex and often expensive, special items can be brought together in a single location. The resultant concentration of resources makes it possible to attract a staff competent in the special techniques required and to keep them busy with challenging problems. The disadvantage of a central facility is that continual vigilance must be exerted to keep it from degenerating into a service organization for other members of the faculty.

At the Harvard Medical School, the Biophysical Laboratory was set up in July, 1946, as a central facility. In this Laboratory we have tried to protect our integrity in two ways. First, we have embarked on a vigorous and continuing program of fundamental biological research. Secondly, though we have trained many of our colleagues in the necessary techniques, and have made numerous radioactive measurements for them, we have always encouraged them to make measurements in their own laboratories as soon as they became competent. By these means we have been able to maintain a central facility that is not simply the servant of others, but is also active in basic research in its own right.

Biophysics is not taught as a discipline to the medical students at this University, and consequently it has been necessary to take special steps to integrate the Laboratory into the fabric of the Medical School. The Laboratory is responsible to the Committee on Medical Research in Biophysics, which includes representatives from many of the preclinical Medical School department.† Furthermore, from its outset,

an active member of the Biochemistry Department has also served as a member of the Biophysical Laboratory, and for the past four years continuing teaching responsibilities in Physiology have been maintained. As a consequence, the Laboratory is uniquely interdepartmental, a circumstance which has proved invaluable in our program.

The first concern in the introduction of radioactivity into medical school research and teaching is the stimulation of interest in the academic community. Interest in the medical use of radioactive isotopes grew rapidly and spontaneously as soon as isotopes became available from the reactor at Oak Ridge in large quantities and at moderate prices. Consequently, the initial problem became not the attraction of interest but the discrimination between those problems for which the use of isotopes provided new and significant information, and those problems in which the use of isotopes could contribute nothing of importance. In order to discriminate wisely, it is necessary to know at first hand the problems involved in accurate measurement of radioactivity. The intelligent application of radioactive isotopes to the solution of a problem demands exact analytical techniques to provide accurate and reproducible radioactivity determinations. In addition, it is generally advisable to make use of other quantitative analytical methods in conjunction with radioactive assays.

PROTECTION OF HEALTH

Next it was necessary to establish a scheme for the protection of all those concerned with the use of radioactivity. Consequently, a set of protection rules was devised as soon as practicable following the establishment of the Laboratory in 1946. Although these initial rules have since been modified,¹ the general principles on which they were based have been retained. In brief, the committee took the position that a sharp line could be drawn between the use of isotopes for specific research problems, and the use of isotopes in the treatment of patients which was properly the concern of the hospital, and not of the Medical School. This decision meant that only small amounts of radioactivity had to be considered, primarily in the microcurie to 10 millicurie range. Consequently, the rules could be devised with a minimum of controls, since the hazards in this range are

* Harvard Medical School.

† The present membership of the committee is: Chairman, A. Baird Hastings, Professor of Biochemistry; George Packer Berry, Dean; Joseph C. Aub, Professor of Medicine; Eugene M. Landis, Professor of Physiology; John L. Oncley, Professor of Physical Chemistry; Shields Warren, Professor of Pathology; Henry Meadow, Assistant Dean; Reidar Sognnaes, Professor of Oral Pathology; and A. K. Solomon, Assistant Professor of Physiological Chemistry.

not disproportionate to the hazards of high voltage, or of poisonous chemicals with which scientists had long been familiar. The resultant freedom from burdensome restrictions was of great importance in encouraging investigators to use radioactive techniques whenever they were applicable.

In order to help further in this direction, we have set up our own institutional film badge service, and have also provided a laboratory monitoring service which is available to all who work with isotopes in the Harvard community.² In return we have required workers to wear film badges at all times when they work with radioactivity and to submit a weekly form which shows the result of the laboratory monitoring, and on which the film badge readings are recorded.

Special problems are involved in experiments in which tracer doses of radioactive isotopes are used in man. These problems have been considered by a subcommittee set up to deal specifically with this subject. The subcommittee has adopted the following statement of policy to guide it in its decisions.

(a) Tracer doses will be kept to the absolute minimum.

(b) Adult humans who are ill and are expected to receive benefit from the procedure shall not receive tracer doses giving off radiation in excess of a total of 4 rep. Children (all patients below 15 years of age) shall not receive more than a total of 0.8 rep.

(c) In any other cases, tracer doses will be limited to radioactive material giving off radiation in an amount less than a total of 1 rep.

(d) These regulations do not apply to alpha-emitting isotopes since they are regarded as particularly dangerous.

The policy statement is set out in a memorandum³ that is given to investigators who want to carry out studies in man. The memorandum contains, in addition to the complete policy statement, instructions for calculating the dose on a conservative basis, illustrated by sample calculations for most of the biologically interesting isotopes.

CONSULTATION ON RESEARCH PROJECTS

We have also found it desirable to help in planning new projects involving isotopes. This includes consultation about the amounts of radioactivity necessary for satisfactory detection, help in the procurement of isotopes, and advice about the best methods for detection. Since the cost of equipment for measurement is so high, it has been expedient to carry out initial measurements in this Laboratory. This has proved of particular value, for under these circumstances an investigator can carry out a pilot study before committing himself either to the long-term use of isotopes, or to the purchase of expensive equipment. With the experience gained in these early experiments, he can determine, with our help, what instruments to buy, and the preferred method of measurement. By this means the use of radioactivity has been introduced to a wide variety of groups both within

the Medical School itself, and to the hospitals in the community.

It seems to us that the responsibility for analytical results belongs in the hands of the investigator himself and not in an outside group. Consequently, the sooner he takes this responsibility, the better. However, if the problem is limited to a short time or a few analyses, all the measurements can be made in our Laboratory. This means that problems requiring the occasional use of radioactivity can be undertaken without the necessity of a major commitment. The policy of decentralization has the result that the central laboratory is not burdened with routine responsibility for thousands of outside analyses and remains free to tackle other problems as they arise and so to encourage new groups.

Since the electronic equipment used in counting is so complex, it is necessary to make provision for servicing it, which cannot be done economically on the basis of a single counter. The concentration of instruments in the Biophysical Laboratory has made it essential to employ a first class electronic technician, whose duties include the maintenance of all the electronic equipment in the Laboratory. In addition, he is available to provide consultation and repair service to other investigators. This, added to our willingness to lend them equipment in times of emergency, assures continuity in a research program that requires radioactivity determinations.

RECEIPT AND DISTRIBUTION OF ISOTOPES

The Biophysical Laboratory also serves as a center for the receipt and distribution of radioactive isotopes. Since many of the isotopes are destined for use in experiments in man, it is necessary to make an independent assay both for the identity of the isotope, and its radioactive strength. Although an isotope has never been received incorrectly labelled as to its chemical identity, we feel that it is essential to make these checks routinely. The method adopted makes use of differential beta and gamma ray counting which serves to identify many of the commonly used isotopes, such as Na²⁴, K⁴², and I¹³¹. Where gamma rays are not emitted, as for P³², differential beta ray absorption is used.

The Laboratory assays of radioactive strength, however, frequently differ from the assays that accompany the isotope when it is shipped. Consequently, we routinely check our assay method by measuring the Bureau of Standards samples which are distributed every few months. Agreement within 10% is considered acceptable, though 5% is desirable. In addition, from time to time, we make absolute determinations of radioactive strength using our own equipment. Upon completion of the assay, the radioactive material is injected into vaccine capped bottles that have previously been sealed. The bottles are labelled to show the time of assay and the assay results, and may then be sterilized without further processing.

Since some of the isotopes which are in great demand have short half-lives, such as Na^{24} (15.04 hours) and K^{42} (12.44 hours) special arrangements must be made to ship them from the reactor to the Laboratory. For some time we chartered an airplane to fly once a week directly to Boston from the nearest reactor, but more recently we have found it possible to make a satisfactory continuing arrangement with a commercial airline. Since the Laboratory acts as a central supply for these short-lived isotopes, it is possible to divide the costs of shipment, which are large, among many users, and thus to supply this material as economically as possible.

INSTRUMENT AND COUNTING TECHNIQUE DEVELOPMENT

The discussion so far has been limited to the service aspects of the Laboratory. Next let us turn to some of the research projects of an applied nature which have helped to bring life into the enterprise. We feel it necessary to develop and build our own new instruments continuously, for only in this way can we keep abreast of the constantly expanding technology. It has been our experience that development of equipment which makes counting easier or more accurate, or which decreases the background is followed by almost immediate biological or medical application. Thus, contrary to the normal fashion in applied research, the instrument development has come first, and the problem has followed after. This unusual approach has meant that the Laboratory is particularly well equipped for the measurement of radioactivity. The instruments which have been developed in this manner have included: a dip counter to measure liquid samples,⁴ a proportional gas flow counter to measure soft beta emitters like C^{14} ,⁵ and a counter for the determination of tritium.⁶ Since radioautographs appeared to be a particularly promising tool for the localization of material in biological systems, we have developed a method for making radioautographs, and have carried out extended studies on the response of photographic film to beta particles.⁷ At the present time we are actively engaged in the development of a low background gamma ray counter, using many of the techniques that have been worked out in connection with C^{14} dating of archaeological specimens.

In addition to our work on instrumental development, we have also found it necessary to explore some other common counting problems. In 1946, the year the Laboratory was started, the initial problems were physical, since it was necessary to determine the basic physical constants of many of the isotopes we planned to use. Consequently, we determined the maximal energy of the beta radiation emitted by C^{14} , S^{35} and Ca^{45} , and later in connection with a biological problem which involved its use, we made an accurate measurement of the half-life of Na^{24} .⁸ Fortunately, this phase of the development of the physical sciences is now past, and accurate values for the disintegra-

tion schemes of most of the isotopes commonly employed in medical and biological work are to be found in the physics literature.

Since much of the counting involves C^{14} , an isotope which is difficult to measure because its beta radiation is so soft, we have been forced to make studies on self-absorption, so that we could be sure that the measurements were accurate. These have included not only studies based specifically on our special instruments, but also basic studies on self-absorption problems.⁹

COOPERATIVE PROJECTS WITH THE DEPARTMENTS OF MEDICINE AND SURGERY

Our interest in instruments has led naturally into the development of techniques of direct clinical application. These have required cooperation with the Department of Medicine at the Peter Bent Brigham Hospital and the Department of Surgery at the Massachusetts General Hospital. The association with the Department of Medicine, with whom we have maintained close relations since the foundation of the laboratory, has been maintained either through joint appointments or through a continuing informal arrangement in which research fellows work cooperatively with both organizations. The development of the Cr^{51} method for red cell and plasma volume measurements was carried out in this Laboratory under such an arrangement.¹⁰ More recently we have been involved in a series of studies on a method for the simultaneous determination of whole body Na with the Department of Medicine.¹¹

Perhaps the most rewarding of these cooperative projects has been the development of the P^{32} method for the localization of brain tumors during operation,¹² carried out in collaboration with the Department of Surgery at the Massachusetts General Hospital. Following a demonstration that P^{32} (injected intravenously as PO_4) is concentrated selectively by many types of brain tumors, it appeared possible to localize the tumor exactly. This required development of a counter so small that it could be inserted into human brain without damage. In this case, contrary to our usual procedure, the counter was designed to fit the problem.¹³ With its use we have been able to localize tumors with great success. The instrument is in commercial production, and the method has been adopted in many hospitals.

BASIC RESEARCH PROBLEMS

Perhaps the most pleasant aspect of a central laboratory is the freedom that it provides for the pursuit of one's own research. Frequently the tools that have been developed for others seem to be particularly applicable to the basic research problems being studied in the Laboratory. These may be divided into biophysical and biochemical areas. In the former we are studying the permeability of cellular membranes, with particular respect to Na and K. The ready availability of these tracers on a weekly basis, as a by-

product of our service to the medical community, makes our research very much easier. Also the method worked out in collaboration with the Department of Medicine for the simultaneous determination of total body Na and K in man, has immediate and obvious applications to our cellular permeability studies. In the biochemical area, our interest lies in the fields of lipid and bacterial metabolism, in which the use of C^{14} is of great importance. The precision attainable in these studies rests upon our exact knowledge of the self-absorption corrections in C^{14} counting, and the sensitivity of the methods depends very greatly on the special low background counters that have been designed and built in the Laboratory.

MEDICAL SCHOOL TEACHING

The necessity to teach undergraduate medical students about radioactive measurements arises from the important contribution to medical knowledge that isotopes have made. We feel that the student should learn some basic facts about radioactivity, but that it is neither necessary nor indeed desirable to provide intensive training except to students who will themselves later make use of isotope techniques.

The introduction to radioactivity is a joint enterprise between biochemistry and physiology. Almost at the outset of the biochemistry course, a special lecture on radioactivity is given to the medical students. This is a broad outline of the subject, with particular reference to tracer problems of the kind encountered in the biochemical literature. Some weeks later a whole laboratory day in the physiology course is devoted to an experiment involving radioactivity. The experiment is divided into two parts. First comes the determination of an unknown volume by the method of isotope dilution using P^{32} as a tracer. The extension of this principle total body composition studies is discussed at some length. The second part consists of a study of membrane permeability, using the transport of one of the alkali cations across a cellophane membrane as an example. During the course of these experiments every member of the class has an opportunity to make radioactive measurements. This first hand familiarity is important in making the students understand both the advantages that can be gained from the use of isotopes, and the pitfalls that come from careless work.

From time to time exceptional medical students indicate a desire to engage in scientific research during their undergraduate years. We try to make a place for such students in the Laboratory, and set them to work on a research problem that can be completed in the limited free time that is available to them during their period of medical training, primarily the summers of their first two years. Although a long enough period has not gone by to permit objective assay of this scheme, it is heartening to note that the first student of this kind who came to the Laboratory six years ago at the outset of his medical training is now returning after completion of his

assistant residency to learn more about basic scientific research using radioactive isotopes.

From what has been said above, it can be seen that the Biophysical Laboratory has two distinct but interconnected responsibilities. One is the training of young students to understand radioactivity and the benefits that can be derived from its use, and the second is the encouragement and vigorous pursuit of research involving radioactive isotopes, not only under its own roof, but throughout the entire medical community.

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Possibilities and Limitations of *In Vivo* External Counting Techniques in Biology and Medicine

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Engineers commonly use the descriptive term "non-destructive testing" for describing testing techniques in which the function of the machine or part under examination is not appreciably affected by the procedure itself. In an analogous sense, it might be said that a goal of clinical medicine is the development of biologically "nondestructive testing" techniques. A new procedure yielding new types of diagnostic information will be widely used if it is simple, inexpensive, and nondestructive, or minimally destructive, in the sense that there is little or no temporary or irreversible destruction of tissue or biological function. As in medicine, a prominent nondestructive method used in engineering is that of radiographic examination. We are all currently aware that radiography and fluoroscopy in medicine is not strictly a nondestructive method, but might more aptly be called minimally destructive, although how much biological destructive action is caused by the necessary dosage of radiation for radiography and fluoroscopy is at present a very moot question.

In a sense, an extension of radiographic and fluoroscopic techniques is that of external counting and scanning of gamma-emitting radioisotopes *in vivo*. Again we find an ill-defined range of administered dose which will produce minimally destructive effects. Usually, a decision must be made on an arbitrary basis of safety after estimation of the infinity tissue dose from the effective half life of the isotope used.

Frequently the maximum allowable dose stringently limits the usefulness of possible techniques either by producing statistical counting variation in the results or requiring an inordinately long time to obtain a statistically significant measurement. This basic limitation could be partially overcome in certain applications by the use of short-lived isotopes from a radioisotope "cow" or directly from a reactor. An important practical limitation of a cow such as one producing I^{132} is that frequently used iodinated compounds such as radioiodinated serum albumin cannot be easily prepared in a pyrogen free form, on the spot for medical applications.

The wider use of short lived isotopes in external counting techniques obtained from a reactor awaits

the day when medical reactors will be closely associated with hospitals and biological and medical research institutions.

The statistical counting fluctuations arising from a limited allowable dose are even more annoying in following with external counting a rapidly changing phenomenon such as presented in cardiac output volume measurement. In fact, such methods have only become practical with the development of highly efficient gamma detectors such as the now commonly used sodium iodide scintillation counter.

In biological research with animals the dose limitation is not so important, especially in short time experiments. One might ask why use external counting techniques in animal experiments, as the animals can be readily sacrificed and the organs or tissues counted in the usual manner? The answer is that a time sequence of events can be determined on one animal without destruction of the animal. This eliminates from the experimental design the usually tremendous statistical variation inherent in an animal population. Also, results are obtained very rapidly with a minimum of effort and animal handling. The smallness of many laboratory animals is frequently a limiting factor in external mapping or localizing of gamma-emitting isotopes. A collimating system used to obtain good spatial resolvability in mapping or picking up activity from a small organ must of necessity respond to only a very small solid angle of radiation emitted from the small region of interest. The small counting rates obtained again introduce the problems of statistical counting fluctuations and long counting times. In many medical applications useful results can frequently be obtained by sacrificing geometrical resolution in favor of higher counting rates as these are intrinsically limited by the allowable dose as previously discussed. We will return later to a more detailed discussion of the collimation problem.

In most cases, but not in all, external counting is limited to a few gamma-emitting isotopes, notably I^{131} , Au^{198} , and in some techniques, Na^{24} , K^{42} , Rb^{86} , Fe^{59} , As^{74} , and in vitamin B-12 combination, Co^{60} . Occasionally a pure beta-ray emitter can be externally measured with a thin window G-M tube, if the maximum energy beta rays do not have to go through a thickness of tissue greater than their range to get

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out through the body surface. For P^{32} , this maximum range is about 7 mm; for some other high energy beta emitters of possible use, it can be more than 2 cm, but in most cases it will be a few millimeters. This external counting technique has been exploited for detecting increased uptake of P^{32} in skin tumors as an indication of malignancy. Another possibility of external counting which has some application is the measurement of a beta emitter such as P^{32} by means of its weak bremsstrahlung. This radiation is so weak that it can only be measured effectively with large doses or by the use of very long counting times. The bulk of the bremsstrahlung are of lower energy and are strongly absorbed in tissue, although they can be efficiently detected with a G-M tube. There is a small higher energy component that can be detected with a scintillation counter.

The optimum techniques for external counting in most cases involve the use of gamma-emitting isotopes. The tissues are not completely transparent to the gamma rays, their absorption characteristics following well established principles. Suffice it to recollect that except at the very high quantum energies where pair production occurs, absorption can be classified either as the "photoelectric" or "Compton scattering" type. The latter is preponderant above about 0.7 Mev for all materials. The former preponderates in higher atomic number elements below this energy, but does not produce much absorption in water and low atomic number materials except considerably below 0.1 Mev. These changes in absorption characteristics with the quantum energy of the radiation mean that I^{131} gammas of 0.36 Mev and Au^{198} gammas of 0.41 Mev, for example, can be efficiently shielded or collimated with much less lead or tungsten than Na^{24} gammas of 2.76 and 1.38 Mev, and K^{42} gammas of 1.51 Mev. Furthermore, before a quantum can be detected it must be absorbed or Compton scattered. A conventional G-M tube efficiently responds to quanta absorbed in parts of its structure, but for high energy radiations most of the quanta go right through the counter without interaction. A typical commercial G-M tube might only show an absorption efficiency of $\frac{1}{2}$ to $1\frac{1}{2}\%$ for I^{131} gammas. This low efficiency has vigorously spurred two other developments applicable to medical external counting: that of the multiple bismuth, tantalum, or other heavy metal plate G-M tube, and more significantly that of the scintillation counter.

The medical scintillation counter obtains increased absorption of incident quanta by the use of a scintillation crystal which is now usually thallium activated sodium iodide.

In the initial development five or six years ago, calcium tungstate was used quite a lot, but although it has its advantages and disadvantages, it is no longer commercially attainable in high grade pieces. Sodium iodide has improved phenomenally and is now universally used on a large scale. Medical counters are roughly classified according to their degree

of collimation as wide angle counters, coarse collimating counters, and high resolution directional counters. Wide angle counters are commonly used for thyroid uptake studies with administered doses of I^{131} of only a few microcuries and for tissue clearance studies to follow the rate of disappearance of the activity of usually only a fraction of a microcurie of radioiodine injected interstitially. Coarsely collimated counters are used in various external tumor detection methods, dynamic circulation studies, and a variety of procedures being experimentally explored, notably dynamic liver uptake and excretion studies with radioiodine tagged dyes, dynamic studies of kidney function, and dynamic studies of ciliary dust clearance of the lungs. High resolution counters are used for mapping distributions of activity and have been the foundation of the scintigram technique of thyroid mapping. They have been used for scintimyelography, that is, the mapping of the distribution of radioiodinated albumen injected into the spinal canal. Blocks and protruded discs can usually be quickly discerned without the necessity of injection and removal of a radio-opaque oil. The exploration of possible external counting techniques is in truth in its very preliminary stages.

There are some simple geometrical principles involved in any external counting or localizing procedure, the understanding of which is essential for intelligent applications. If a detector were sensitive only in a very small volume so that this volume could be considered practically as a point compared with its distance from active sources of gamma rays, then for a point source of gamma rays the net count per second will read inversely as the square of the distance between the source and the detector. Of course, this only holds when there is no appreciable absorption of radiation between them. Neglecting absorption for the time being, we must now consider the behaviour of a point detector with an extended source, and an extended detector with a point source, and that of both extended detectors and extended sources. Obviously, if an extended source is far enough away from a point detector so that the distance is large compared with the extent of the source, the inverse square law will still hold. As the extended source is moved in closer to the detector, the net counts per second will start falling off from the inverse square law referred to the nearest point in the source. This is simply because other points of the source, each of which follows an inverse square law individually, are further away than the nearest point. The larger the extent of the source the longer the distance at which measurable deviation from the inverse square law will occur. In fact, for a very extensive—practically infinite—uniform sheet of activity there will be no distance where the inverse square law holds, and again neglecting absorption, the net count per second will stay constant at all distances. This simple, but to some, remarkable result follows from the fact that there is exact com-

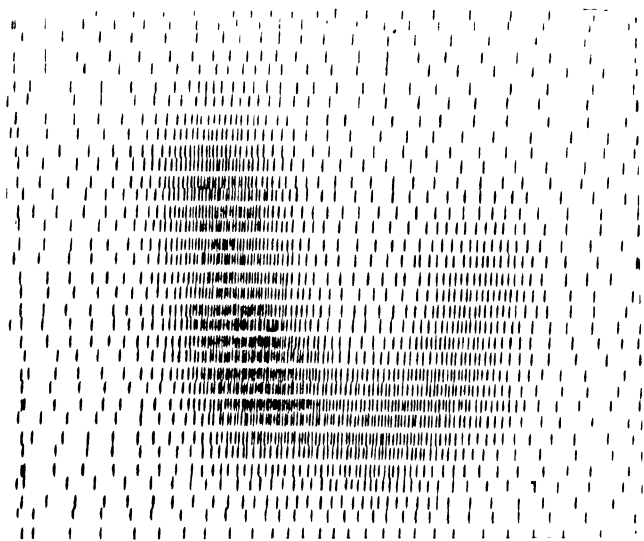


Figure 1. Scintigram of a patient with hyperthyroidism. Thyroid gland diffusely enlarged with the right lobe thicker and higher, which is normal (No. 4111)

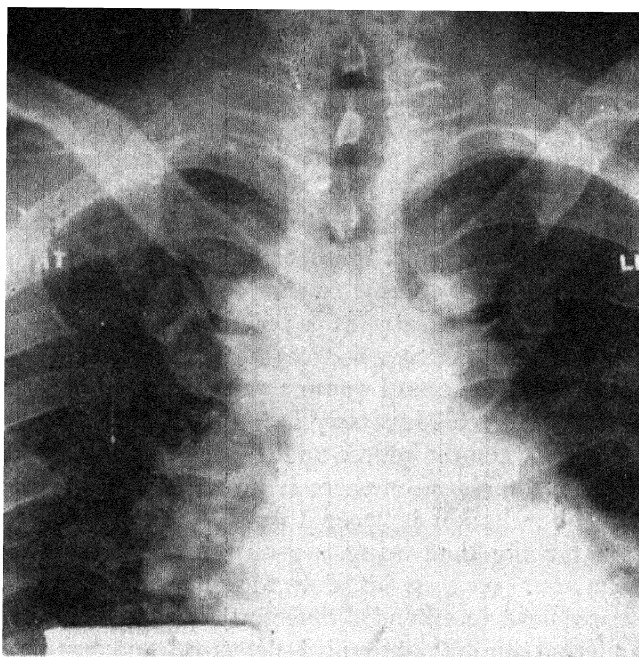


Figure 3a. Roentgenogram of chest. Note mass in superior mediastinum to the right of the aortic arch (No. 4107)

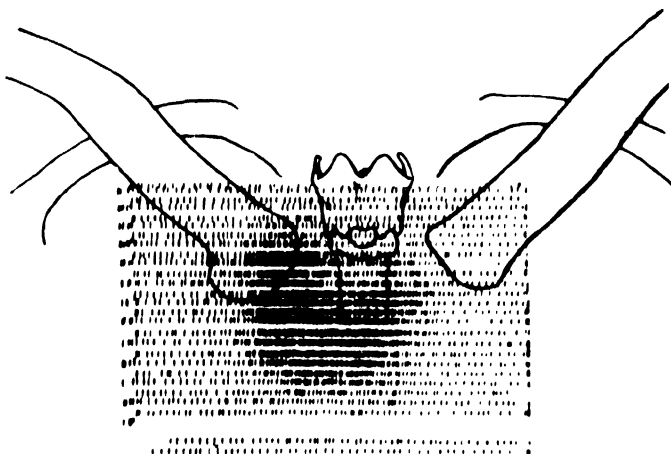


Figure 3b. Scintigram of the same patient with larynx, clavicles, and first ribs drawn diagrammatically. Substernal thyroid gland (No. 4107)

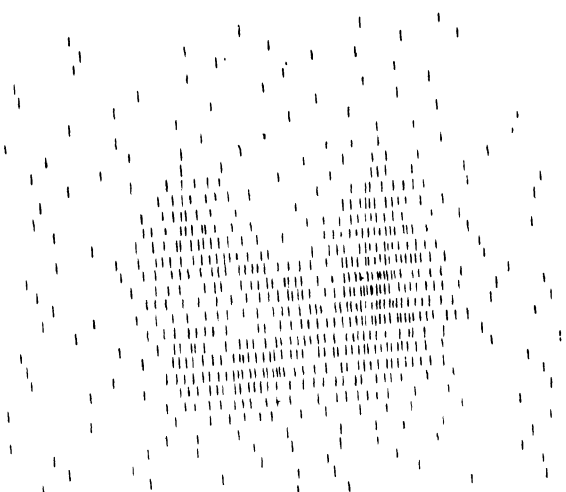


Figure 2. Scintigram of a patient with papillary adenocarcinoma of the thyroid. Note the "non-functioning" area in the middle of the right lobe of the thyroid, which corresponded to a palpable node (No. 4755)



Figure 4a. Scintigram of a patient who had undergone total thyroidectomy for thyroid carcinoma. Tracer dose: 500 microcuries ^{131}I preceded by 10 units of thyroid stimulating hormone intramuscularly. Radioiodine concentrating area was considered to be a thyroid remnant (No. 4753)



Figure 4b. Scintigram of the same patient three days later, following a therapeutic dose of 25 millicuries ^{131}I , again preceded by 10 units of TSH intramuscularly. Many metastatic lesions are now visible (No. 4753)

pensation for the fall off in intensity from each square cm of the sheet by the increased number of square cms seen by the detector in the same solid angle as its distance is increased.

A reciprocal situation exists with a point source and an extended detector, if distances are measured from the nearest point of the detector. The larger the extent of the sensitive element of the detector, the greater the distance of the point source that deviations from the inverse square law become apparent.

For an extended source and extended detector a very complex geometrical situation exists, requiring mathematically a double integration to evaluate. However, it is still plain that if nearest points in detector and source are chosen as reference points, there will be a fall away from the inverse square law as the distance is decreased, although in some geometries this might not be as rapid as when one of the elements is a point.

These considerations are important in accurate I^{131} uptake studies by external counting of minimally destructive administered doses. Thyroid glands are of various sizes. It is important to use a counter that can equally efficiently see the whole of even large glands. Many commercial counters are collimated sufficiently so that they do not do this at the short distances where they give high counting rates with small doses. Even a so-called wide angle counter should be tested with a point source for equal counting efficiency over a wide enough angle. When the counter is placed near enough to the neck so that the inverse square law does not hold one method of calibration can be most effectively attained by use of high activity models of various size ranges that can be measured close and also at a distance where the inverse square law holds. Unfortunately, there is as yet no standardized procedure in general use. Good results can be obtained with adequate care with administered doses of $1.0 \mu\text{c}$ to $5.0 \mu\text{c}$. It appears that there is no objection to using this dosage range on the general public.

In a collimated detector system the sensitive element can "see" only a small portion of the source distribution through a hole or tunnel or group of apertures in a radiation absorbing shield. The smaller the area "seen"—that is, the smaller the resolution—the smaller the net counting rate. This means that to get very fine resolution with a reasonable counting rate requires either a high activity per unit projected area or a very high sensitivity of the pick-up element, or both. Sensitivity can be gained at the sacrifice of resolution and vice versa. Any collimator used with a non-point crystal assembly does not enable the crystal to either "see" or not "see" a given area of the source. There is always a penumbra area surrounding the region seen by the whole crystal, which area is only seen by part of the crystal. A long collimating channel reduces the size of the penumbra zone, but requires putting the crystal further away from the active region, thereby reducing



Figure 5a. Oil myelogram of a patient with protruding intervertebral disk (No. 4952)

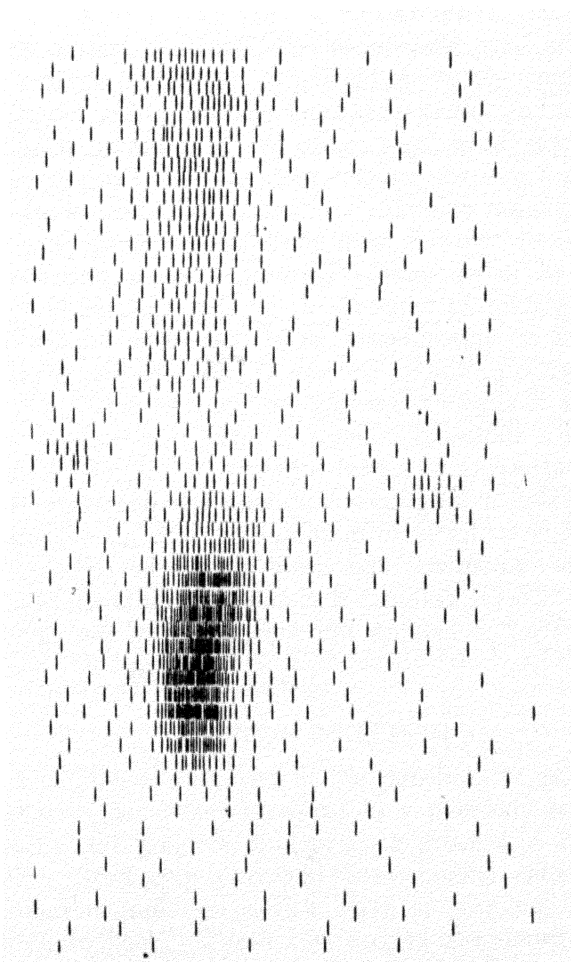


Figure 5b. Myeloscintigram, using 150 microcuries of radioiodinated human serum albumin. Note the defect in the column of radioactivity. The two "hot" spots to each side of the defect are due to small lead buttons with 10 microcuries antimony-125; these buttons are taped to the patient's back at the level of the fourth lumbar interspace (No. 4952)

intrinsic sensitivity. Conically converging collimators have been suggested and are being developed. They should map out the activity distribution in a section, but if the distribution has appreciable depth, the smeared background will be high, tending to destroy contrast. Some contrast could be restored by means of a constant background subtractive technique.

The optimum design of a scanning system requires a practical compromise of spatial resolution, contrast, dose level, time required to scan a given area, and regularization ratio of the recorder to the incoming counts. The higher the number of counts required to give one pulse to the recording system the more visually interpretive the record is. This regularization removes statistical fluctuation phenomena from the record. To anyone who has seen thyroid scintigrams it is obvious that the design compromise, although good enough to often give useful information in a reasonable length of time, sometimes is on the verge of not giving a clean cut answer. On scanning larger low contrast areas such as the liver or brain, the design compromise suitable for thyroid scanning is no longer suitable. On account of a large amount of scattered radiation getting into the collimated channel from surrounding regions of activity, the contrast of the record is extremely low. Some attempts have been made to reduce the scatter effect by sharp electronic biasing of the crystal against the somewhat softened scatter radiation. Other extensive attempts have been made in brain tumor scanning using a positron emitter such as As^{74} . Two opposing scintillation counters produce coincidences when affected by two 0.5 Mev annihilation quanta that are emitted in opposite directions when a positron is annihilated. It appears so far that this method requires an inordinately large dose of a hard-to-get cyclotron produced isotope. When very low contrast exists it appears that the best way to detect it is to make a thousand counts or more over each of the compartments of a grid network. If the grid is fine, this takes a long time, although the necessary time could be reduced to a minimum with an automatic system. Required time could always be reduced by using several collimated channels simultaneously. It is difficult to balance the sensitivities of the various

channels and the cost of the method rises almost in proportion to the number of channels. Scanning recorders have been developed in the form of printers and by the photography of a scope screen or flashing neon lights. It appears that the simplicity of a mechanical printer has much to recommend it.

There is still much scope for ingenuity and inventiveness in external counting instrumentation. The medical profession will most heartily welcome any development of "non-destructive" clinical testing devices.

The practical applications of the foregoing are exemplified by the diagnostic procedures used in a large General Hospital:

The scintillation tube is used for the determination of thyroid function and in the diagnosis of morphologic abnormalities of the thyroid gland. The latter procedure was developed by the authors. It has proven of great value in the estimation of the size of the thyroid gland in hyperthyroidism, non-toxic goiter, aberrant thyroid tissue and the nature of solitary thyroid nodules. Thus if a solitary thyroid nodule is found to accumulate radioiodine, malignancy can, as a rule, be excluded.

For the detection of intracranial neoplasms, radioiodinated human serum albumin is used as a tracer agent. The differences in the concentration of radioactivity measured by external counting with a scintillation tube between the two hemispheres are recorded. A specially designed G-M tube (Selverstone-Robinson) is used in the operating room for the detection of brain tumors. Radioactive phosphorus is the tracer agent and the probe is inserted into the brain in the search for high counting areas which indicate rapid growth and metabolism and thereby suggest tumor.

For the detection of intra-ocular neoplasm such as malignant melanomas, a specially constructed G-M tube (Anton) is used. Radioactive phosphorus is the tracer agent. Malignant melanomas give considerably higher counts than the corresponding areas of the healthy eye. Benign nevi of the skin can be differentiated from malignant melanomas by external counting with a G-M tube, radioactive phosphorus being the tracer agent.

Scanning of Positron-emitting Isotopes in Diagnosis of Intracranial and Other Lesions

By G. L. Brownell and W. H. Sweet,* USA

Radioactive isotopes have proved to be of value in the diagnosis of focal disorders of the brain. The biological behavior which permits these techniques to be used is a fundamental and poorly-understood property of the normal brain to exclude or to admit slowly a wide variety of substances ranging from normal intracellular ions to large organic molecules. This property has been described as a "blood-brain barrier", although the existence of a physical barrier is by no means proven. Thus, the following ions, P^{32} , K^{42} , Mn^{52} , Cu^{64} , As^{74} , and Rb^{84} , have been shown¹ to enter the normal cerebrum of mice much more slowly than spleen, liver, kidney, pancreas, adrenal, muscle or transplanted subcutaneous glioma. Our studies in man with radioactive substances have shown that trace ions, such as Mn^{52} , Cu^{64} , and As^{74} , concentrate in brain tumors to a substantially greater degree than in normal brain; ratios of 10:1 or more are common. Labeled compounds such as di-iodo-fluorescein² and iodine labeled albumin³ also have similar properties. In our laboratory the normal intracellular ions, phosphate and potassium, have been found to concentrate to a greater degree in human brain tumor relative to normal brain than any other substances.

Numerous practical techniques have evolved from these observations. Selverstone and White⁴ have demonstrated the use of P^{32} in localizing tumors at operation, and Selverstone, Sweet, and Robinson⁵ refined the techniques by using a fine Geiger-Mueller probe counter of 2 mm diameter for the localization. Moore² reported first the use of external counting for the localization of brain tumors, and numerous others^{6,7,8} have reported observations with gamma-ray emitting isotopes with varying results.

Fundamentally, the problem of external localization of isotopes is twofold; obtaining a suitable resolution of measurement of activity within the head while maintaining the intensity, and presenting the information in a usable manner. The use of positron-emitting isotopes appeared to offer considerable promise for obtaining high resolution while maintaining the sensitivity at an appropriate level. Automatic scanning of the head and recording of data

appeared useful in the presentation of results. The development of such a device, called the positron scanner, and preliminary results, have been described in previous reports.^{11,12}

We discuss here the result of positron scans on 719 patients, most of whom have received radioactive arsenic, As^{74} . These have been so encouraging for the detection of brain tumors that the method has been tried in numerous other situations where one wishes to know the distribution of an isotope deep within the body. Preliminary results of scanning of other organs in animals will be discussed.

THEORY OF SCANNING

A relationship between optimal concentration of isotope, resolution, and efficiency of detection may be derived. Thus, the required concentration in disintegrations per min per cm^3 (dpm/ cm^3) is:

$$C = \frac{400 A}{\pi d^4 L \epsilon T}$$

where A = area to be scanned in cm^2 , d = resolution of apparatus in cm, L = width of object to be scanned in cm, ϵ = efficiency (cpm of detector per dpm of isotope located at center), T = time available for scan in min.

The required concentration of isotope is seen to be strongly dependent on the resolution of the device regardless of the physical method of detection. As the maximum concentration is limited by biological considerations and is very roughly the same for most useful isotopes, the optimal resolution is primarily dependent on the efficiency.

POSITRON DETECTION

The physics involved in the detection of positron radiation has been discussed in a previous report.⁹ Briefly, the positron undergoes a process of annihilation, producing two 0.51 Mev quanta called annihilation radiation which leave at 180 degrees or "back to back." These two quanta are detected by two scintillation counters located on either side of the head and connected to a coincidence circuit.

The advantage of the coincidence method is that it allows increased resolution without loss of intensity. Figures 1(A) and (B) show scans on a geometrical pattern of activity in the form of a six-

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pronged star. The actual inner diameter of the tubes containing activity is only 2 mm and is small compared to the resolution. In (A) a pattern of As^{74} is scanned using coincidence detection with $\frac{3}{4}$ " apertures, while in (B) a pattern of I^{131} is scanned with a single detector with a collimator 6" in length and

$\frac{5}{8}$ " diameter. The pulses from the single detector are analyzed and only the 360 kev photopeak is retained for plotting the scan. The increased resolution of the coincidence scan is obvious, whereas the efficiency of the coincidence system is still greater than that of the single channel system.

Resolution, as used in this report, is given the following arbitrary definition. If a point source of activity is moved transverse to the axis of the detector at a plane corresponding to the middle of the object being scanned, a characteristic curve is obtained having a maximum at the center and decreasing rapidly on either side. The distance between the two points at which the response has decreased to one-half its maximum value is called the resolution. Figure 2 shows the effect of aperture diameter on resolution for both a coincidence detecting system and a single channel system with a collimator 6 inches long. When all factors are considered, it appears that for equal resolution, the efficiency of the coincidence system is about 10 times that of the single channel system. Stated in other words, to produce a scan of a given resolution in a given time, the single channel techniques will require about 10

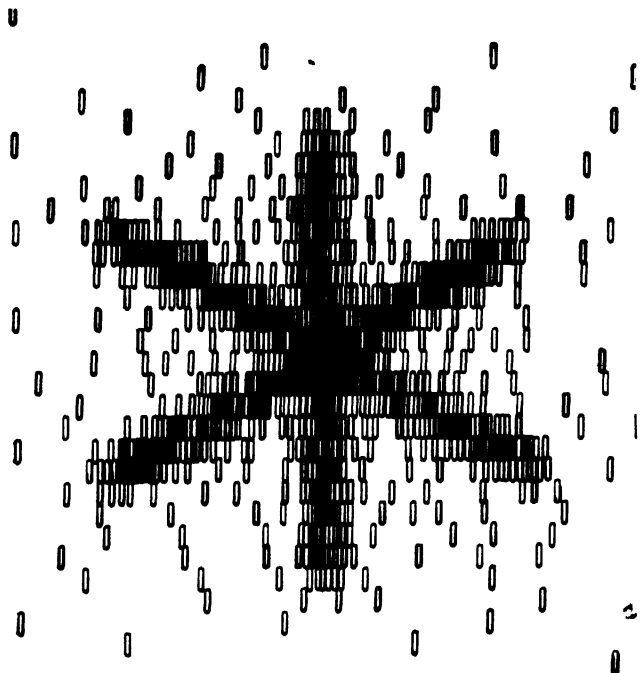


Figure 1A. Scans of star-shaped phantom consisting of 6 tubes of 2 mm id containing a radioactive solution. As^{74} scanned by means of coincidence detection with two detectors of aperture $\frac{3}{4}$ in.

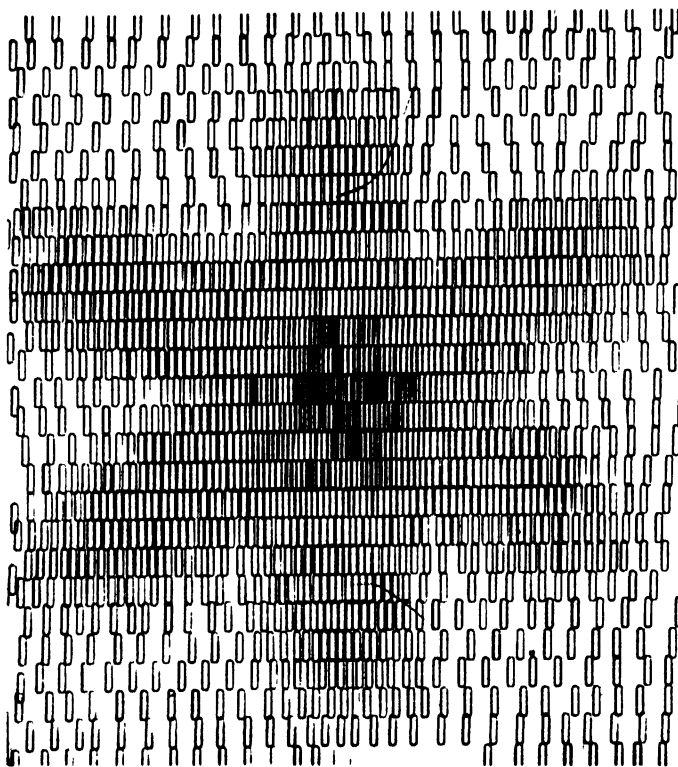


Figure 1B. Scans of star-shaped phantom consisting of 6 tubes of 2 mm id containing a radioactive solution. I^{131} scanned by means of a single-channel collimator of $\frac{5}{8}$ in. diameter and 6 in. length. Pulse height discrimination used to select the 360 kev photo-peak. Length of symbol in both cases is 1 cm

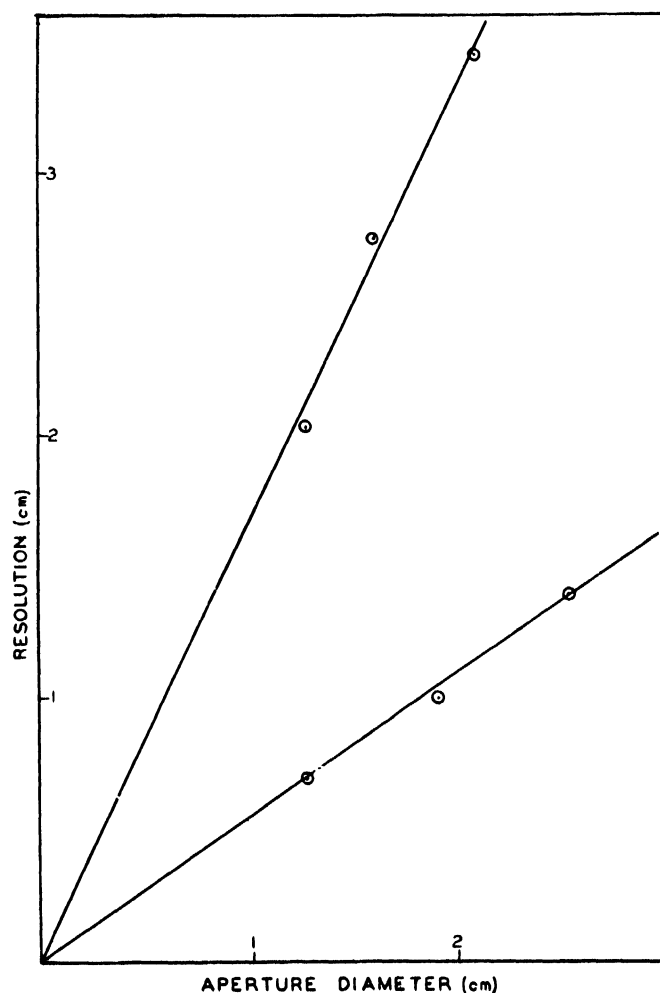


Figure 2. Dependence of resolution on aperture diameter. The upper curve refers to a single-channel collimated detector of length 6 in. The lower curve refers to coincidence detection of positrons. For a given resolution, the aperture diameter for the coincidence system is about 3 times that of the single channel system

times the dose of isotope required by the coincidence technique using positron-emitting isotopes.

BRAIN TUMOR LOCALIZATION

The new version of the positron scanner will be described in detail elsewhere.¹⁰ Most of the scans to be reported here have been obtained with the original model which has been previously discussed.¹¹ Both devices produce two scans, a positrocephalogram, or PCG, which shows the distribution of activity throughout the head, and an asymmetrogram, or AGG, which shows any asymmetry in activity from right to left within the head. These two scans, obtained simultaneously, provide information on the exact location and extent of an abnormal region. All patients in the present group have re-

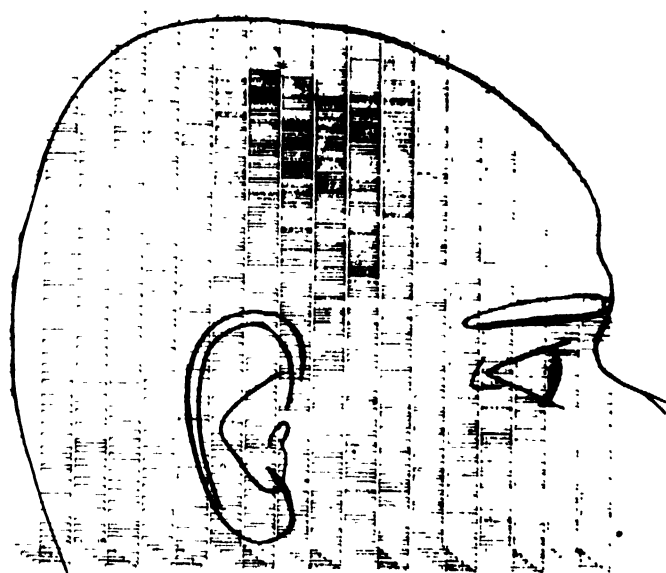


Figure 3A. Typical scans on a patient with a right-sided meningioma. PCG at 1 hour shows the lateral extent of the tumor

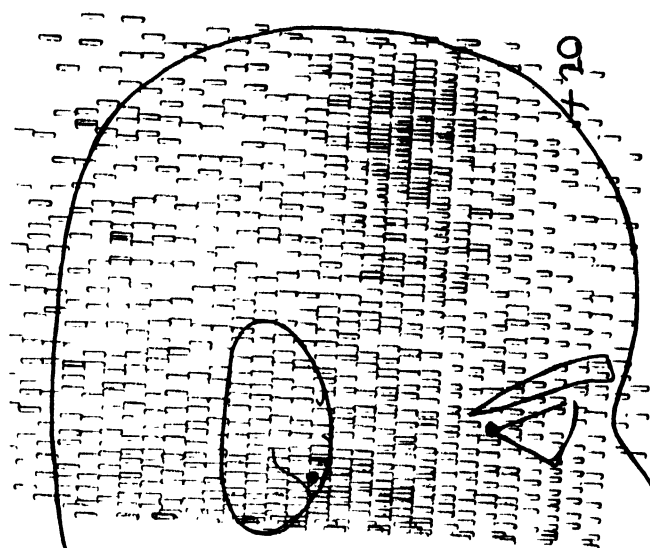


Figure 3C. Typical scans on a patient with a right-sided meningioma. PCG of following day, taken on new machine shows effect of higher resolution. The dumbbell-shape of the tumor is more apparent from this scan

ceived As⁷⁴ and in general scans are obtained one hour following the intravenous injection and again on the following day. Both the arsenate and arsenite ions have been used and our usual dose is 20 μ c/kg body weight.

The normal PCG shows an increase in isotopic content in the region of the face and around the periphery of the head. The area over normal brain shows a uniform and low content of activity. The normal AGG should in principle show no symbols, but usually shows a slight right on left unbalance over a portion of the scan. A tumor or abscess produces an abnormal area of isotopic concentration and many produce an indication on the AGG scan if the area is situated to one side of the midsagittal plane. Figures 3(A) and (B) show scans on a patient with a right-sided meningioma. The lateral extent is indicated by the marked area of concentration on the PCG, and the fact the tumor is on the right is indicated by the cluster of curved symbols on the AGG. A left-sided tumor would be indicated by a cluster of straight symbols on the AGG. Note that the area covered by the curved symbols in the AGG, Fig. 3(B), is much larger than the actual area of the tumor which is shown faithfully by the PCG, Fig. 3(A). One day later on our new machine the PCG of Fig. 3(C) was obtained. The dumbbell-shape of the tumor revealed at operation is visible only on this new scan at higher resolution.

RESULTS OF PATIENT SCANS

Table I summarizes the diagnostic accuracy of the method in patients with tumors; Table II covers the other categories of disease. For this study we have reviewed our scans on all 719 patients without reference to our previous diagnostic descriptions or any clinical data. In certain cases several sets of scans have been obtained for one patient. We have classified our diagnoses into four groups:

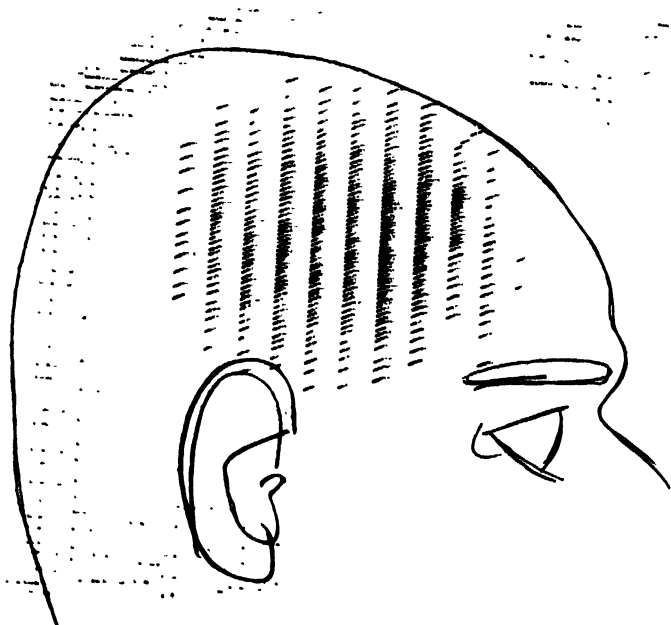


Figure 3B. Typical scans on a patient with a right-sided meningioma. AGG at 1 hour, taken simultaneously with the PCG (Fig. 3A), shows a cluster of curved symbols (right unbalance) over the tumor area

- 1 = abnormal isotopic concentration
- 2 = probably abnormal isotopic concentration
- 3 = probably normal isotopic concentration
- 4 = normal isotopic concentration

If the size and locus of the abnormality appear unequivocal and especially if there is agreement in all four scans, PCG and AGG on two successive days, we place the case in group one. If this data is consonant with a clinical history and findings indicating a space-taking lesion, we are omitting the slightly hazardous surgical diagnostic measures of pneumography and arteriography. When we consider that there is a probability but not a certainty of abnormality in the scans (group 2), full diagnostic measures, including appropriate surgical studies, have usually proven advisable. Diagnosis of the probable normals (group 3) and normals (group 4) is derived from other data, but especially in group 3 repetition of the scan may prove fruitful. Although we have classified the group 3 scans as misses when a tumor was present, the suspicious area in the scan has often corresponded to the position of the tumor. The position of the abnormal uptake shown in the pre-operative PCG has proven to correspond to the location of the intrinsic lesion—tumor or abscess—rather than to surrounding or distant edema. Hence, the 1:1 reproduction given by the scan permits the surgeon to place his cerebral incision as well as his bone flap properly:

Tumors

Our greatest accuracy by far has been in the meningiomas which have a high uptake of the As^{74} even among the slowest growing, least vascular examples. We could, moreover, be certain of the abnormality in 32 of our 33 positive diagnoses. Two of these tumors were diagnosed and localized at a time when both arteriography and pneumography had yielded technically good, normal films and the abnormal PCG and AGG were crucially important in leading to early effective surgery. The only one we are aware of having missed was a tiny 6 gm tumor arising from the tuberculum sellae.

Our next highest percentage of accuracy is in the group of glioblastomas. In at least two of our six misses in these patients there was within the tumor a sizeable quantity of cystic fluid which often takes up even less isotope than does normal brain. The use of our new equipment with greater resolution may improve our accuracy both in these patients and in the substantial number, 16 of the 31 metastatic lesions (52%), in whom the total mass of tumor was too small for detection. However, even a small nodule may be detected if near the surface of the brain, and we have one example in our series of a decisively positive scan despite a normal arteriogram and pneumogram. Many of the metastases lie in the posterior fossa, a problem site for this method because of the thick muscular covering with a moderate As^{74} content. For example, we detected most of the

supratentorial metastatic carcinomas and only one of the 5 in the posterior fossa. The corresponding figures for astrocytomas are 13 of 20 supratentorial (65%) and only 1 of the 3 infratentorial lesions of this type. We also missed the 4 medulloblastomas and the 3 hemangioblastomas in the posterior fossa.

When the tumor is so situated that a small number of cells yields symptoms, such as occur in the brain stem, third ventricle, and sellar region, our accuracy also drops sharply. Happily here and in the posterior fossa other methods such as visual fields and/or pneumography are at their best so that this method is complementary to those now in general use. In

Table I. Tumors

Category	Isotopic concentration			
	Abnormal		Normal	
	1	2	3	4
Gliomas				
Astrocytoma	7	7	2	7
Ependymoma	0	2	0	1
Glioblastoma	44	15	6	0
Medulloblastoma	0	0	2	2
Oligodendroglioma	1	0	1	1
Brain stem (usually unverified)	1	2	3	3
Meningioma	32	1	0	1
Acoustic neuroma	0	0	1	3
Hemangioblastoma, cerebellar	0	0	1	2
Craniopharyngioma	1	3	0	3
Pituitary adenoma	1	3	3	0
Epidermoid	0	0	1	0
Third ventricle (usually unverified)	1	1	0	3
Melanoma	2	0	0	0
Metastatic malignant	10	5	8	8
Lipoma of corpus callosum	0	0	0	1
Unclassified	2	1	3	0
Total	102 (49%)	40 (19%)	31 (15%)	35 (17%)
	142		66	
Grand total	207	Abnormal concentration 69%		

Table II. Non-neoplastic Disease

Vascular				
Aneurysm	0	1	1	4
Arteriovenous malformation	1	1	1	2
Cerebral vascular accident	2	7	14	10
Intracerebral clot (verified)	1	0	0	2
Subdural hematoma	2	1	1	0
Abscess	3	4	1	1
Convulsive seizure, not due to above causes	4	7	25	27
Paget's disease	4	0	0	0
Orbital tumors	1	0	0	3
Other non-neoplastic disorders	2	13	43	99
	20	34	86	148

the slow growing gliomas, the acoustic neuromas, and the "congenital rest" tumors, we do poorly no matter where they are situated because of the low differential uptake of the isotope. The major problem here is to secure some compound or ion of some isotope which will avidly enter these lesions.

In general, our best results are obtained in tumors of the cerebral hemisphere, especially those near the surface. It is in just this type, which is likely to cause a few convulsions at an early stage, that prompt diagnosis has been out of reach. We anticipate that our method may make its greatest contribution in this field both for the initial diagnosis and the apprehension of recurrences. Serial scans as often as indicated are harmless to the patient, a feature which is also valuable in the critically ill who may tolerate poorly intra-ventricular or intra-arterial injections.

Postoperatively, it is not fruitful to scan again for at least one month. Presumably, post traumatic breakdown of the blood-normal brain barrier permits entry of isotope at the operative site even when tumor has been removed. Late scans may show asymmetry with higher uptake on the normal side just opposite the earlier site of tumor. We attribute this to the greater bulk of tissue on the normal side with a higher uptake than cerebrospinal fluid. These factors all make it necessary to have for comparison a late postoperative scan in order to diagnose early a recurrence of tumor.

Abscess

An unexpected dividend of the method has been the accurate localization in 7 of 8 scans of supratentorial abscesses. Even in a well encapsulated lesion, we have found that the pus as well as the capsule may contain large amounts of As^{74} . We missed one small parietal abscess and one within the petrous portion of the temporal bone.

Vascular Lesions

Under the heading of cerebral vascular accident we have grouped the thrombotic and the few hemorrhagic lesions we have scanned. It will be noted that in 9 of the 38 patients in this group the scan was positive, but unequivocally so in only 2. In each of these the ictus was recent and later scans returned toward normal. The method is consequently proving a real help in spotting the patient with a tumor who improves markedly after an initially severe bout of symptoms. In this patient the original scan is more strikingly abnormal and it improves but little on repetition. Of the other vascular disorders we note here only that we had 3 positive scans in unilateral subdural hematomas and the only negative scan occurred when these lesions were bilateral and symmetrical.

Convulsive Seizures

If our study is carried out within 48 hours of a convulsion, especially a focal one, a positive scan

may ensue. The 11 positives out of 63 patients (with seizures not caused by one of the foregoing diseases) were found mainly in the early post-ictal phase. Hence, it is advisable to defer the study for two days after a seizure, if feasible, in order to avoid the temporary clouding of the diagnostic inferences by the transitory breakdown of blood-brain barrier in the region of epileptically discharging cortex.

Page's Disease

There is a marked uptake of As^{74} in the diseased skull in this disorder. All 4 scans showed diffuse dense marking, especially in the frontal regions.

Orbital Tumors

In the 4 lesions studied, all of which produced exophthalmos, only one, a subfrontal mucocoele, yielded a positive scan.

Other Non-Neoplastic Disorders

This comprises a heterogeneous group of nearly all other diagnostic categories in which an intracranial mass lesion might be suspected. A number of these patients had no organic disease of the central nervous system, had e.g., Meniere's syndrome, labyrinthitis, psychoneuroses, or psychosis. They comprise as normal a group as we have. As the table shows, in only about 10% of these is the scan suggestive of a focal lesion and in only 2 patients was there an unequivocal abnormality of the distribution of isotope. In one of these, the abnormal scans corresponded with bizarre clinical findings, but the patient improved and the nature of the disease was not determined.

PRELIMINARY RESULTS FOR OTHER ORGANS

Studies have been carried out on scanning other organs of the body such as liver, pancreas, kidney, and lung with various positron-emitting isotopes, including Cu^{64} and Zn^{62} . The preliminary results offer promise that successful tests will be developed for diagnosing disorders of these organs.

Figure 4 shows a typical scan of the liver of a dog obtained one-half hour after an intravenous injection of Cu^{64} . The liver concentrates nearly 80% of the dose of Cu^{64} and quite detailed scans may be obtained.

SUMMARY

We have automatically scanned the head after intravenous injection of As^{74} so as to yield simultaneously two types of data. That which we denote the *positrocephalogram*, PCG, localizes any abnormal uptake in the sagittal plane. The other the *asymmetrogram*, AGG, indicates the degree of removal from the midline toward one side or the other of such abnormality. The data evolve pictorially and preparation and interpretation require but a few minutes. The painless, harmless character of the procedure permits its use when the index of suspicion of tumor is low. Nearly all meningiomas and the great majority of tumors and

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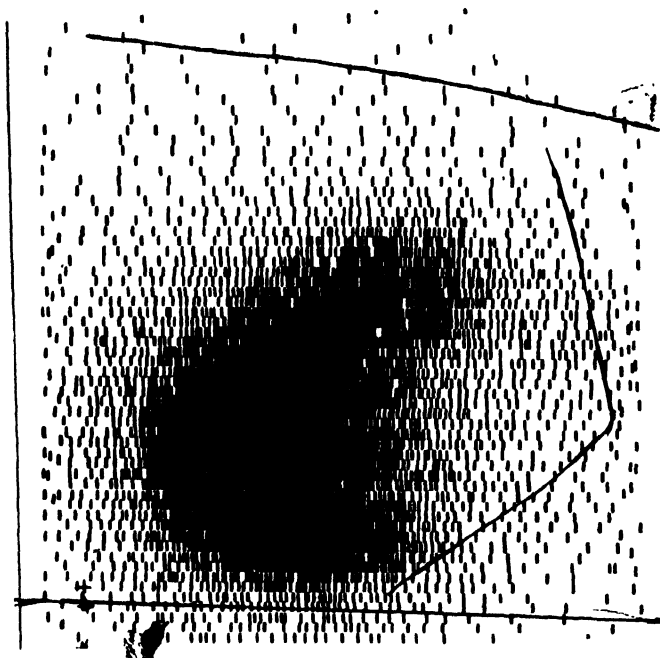


Figure 4. Scan of liver of dog following intravenous injection of Cu^{64} . The scan is a lateral view of the dog

abscesses of the cerebral hemisphere are delineated so precisely that the surgeon may often proceed without arteriogram or pneumogram. With the exception of fortuitous arteriographic demonstration of neoplastic vessels no other method directly portrays the lesion itself. For other neoplastic types and loci, our method is much less accurate. It does, however, aid in differentiating tumor, abscess or subdural hematoma from cerebral vascular thrombosis or hemorrhage.

Development of the method for scanning other organs in the torso with As^{74} , Cu^{64} , and Zn^{62} is in progress.

The Dynamic Aspects of Erythropoiesis in Normal and Pathologic States as Indicated with the Radioactive Isotope Tracer Technique

By J. F. Ross* and A. Miller,† USA

The unique contribution of the radioisotope tracer technique of medical investigation has been the clarification of the *dynamic* aspects of metabolic and physiologic processes in the intact, undisturbed organism. This method has made possible, in normal and in diseased individuals, the determination of the actual *rates* at which vital functions are proceeding; the localization of the *sites* in the body where they occur; and the quantitative measurement of the *amounts* and *distribution* of substances in the body. Such studies have led to complete revision of the concepts of the pathogenesis of certain human diseases; have indicated promising new avenues of investigation; and have resulted in improved methods of therapy.

The radioisotope tracer method has been particularly fruitful in studies of the mechanisms of erythropoiesis and iron metabolism, and of the influence of disease processes upon these mechanisms. The simultaneous application of radioiron and radiochromium tracer techniques to hematologic problems provides a comprehensive picture of the dynamic aspects of the entire "red cell cycle": erythropoiesis, duration of erythrocyte survival, and erythrocyte destruction. Furthermore, it makes possible localization in the intact organism of the actual anatomic site where these processes occur, and enables studies of the metabolic turnover of iron containing tissue enzymes. Such studies have led to a better understanding of the physiology of iron metabolism, and of the erythron, and have provided invaluable information concerning the pathogenesis of various types of anemia. We report herewith studies of the mechanism of the "secondary anemia" of neoplastic disease.

THE PATHOGENESIS OF THE "SECONDARY ANEMIA" OF NEOPLASTIC DISEASE

Traditionally, this type of anemia has been assumed to be due to a depression of erythropoiesis by a

"crowding out" of the bone marrow by tumor, or to an "inhibition" of marrow function by a hypothetical "toxin."

We have studied the pathogenesis of this type of anemia in 25 patients with leukemia, or malignant lymphoma, and in 38 patients with cancer, selecting cases in which blood loss, overt hemolytic anemia, and nutritional factors could be excluded as contributing to the anemia. Our studies were directed toward determining: (1) the rate of formation of erythrocytes; (2) the duration of survival of erythrocytes; (3) the rate of destruction of erythrocytes; and (4) the metabolic pathways by which iron is distributed and utilized in the body.

Correlation of these factors has presented a clear picture of the mechanisms producing anemia in these patients.

PROCEDURE

Hematological studies were carried out according to standard methods. Quantitation of fecal urobilinogen excretion was made on 4 day collections of stools according to the method by Schwartz¹ and the hemolytic index was calculated² employing erythrocyte volumes experimentally determined with P³² tagged erythrocytes, or calculated from Evans Blue-dye Plasma volume measurements and venous hematocrit values corrected by the factor 0.92.³

Erythrocyte survival was determined by employing: the differential agglutination technique of Ashby as modified by Young⁴ and by Emerson⁵ NoQ values were calculated by the method of DeGowin.⁶ Red blood cell survival of the subject's own cells within his circulation, as well as the survival of his cells in the circulation of a normal individual, and the survival of normal cells in the subject's circulation was determined employing the radioactive chromium method.⁷

Plasma iron concentration was determined by the method of Kitzes.⁸ Tissue iron concentration was determined by histological study of Turnbull blue stained autopsy and biopsy specimens of liver, spleen, and bone marrow.

Radioiron studies were carried out employing radioactive Fe⁵⁹ ammonium citrate with a specific

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† Robert Dawson Evans Memorial, Massachusetts Memorial Hospital.

activity of 2 to 5 microcuries per microgram. This was prepared for intravenous injection by sterile incubation with normal human plasma to effect transferrin binding. A 10 ml aliquot of this plasma containing 2 to 8 micrograms of iron with 7 to 11 microcuries of activity was injected intravenously.

(a) "Plasma iron disappearance half-time" was determined by quantitation of radioactive iron in samples of blood drawn serially at intervals during a period of 240 minutes following the intravenous injection of the tracer dose, and, by then, relating the logarithm of the concentration of Fe^{59} in these samples with time. The rate of disappearance of Fe^{59} was exponential between 20 and 180 minutes, and the time required for the concentration of radioiron to fall to 50% of the initial concentration of radioactivity was designated as the "plasma iron disappearance half-time" or "PDT $_{1/2}$ ". It is recognized that this value may not be an absolute index of the rate of plasma iron turnover, but it is believed that it has biological significance, particularly when the PDT $_{1/2}$ in patients with diseases differs too markedly from that of normal subjects.

(b) Plasma iron turnover defined as the total amount of iron in milligrams leaving the plasma iron pool per hour was calculated employing the formula of Huff.⁹

$$\text{Plasma iron turnover} = \frac{0.693 \times \text{serum iron concentration } (\mu\text{g Fe/ml}) \times \text{plasma volume (ml)}}{\text{PDT}_{1/2} \text{ (in hours)} \times 1000}$$

This value also was expressed as mg iron/hour/liter of blood cells in the subjects circulation.

(c) Incorporation of radioiron into erythrocytes was determined by quantitating the radioactive iron in samples of blood drawn at intervals of 1 to 4 days during a period of 18 to 20 days following the intravenous injection of the tracer dose. The fraction of the injected Fe^{59} present per unit mass of erythrocytes multiplied by the total erythrocyte volume indicated the percentage of the administered Fe^{59} utilized for hemoglobin formation. Customarily this reached a maximum within 15 to 18 days, and this maximum was termed "Per cent red cell utilization of Fe^{59} ."

(d) The rate of red cell production was then calculated from the formula: mg iron utilized to form erythrocytes = mg plasma iron turnover $\times$ % red cell utilization of Fe^{59} . This has been termed "red cell iron turnover" and has been expressed as mg iron/hour/liter red cells in the subject's circulation.

(e) When this value was divided by the total amount of iron present in the subject's circulating red cell mass, an indication was given of the percentage of total red cell mass produced per day. This we have called the "red cell renewal rate." This is equal to the rate of erythrocyte destruction in subjects with a stable erythrocyte level in the peripheral blood.

The distribution and movement of radioiron in the body was measured at intervals following the injection of the tracer dose of Fe^{59} with a shielded one-inch diameter by one-inch deep sodium-iodide-thallium activated scintillation crystal counter applied directly to the skin overlying various organs. Serially determined radioactivities indicated varying concentrations of radioiron in the different organs and followed certain characteristic patterns of distribution. Such measurements and distribution patterns do not indicate quantitatively the distribution of iron in any given organ, since the influence of variable geometry of counting and of back-scattered radiation prevents any absolute quantitative relation of the radioactivity with that of the injected dose of Fe^{59} .

Radioactivity Detection Methods

Beta counting of P^{32} labeled erythrocytes employed in blood volume measurements was carried out on dried samples of erythrocytes of standard mass compared with aliquots of a standard of identical mass, employing a thin mica-window Geiger-Müller counter. The samples counted 20 to 30 times background and multiple series of 4096 counts were made on each sample. The counting error was less than 1%. Gamma counting was employed for quantitation of Fe^{59} and Cr^{51} , all measurements being made on liquid samples of 4-ml volume in standard tubes of 125-mm diameter. In samples containing red blood cells uniform distribution of radioactivity within the sample was achieved by producing hemolysis with saponin. All samples were compared with standard aliquots of the injected radioactive iron or radioactive chromium, standards being counted interchangeably with the experimental samples. Radioactivity was measured with a well-type sodium-iodide-thallium activated scintillation crystal counter, with a well 1½-inches deep surrounded by ½ inch of crystal. A photo-multiplier tube, linear amplifier, and scaling circuit were employed to register radioactivity. The counting efficiency of this equipment was 18% for Fe^{59} and Cr^{51} . Samples usually counted 5 to 15 times background and sufficient total counts were taken to give a statistical counting error of 1.5%. Plasma samples taken 3 to 4 hours after injection of Fe^{59} had such low activity that statistical counting error was 5% to 10% even though samples were counted for periods as long as two to six hours.

External monitoring of radioactivity over various body sites was carried out with a shielded one-inch diameter, one-inch deep sodium-iodide-thallium activated scintillation crystal counter applied directly to the skin. Each site was counted for several one minute periods while the background was counted for 10 minutes. The background ranged from 600–1000 counts per minute, and body counts ranged from 6000 to 18,000–counts per minute. Radioactivity measurements were made at 30 to 60 minute intervals during the day of the injection, and at 1 to 3 day intervals thereafter. The gross counting

rate over each organ was corrected by subtracting the counting rate determined on the same day over the thigh muscles to give the net counting rate for each site. An appropriate Fe^{59} or Cr^{51} standard was counted alternately with measurements of body sites to make possible inter-comparison of radioactivity from day to day.

OBSERVATIONS

Hematologic Status

All 25 patients with leukemia and lymphoma, and 28 of the 38 patients with cancer exhibited a moderate to severe normochromic, normocytic anemia. Four of the ten patients without anemia had quiescent disease, and despite extensive bone metastases one of the 10 had normal hemoglobin values. The reticulocyte count was 3–5% in 4 patients. The Coombs test was negative and the serum bilirubin within normal limits in all patients studied.

The iron content of the liver, spleen, and bone marrow was normal or increased in autopsy or biopsy specimens in 19 of 20 cases in which these studies were performed, confirming the belief that iron deficiency did not contribute to the etiology of the anemia or to the pattern of iron metabolism in these subjects.

Radioiron Metabolism and Rates of Erythropoiesis

Plasma Iron Disappearance Half-Time ("PDT_{1/2}")

The PDT_{1/2} of normal subjects showed a range of 1.18 to 2.18 hours, while in 37 studies in 33 patients with cancer, the range was 0.35 to 2.25 hours with a median of 1.08 hours (Fig. 1). In 27 to 37 studies, the PDT_{1/2} was more rapid than normal. Decrease in the PDT_{1/2} correlated well with a decrease in the serum iron level, the coefficient of correlation being 0.690. However, a rapid PDT_{1/2} did not indicate an increased rate of erythropoiesis, since in 17 studies with rapid PDT_{1/2}'s ranging from 0.35–1.10 hours the rate of red cell production either was normal or actually decreased as measured by red cell iron turnover.

The relationship between PDT_{1/2} and serum iron concentration was further emphasized when radio-

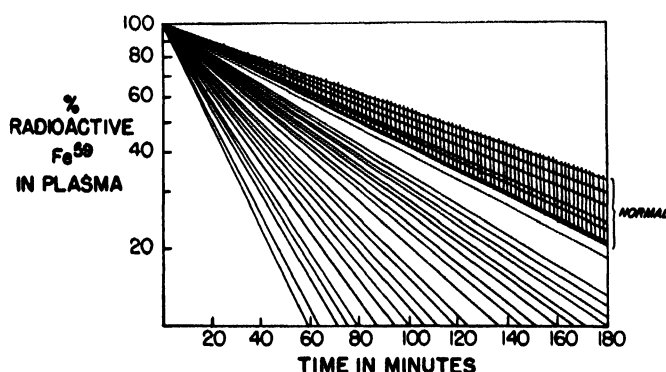


Figure 1. The rate of disappearance of tracer amounts of radioactive iron from the plasma of patients suffering from cancer. The rate of disappearance was more rapid than normal in 3/4 of the cases studied

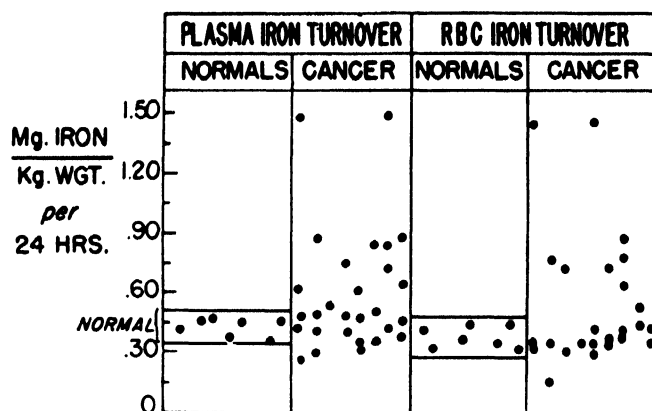


Figure 2. The rate of plasma iron turnover and the rate of red blood cell iron turnover in patients with neoplastic disease. These rates were normal or increased in the majority of subjects

iron studies were repeated on the same subjects during the progression of neoplastic disease. As the disease grew worse, the plasma iron level decreased and the PDT_{1/2} was markedly accelerated.

In only 9 of the 37 studies was the PDT_{1/2} within normal limits. Four of these patients had localized or quiescent cancer, and only three of the nine cases had anemia. One patient with an elevated serum iron and decreased red cell production had a slower than normal PDT_{1/2}.

The Plasma Iron Turnover

This indicates the amount of iron leaving the plasma per unit of time ranged from 0.465 to 4.520 mg/hour/liter circulating erythrocytes as compared to the normal range of 0.569 to 1.149 mg iron/hour/liter circulating erythrocyte (Fig. 2). The median of 37 studies on cancer patients was 1.025 mg/hour/liter circulating erythrocytes, and in 21 studies the plasma iron turnover was within the normal range despite the fact that in 17 of these the PDT_{1/2} was accelerated. In 11 patients, the plasma iron turnover rate was increased over normal to as much as 4 times normal. In only 2 of 37 studies was the rate decreased.

The Red Cell Utilization of Injected Fe^{59}

The incorporation of the injected Fe^{59} into newly formed erythrocytes was normal (80% to 100% in

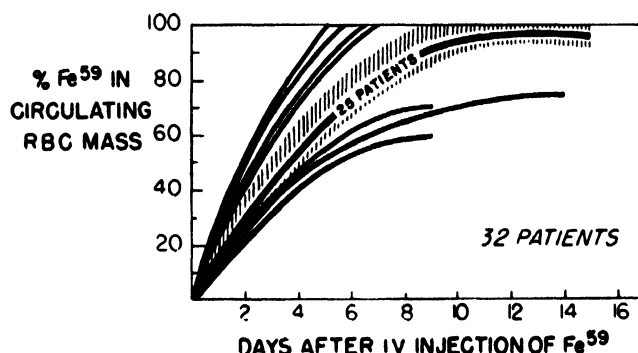


Figure 3. Incorporation of radioactive iron into red blood cells in cancer patients. The rate and percentage of incorporation was normal or increased in the great majority of patients

10 to 21 days) in 32 of 37 studies. In only 5 of 37 studies was the red cell utilization of Fe^{59} decreased (Fig. 3).

The Red Cell Iron Turnover

This indicates the amount of iron entering into newly formed red blood cells per unit time. It ranged from 0.114 to 4.520 mg iron/hour/liter red cells with a median of 0.908 mg iron/hour/liter red cells as compared with the normal range of 0.569–1.033 mg iron/hour/liter red cells (Fig. 2). The red cell iron turnover was normal in 21 of 37 studies, in spite of the presence of anemia in 14 of these 21 patients. It was increased in 11 cases to as much as 4 fold normal, and was increased in several patients, who were only mildly anemic, indicating that in these subjects the rate of blood destruction was increased, but that there was a compensatory increased erythropoiesis. Decreased red cell iron turnover occurred in 4 patients, of whom 3 showed metastases to bone. In one patient with complete marrow replacement by lymphosarcoma, the turnover was very low, 0.114 mg/hour/liter.

The Red Cell Renewal Rate

This ranged from 0.5% to 10.8% as compared with a normal range of 1.1% to 1.8% in our control subjects. In 20 studies performed on 18 patients, the red cell renewal rate was increased over normal, being 4 to 8 times normal in several anemic subjects. In two subjects with minimal degrees of anemia, the red cell renewal rate was twice normal. In all 20 studies with increased red cell renewal rates, metastases were present, whereas metastases were present in 9 of 16 studies in which renewal rates were normal. Anemia was present in 18 of 20 patients with increased red cell renewal rates, whereas it was present in only 7 of 16 studies with normal red cell renewal rates.

Patterns of Distribution of Iron in Body Organs

External monitoring made possible graphic representation of changes in distribution of radioactivity over various organ sites with the passage of time. This pattern of distribution was normal in five subjects with cancer: as Fe^{59} left the plasma, there was a rapid accumulation of Fe^{59} in the sacral region (an anatomic site representative of active erythropoietic bone marrow), a maximum radioactivity being reached in 1 to 2 days, by which time plasma radioactivity had fallen to a negligible level. Then as the Fe^{59} began to be released in newly formed red blood cells, the concentration of Fe^{59} in the sacrum decreased, reaching a stationary low level between the sixth and the tenth day. At this time, the concentration of Fe^{59} in the erythrocytes had reached its maximum concentration. In five cancer patients, as in normal subjects, the concentration of Fe^{59} in the liver, spleen, and other tissues showed no significant changes when compared with a "neutral area" such as the thigh.

A patient with widespread bone marrow (including the sacrum) metastases from cancer of the breast, had an abnormal distribution of Fe^{59} . After injection the Fe^{59} rapidly disappeared from the plasma, but the rise in radioactivity over the sacrum seen in normals did not occur. The liver revealed a slight increase in the concentration of Fe^{59} at 2 hours, after the injection of Fe^{59} , this was maintained for the next 24 hours and the concentration of Fe^{59} then returned to its initial level. Other tissue sites revealed no abnormalities in localization. Despite the failure of sacrum to concentrate and discharge the Fe^{59} , 100% red cell utilization of the injected Fe^{59} occurred suggesting that other marrow sites less involved by the cancer were functioning erythropoietically.

Another abnormal pattern of Fe^{59} distribution was observed in a second patient with metastases from cancer of the breast. In this patient the onset of anemia had occurred a few months before the study, and was concomitant with the appearance of pulmonary metastases. Spleno- and hepatomegaly were never present. Evidence of increased red cell destruction was secured from radioiron and fecal urobilinogen studies. After the injection of Fe^{59} , the sacrum failed to concentrate or discharge Fe^{59} as would have occurred in a normal individual. From 2 hours after the injection, the spleen site had the highest concentration of Fe^{59} which it maintained for the succeeding 9 days despite the 60% red cell utilization of the injected Fe^{59} by the seventh day. The concentration over the liver site was relatively stable during the first 6 days, but on the 6th day the concentration of Fe^{59} increased and remained elevated. At 23 days after Fe^{59} injection, the spleen site concentration had greatly decreased approximating that over other tissues; whereas the liver now had the highest concentration of Fe^{59} . The patient died a few days later, but autopsy was not secured.

Another abnormal pattern of Fe^{59} distribution was found in a patient with lymphosarcoma who showed, at autopsy, diffuse lymphosarcomatous replacement of the bone marrow. Hepatosplenomegaly, anemia and a marked decrease in red cell production were present at the time of study. After injection, the Fe^{59} disappeared slowly from the plasma with the $\text{PDT}_{1/2}$ 2.25 hours. Concentration of Fe^{59} in the sacrum site did not occur, whereas the liver site showed a progressive rise in concentration of Fe^{59} beginning at 2 hours and reaching a maximum at 36 hours. The high liver site concentration of Fe^{59} was maintained for 4 days after the injection. At 6 days, only 5% red cell utilization of the injected Fe^{59} had occurred.

In two patients, one with a fibromyxosarcoma in the right scapular region, and the other with cancer of the left breast, external monitoring directly over the tumor sites revealed no appreciable concentration of the injected Fe^{59} in the tumor areas. In a patient

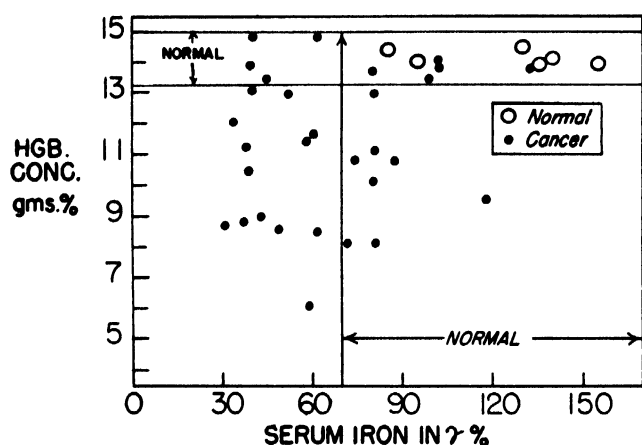


Figure 4. Serum iron concentration was decreased in many patients with cancer, but there was no apparent correlation between degree of depression of serum iron and hemoglobin level

with bronchogenic carcinoma and a patient with adenocarcinoma of the bronchus, Fe^{59} was injected intravenously 24 hours before surgery. From the resected tumor 4 gm aliquots representative grossly of the entire tumor specimen were placed in test tubes and assayed for radioactivity in a well counter. Blood concentration of Fe^{59} was negligible at this time. No appreciable concentration of Fe^{59} was found in either of these tumors.

Iron Metabolism

Serum Iron Concentration

The serum iron concentration ranged from 30.0 to 165.0 micrograms/100 cm^3 with a median of 65.0 micrograms/100 cm^3 in 33 patients as compared with normal range of 80 to 155 micrograms/100 cm^3 for males and 70 to 150 micrograms/100 cm^3 for females (Fig. 4). On an individual study, the incidence and the magnitude of the serum iron depression was poorly correlated with the presence of widespread metastases. Thus 11 of 17 patients with normal serum iron levels had widespread disease as compared with 12 of 17 patients with widespread disease who had depressed serum iron values. However, when repeat studies were performed on the same patient during the dissemination of the disease process, the serum iron concentration tended to fall progressively. This phenomenon is particularly well shown in a patient with metastatic carcinoma of the breast, who, when first studied, was asymptomatic and had a normal serum iron. Seventy-three days later when widespread dissemination of the disease process occurred, the patient's serum iron had decreased to 34.6 micrograms/100 cm^3 . In a patient with disseminated cancer of the breast followed during the gradual progression of her disease process over a 1½-year period, serum iron levels declined gradually. Five other patients also demonstrated similar lowering of serum iron levels with progression of their disease. Four of above patients had been on oral cortisone at the time of repeat serum iron studies. Two non-cancer patients on long continued cortisone treatment did not show a lowering of serum iron levels. It appears

unlikely that cortisone therapy was responsible for the depression of the serum iron in the cancer patients. With improvement in the clinical state of the patient, serum iron levels tended to rise towards normal. Thus in a patient with a bronchogenic carcinoma without metastases, the serum iron was 35.0 micrograms/100 cm^3 at the time of the first study. Complete surgical removal of the carcinoma was achieved, and 8 months later when the patient was asymptomatic, the serum iron had risen to a low normal level of 89.7 micrograms/100 cm^3 .

The simultaneous presence of anemia and depressed serum iron levels occurred in 13 of 33 patients (Fig. 4). Anemia with normal serum iron concentration was found in 10 patients while depressed serum iron levels without anemia occurred in 4 other patients. Normal serum iron values without anemia were found in 6 patients.

Determination of Storage Iron

A semi-quantitative assessment of body storage iron was accomplished in 10 patients by evaluation of Turnbull's blue-stained sections of liver, spleen and bone marrow of patients dying of their disease. The iron demonstrated by this technique is primarily hemosiderin. The amount of histological stainable hemosiderin in the reticulo-endothelial system of these organs is roughly representative of the amount of storage iron present. In 8 of 10 patients normal amounts of storage iron were present in these organs, and in 1 of the 10, a moderately increased amount was present. In only one patient was there a definite decrease in the amount of storage iron, although even in this case, hemosiderin still was demonstrable. In 6 of these 10 patients, decreased serum iron levels were present despite the presence of normal or even increased amounts of storage iron. The one patient with the decreased amounts of storage iron had a low normal serum iron level.

Fecal Urobilinogen Studies

The hemolytic index was elevated in 13 of 18 cases of leukemia or malignant lymphoma, and in 6 of 14 studies in cancer patients (Fig. 5). In five of these six patients, increased rates of red cell destruction also was evidenced from red cell survival

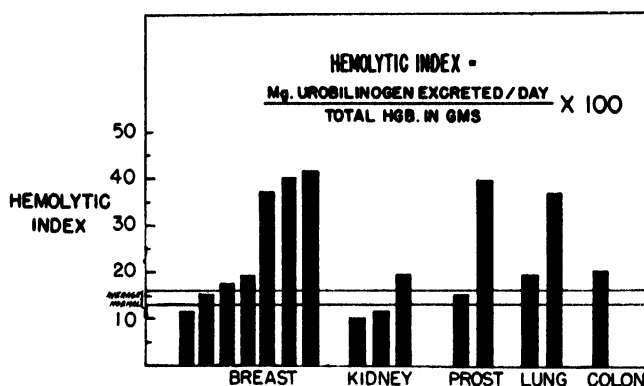


Figure 5. The hemolytic index in patients with cancer. Significant elevation is seen in five of these cases

and Fe^{59} studies. Four of 8 patients with normal hemolytic indices had evidence for increased rates of red cell destruction from radioiron and red cell survival studies.

Red Cell Survival Studies

The survival of normal transfused red cells was decreased in 12 leukemia-lymphoma patients and in 6 cancer patients with the survival of the transfused cells ranging from 25 to 85 days as compared with the normal 120 days (Fig. 6). Five of the 6 patients died before completion of the study; however, in all 5 the evidence of shortened survival was evident at the time of death. Anemia and metastases were present in all 6 of the patients with the shortened red cell survivals.

A constant state of random red cell destruction was present in 5 of 6 patients. Thus, the NoQ values of these patients form a line sloping to the right, and are representative of other patients in this group.

Normal survival of transfused red cells was found in 4 patients. Anemia and metastases were present in all 4 with bone metastases present in 3 out of the 4. Despite the presence of metastases, 3 of the 4 patients were alive 2 to 24 months after the completion of the transfusion study. One patient with severe anemia and widespread disseminated cancer had a normal red cell survival during the 21 day period before expiring of his disease.

The survival of normal transfused and the patient's own red cells was studied by following the survival of normal transfused red cells by the differential agglutination technique and patient's own cells by the Cr^{51} technique. In 2 patients, the survival of the patient's own and normal donor cells were identical and normal.

Observations During the Progression of Cancer

(a) One patient had had a radical mastectomy for cancer of the breast three years previously. Since operation, her disease had been relatively quiescent, with a solitary metastasis to the left orbit, first noted 8 months previous to the first Fe^{59} study. This was the only evidence of dissemination of the cancer. At the time of the first Fe^{59} study, the patient was asymptomatic, with serum iron, hemoglobin levels, $\text{PDT}_{1/2}$, red cell production and destruction all normal. Seventy-three days later, at the time of repeat studies, obvious dissemination of the cancer to lungs, liver, and femur had occurred. Repeat studies revealed the development of anemia, a fall in serum iron, and a rapid $\text{PDT}_{1/2}$. Red cell iron turnover was now slightly increased, but red cell renewal rate had not changed. A red cell survival study demonstrated increased red cell destruction with the lifespan of normal transfused red cells about 75–80 days. The patient died 48 days after the transfusion study had started.

(b) A second patient, age 72, had had a radical mastectomy for a cancer of the left breast in 1948. At the time of the first study, widespread bone

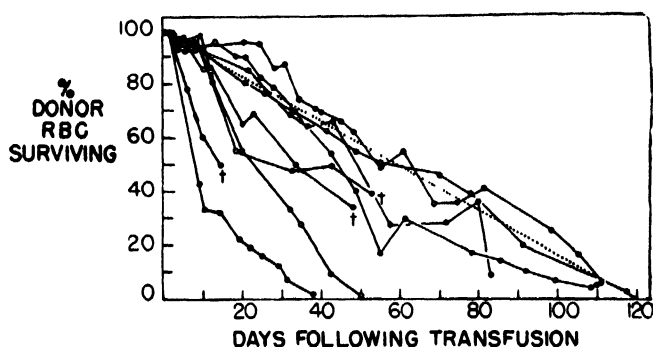


Figure 6. Survival of normal donor erythrocytes in the circulation of patients with cancer as indicated by the Ashby differential agglutination technique. A shortening of survival time is present in the majority but not all of these patients

metastases were present. An anemia, slightly depressed serum iron levels, a moderately rapid $\text{PDT}_{1/2}$, normal red cell production and a questionable slight increase in red cell destruction were observed. Four months later, the patient was restudied when her disease had not progressed. The only changes were a slight further decline in hemoglobin and serum iron levels and a slightly more rapid $\text{PDT}_{1/2}$. A survival study demonstrated normal life-span of transfused red cells. Eleven months later when the patient was restudied, new skin and bone metastases were present. The serum iron showed further depression concomitant with a further shortening of the $\text{PDT}_{1/2}$. Despite the further dissemination of the disease, repeat red cell survival study of transfused as well as patient's own cells was normal.

DISCUSSION

Association of Anemia and Cancer

All of our patients with leukemia or malignant lymphoma and 75% of those with cancer were anemic—both as indicated by reduction in blood hemoglobin concentration, and by decrease in circulating red blood cell volume. Blood loss, chronic infection and iron deficiency were excluded as possible causes of anemia in our patients, and we believe that their anemia truly may be called “the anemia of cancer.” Since our patients were carefully selected and were not consecutive hospital admissions, our studies do not indicate the actual incidence of this type of anemia.

In general, anemia was present when the neoplastic process was widespread, and the patient seriously ill, but tended to be absent or mild when the disease was localized or quiescent. As neoplastic disease progressed in severity, anemia became more marked. Morphologic studies of the bone marrow confirmed the reports of other investigators that even in patients with severe anemia erythropoietic activity was normal or actually increased.

The Rate of Formation of Erythrocytes

Our radioiron tracer studies have demonstrated that the majority of subjects with neoplastic disease are utilizing a normal or increased amount of iron

for hemoglobin and erythrocyte formation. Far from a "marrow failure" being present in these patients, there actually is a normal or increased rate of erythropoiesis. This is not invariably the case, since in 4 leukemia and lymphoma cases, and 3 cancer patients myelofibrosis or complete tumorous replacement of the bone marrow existed, and in these patients the rates of erythropoiesis were markedly decreased.

Tracer studies have shown a fairly characteristic change in the dynamic aspects of iron metabolism in the majority of patients with neoplastic disease—the plasma iron concentration tends to decrease with progression of the disease, and with this decrease, there is an acceleration in the "rate of disappearance" of a tracer dose of radioactive iron from the plasma. This acceleration in plasma iron disappearance appears to be compensatory for the decrease in plasma iron concentration since in most patients the total amount of iron passing through the plasma pool per unit time is maintained at a normal or even at an increased amount.

Which is primary and which secondary—the decrease in plasma iron concentration or the acceleration in rate of plasma iron disappearance is problematic. It may well be that these two factors are intimately interrelated and that a feed-back mechanism is operating to maintain an optimum total plasma iron turnover. It is of importance that storage iron in these subjects is not diminished, and that iron deficiency apparently is not responsible for reduction of plasma iron concentration, or for the acceleration of plasma iron disappearance.

What factors may be responsible for these changes in the kinetics of iron metabolism is of considerable importance, since it probably is intimately related to the fundamental effect of the disease process on the host organism. From the data obtained in these investigations, it appears that the decrease in the survival of the erythrocytes may be one of the major factors responsible for these striking changes in iron metabolism. It must be emphasized that this may be only one of several contributing factors. Other studies suggest that neoplastic disease accelerates the turnover rate of iron containing tissue enzymes (e.g., cytochrome-C, catalase) which would contribute to increase in total iron turnover. It also is quite possible that increased reticulo-endothelial activity, or unknown factors may contribute. Investigation of these possibilities may be expected to be very fruitful.

In general, there was a positive correlation between the shortening of erythrocyte survival, (and increased rate of erythrocyte destruction) and the decreased plasma iron concentration—accelerated plasma iron disappearance suggesting that the need for iron for new hemoglobin formation was of considerable importance in modifying iron metabolism. This was reflected by a positive correlation between plasma iron turnover and the rate of erythropoiesis. That other factors also were operating was clearly

demonstrated by the cases described in which no increased rate of hemolysis could be demonstrated, but $PDT_{1/2}$ was accelerated and plasma iron turnover was increased. In such cases, tissue iron turnover or other factors probably are of major importance in modifying iron metabolism.

The above-discussed changes in serum iron concentration, plasma iron disappearance, and plasma iron turnover did not occur in all patients with neoplasia—even in those in the advanced stages of the disease. Why this should be so is most intriguing. In most cases there were no clinical features to distinguish them from patients with the more usual changes in iron metabolism. In a few such cases, the explanation of this aberrance was evident—complete destruction of erythropoietic tissue removed the major source of demand for iron and thus serum iron concentration remained normal or high, plasma iron disappearance was slow and plasma iron turnover was decreased.

Incorporation of Iron into Erythrocytes

The capacity of these patients to manufacture new hemoglobin and erythrocytes was quantitatively within the normal range or increased in the majority of patients. In addition, this process appeared to proceed in an unimpaired normal fashion, since the quantity of the tracer dose incorporated into red cells was perfectly normal, and the rate at which these red cells were released into the circulation was accelerated in many cases.

Erythrocyte Survival

Probably the fundamental abnormality leading to the development of anemia in patients with neoplastic disease is a marked shortening of the lifespan of the erythrocytes. In many patients the mean lifespan of the red cells was considerably less than half normal. In general, this decrease in erythrocyte longevity varied directly with the severity of the neoplastic process; duration of red cell survival progressively decreased with progression of the disease, and was prolonged when response to therapy was favorable. However, there were striking exceptions to this generality. In several patients, a normal erythrocyte survival was maintained in the face of fatal, widely disseminated cancer. There were no clinically distinguishing features in these cases, but their existence is most significant and indicates the necessity for further study in an attempt to ascertain what factors made possible normal erythrocyte survival and caused them to differ from the majority of patients with neoplasia.

Why red cell survival should be shortened in patients with neoplastic disease is unanswered. Certain important facts relating to the problem are known, however. By employing radiochromium tagged cells and the Ashby differential agglutination technique, it has been possible to show that both normal red cells transfused into a patient with cancer, as well as

the patient's own red cells have a similar decreased longevity in the circulation of the patient. This indicates that the shortened survival is due to an extra-corporeal influence which effects normal, as well as patient's cells. By retransfusion into normal subjects of normal cells that initially had been transfused into and had remained in the circulation of cancer patients for variable periods of time, it was shown that exposure of these cells to the influence of the "neoplastic milieu" for periods as brief as 7 days resulted in irreparable changes in the erythrocytes and consequent shortening of survival.

What effect the "neoplastic milieu" may have on the erythrocyte remains to be determined. The Coombs test consistently has been negative in these cases, and all attempts to demonstrate agglutinins, hemolysins, or fragility changes have been unsuccessful. It is possible that substances present in the plasma of patients with neoplastic disease may adversely effect the metabolism of the erythrocyte in a fashion analogous to the recently demonstrated metabolic abnormalities which exist in the erythrocytes of certain congenital and acquired types of hemolytic anemia. Such metabolic abnormalities could account for the apparent acceleration of the over-all rate of senescence, as well as the increased rate of random erythrocyte destruction seen in these patients.

Erythrocyte Destruction

The hemolytic index was considerably increased in most of the cases with leukemia or lymphoma, but in less than half of the cancer cases. In several of the latter patients, there was increased blood destruction as evidenced by cell survival and Fe^{59} studies, but no increase in urobilinogen excretion or of the hemolytic index was observed. It is believed that technical errors do not account for the lack of correlation between the hemolytic index and obvious increased rates of hemolysis. The possibility cannot be excluded that degradation of hemoglobin in these patients may not be reflected by quantitative excretion of urobilinogen.

Failure of Complete Erythropoietic Compensation

Although our studies have clearly indicated that erythropoiesis proceeds at a normal or increased rate in most patients with neoplastic disease, the fact that such patients become anemic plainly shows that erythropoiesis has not accelerated to an extent sufficient to compensate completely for the increased rate of erythrocyte destruction. The shortened erythrocyte lifespan increases the demand for red blood cells by a factor which ranges from two to six fold normal. The normal human organism is quite capable of meeting an increased demand for erythropoiesis of this magnitude, and persons with various types of hemolytic processes may considerably exceed this rate of erythropoiesis.

It thus appears that the erythropoietic response of patients with neoplasia is not increased in pro-

portion to demand, nor to an extent that would be expected in a normal individual. Why this should be so is yet to be determined.

Metabolic Pathways of Iron

The normal body pathway of iron—plasma to bone marrow to erythrocyte—was observed in the majority of cancer patients studied. However, external monitoring demonstrated marked abnormalities in a few patients. Thus, in several cases with massive bone marrow metastases no uptake of Fe^{59} occurred in the sacral bone marrow. An extra-medullary erythropoietic site in the spleen was readily demonstrated in one patient. In a case in which essentially no erythropoiesis was proceeding the iron was deposited in the liver and remained there.

CONCLUSION

Application of radioisotope tracer methods in conjunction with classical hemotologic techniques have shown that many cases of neoplastic disease develop anemia as a consequence of a shortening of the lifespan of the erythrocyte—probably due to extra-corporeal factors existing in patients with neoplastic disease. Erythropoiesis in the majority of these patients proceeds at a normal or increased rate, but does not increase to a sufficient degree to compensate completely for the increased rate of erythrocyte destruction. Why erythropoiesis does not increase to a degree sufficient to be completely compensatory is not known.

Certain patients do not conform to the general pattern outlined above—red cell survival is normal in some and the rate of erythropoiesis may not be increased in others. Why these patients should be different from the usual case with neoplastic disease is not clear.

Marked changes in the dynamic aspects of iron-metabolism occur in patients with neoplastic disease. These changes probably reflect the influence of neoplasia on the host organism.

SUMMARY

1. The dynamic factors leading to the development of anemia in patients with neoplastic disease were studied with radioisotope tracer techniques.

2. Decrease in erythrocyte lifespan, and increase in rate of erythrocyte destruction, was observed in the majority, but not all of such patients.

3. Decreased erythrocyte lifespan was attributable to extra-corporeal factors.

4. Rates of erythropoiesis were normal or increased in the majority of patients with neoplastic disease.

5. This increased rate of erythropoiesis was not adequate to compensate completely for the increased rate of erythrocyte destruction, and, thus, anemia developed. Why erythropoietic response was inadequate is not known.

6. Marked changes in the dynamic aspects of iron metabolism occur in patients with neoplastic disease.

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The Use of P^{32} in the Identification of Intraocular Tumors

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The fact that certain radioactive materials and particularly radioactive phosphorus are taken up selectively in rapidly growing neoplastic tissue has been well known for some time. The practical significance of this has not been great since the identification of malignant tissue can be most positively effected by histologic examination. It was, however, realized that the increased uptake of P^{32} in malignant tissue could be used to advantage in certain specific conditions. The first of these, and the one which is being considered in the present paper is the identification of malignant tumor of the eye.^{2,3,6,7} Histologic examination is only possible after enucleation of the eye and thus the possibility of using a radioactive material which will allow identification of malignant tissue *in vivo* is extremely promising. The major problem in the identification of intraocular tumors is that of differentiation between detachments of the retina caused by underlying fluid or benign inflammatory condition and those which are caused by malignant growth. It will be shown that in the majority of cases encountered, these three types of conditions; fluid detachments, detachments due to inflammatory conditions, and detachments due to malignant growth of tissue are differentiable with the aid of the present test. It should be stressed, however, that this method, carried out as a single procedure, does not provide a positive means of identifying any of these three conditions. Actually the test is a valuable tool and when used along with usual diagnostic procedures, allows for more accurate diagnosis of the above mentioned conditions.

Because of the fact that intraocular tumors can be accurately located by ophthalmoscopic examination and because these tumors lie very close to the outside surface of the eye, it has been possible and particularly advantageous to use a beta emitter for their identification. The advantage of using beta rays is that the measurement obtained by counting directly over the suspected area is essentially unaffected by the radioactive material in the surrounding normal tissue. This eliminates the masking effect of background due to normal tissue which complicates identification and localization measurements in which gamma emitters are used. The beta emitter chosen for the present study is P^{32} . The most important reason for the choice of P^{32} is that previous studies by

other investigators^{1,4,5} have shown that P^{32} is selectively taken up by practically all types of malignant tissue in concentrations of from two to 500 times that found in normal tissue. Other reasons for the use of radioactive phosphorus are: (1) easy availability, (2) long enough half-life to allow ease of use, and (3) beta particle energy such that particles can penetrate about 8 mm of tissue (3 mm is the average range and probably constitutes the maximum thickness of tissue through which increased concentrations can be *easily* identified.)

COUNTING APPARATUS

The counting apparatus used consists of three types of special Geiger tubes. The first is a small end-window tube with the end window mounted at right angles to the axis of the tube. This counter allows measurement over areas on the anterior hemisphere of the eye and in some areas posterior to the equator when extreme rotation of the eye is used. The second type of tube is essentially the same as the first, with the exception that the window is mounted at an angle of 45 degrees to the axis of the tube. This allows for measurement over more posterior portions of the eye. The third type of tube is one which has been quite recently designed by the present authors⁸ and which allows measurement at all posterior areas of the globe. These three special Geiger tubes are shown in Fig. 1.

COUNTING PROCEDURES

1. The patient is prepared by installation of 1% tetracain hydrochloride or 4% cocaine hydrochloride into the conjunctival sac.

2. A thorough ophthalmoscopic examination is carried out to determine the exact location and extent of suspected tumor.

3. After a check has been made to determine that the counting apparatus is working properly, 500 μc of P^{32} (about 7 $\mu\text{c}/\text{kg}$ body weight) in sterile isotonic saline is injected intravenously (500 μc of P^{32} for a 70 kg individual is far below the level of radioactivity which might produce manifest injurious effects; it is however greater than the generally accepted permissible dose. The exposure is less than that received during the course of most diagnostic X-ray procedures.)

4. Immediately after injection the counting procedure is started by placing the counting tube directly

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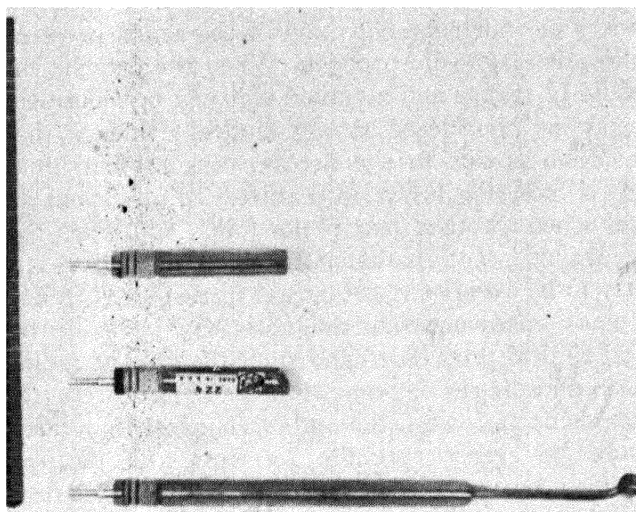


Figure 1. Geiger tubes

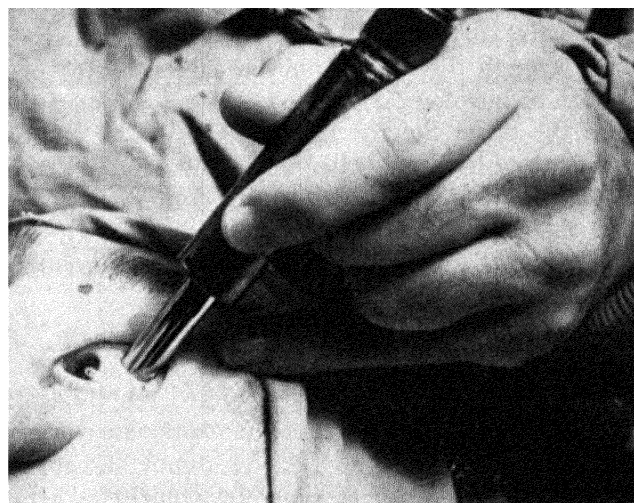


Figure 2. Counting tube in contact with the eye

in contact with the eye immediately over the suspected area (see Fig. 2).

A measurement is made for $\frac{1}{2}$ to 1 minute in this location and the count is recorded. The counter is then moved to a normal portion of the same eye as far removed from the tumor site as is possible, and a similar count is taken. This procedure is continued by alternately counting over the suspected tumor and the normal tissue for approximately 15 minutes. Additional series of counts are then made over these areas at one-half hour and one hour after injection.

In some cases an additional series of counts is made after 1, 2 or 3 days.

5. The variations in counting rate with time for the tumor and normal tissue are plotted on ordinary graph paper. The ratio of tumor to normal counts is determined for various times and is recorded. Various *in vivo* ratios are listed in Table I. The ratio which has been listed in previous papers and the one which we feel is most useful is the peak to peak ratio; that is the ratio of the peak of the tumor curve to the peak of the normal curve.

Table I. Positive Tumors

Case no.	Date	Peak	In vivo ratios			In vitro ratio	Pathological report or clinical findings
			$\frac{1}{2}$ hr	1 hr	Late		
Anterior tumors							
2	1/51	1.50		1.50		No specimen	Melanoblastoma choroid
3	2/51	2.30				5.62 (wet)	Melanoma choroid
4	3/51	1.66				4.68	Melanosarcoma choroid
5	3/51	1.65		1.65	1.44 (24 hr)	4.89	Melanosarcoma choroid
7	7/51	2.26	1.85	2.26		3.49	Melanoma choroid
9	9/51	1.42	1.20		1.42 (24 hr)		Metastatic C.A. (breast)
22	6/52	1.94 (15 min)			1.31 (24 hr)	6.85	Melanoma choroid
27	7/52	1.74	1.74	1.68		6.23	Melanoma choroid
33	11/52	1.47 (15 min)	1.34	1.21			Metastatic C.A. (breast)
35	1/53	1.41 (15 min)	1.32	1.23		40.0	Melanoma choroid
38	3/53	1.79	1.69	1.79		1.91	Melanoma of ciliary body
53	12/53					75.8	Melanoma choroid
54	12/53	1.84		1.84		2.27	Melanoma choroid
58	2/54	1.67 (3 day)	1.23	1.59	1.67 (3 day)	4.5	Melanoma choroid
62	4/54				2.88 (1 day)	No specimen	Melanoma choroid
Posterior tumors (early type counter)							
14	2/52	1.27 (13 min)	1.23			No specimen	Melanoma choroid
17	5/52	1.21	1.21	1.15		3.82	Melanosarcoma choroid
18	5/52	1.24	1.17	1.24		2.17	Spindle cell sarcoma
Retinoblastoma							
37	2/53	0.95 (20 min)				5.4	Retinoblastoma (glioma)
44	5/53	1.16 (15 min)	1.16		1.66 (3 day)	2.15	Retinoblastoma
52	10/53					3.63 (4 day)	Retinoblastoma
57	2/54					5.5	Retinoblastoma
63	4/54	2.03		2.03		2.36	Retinoblastoma

In addition to the above-mentioned *in vivo* procedure, in all cases where enucleation is deemed necessary and carried out, the eye was sectioned directly through the tumor and one-half used for preparation of histological sections and radioautographs. From the other half of the eye samples of tumor, sclera, retina lens and other important sections were obtained for assay of radioactivity with the conventional laboratory Geiger counter apparatus. The results (*in vitro* ratios) are recorded in corrected counts per minute per gram of sample.

RESULTS

A total of 63 cases has been studied. Of these 23 were tumors (Table I); 18 of the melanoma variety and 5 retinoblastomas. In the non-tumor categories (Table II) there were 12 fluid detachments, 15 inflammatory conditions, and 3 miscellaneous. In addition to these, there were a total of 10 cases in which the results are inconclusive for various reasons (Table III). The tumor results are best divided into two parts, anterior and posterior. There were 15 anterior tumors for which the peak ratio varied from 1.41 to 2.88 with an average of 1.82. There were 3 posterior tumors having an average ratio of 1.24 and ranging from 1.21 to 1.27. The results of retinoblastomas are very erratic and questionable as yet. The range of ratios was 0.95 to 1.20 with an average of 1.05. For inflammatory conditions the range is from 0.98 to 2.40 with an average of 1.33. The three miscellaneous cases include two benign tumors and one hemorrhage. The ratios range from 0.8 to 1.20. All of the ratios stated above are the peak ratios which occurred for the various conditions. Ratios at times other than that at which the peak was achieved are also listed in Tables I, II and III, and should be noted especially for the inflammatory conditions. In these cases, although the peak ratio may be quite high, the ratio falls to normal or non-tumor levels usually in one hour and in all cases within 3 days. It is also noteworthy that the ratios for posterior tumors fall between the non-tumor range and the range in which the anterior tumors have been found. Only three such cases are included and these were measured in early studies with the older type counters which could not be properly placed over the suspected posterior tumors. Because of this, the low ratios were fairly conclusive even though they did fall between the range of the non-tumors and the definite anterior tumors.

DISCUSSION

This study indicates that the measurement of P^{32} uptake in suspected intraocular tumors is of value in establishing the diagnosis. It is effective in determining whether retinal separations are produced by non-neoplastic or neoplastic disease. There are many cases in which the appearance of a retinal lesion will permit satisfactory diagnosis by usual diagnostic procedures carried out by a trained oph-

thalmologist. There remain, however, numerous instances in which the elevation of the retina presents a difficult diagnostic problem. When the detachment is fluid in nature and a retinal tear can be identified, it may be considered strong clinical evidence that the lesion is due to a collection of subretinal fluid and is non-neoplastic in nature. In questionable cases when a retinal tear is not found and when the appearance is not characteristic, the present test appears to be a very important aid in diagnosis. Since in many cases accurate diagnosis can be made by usual clinical procedures and since the dose of radioactive phosphorus is somewhat above the presently recommended dosage, the authors suggest that cases should be rather carefully screened and positive diagnosis be made where possible by usual clinical procedures and the test only used in cases where there is question as to the diagnosis.

The success of the test is largely dependent upon determination of the exact location and extent of the suspected tumor. Ophthalmoscopic examination must be carried out under the effect of a mydriatic in order to permit the most careful localization. Since the maximum range of the P^{32} beta particles is about 8 mm in tissue, it is necessary to be certain that the counter is in as close proximity to the tumor as possible. For practical purposes 5 mm or more between the tumor and counter is very unsatisfactory. Therefore when difficulty in properly placing the counter in apposition to the tumor is experienced, the significance of the result, particularly if it be a negative one, is doubtful. Because of this, it was in the past very difficult to study tumors situated in the posterior quadrant since the two early types of Geiger probes described above could not be applied with any certainty in the posterior portion of the globe. The newest type of probe design offers hope that these posterior tumors may be properly measured by placing this counter directly over the area where the suspected tumor lies. This will in general entail an operative procedure whereby the capsule must be opened and the counter placed immediately against the outside surface of the eye. It is felt that best positioning of this counter will be obtained when a transilluminator (light pipe) can be attached to the counter itself, and then by means of ophthalmoscopic examination the light transmitted down the transilluminator can be used to properly position the counter. This device has been designed but has not as yet been attached to the counter for use.

It should be mentioned that the present test differs from tumor studies carried out by other individuals in that measurements are made within a very short period of time following injection, rather than after 2 to 4 days. Preliminary studies were made of the early accumulation of P^{32} in neoplastic and normal tissue. A number of patients with superficial malignant tumors were studied using the following procedure. (Patients with superficial malignant tumors were used so that continuous recording of counts

Table II. Non-tumors

Case no.	Date	In vivo ratios				In vitro ratio	Pathological report or clinical findings
		Peak	1/2 hr	1 hr	Late		
Fluid detachments							
1	12/50	0.98	0.98	0.98		1.00	Fluid detachment
6	6/51	1.06	0.98	1.06			Reattachment
11	11/51	1.20	1.20				Reattachment
20	6/52	1.02		1.02	0.96 (24 hr)		Reattachment
25	7/52	1.05 (10 min)	0.83				Reattachment
30	9/52	0.95		0.95			Fluid
31	9/52	1.08	1.08	1.05			Reattachment
32	10/52	1.09	1.09	1.09			Reattachment
41	4/53	1.06	1.06	1.0			Fluid
45	6/53	0.98 (15 min)	0.88				Reattachment
48	8/53	1.09 (15 min)		1.02			Fluid
55	1/54	0.99	0.83	0.99			Fluid
Inflammatory lesions							
8	9/51	1.02					Uveitis
10	10/51	1.03	1.03				Inflammatory
13	1/52	1.10	1.10	1.06			Uveitis
15	4/52	2.4 (15 min)	1.53	1.16	1.08 (24 hr)		Inflammatory
19	6/52	1.05	1.05				Uveitis
28	7/52	0.98	0.98				Inflammatory
29	7/52	1.32	1.19	1.32			Inflammatory
36	2/53	1.10 (20 min)		1.00	1.04		Inflammatory
40	4/53	1.39 (15 min)	1.23	0.92			Uveitis
46	7/53	1.23	1.23	0.99			Uveitis
47	7/53	1.47 (10 min)	1.50	1.28	0.94 (2 days)		Inflammatory
49	8/53	2.02 (15 min)	1.30	1.34	1.08 (3 days)		Inflammatory
50	9/53	1.20	1.20	1.02			Uveitis
56	2/54	1.23 (10 min)	0.94				Inflammatory
61	4/54	1.37 (1/2 hr)	1.37	0.93			Inflammatory
Miscellaneous							
23	7/52	0.72		0.72			Benign tumor
24	7/52	1.16	1.16				Benign tumor
26	7/52	1.20		1.20			Hemorrhage

Table III. Inconclusive Studies

Case no.	Date	Peak	In vivo ratios			Clinical findings	Remarks
			1/4 hr	1 hr	Late		
Posterior location (old type counter used)							
12	12/51	1.10 (15 min)	1.08			Possible tumor	Posterior incon.
21	6/52	1.15	0.97	1.15		Possible tumor	Probable fluid
34	12/52	1.28	1.28			Possible tumor	Posterior incon.
59	3/54		0.95	0.70		Possible tumor	Posterior incon.
Did not return or refused enucleation							
16	4/52	1.61 (45 min)	1.41			Probable tumor	Refused enucleation
42	4/53	1.58	1.31	1.58	1.29 (3 days)	Probable tumor	Did not return
51	9/53	1.62	1.31	1.62	1.12 (3 days)	Probable inflammatory	Did not return
Retinoblastomas (being followed)							
39	3/53	1.68 (15 min)	1.56	1.37	1.14 (3 days)	Probable inflammatory	Being observed
43	4/53	1.15	1.15			Possible tumor	Count again
60	4/54				1.27 (3 days)	Probable tumor	Being observed

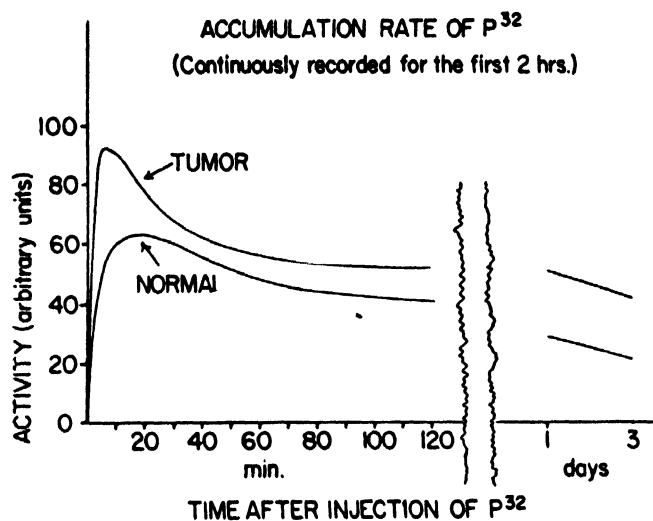


Figure 3. Accumulation rate of P^{32} (continuously recorded for the first 2 hr)

could be made. It has been subsequently borne out that intraocular tumors exhibit the same sort of curve.) Immediately after the injection of 500 μ c tracer dose of P^{32} , continuous recording of counts over the tumor and over normal tissue were made. These measurements were continued for a period of one or two hours. Additional counts were made the following day and at subsequent times up to 3 or 4 days. A graph showing the composite variation of counts with time was prepared from these data and reproduced here as Fig. 3.

Examination of this curve shows that at a very early time (less than 15 minutes for the patients studied) the ratio of tumor counts to normal counts reaches a maximum and that this ratio decreases for one or two hours following injection. After this decrease, a subsequent increase in ratio becomes evident and continues over the period studied probably reaching a maximum in 4 or 5 days. The cause of increased activity in the tumor tissue in the early stages is uncertain, and is now under further investigation. It may be due to one or all of the following: (1) increased vascularity of the tumor, (2) more rapid transport of the inorganic phosphorus across the vascular membrane into extra-cellular space, (3) more rapid transport of P^{32} across cellular membrane into intracellular space, (4) more rapid incorporation of P^{32} into nuclear and other cellular

constituents. Whatever the mechanism may be for producing higher counts in neoplastic tissue after administration of P^{32} , it should be noted that measurements as made in this study have shown that neoplastic tissue may be identified at an *early* period. In cases where the diagnosis has been questionable or borderline at early periods, patients are brought back in 2 or 3 days and further studies are made. In the late studies it is fairly certain that the P^{32} which is being measured is actually incorporated in the nucleus and other cellular constituents. It is probably the best procedure to make all measurements at 2 to 3 days; however, in clinical studies it has been found that the early measurement has an advantage in that it can be done in a very short period of time and that the patients need not be asked to return for late counts.

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The Speed of Iodine Metabolism

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The use of the radioactive isotopes of iodine has made simple the study of many aspects of iodine metabolism in man; and perhaps the most valuable information has been of the speed with which iodine is metabolised in various phases of its cycle, and in different types of normal and abnormal thyroid tissue.

Hitherto it is the uptake of iodine into these tissues, rather than its discharge from them, that has been most fully studied. It is easy to measure the speed with which iodide, given in radioactive form, is removed from the circulating blood and appears either in the urine or in the thyroid gland. The activity of the kidney in clearing iodide from the blood plasma into the urine is commonly expressed by estimating the total volume of plasma from which radioiodide is removed into the urine each hour. This value is obtained by a simple comparison of the radioactivity of the urine with that of a plasma sample, since for the first few hours after a dose the iodine is still largely in the form of iodide. It is found that the hourly urinary excretion is normally equal to the iodide content of 2 litres of plasma. Other excretory losses from the body are very much smaller.¹

The thyroid activity can be estimated in an analogous manner. If we know the rate at which radioiodine is accumulating in the thyroid gland, and the isotope concentration in the plasma at the same time, we can estimate the thyroid activity in terms of the volume of plasma being cleared of its iodide each hour by the thyroid gland; and the normal thyroid clears about 1 litre of plasma per hour.

Radioiodide is thus normally withdrawn from the circulation by the kidney, clearing 2 l/hr, and by the thyroid, clearing 1 l/hr. It is evident that the ratio of these clearances will determine the final distribution, the thyroid containing about one-third of the dose given. It is also apparent that the speed of withdrawal of radioiodide from plasma will depend on the sum of these clearances, and, in fact, if the isotope is distributed throughout 30 litres of which 3 litres are being cleared per hour, the plasma concentration will fall by about 10% per hour or with a half-period of about 7 hours.

In patients suffering from Graves' (or Basedow's) disease in which the thyroid is overactive, the thyroid clearance has an average value of 15 litres per hour,

the renal clearance remaining normal at 2 l/hr. The thyroid, therefore, collects about 90% of the dose and the plasma radioiodide concentration falls rapidly, with a half-period of an hour or two. It is evident that a variety of simple tests will reflect this greater speed and extent of thyroid collection of an administered dose, whether they are based on final thyroid uptake, total renal excretion, speeds of uptake and excretion, thyroid clearance rate, fall of plasma radioiodide or combinations of such measurements. Many of the diagnostic tests that have been suggested appear to have about equal clinical value and the choice often rests on the speed and convenience of the test and its freedom from undue radiation and from sources of accidental error. The following test gives a measure of thyroid activity and gives only 0.04 rep of irradiation to the thyroid in the process with levels 100 times smaller in the rest of the body. The patient receives a 6 μ c dose of I^{132} and returns 2 hours later when two 5-minute counts are made simultaneously: one by a ring of shielded counters round the neck to determine the amount of thyroid uptake by this time; and the other at the thigh to estimate the proportion of the dose still remaining in the body. The ratio of these counts gives a good estimate of thyroid function, and correlates well with the thyroid clearance rate, since the more active the thyroid, the more radioiodine will be in the gland after 2 hours and the less will be left in the rest of the body. The test is rapid and free from the errors involved in collection of specimens and the use of glassware and, since its result depends upon a ratio of counting rates, it is not even necessary to make an exact calibration of the dose given.

The efficiency with which the overactive gland removes iodine from the bloodstream must be very high and in many cases the volume of plasma cleared per hour must be as great as the whole volume flowing through the gland in that time. We have no good figure for the efficiency with which the normal gland removes iodide from the plasma reaching it, but a very rough estimate has given a value of 20%.²

It has long been known that some cancers of the thyroid and particularly those with a cell arrangement resembling that of the normal thyroid have, or may develop, the ability to concentrate iodine. This ability is, of course, of the greatest importance since it offers an opportunity for treatment with radioiodine; and its quantitative study has therefore be-

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come important. It is sometimes possible to study the clearance rate of a particular mass of tumour tissue, in just the same way as for the thyroid gland, and to estimate the activity of such tissue in terms of its clearance rate per gram of tumour. When this is done, it is found that tumours rarely have one-fifth the iodine concentrating activity per gram that normal thyroid tissue has. In contrast with this, hyperthyroid glands average about five times the normal activity per gram.

Hitherto we have discussed the removal of iodide from the bloodstream into thyroid tissue in different states of activity, and have seen that the speed of removal is determined by measurable thyroid and extrathyroid factors. What is the fate of the iodine when it becomes concentrated in the gland, and for how long does it remain there?

It has been found that radioiodine is discharged at very different rates from the thyroids of animals of different species, although the influence of experimental conditions of dietary iodine intake, size of radioiodine test dose, and recirculating iodine have not yet been adequately explored. It appears, however, that the thyroid glands of the rat, the chick, the mouse, the rabbit and the mule discharge iodine rapidly under the conditions in which they have been studied, whereas those of the guinea pig, horse, cow and man discharge it much more slowly. While these differences may merely reflect different experimental conditions, they may offer a useful field of enquiry, to identify what factors of pituitary activity or of thyroid cytology are associated with a rapid or with a slow discharge of secreted hormone.

Iodine leaves the normal human thyroid very slowly, an iodine atom probably having a mean stay in the gland of about 6 weeks. By contrast, discharge is rapid from the hyperthyroid glands, from iodine deficient goitres and from thyroid cancers, the mean stays being of a few days only.

The discharge rate of radioiodine cannot be measured simply by successive determinations of the radioactivity of the gland, because the thyroid radioiodine content is influenced not only by discharge from the gland but also by any continuing entry into the gland of radioiodine present in the circulation. The iodine which is entering the gland can, however, either be prevented from doing so by the use of suitable drugs which do not affect the discharge rate, or can be estimated by simultaneous measurement on the urinary excretion and by a knowledge of the ratio in which iodide is shared between thyroid and kidneys.

When patients with thyroid over-activity are examined in this way, the discharge rate is consistently high and indicates a mean iodine stay in the gland of about 4 days. The discharge of iodine may indeed be so rapid that half of the dose may be circulating in hormonal form at one time. From this state, it is broken down in the tissues to iodide, and it has been shown that the mean life of circulating thyroxine

molecules is inversely proportional to their concentration in the plasma.¹ In the hyperthyroid patient, therefore, each phase of the iodine metabolic cycle is accelerated: the removal of iodide from the bloodstream into the thyroid, owing to the gland's increased clearance rate; the discharge of hormonal iodine from the gland; and also the breakdown of this hormonal iodine to iodide and its return to the circulation. Moreover, owing to the efficiency with which the hyperplastic thyroid takes up iodine, the hyperthyroid patient will use most of her dietary iodine many times over in hormonal synthesis, whereas in the normal subject only 30% of it will ever be so used.

When the human diet is deficient in iodine, it has been shown that the activity of the thyroid in concentrating this element increases correspondingly so that a large percentage of a test dose of radioiodine is rapidly concentrated there. Further, if the re-entry of iodine into the gland is blocked by means of anti-thyroid drugs, it is revealed that the rate at which the iodine is discharged from the gland is abnormally rapid, although capable of being accelerated still further by thyrotropic hormone. If re-entry of radioiodine is not so blocked, the thyroid content remains about constant since the radioiodide discharged from the gland is re-utilised with great efficiency.³

A functioning thyroid cancer resembles the over-active and the iodine-deficient glands in its rapid discharge of iodine, and we have found the mean stay of iodine to be about 4 days in a series of patients with such tumours, in whom daily quantitative measurements could be made of the radioactivity of one or more tumour deposits. The functioning cancer contrasts sharply with the overactive gland, however, in having a much reduced, instead of a much increased, efficiency of uptake, as measured by the clearance rate per gram of tissue.

The inefficient uptake and the rapid discharge of radioiodine by such tumours both require investigation, since both diminish the therapeutic irradiation that can be achieved in these tissues by a given dose of administered radioiodine. It has repeatedly been shown that significant uptake by a tumour may only develop at some months after the function of the patient's normal thyroid tissue has been abolished. In some cases, however, the tumour can be shown to be concentrating iodine even in the presence of a normally functioning thyroid gland, and sometimes to be producing hormone in excess of the body's needs. There is the suggestion here that the function of the tumour can be stimulated, but cannot be suppressed, in response to the body's metabolic needs. When, therefore, we find that most tumours discharge rapidly such iodine as they concentrate, it might be supposed that this was due to stimulation of the tumour, perhaps by the thyrotropic hormone of the pituitary, to the fastest possible production of thyroxine. Evidence has been produced, however, that in conditions in which the release of thyrotropic hor-

mone should be minimised, the discharge of iodine from these tumours is not reduced.⁴

Several other reasons may be suggested why the discharge of radioiodine from tumours might be rapid. Firstly, hormone synthesis may not proceed normally and abnormal organic iodine compounds may be discharged prematurely. This possibility cannot be excluded and requires more investigation of tumour thyroglobulin and of the compounds liberated into the bloodstream. A simple arrest of iodide utilisation can, however, be excluded since drugs which should discharge any of this ion from the tumour, do not in fact diminish its radioiodine content. Secondly, the effect might be due to increased metabolic activity of tumour tissue such as is observed for glycolysis, nucleoprotein metabolism and other processes studied in various tumours. Thirdly, it has been shown that the turnover of iodine should be most rapid for a given tissue in follicles having the smallest diameters, if it is assumed that the cells lining all follicles have an equal activity.⁵ This is a suggestive point, since the follicles of tumour tissue are characteristically small from which, on *a priori* grounds, iodine discharge should be rapid. Fourthly, it is known that the iodine taken up by normal thyroid cells rapidly leaves them, to be stored in the lumen of the follicle which these cells surround. A tumour consisting of thyroid cells which lack the normal arrangement in follicles, might therefore possess the capacity of concentrating, but not of retaining, iodine. The rapid turnover might therefore reflect an abnormality of tumour structure in that its cells were arranged less frequently in follicles, and more often in papillae or in compact masses, than in the normal thyroid.

Some support for this last possibility is obtained by examining the curves of radioiodine discharge from functioning tumour metastases. In many instances, it is found that a large proportion of the radioiodine initially concentrated, is lost very rapidly, with a half-period of about 12 hours which is similar to that of the plasma radioiodide concentration. The remainder, however, is lost much more slowly, with a half-period of perhaps 5 days. The first of these components may represent concentration by cells without storage in follicles; and the second, the radioiodine which enters follicles and is stored.

As yet we know very little about the fate of thyroid hormone after it has left the gland or the tumour tissue, and many different factors may alter its rate of removal. I have mentioned already that the life of thyroxine molecules in the circulation appears to

be longer, the lower their absolute concentration there, the relationship being consistent with a bimolecular reaction in their removal. The mechanism of this removal is, however, little understood. Thyroxine very rapidly becomes distributed throughout the liver in high concentration relative to the plasma, this distribution being nearly complete within half an hour of giving radioiodine-labelled thyroxine in man; and presumably, in equilibrium conditions, it leaves this organ as rapidly. The rate of loss into the bile draining from the liver, or into the faeces, is, however, much slower, only 2 or 3% of the circulating thyroxine appearing daily in the faeces. Of the tissue metabolism of thyroxine we know only that the iodine returns from the tissues as iodide, and the time interval between disappearance of circulating thyroxine and the element's reappearance as circulating iodide is probably short.

It is not yet known in man what proportion of the iodine leaving the thyroid gland does so in the form of thyroxine, or, for example, how much tri-iodothyronine is also discharged from normal, hyperthyroid or cancerous tissue. Tri-iodothyronine is known to be metabolised more rapidly than thyroxine, and the proportion of these substances leaving the gland may influence the mean circulating time of the hormonal iodine, as well as the metabolic effectiveness of a given discharge rate.

It will be evident that the radioactive isotopes of iodine when used in tracer doses are yielding information about the kinetics of normal and abnormal human thyroid function which could not have been obtained otherwise; and that the results of present and future work should increase considerably our understanding of the metabolism of iodine in the thyroid gland and in cancers arising from it.

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Further Clinical Studies of Thyroid and Salivary Gland Function with Radioiodine

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Radioiodine tests of thyroid function are based on the well-established ability of the thyroid gland to concentrate iodide ion given either orally or intravenously, to convert it more or less rapidly into thyroxin and finally to secrete thyroxin into the blood stream. Although each radioiodine test used today is designed to measure thyroid activity, it is obvious that no one test can measure all aspects of thyroid function. At best, each test will measure the rate at which some phase of the iodine cycle takes place. The most commonly used radioiodine pick-up tests, for example, measure in the first instance the ability of the thyroid gland to trap iodide ion, the tests being made a few hours after the administration of a tracer dose. On the other hand, the usual twenty-four hour pick-up test will in addition measure the ability of the thyroid gland to store iodine in the form of thyroxin.

Uptake or pick-up tests do not, however, measure the rate of secretion of thyroxin into the blood stream, and since the uptake does not always correlate with the clinical condition of the patient, it seems clear that the radioiodine uptake and the output of hormone do not always bear a constant relation. A number of tests is therefore designed to measure the rate of output of thyroid hormone. Still other tests are concerned with the amount of protein-bound radioiodine in the plasma. For example, the so-called iodine conversion ratio measures the proportion of the total plasma radioactivity in the organic fraction at twenty-four hours. It is our experience and the experience of others that this test of thyroid function correlates better with the clinical condition of the patient than does the twenty-four hour pick-up test.^{1,2,3}

Although pick-up and conversion ratio tests are approximately 85 and 95 per cent accurate respectively in the diagnosis of hyperthyroidism, both tests are disappointing in the diagnosis of hypothyroidism.^{2,3} It is obvious, therefore, that these tests and slight variations of them do not give the full story. It is well known, of course, that the pituitary secretion of the thyroid stimulating hormone (TSH) plays an important role in controlling the level of thyroxin in the plasma. Jeffries *et al.*,⁴ recognizing the need

for a better test for hypothyroidism, investigated the effect of TSH on the radioiodine pick-up test in euthyroid, hypothyroid and hyperthyroid patients. It was found that the pick-up did not increase for primary hypothyroid patients after a dose of TSH as it did for others. This test has proved to be very useful in the diagnosis of hypothyroidism.

There are, however, still other phases of the iodine cycle which appear to have a bearing on thyroid function. Recent biochemical and clinical studies indicate that the salivary glands are concerned in the iodine cycle in the body. For example, the discovery of Fawcett and Kirkwood^{5,6} of our University that the thyroid enzyme, tyrosine iodinase, responsible for the iodination of tyrosine to monoiodotyrosine is also present in relatively high concentrations in the salivary glands, indicates a direct relationship between the two gland systems. Their further studies with labelled diiodotyrosine give positive evidence that the salivary glands may be responsible for the degradation of thyroxin in the body and the recycling of the iodine to the thyroid gland as iodide ion.⁷ It has, of course, been known that the salivary glands concentrate iodide ion into the saliva, the concentration in the saliva being about 30 times that of the blood plasma. But these results taken together indicated a definite relationship between thyroid and salivary gland function and suggested to us that radioiodine tests on the saliva of euthyroid, hyperthyroid and hypothyroid patients might correlate with and supplement conventional thyroid tests.

Our first results obtained on 76 patients reported earlier^{8,9} showed a remarkable correlation between the radioiodine secreted by the salivary glands and thyroid function. For example, the saliva radioactivity varied from 83 counts per minute (cpm), 3 cm³ samples, for a hyperthyroid patient to 24,000 cpm for a hypothyroid patient, twenty-four hours after administration of 50 μ c of I¹³¹, the average values being 493, 2393, and 12,172 for the hyperthyroid, euthyroid and hypothyroid groups respectively. When the saliva activity (S) was compared with that in the blood plasma (P) (S-P ratio), an equally good correlation with thyroid function was obtained. However, a very much better indication of thyroid function was obtained when we compared the saliva activity in relation to the protein-bound

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iodine (PBI) activity in the plasma (S-PBI ratio). An excellent diagnostic test for thyroid disorders was therefore indicated, as well as clinical evidence for the salivary-thyroid gland relationship. It should be pointed out that Freinkel and Ingbar¹⁰ reported no correlation between thirty-minute saliva-plasma activity ratios and thyroid function. The difference between the thirty-minute and the twenty-four hour saliva tests has not been fully explained.^{8,10}

Radioiodine saliva tests have now been carried out on an additional 350 patients to evaluate their usefulness more fully. Also, saliva tests have been made on patients receiving labelled *L*-thyroxin tracer in order that the role played by the salivary glands in the iodine cycle might be further elucidated. The results of these investigations are reported in this paper.

EXPERIMENTAL OBSERVATIONS

Details of the radioiodine pick-up, conversion ratio and saliva tests as performed by us have previously been described.^{2,8} The radioiodine pick-up and conversion ratios are measured with especially designed scintillation counters twenty-four hours after the administration of a 50 μ c dose of radioiodine. In the latter test the protein-bound fraction of a 3 cm³ aliquot of blood plasma was precipitated with trichloroacetic acid, washed free of serum, redissolved with sodium hydroxide and finally diluted to the original 3 cm³ volume. The radioiodine activity of the 3 cm³ sample was then compared with the activity of a 3 cm³ aliquot of the original plasma, the ratio of activities being called "conversion ratio".¹ The sample activities were measured wet in plastic dishes mounted over a crystal detector well-shielded by lead. Finally, 3 cm³ aliquots of saliva obtained from the patients by simple expectoration twenty-four hours after administration of tracer doses of radioiodine were measured into plastic dishes and the activity determined as before.

The samples were counted with the scintillation counter for five minutes. With this counting time the

natural background of the counter, 80 to 100 cpm, could be determined to 5 per cent. Since the scintillation counters were used to count gamma rays with wet samples, self-absorption problems were negligible. Further, there was no evidence of radioiodine losses in the samples dishes even on long standing.

RESULTS

Since our last report,⁹ 350 new patients have been investigated to compare the results of I¹³¹ tests. Seventy of these were hyperthyroid, forty hypothyroid, twenty represented tests of progress at various times after I¹³¹ therapy and the balance were normal controls or euthyroids. The diagnosis in each case was first based on clinical assessment, radioiodine pick-up and conversion ratio tests. Tables II and III give the results obtained for a representative

Table II. Twenty-four Hour Radioiodine Tests of Salivary and Thyroid Function in Patients with Diagnosis of Hyperthyroidism*

Patient no.	Total pick-up, %	Conversion ratio, %	Saliva-iodide ion† activity ratio	Saliva activity, cpm/3 cm ³	Saliva-PBI‡ activity ratio
86	82	81	—	71	0.14
237	60	98	21.0	496	0.50
260	74	96	15.0	216	0.70
230	73	80	3.7	179	1.00
215	64	64	1.9	81	1.10
221	61	76	3.7	136	1.20
137	83	97	81.0	163	1.60
216	65	79	7.8	238	2.00
102	78	95	46.0	370	2.60
292	83	67	6.5	295	3.20
105	71	92	41.0	205	3.80
194	53	91	46.0	969	5.40
147	72	78	24.0	289	6.90
181	63	81	34.0	553	8.30
206	64	66	25.0	150	12.50
140	39	50	18.0	513	17.00
192	44	50	25.0	343	24.60
212	52	66	59.0	1429	29.80
177	8	14	5.9	252	36.00
113	37	31	19.0	660	44.00
Averages	61	73	25.4	380	10.10

* All tests made twenty-four hours after oral administration of a 50-microcurie dose of radioiodine as iodide ion.

† Refers to iodide ion activity in 3 cm³ plasma.

‡ Refers to protein-bound iodine activity in 3 cm³ plasma.

Table I. Twenty-four Hour Radioiodine Tests of Salivary and Thyroid Function.* Normal Subjects (Euthyroid)

Patient no.	Total pick-up, %	Conversion ratio, %	Saliva-iodide ion† activity ratio	Saliva activity, cpm/3 cm ³	Saliva-PBI‡ activity ratio
19	28	11	18	1123	160
28	23	18	37	3726	170
65	18	17	35	2306	177
22	26	13	26	1601	178
34	31	23	59	4180	199
30	32	19	47	1990	199
20	35	16	43	1878	235
Averages	28	17	38	2393	188

* All tests made twenty-four hours after oral administration of a 50-microcurie dose of radioiodine as iodide ion.

† Refers to iodide ion activity in 3 cm³ plasma.

‡ Refers to protein-bound iodine activity in 3 cm³ plasma.

group of hyperthyroid and hypothyroid patients respectively. The total number of patients was then listed in groups in order of magnitude of saliva-PBI activity ratio, or according to thyroid activity, and the tables include the data of every second or third patient, etc. The data is therefore truly representative. Table I includes results obtained for a group of normal individuals for comparison. The second and third columns of the tables give the twenty-four hour radioiodine pick-up and conversion ratio tests. The fourth, fifth and sixth columns give the ratio of the saliva activity to plasma-iodide ion activity (S-iodide

Table III. Twenty-four Hour Radioiodine Tests of Salivary and Thyroid Function in Patients with Diagnosis of Hypothyroidism*

Patient no.	Total pick-up, %	Conversion ratio, %	Saliva-iodide ion† activity ratio	Saliva activity, cpm/3 cm ³	Saliva-PBI‡ activity ratio
186	18.0	11.0	52	4950	413
233	8.5	8.9	42	7760	431
285	22.0	7.7	38	19,180	456
244	2.0	6.2	34	2547	509
256	19.0	7.4	43	11,365	541
289	13.0	9.6	61	13,590	566
172	17.0	14.0	100	14,100	613
187	2.0	16.0	124	10,500	657
247	22.0	10.0	82	16,330	742
218	19.0	13.0	123	14,420	849
255	6.3	3.2	28	6900	862
116	8.0	13.0	148	34,100	971
303	20.0	6.8	81	24,650	1121
240	8.7	6.8	94	33,600	1300
301	7.3	5.5	112	76,000	1900
Averages	13.9	9.4	77	19,333	795

* All tests made twenty-four hours after oral administration of a 50-microcurie dose of radioiodine as iodide ion.

† Refers to iodide μ activity in 3 cm³ plasma.

‡ Refers to protein-bound iodine activity in 3 cm³ plasma.

ion ratio), the saliva activity, and the ratio of the saliva activity to activity of protein-bound iodine in the plasma (S-PBI ratio) respectively.

In a number of cases the conventional radioiodine tests did not agree with each other, in which case the diagnosis was decided on the basis of clinical symptoms. In the case of the hypothyroids, the diagnosis was much more difficult due to the fact that conversion ratio and pick-up tests are often not indicative. However, when the patients are listed according to saliva activity and in particular according to the saliva-PBI activity ratio (as in Tables I, II and III), it is found that saliva-PBI activity ratios above 400 and ranging to 1900 included all patients with either low pick-up or a low conversion ratio and all who showed clinical symptoms suggestive of hypothyroidism. Furthermore, a number of patients whose saliva tested "hypothyroid" and whose clinical condition was in some doubt was tested

further using Jeffries' TSH (thyroid stimulating hormone) test for obscure hypothyroidism.⁴ Each case, suspected by saliva test, was found to be hypothyroid with the TSH test. The results therefore confirm our preliminary work reported earlier that the saliva-PBI activity ratio is an excellent index of thyroid function and this is particularly true for hypothyroidism. In general, the saliva activities twenty-four hours after the administration of a tracer dose of radioiodine are low, intermediate and high for hyper-, euthyroid and hypothyroid respectively; the saliva-PBI activity ratio which appears to be a better index of thyroid function ranges from 0.14 to 44 for hyperthyroids, from 150 to 250 for normal subjects and from 400 to over 1900 for hypothyroids; the borderline-ranges between the three groups, from 50 to 150 and from 250 to 400 respectively, probably include in addition to euthyroids, patients who are mildly hyper- and hypo- respectively.

The saliva tests have been found by us to be a most useful aid in the diagnosis of thyroid disorders. Frequently these tests have indicated hypo- or hyperthyroid states when other tests indicated normal function contrary to clinical signs or symptoms. Also, the S-PBI test seems to represent the clinical progress more accurately than others when done in tests of progress after treatment.

¹³¹I Labelled L-Thyroxin Tests

In the normal radioiodine tracer tests the radioiodine is given in the form of inorganic iodine, which is removed from the circulation by the thyroid gland and then re-introduced into the blood stream in the form of labelled hormone. In two experiments, normal subjects were given tracer radioiodine in the form ¹³¹I labelled L-thyroxin direct by intravenous injection. To follow the circulation and degradation of L-thyroxin, 3 cm³ samples of blood and saliva were taken and the per cent total pick-up in the thyroid determined at intervals of time. Results obtained approximately 2, 20 and 140 hours after the administration of the ¹³¹I labelled L-thyroxin are shown in Table IV. Many additional pick-up and saliva tests were made during the period of the experiment. The per cent total pick-up in the thyroid rose for

Table IV. Radioiodine Labelled L-thyroxin Tracer Tests.* Normal Subjects

Subject	Elapsed time, hr	Plasma		Total pick-up, %	Conversion ratio, %	Saliva-iodide ion† activity ratio	Saliva activity, cpm/3 cm ³	Saliva-PBI‡ activity ratio
		Total activity, cpm/3 cm ³	PBI activity, cpm/3 cm ³					
1	2.3	4587	4197	0.6	92	7.3	2865	0.68
	20.3	2355	2202	5.2	94	7.2	1095	0.50
	139.3	708	608	9.0	91	4.4	294	0.46
2	2.0	34,515	34,477	1.5	100	—	6930	0.20
	20.0	9062	8941	5.7	99	—	4150	0.46
	139.0	3637	3283	11.0	90	5.5	1950	0.59

* Subjects 1 and 2 given intravenous injections of 50 microcuries and 200 microcuries of ¹³¹I labelled L-thyroxin respectively.

† Refers to iodide ion activity in 3 cm³ plasma.

‡ Refers to protein-bound iodine activity in 3 cm³ plasma.

the first 100 hours and then appeared to level off at 9 and 11 per cent for patients 1 and 2 respectively. After 140 hours, about 10 per cent of the activity in the blood was in the form of inorganic iodine as determined by precipitation with trichloroacetic acid.

Some indication of the rate of hormone turnover can be deduced from the decrease in PBI activity in the blood with time. After the first 20 hours, the PBI activity is seen to fall off at about 16 per cent per day. Most important from the point of view of our saliva tests is the low saliva-PBI activity ratio which is seen to be of the order of 0.5 to 0.6 for normal subjects. Recently Freinkel and Ingbar¹¹ performed similar tests in subjects with diverse states of thyroidal activity and report twenty-four hour saliva-PBI activity ratios of from 0.08 to 1.27. Although this variation between patients with diverse thyroid activities is in itself indicative of some relationship between thyroid and salivary gland function, it is nevertheless clear that the very high saliva activities and the high saliva-PBI activity ratios obtained for patients receiving inorganic iodine tracer (see Tables I-III) are largely due to the ability of the salivary glands to trap and concentrate iodide ion into the saliva. Only the patients with very active Graves Disease have saliva-PBI ratios as low as or lower than 0.5.

DISCUSSION

Value and Significance of Saliva Tests

Saliva tests have been made on over four hundred patients with varied thyroid activity and it is quite clear that the saliva activity and the saliva-PBI activity ratio obtained twenty-four hours after the administration of a tracer dose of radioiodine as iodide ion is a remarkably good index of thyroid function. The question arises as to the significance of the saliva tests and the role played by the salivary glands in the iodine cycle. It is well known that iodide ion is removed from circulation by the salivary glands and concentrated into the saliva.^{12,13,14} Therefore, regardless of whether they also degrade thyroxin as suggested by Fawcett and Kirkwood,^{6,7} the majority of the iodide concentrated must arise from the inorganic iodide in the blood. Thus, insofar as the salivary gland function is not altered in any way, the saliva activity tests will reflect the iodide ion level in the blood stream. Also, in a general way, it will be true as suggested by others¹¹ that there will be a more or less high and low iodide ion level in the blood 24 hours after a tracer dose of iodide ion for hypo- and hyperthyroids respectively, even though this picture is complicated by such factors as thyroid gland "hold-up" of iodine which depends on the size and nature of the gland, etc. It is apparent, however, from our results that the degree of functional activity of the salivary gland is altered and the accurate saliva tests for thyroid function are reflecting a connection between these two gland systems. It is seen from Tables I, II and III, column 4, that

the saliva-plasma iodide ratio which is a measure of one aspect of salivary gland function is far from constant. There is considerable overlapping of this ratio between groups of patients and the average value of the ratio for hypothyroids is about double that for normals. It is, of course, noted that in normal and hypothyroids both the saliva and plasma iodide samples are high in activity and can be determined accurately whereas for hyperthyroids the iodide ion level in the plasma is often low and the saliva-iodide ratio is not always so significant. It seems clear therefore that the degree with which the salivary glands concentrate iodide ion from the blood stream through to the saliva varies widely from patient to patient within each group and is in general altered with thyroid activity. The saliva tests of thyroid function must therefore reflect a connection between thyroid and salivary glands as well as iodide ion level in the blood. Saliva flow rates will, of course, have a bearing on the saliva tests. For example, high flow rates might be expected to show low iodide ion activity and vice versa. However, the saliva flow rate must reflect an important aspect of the function of the salivary glands which, like their ability to concentrate iodide ion, might well be altered by thyroid gland disorders.

Certainly, the most direct evidence of a connection between the salivary and thyroid glands is the presence of the thyroid enzyme, tyrosine iodinase, in the salivary glands in relatively high concentration and in large amounts first demonstrated by Fawcett and Kirkwood⁶ in our laboratories. They have shown that this enzyme will catalyze the synthesis of mono-iodotyrosine (MIT) as well as its deiodination in controlled *in vitro* experiments depending on the redox potential. In the thyroid gland its role is obviously a synthetic one and, since there is no evidence for the synthesis of organically bound iodine in the salivary glands, they have suggested the salivary glands as a seat of the deiodination process which is known to take place in the body. Recently they published data which provide direct evidence that the salivary glands do function in the metabolism of organic iodide⁷ and preliminary results in our laboratories indicate for the first time the existence of organically bound iodine in the saliva.¹⁵ If the salivary glands are a seat of deiodination of thyroxin, then alterations in salivary gland activity such as our saliva tests reflect, are to be expected. It is clear also that, in the saliva tests reported, any iodide in the saliva due to degradation of thyroxin would in most cases be masked by the larger amount of iodide ion normally concentrated from the blood into the saliva, since the degradation rate is only between 10 and 15 per cent per day.

Although perhaps less direct, there is much additional evidence in the literature suggesting a connection between the salivary and thyroid glands. For example, Hammerli¹⁶ has reported that sub-maxillary weight increases as the thyroid weight in

goiter patients and Albright¹⁷ has recently reported hyperthrophy of the salivary glands during the treatment of myxedema with triiodothyronine. Also, Rawson¹⁸ has reported swelling of the submaxillary and parotid salivary glands in a patient treated with thiouracil. This patient showed a marked decrease in thyroid function when under treatment with thiouracil and one of her parotid glands underwent permanent atrophy. These results are similar to those of Leblond and Grad¹⁹ and Arvy *et al.*²⁰ who showed that thyroidectomy and treatment with thiourea both result in atrophy of the submaxillary salivary glands of rats.

There are many clinical conditions with varying degrees of hypo- or hyperthyroid activity. Each I^{131} tracer test adds something to our knowledge of thyroid function. Any clinician-isotopologist, however, will admit that each test devised, and often a combination of two or more, will not infrequently fail to clearly point up the thyroid state to his satisfaction. It is to be noted, therefore, that we do not rely on the S-PBI test alone to indicate final diagnosis—we will continue to do our other tests and we will add the more recent “two-hour pick-up” and the “triiodothyronine suppression” as well as the “TSH” tests to our “battery” when indicated. As clinicians and scientists, however, we are impressed with the aid received from the S-PBI test, especially since it requires so little more of the patients’ time, no extra visits, and is simply performed in any laboratory doing plasma radioiodine measurements.

SUMMARY

Radioiodine saliva tests for thyroid function have been evaluated on over 400 patients. Not only was the S-PBI ratio test found to be almost 100 per cent accurate, but in many cases where other radioiodine tests gave inconclusive results the S-PBI test gave a clear-cut answer more consistent with the final clinical diagnosis. Advantages of the S-PBI test for thyroid function appear as follows: (a) equally accurate with radioiodine pick-up and conversion ratio tests for hyperthyroids; (b) S-PBI test gives accurate indication of hypothyroidism whereas other tests give disappointing results (thyroid stimulating hormone [TSH] test excepted); (c) the S-PBI test seems to follow clinical symptoms more closely

for patients under treatment with radioiodine, propyl thiouracil and other therapy.

Finally, the excellent correlation between saliva tests and thyroid function provides strong clinical evidence for a connection between the salivary and thyroid gland systems and supports the evidence of Fawcett and Kirkwood of our University that the salivary glands play an important role in the iodine cycle in the body—perhaps in the degradation or organically bound iodine.

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The Part Played by the Genetic Factors in the Development of Congenital Myxedema

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Congenital myxedema is due to the complete absence of the thyroid gland in the newborn child. In some cases, there will exist, at birth, rudimentary fragments of the gland, the regression of which will then continue, leading, over several months or years, to the total disappearance of the gland. This process of glandular atrophy, which is completed, or nearly completed during intra-uterine life may be due to a genetic factor, to an extrinsic factor which acted on the fetus during intra-uterine life, or to a combination of both factors. We have looked for the part which may have been played by a genetic factor, and investigated the families of children affected by congenital myxedema.

PRELIMINARY RESEARCH ON THE PART PLAYED BY GENETIC FACTORS

Few papers have been published on the subject, and it is difficult to draw any definite conclusions from them. The existence of two or three cases of congenital myxedema in the same family has been reported. We succeeded in finding published cases, in which this occurrence was observed, and which are to be grouped as follows: two myxedematous sisters, one brother and one sister, two sisters and one brother, one niece and one uncle or aunt, two distant male cousins.

The existence of Basedow's disease, or of simple goiter, in the families of myxedematous children, is more frequent. Some authors have been struck by the frequency of such a family tendency as regards the affections of the thyroid gland.

Such findings in twins are rare and contradictory: both identical twins in some instances have been observed to suffer from myxedema, and other pairs were found with only one twin affected. The determination of monozygotism has not always been made with the required accuracy.

We gathered five observations of young women suffering of congenital myxedema, and who none the less were able to carry pregnancies to full term.

Seven pregnancies out of eight led to the birth of a normal child, and only one to that of a myxedematous child.

Finally, it is important to note that congenital myxedema, along with the other affections of the thyroid, appears to have a marked preference for the female sex. The mean ratio, among the cases published, is about eight girls to one boy.

The authors that have been especially interested in the hereditary aspects of thyroid diseases, particularly E. D. Bartels, believe that the published genealogies strongly suggest the influence of genetic factors in the development of congenital myxedema. On the other hand, many other authors, in view of the fact that myxedema usually appears only as an isolated case in one and the same family, reject the theory of a role of genetic factors and think, rather, of a cause external to the fetus which has impeded the development of its thyroid body during intra-uterine life.

STARTING POINT FOR THE INVESTIGATION

We have taken, as our starting point, 20 children or adolescents suffering from myxedema. These subjects, whose ages now range from 3 to 27 years, have all been followed up for many years at the Department of Infants' Diseases in Lyon, and a diagnosis of congenital myxedema was established before any reached the age of three. Neither the initial clinical findings, nor the results of thyroid extract therapy left any doubt as to this diagnosis.

For 17 children of this group, we carried out the radioactive iodine tests, and our findings, for 14 of them, were as follows: (1) total absence of cervical fixation of the tracer iodine (less than 3% of the ingested dose), (2) urinary excretion which, within five days, reached almost the whole of the ingested dose, and (3) total absence of iodized radioactive protein compounds from the serum. This picture shows the total absence of functional thyroid tissue.

In the other three children we found: (1) a low but significant degree of cervical fixation: 5 to 7% of the dose, and (2) the presence of iodized radioactive protein compounds in the serum. These children still had some remaining functional tissue.

Determination of stable protein iodine showed, in every case, a figure lower than 4 μg per 100 cm^3 .

Original language: French.

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Finally, the injection of TSH (100 guinea pig units per day, for five days), made for eight children, did not bring about any change in the above mentioned results.

We are dealing, therefore, with a homogeneous group of children who have no functional thyroid tissue at all, or just traces of it. There is no problem having to do with any lack of stimulation from the pituitary.

Approximately half of our patients' fathers and mothers come from regions where goiter is moderately endemic. Conversely, the myxedematous children were born and have lived in regions where the disease is not endemic.

CLINICAL STUDY OF THE MYXEDEMATOUS CHILDREN'S FAMILIES

We were able to make a close study of the myxedematous child's siblings, his or her parents, his or her grandparents, his or her uncles and aunts on the paternal and maternal sides, and his first cousins on both sides. Only on rare occasions did we obtain useful information about the more distant relatives.

In twenty families studied, our findings were as follows: 14 families, one or several members of which showed unequivocally positive thyroid disorder histories; 6 families, in which no such thyroid history existed; among these, two families could be studied only incompletely.

The positive histories found are distributed in the following manner: 5 cases of Basedow's disease affecting the mother of the affected child; 2 cases of simple goiter affecting the mother; 2 cases of Basedow's disease affecting the maternal or paternal grandmother; 1 case of Basedow's disease affecting the maternal great-grandmother; 2 cases of Basedow's disease affecting a maternal or paternal aunt; 1 case of simple goiter affecting a maternal aunt; 1 case of Basedow's disease affecting a maternal grand aunt; 2 cases of simple goiter affecting a maternal and a paternal grand aunt; 1 case of simple goiter affecting a maternal great-grand aunt.

We met with only 2 cases of myxedema in the family, affecting a father's first cousin and a mother's sixth female cousin of the myxedematous child.

We never found an affection of the thyroid for our patients' siblings. However, we did note a high rate of stillbirths and a high rate of mortality among them during the first year of life, predominantly in the boys. The reasons for this mortality rate are somewhat obscure.

In 10 families we found, apart from the subject affected by myxedema, but one single member of the family affected by thyroid disorder.

In four families, we found several cases of thyroid disorder:

Family No. 5: Basedow's disease affecting the maternal aunt and the maternal grand aunt; myxedema affecting a distant female cousin on the maternal side.

Family No. 13: Basedow's disease affecting the mother and the maternal great-grandmother; goiter affecting a maternal great-grand aunt.

Family No. 15: Basedow's disease affecting the mother; goiter affecting a maternal grand aunt.

Family No. 19: Basedow's disease affecting the mother and a maternal grand aunt.

A positive pathological history was to be found in 10 families on the maternal side, and in four families on the paternal side.

The clinical results of our investigation suggest a genetic influence, but do not warrant definite conclusions. As other authors have already pointed out, many more cases of Basedow's disease or simple goiter in the family will be found than myxedemas in the strict sense of the term.

BIOLOGICAL INVESTIGATION OF CLINICALLY NORMAL PARENTS

With the partial result of this investigation in mind, we studied thyroid function in nine pairs of our patients' parents, who were all clinically normal. In their history and at the clinical examination which went along with our examination, these parents did not show any symptoms of thyroid disorder, particularly no hypertrophy of the thyroid. We used the following research methods:

I^{131} Test

After ingestion of the tracer dose (100 microcuries of I^{131} + 100 micrograms of carrier I^{127}) by the subject before meals, we studied the thyroid fixation at the 6th, 24th and 48th hour; the urinary excretion for 3 to 5 days; the radioactivity and the rate of radioactive protein iodine of the plasma, 24 hours after ingestion of the dose.

Measuring of Stable Protein Iodine in the Plasma

We used Barker's method¹—calcination of the protein precipitate with sodium carbonate at 600°, recovery of the residue by means of HCl, and measuring of the iodine content of the solution obtained in this way by catalytic action on a ceric sulphate + arsenious acid mixture. In our laboratory, this method yields normal figures somewhere between 4 and 10 micrograms for 100 cm³ of plasma.

In all parents investigated by this method, we found very definite abnormalities of iodine metabolism. These are shown on Table I, and they may be grouped according to two types.

The first type (11 cases: 4 mothers, 7 fathers) is characterized by a high rate of I^{131} fixation by the thyroid, low urinary excretion, a high conversion factor of plasma I^{131} (ratio of protein iodine of the plasma to total iodine between 59 and 100%), and a rate of stable protein bound iodine which reaches high figures in the serum, as are usually considered characteristic for hyperthyroidism. This metabolic type corresponds to *functional hyperactivity* of the thyroid gland. It is significant that the results of the various examinations are in agreement in this respect.

Table I. Biochemical Testing of the Normal Parents

Name of the child	Sex and age (years)	Relationship	Parents age	Thyroid fixation (% of dose)			Urinary excretion (% of dose)		I^{131} serum 24th hr		Protein I^{131} (gm/100 cm ³ of serum)
				6th hr	24th hr	48th hr	24th hr	48th hr	% of dose per liter	protein fraction	
1 Dan. GER.	Girl 3	Father	42	31	—	59	33.6	35.3	0.05	100%	13.6
		Mother	36	36	—	53	37.0	38.6	0.07	79%	9.3
2 Hél. GUI.	Girl 5	Father	45	36	51	—	36.0	38.8	0.15	84%	12.0
		Mother	42	27	—	42	58.0	59.8	0.11	100%	12.7
3 Jos. LEC.	Girl 5	Father	35	35	44	41	37.6	38.0	0.11	83%	13.8
		Mother	27	48	62	58	—	—	0.07	84%	8.0
4 Mic. PER	Girl 5	Father	30	41	67	—	29.3	—	0.11	58%	8.0
		Mother	26	37	64	—	33.3	—	0.14	52%	6.0
5 Mar. VIR.	Girl 17	Father	46	27	47	54	—	—	0.09	82%	5.9
		Mother	44	24	53	60	—	—	0.10	31%	4.4
6 Eli. VAR.	Girl 12	Father	40	49	61	—	—	—	0.16	86%	14.0
		Mother	40	41	57	—	33.1	33.7	0.35	85%	12.9
7 Mar. GAR.	Girl 12	Father	55	40	51	—	40.7	—	0.07	59%	13.7
		Mother	45	51	70	76	—	—	0.24	93%	15.8
8 Mau. LAT.	Boy 19	Father	60	50	62	66	40.3	40.4	0.06	48%	16.9
		Mother	51	44	55	55	—	—	0.28	64%	14.9
9 Mic. MA.	Girl 22	Grandmother	60	28	48	—	39.8	42.1	0.25	18%	14.7
10 Jea. PAR.	Girl 27	Father	65	10	21	22	66.4	77.3	0.74	8%	14.4

The second type (six cases: 4 mothers, 2 fathers) is characterized by a high rate of I^{131} fixation by the thyroid, low urinary excretion, a normal conversion factor for plasma I^{131} , and a normal level of stable protein bound iodine in the plasma. We will call this second type *simple hyperfixation*.

The figures obtained are quite significant. For instance, we find a mean rate of fixation of 55.2% of the dose at the 24th hour for the mothers, and a mean rate of fixation of 51.5% at the 24th hour for the fathers.

In 229 normal subjects Seed² found a mean fixation rate of 29%, and in 86% of the subjects a fixation rate of somewhere between 15 and 45% (at the 24th hour).

In 1000 normal subjects Werner³ found a mean fixation rate of 24.4%, and in 90% of the cases, a fixation rate of the dose somewhere between 10 and 40%. E. Quimby⁴ studied 1050 normal subjects: in 989 cases, fixation at the 24th hour was between 10 and 40%, and no fixation higher than 51% was observed. The normal results obtained in our laboratory are comparable to those of E. Quimby.

Similar considerations are to be applied to the figures concerning urinary elimination, radioactive protein iodine and stable protein iodine in the serum.

The fact which struck us most of all was that all the fathers of myxedematous children showed anomalies which were even more clearly in evidence in the fathers than in the mothers.

We examined the maternal grandmother of one of our children (Mic. MA., Family No. 9) a girl whose mother was deceased and whose father was unknown. In this woman of 60, we found the abnormalities characteristic of functional hyperactivity.

Finally, we made a somewhat closer study of the VIR. family. (Family No. 5, Table II). In this family, the little myxedematous girl is one of seven siblings, 3 sisters and 4 brothers: 3 boys died suddenly at the age of one month and no accurate diagnosis could be made, while 2 sisters and 1 brother, who are clinically normal, show a simple hyperfixation, as found in the father and the mother.

In those two last named cases, the figures of the serum bound protein iodine are at the lower limit of normality. Furthermore, a maternal aunt and

Table II. VIR Family

Name	Degree of relationship	Age (years)	Thyroid fixation (rate of % of dose)			I^{131} serum 24th hr		Protein I^{131} μ g/100 cm ³ of serum
			6th hr	24th hr	48th hr	% of dose per liter	protein fraction	
Marie	Myxedematous	17		2	1	1.28	5%	3.1
Jeani.	Normal sister	16		53		.09	49%	7.3
Thér.	Normal sister	10		50		.10	27%	8.2
Roger	Normal brother	13		75				8.5
Jean M.	Normal father	46	27	47	54	.09	82%	5.9
Claud.	Normal brother	44	24	53	60	.10	31%	4.4

grandaunt are affected by Basedow's disease, and a female cousin on the maternal side is myxedematous.

DISCUSSION AND INTERPRETATION OF THE RESULTS

1. A first question will arise concerning the biochemical tests: can it be stated that the anomalous conditions found reveal a constitutional and permanent condition of these subjects' thyroid glands, or do they just show a passing reaction in a situation which is apt momentarily to create thyroid hyperactivity. We are strongly inclined to support the first one of these hypotheses for the following reasons:

(a) None of these subjects shows symptoms of a thyroid disorder, particularly no hypertrophy of the gland.

(b) There is nothing, in them, to show a situation apt to create thyroid hyperactivity: all are adults who are well over thirty, the women have been examined some time after their latest pregnancy, some are well past the menopause; these subjects do not suffer from any lack of iodine.

(c) Finally, in particular, these abnormalities are always to be found in the father; now, in the adult man, the function of the thyroid is a very stable one.

2. When we add the cases where the parents show a clinical thyroid affection to those where they show only biochemical abnormalities, we find such abnormalities in the parents of 16 children.

As regards the other four children of our group, we could not yet examine the parents of three of them, while the last one was born of an unknown father and has lost his mother (the abnormality was found in the maternal grandmother).

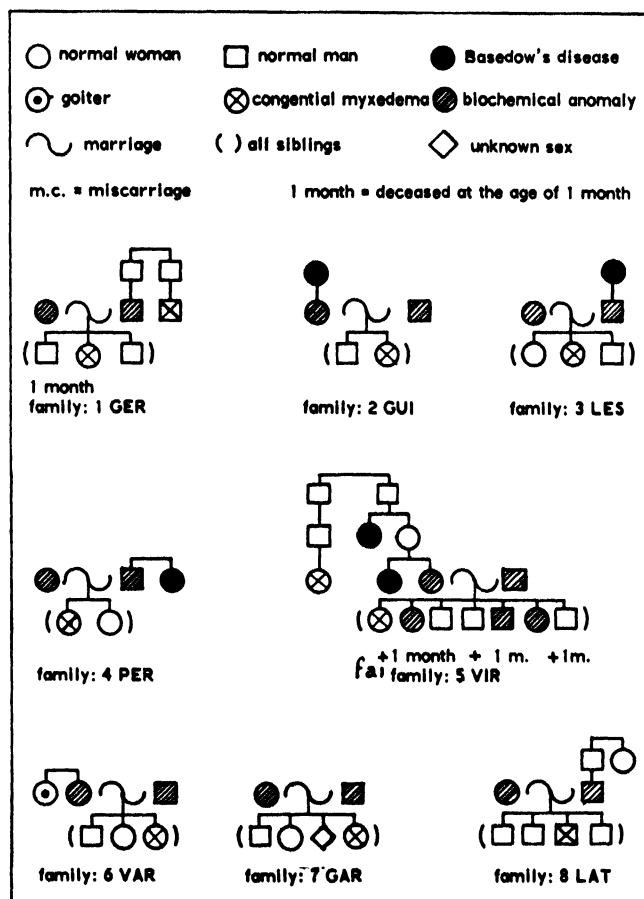
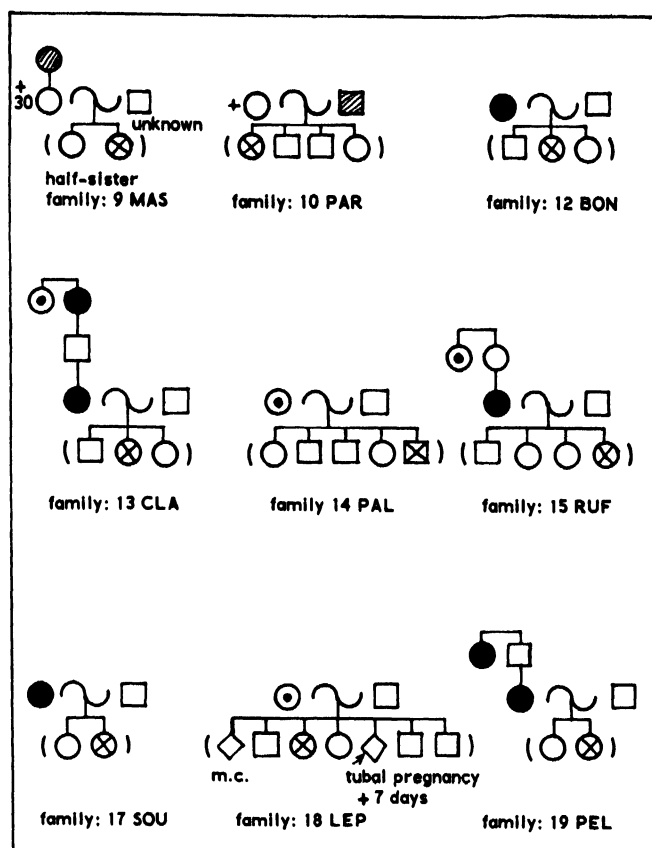
These results show the part played by a genetic factor in the development of congenital myxedema. The clearest evidence is supplied by the fact that the fathers will always show abnormalities.

The part played by the mother may likewise express the transmission of a genetic factor, or simply an endocrinological interaction between the mother and fetus during the intra-uterine life. The noxious part of this interaction would be favored by the abnormal gene transmitted by the father.

We cannot come to any definite conclusion on this point, but the most likely eventuality, it would seem to us, would be the transmission of a genetic factor by the mother. This leads us to think that the subject suffering from congenital myxedema is *homozygous* with respect to the defective gene.

3. Once the intervention of a genetic factor has been conceded, it will be difficult to explain clearly how it acts, since we see that abnormalities of the hyperthyroid or functional hyperactivity type lead to the exact opposite.

In his notable work on the part played by genetic factors in the development of Basedow's disease, E. D. Bartels,⁵ after very extensive investigation, comes to the conclusion that the same gene is liable to cause the distinct types of thyroid diseases: Graves' disease, toxic adenoma, simple goiter, and



The subjects marked as normal have been examined from the clinical point of view only, and have not yet been investigated biochemically

possibly, myxedema as well. He brings out convincing arguments for the first three of these diseases, and he hesitates as to myxedema, in view of the basic opposition between this latter disease and the others.

The functional hyperactivity observed in the parents of our myxedematous children will resolve the difficulty and enable us to think of a way of having this disease taken over in the over-all cycle.

We may therefore take up Bartels' hypothesis by giving it the following form:

(a) Graves' disease, toxic adenoma, simple goiter, and congenital myxedema show a correlation, for their development, with the prior existence of an abnormal gene in the affected individual, which is the same for these various types of diseases.

(b) Frequently, the gene concerned will be apparent only in the form of functional abnormalities of the thyroid body, without any clinical symptoms, which can be revealed by suitable laboratory tests. The abnormality which consists of functional hyperactivity is a minor manifestation of the pathologic gene. This occurrence is particularly frequent in the male and explains the apparent restriction to the female sex.

(c) Non-genetic circumstances (puberty, pregnancy, slight lack of iodine, etc.) cause the effects of the gene to change from the inapparent to the clinically apparent form.

The part played by this genetic factor should not allow us to forget the existence of various types of goiter, the development of which is mostly linked to an extrinsic factor, such as endemic goiter, goiter caused by ingestion of antithyroid substances, etc.

As regards the mode of transmission of the single gene responsible for thyroid affections, Bartels can see two possibilities: (a) dominance, with absence of manifestations in a certain percentage of the cases, and restriction to the female sex; (b) recessiveness, with marked limitation to the female sex, in which the frequency of occurrences is from 70 to 80%.

His records do not allow him to make his choice between the two hypotheses, (a) the single gene responsible for thyroid diseases is *dominant*; (b) in the heterozygotes it causes either a minor biochemical manifestation, or a simple goiter, or then Graves' disease or toxic adenoma; (c) in the homozygotes, it causes the appearance of myxedema.

Our results regarding myxedema might be explained by recessive transmission which would solely apply to this disease, but it cannot be seen how the

explanation might be extended to cover thyroid diseases as a whole. Now, the genetic relationship of the various thyroid affections appears more and more likely to be a well established fact.

These last hypotheses go beyond the facts we have observed. In order to be discarded or confirmed, they would require that our investigation be extended to the families of the subjects affected by Graves' disease or by simple goiter, and the grouping of a larger number of myxedematous families. This is the aim pursued by us at the present time.

SUMMARY

1. We have studied the families of 20 children affected by congenital myxedema, for the purpose of showing the existence of a genetic factor at the origin of this affection.

2. In 14 families, we found one or several persons affected by diseases of the thyroid, usually by Graves' disease or by simple goiter.

3. Taking 9 couples of parents who were entirely normal, from the clinical standpoint, we carried out I^{131} tests and measured serum levels of stable protein bound iodine. In each and every case we found abnormalities in the iodine metabolism of the type associated with functional hyperactivity or simple hyperfixation. We were struck by the presence of these anomalies in all the fathers examined.

4. These clinical and biochemical results warrant the conclusion that a genetic factor comes into play in the development of congenital myxedema.

5. By comparing our results with those of Bartels, we come with him to the conclusion that thyroid disease forms a genetic unity.

6. We discussed the possible mode of transmission of the pathologic gene concerned.

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The Dynamics of Radioactive Iodine in Normal and Pathological Thyroid Function

By G. Joyet, Switzerland

The experience of the last ten years clearly shows that the measure of thyroid activity at a given "standard" time following the administration of radioiodine (3, 6, 24 or 48 hours) does not permit a systematic distinction between normal and pathological functions.^{1,2} The same is true of urinary excretion measurement, which only gives information on hypofunctioning,^{1,2,3} but does not lend itself to a separation as between normal and hyperfunction.

Some authors⁴⁻⁸ clearly saw that the static activity of the thyroid at a given time was the result of several migrations of radioiodine inside the organism, some of which were related to the activity of thyroid function, while others were independent. The urinary excretion rate, in particular, is very variable but probably has no connection with thyroid function. In order to characterize the activity of the latter, it is necessary, therefore, to measure the speed of the elementary movements which depend on it, to the exclusion of those which are independent. The thyroid, blood, and intercellular space, thus appear as containers connected by currents of organic or inorganic iodine having different rates of flow. One should remember, at all times, that only mathematical analysis can make discrimination possible between those outputs and indicate to us how they can be measured.

The most important effort, in this direction, was made by Rotblat and Marcus⁶ who considered, following intravenous injection, four basic iodine currents or migrations: ascent into the thyroid, renal excretion, flow of blood iodine to the tissues and finally, back flow from the tissues to the blood. Each of the four movements is characterized by its parameter, the values of which determine the volume of relative displacement. The authors have been able to compute, as functions of these parameters, the activity equation corresponding to the blood, the thyroid and renal excretion. Leaving thyroid activity aside, it is with the help of the renal excretion curve, plotted by means of a series of closely spaced points during the first few hours following the injection, that a determination of the four parameters is made for each patient. We find from the statistics drawn from 58 tests that, out of the four parameters, the value of thyroid fixation λ_T is the only one which can be used for differentiating between normal and pathological

functions. The authors find for λ_T the following values:

Normal function	$0.10 \pm 0.025\%$ (standard deviation per hour)
Hyperfunction	0.37 to 1.50%
Myxedema	0.04%

THREE PARAMETER THEORY

In an attempt similar to that of Rotblat and Marcus, we have developed a first theory⁹ which takes into account for the first time the flow of organic iodine from the thyroid to the blood, which had not been allowed for by other authors. In addition, we do not separately take into account the migrations of iodine from the blood toward the tissue and, conversely, from the tissues to the blood. A certain number of papers¹⁰⁻¹³ have shown that the blood and intercellular space (iodine space) can be considered, in so far as inorganic iodine is concerned, as being a single space, in which the latter very rapidly diffuses. Our theory thus rests on the following basis, which can be considered as experimentally established:

1. Following intravenous injection, inorganic radioactive iodine takes up the whole of the iodine space in a few minutes. Figure 1 shows, for seven different functions, the relative activity measured on the surface of a thigh, with the help of a lead collimator equipped counter. In every case, ten minutes after intravenous injection, the maximum or 90% of it is already reached. Figure 2 shows the same curves for six of the patients following absorption of iodine by the oral route. It will be seen that the moment at which the maximum appears is very variable, occurring somewhere between 20 minutes and 3 hours. *The oral route is to be ruled out and intravenous injection alone is feasible, if it is desired to achieve well-defined initial conditions for the ascent of radioiodine to the thyroid.*

2. We assume as Myant, Pochin and Goldie¹⁴ have experimentally verified, and as other authors^{4,5,6} have also assumed, that the thyroid fixation and renal excretion of inorganic radioiodine are, at any given time, proportional to the concentration of radioiodine in the iodine space or, in other words, they are proportional to the total amount of radioiodine localized in the iodine space.

3. One can verify experimentally on man that 95% of the iodine injected passes through the thyroid or can be found again in the urine during the first days. On the other hand, radioactive protein bound iodine, which is produced by the thyroid, and 90% of which consist of thyroxine, is at first practically neither excreted by the kidneys nor taken up by the thyroid^{15,16,17} in subjects with normal or hypofunction of the thyroid. Only when thyroxine is disintegrated, with a half-life of about ten days,^{9,16,18} can it go through the primitive routes of thyroid fixation and renal elimination. It is known, on the other hand,¹⁹ that, in hyperfunction, protein excretion may reach 12 to 40% until the third day.

During the first days following injection, it can be admitted, for the subjects in hypo and normal function, that the major renal excretion and thyroid fixation phenomena take place essentially in an inorganic form, and that the organic form can be left out of the computations as a first approximation.

4. Finally, we assume, as all the other authors of similar theories,^{4,5,6,20} but without any further demonstration than experimental proof of the calculations, that the total organic iodine fraction which flows from the thyroid to the plasma is, at all times, proportional to overall thyroid activity.⁹

With these working hypotheses, the following method of computation can be developed:

Let S be the total activity in the blood and extracellular fluid (iodine space), T total thyroid activity, and U the activity of the urine excreted from time 0 to time t . Let a be the rate of inorganic plasma iodine fixation by the thyroid, η the urinary excretion

rate, and λ the output of organic iodine delivered by the thyroid to the plasma. The following differential equations can readily be set down:⁹

$$dT = aSdt - \lambda Tdt \quad (\text{thyroid}) \quad (1)$$

$$dS = aSdt - \eta Sdt + \lambda Tdt \quad (\text{iodine space}) \quad (2)$$

$$dU = \eta Sdt \quad (\text{urine}) \quad (3)$$

$$S_0 = T + U + S = \text{constant} \quad (4)$$

S_0 being the activity injected at the starting (0) time.

These equations lead to the following partial differential equation:

$$\frac{d^2T}{dt^2} + (a + \eta + \lambda) \frac{dT}{dt} + \eta\lambda T = 0 \quad (5)$$

which can be integrated to give:

$$T = \frac{aS_0}{2b} \left[e^{-(a-b)t} - e^{-(a+b)t} \right] \quad (6)$$

with

$$2a = a + \eta + \lambda \quad (7)$$

$$b = \sqrt{a^2 - \eta\lambda} \quad (8)$$

Similar functions are found for the blood activity S , and that of excreted urine, U .⁹

The thyroid activity curve thus appears as the difference between two exponentials, the dampenings of which make it possible to determine constants a and b . We succeeded in confirming this fact in 49 out of 60 patients, on whom measurements were taken for times varying from 4 to 8 days, with devia-

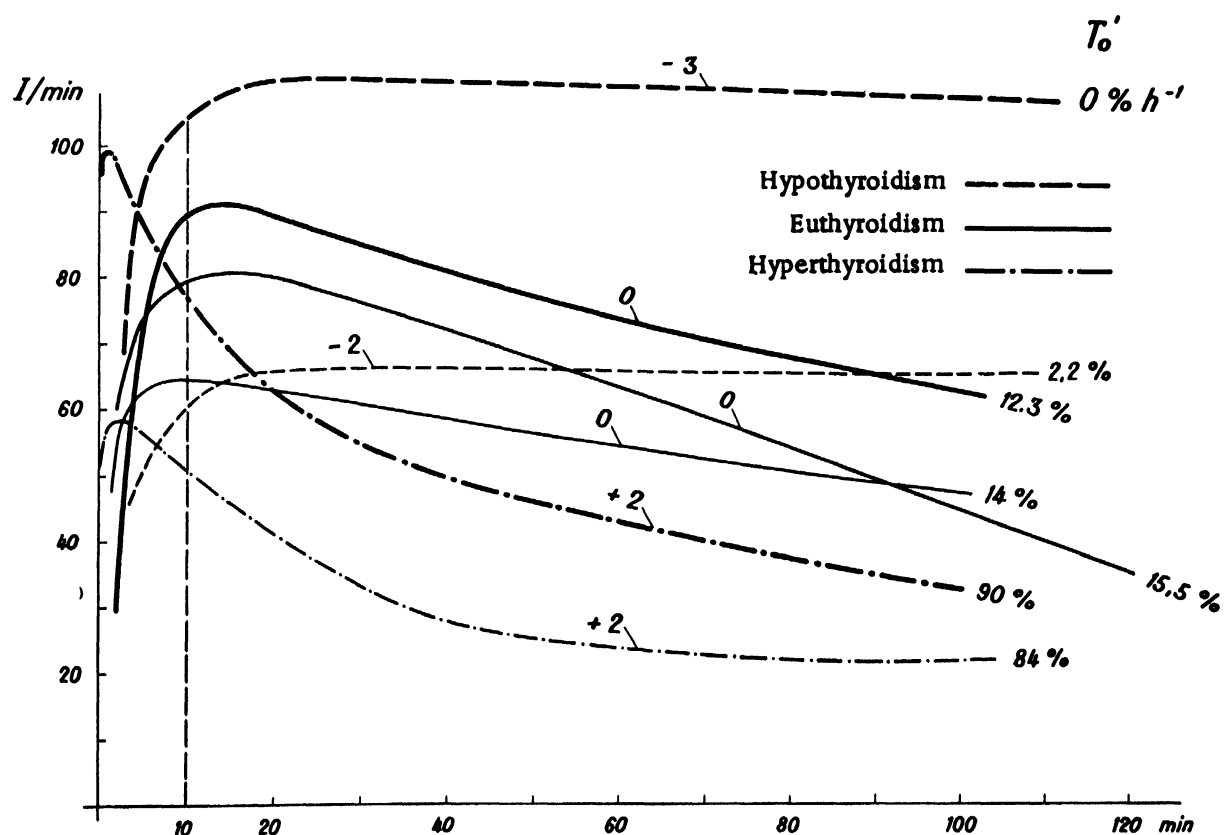


Figure 1. Relative activity on thigh, after intravenous injection

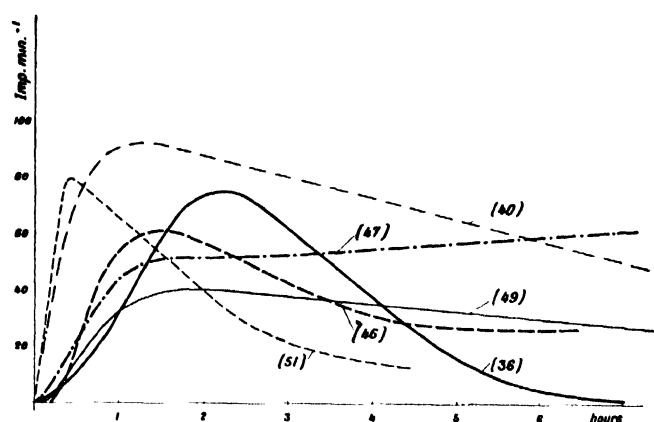


Figure 2. Relative activity on thigh, after oral dosage

tions smaller than 5%. In seven cases, all of hyperthyroidism, for which the theory applies but partially, the differences were between 5 and 10%. In four cases only (2 treated thyrotoxicoses, 1 severe case of Graves's disease and one marked myxedema), the deviations exceeded 10%.

Finally we showed⁹ the remarkable relationship:

$$T_0' = aS_0$$

namely, that the initial tangent to the ascending branch of the curve is equal to the rate of fixation of plasma inorganic iodine by the thyroid. ($S_0 = 1$ or 100%, being the relative activity of the injected solution, measured with the same geometry and diffusion conditions as the thyroid activity, and related to the same time for taking the measurements.)

The computation also shows⁹ that the maximum of the activity curve of the thyroid generally is a complex function of a , η and λ , namely of the three iodine displacements under review. *This maximum has no well defined biological significance.* Where λ is small (hypofunction and normal function):

$$T_{max} = \frac{a}{a + \eta}$$

a formula similar to that of Myant and his group

$$T_{max} = \frac{g}{g + r}$$

in which g and r , respectively, are the thyroid and renal clearances.

Finally, it is shown that parameter η is equal to the zero point tangent to the urine excretion curve.

APPLICATIONS OF THE THEORY

In cooperation with the clinic of the University of Zurich (Prof. Loeffler), the Radiotherapeutics Clinic (Prof. H. R. Schinz), and the Neumunster Hospital (Prof. F. Koller), we have applied the above theory to approximately 150 cases of euthyroidism, hypothyroidism and hyperthyroidism. A clinical report on these cases will be published in the near future in the bulletin of the Swiss Academy of Medical Sciences, in collaboration with Dr. Prof. Koller, Dr. Morandi, and Dr. Siegenthaler.

To these authors go the credit of having defined the biological activity of the thyroid from the clinical standpoint alone, quite independently of laboratory analysis. The patients were divided into classes +1 to +3 for Graves's disease patients, according to the intensity of the dysfunction, -1 to -3 for hypofunctioning, euthyroid cases being in class 0.

By systematical examination of the significance of parameters a , η and λ , as well as that of maximum increase T_{max} , we already have obtained, with Madame R. Gautier,⁹ the following results:

1. The urinary elimination coefficient, measured by the tangent to the excretion curve at point 0 is completely devoid of meaning as to the biological intensity of thyroid function.

2. Passing from marked hypofunctioning to normal activity and from there to hyperfunction, it will be observed that the maximum rise T_{max} ranges from 10% on an average to close to 90%. There is a general tendency to an increase in maximum deposition or fixation with the intensity or activity of the biological function. However, it will be observed that the deviations are very large and that, in normal subjects, T_{max} varies from 40 to 75%; for Graves's disease patients, from 50 to 90% approximately. Thus, the maximum rise does not permit any distinction between normal function (euthyroidism) and hyperfunctioning. At the most, it can make it possible to distinguish marked hypofunctions.

3. The rate of thyroid flow characterized by coefficient λ also tends to increase as one passes from normal to Graves's disease patients. However, the area pertaining to the latter, ranging from about 1% to over 60%, covers almost all the values which pertain to normal subjects. Similarly, the values of λ for the hypofunctions are not to be distinguished from those of normal subjects. *Therefore, the rate of thyroid flow is not a suitable index of thyroid function activity.*

Bearing in mind that thyroid flow is essentially made up of organic iodine and that 90% of this iodine is to be found in the thyroxine,^{15,16,17} the above results might, at first, appear surprising. They will readily be clarified by remembering that thyroid flow is not equal to λ , but rather to $J\lambda$ in mg per hour, in which J is equal to the total weight, in mg, of the iodine contained in the thyroid. J probably varies a great deal from one subject to another and, since J is the expression which characterizes, by giving the output of organic iodine, the true intensity or activity of the function, coefficient λ , *per se*, has no systematic significance.

THE SIGNIFICANCE OF THE THYROID FIXATION RATE OR OF THE INITIAL TANGENT TO THE ASCENDING BRANCH OF THE CURVE

In 58 cases, for which thyroid activity was measured for a time lasting from 4 to 8 days, the rate of thyroid fixation a was determined at the same time as η and λ by the ascending branch of the curve and

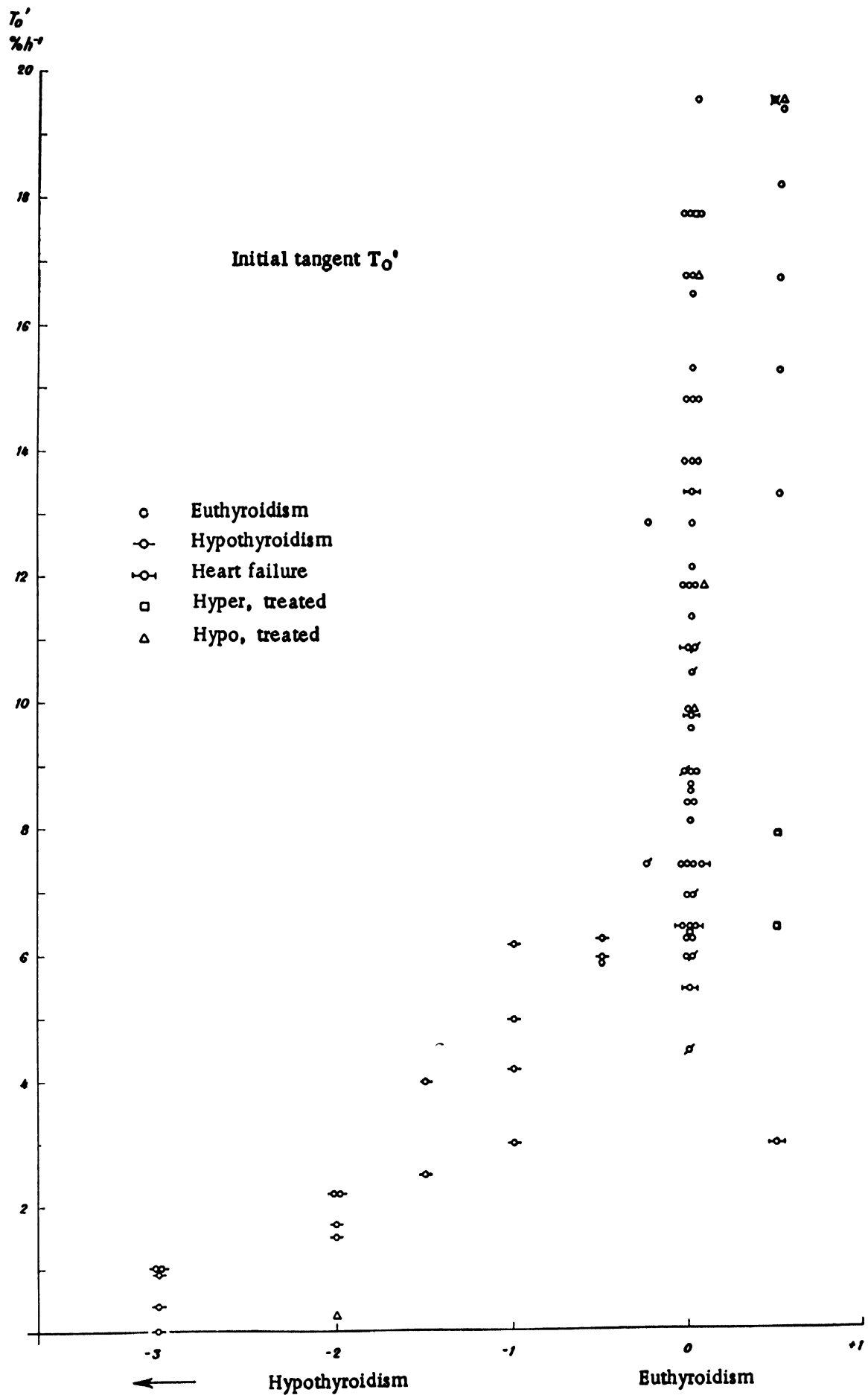


Figure 3. Euthyroidism and hypothyroidism

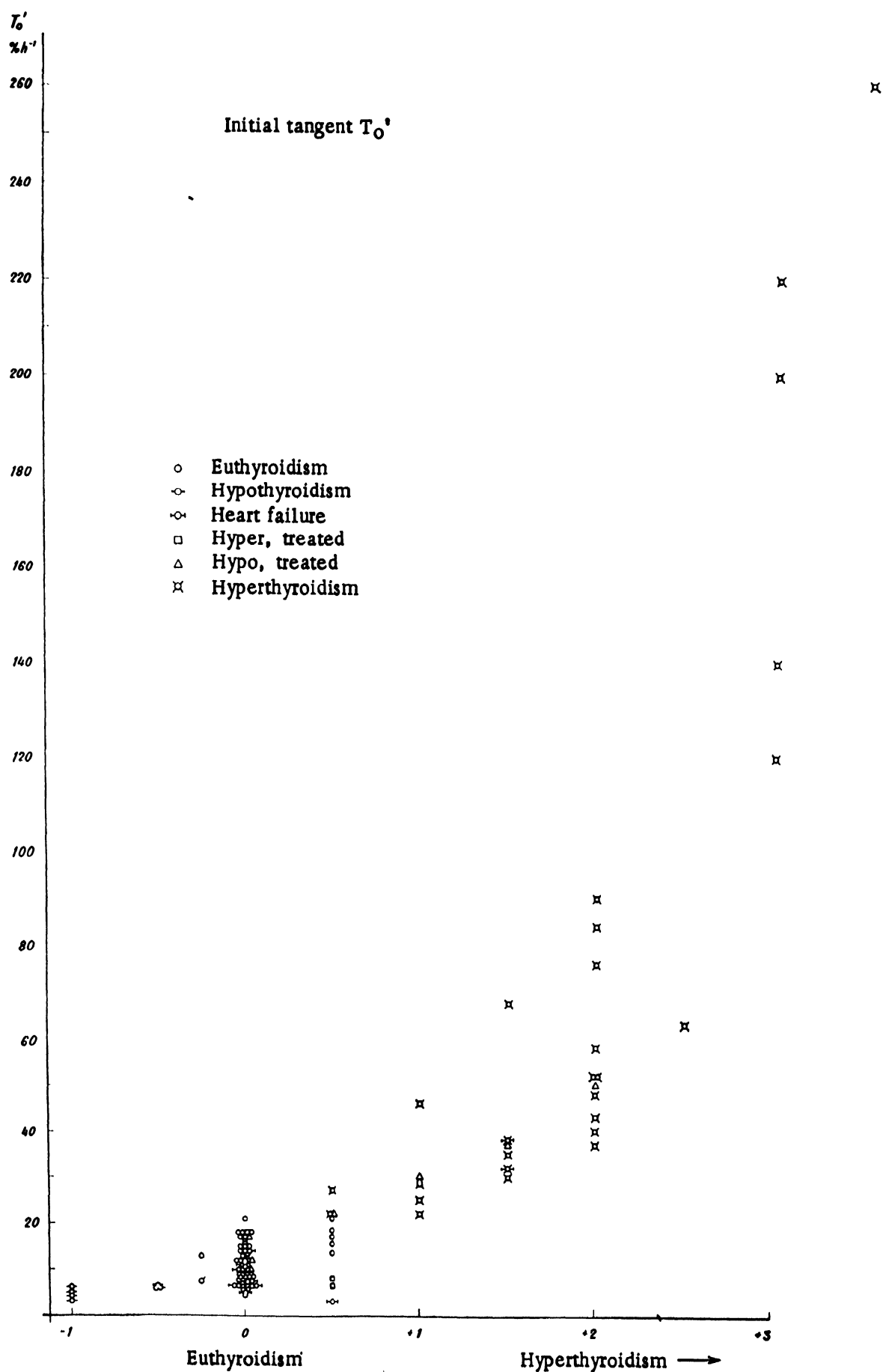


Figure 4. Euthyroidism and hyperthyroidism (Fig. 3, with different ordinate scale)

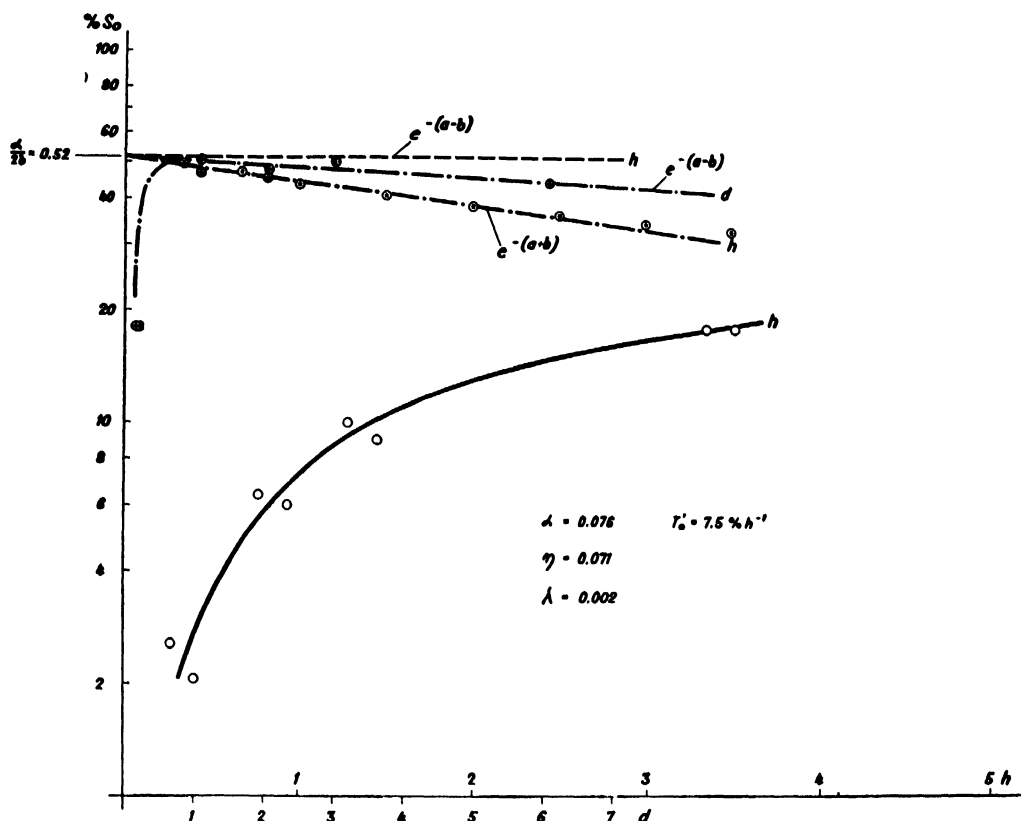


Figure 5. Euthyroidism

T_0' by the initial tangent to that curve. The statistics compiled show that a and T_0' differ, on an average, only by about $\pm 15\%$, and that the correlation between the two values is excellent. Within the possibilities allowed by the precision of the measurements, one can consider, as required by theory, that the initial tangent to the ascending branch of the curve is equal to the rate of fixation of the inorganic iodine in the blood by the thyroid.

Figures 3 and 4 show (on two different ordinate scales) and for 117 patients, the distribution diagram of the initial tangent T_0' as a function of the clinical intensity of the deviation.

The first figure includes euthyroidism and hypothyroidism, the second, euthyroidism and hyperthyroidism. One can thus see the enormous sensitivity of the test, which varies from some points per thousand, in hypothyroidism, to over 200% an hour in Graves's disease. It also permits nearly perfect separation between the normal and pathological functions. The latter can be classed as follows:

T_0'

Euthyroidism	7 to 18% $S_0 h^{-1}$
Hypothyroidism	less than 5% $S_0 h^{-1}$
Graves's disease	more than 23% $S_0 h^{-1}$

The initial tangents, from 5 to 7% h^{-1} , and from 18 to 23% h^{-1} now correspond to euthyroidism and then to slightly abnormal thyroid function. Naturally, there is a certain measure of scattering in the distribution of the points, and the clinical designation of the classes may entail an error having an order of magnitude of about half a class.

Let it be specified that the 12 aberrant cases were not shown on the distribution diagrams. They all refer, with one single exception, to patients who received large amounts of stable iodine during the weeks or two or three months which preceded the test, in which instances T_0' was brought down to zero, or to the level of hypothyroidism. The iodine preparations injected into the blood-stream (Joduron, Jodibil) for an X-ray or a cholecystography, or used for a pyelogram, a gynecological examination or on the occasion of the extraction of a tooth, are the usual causes of thyroid saturation. The every day diet does not seem to play any role.

Thus, prior to testing, the patient should be questioned and, if need be, the test should be repeated three months later.

In the clinical paper now in preparation, we shall finally show that the initial tangent is modified by pituitary extracts, TSH and the drugs used in the treatment of Graves's disease. In every case, following treatment, it shows the intensity or activity of the function at the moment considered.

THE TERM λT AND THE THYROXINE HALF-LIFE

It has been seen that, while the decomposition of the ascending branch of the curve into two exponentials does apply to hypothyroidism and euthyroidism, this no longer is the case in hyperthyroidism beyond the first few hours following the injection. Figures 5 and 6 show this difference. On Fig. 5, which refers to the case of normal function, the ascending branch of the curve is perfectly suitable for decomposition into two exponentials. This, however,

no longer is true, in the case of Fig. 6, which applies to hyperthyroidism, beyond the first hours following the injection. This is due to the high value of thyroid flow λT in the case of Graves's disease.

In Equation 2 all the terms have to do with inorganic iodine, except the term $\lambda T dt$, which is relative to organic iodine. The significance of the equation is falsified by this term, for only inorganic iodine can be newly fixed or excreted in appreciable quantities, by the thyroid and kidneys respectively. The term $\lambda T dt$ must be replaced, in the equation, by the fraction of the organic flow which is disintegrated at the same rate as the biological half-life, Θ , of the thyroxine.

Let θ be the disintegration probability of a thyroxine molecule outside the thyroid during the unit of time.

$$\theta = \frac{0.693}{\Theta}$$

In Fig. 7, the fraction of elementary organic flow $\lambda T dt$ which appeared at time t and was disintegrated to inorganic iodine up to time t is equal to

$$\lambda T \left[1 - e^{-\theta(t-t_1)} \right] dt_1$$

and its differential during dt

$$\theta \lambda T e^{-\theta(t-t_1)} dt_1 dt$$

The total quantity of thyroxine disintegrated during time dt is thus:

$$\theta \lambda T e^{-\theta t} dt \int_{t_1=0}^{t_1=t} T e^{\theta t_1} dt_1$$

and the differential equation for blood (iodine space) thus becomes,

$$dS = -aSdt - \eta Sdt + \theta \lambda T e^{-\theta t} \times dt \int_0^t T e^{\theta t_1} dt_1 \quad (2')$$

in which all terms are now inorganic. Equations 1 and 3 are not changed. This system, taken as a whole leads to the partial derivation equation

$$\frac{d^2 T}{dt^2} + (a + \eta + \lambda) \frac{dT}{dt} + (a + \eta) \lambda T = \lambda a \theta e^{-\theta t} \int_0^t T e^{\theta t_1} dt_1 \quad (5')$$

the solution of which is no longer a sum of two, but rather of three, exponentials from which we hope we may derive half-life $\Theta = 0.693/\theta$ of thyroxine.

EXPERIMENTAL DETERMINATION OF T_0'

This determination⁹ is made with the help of two counters, each of which is equipped with a suitable collimator yielding a protection of 25 to 30 mm of lead outside the measuring field of each. Scintillation counters using NaI(Tl) will be pre-

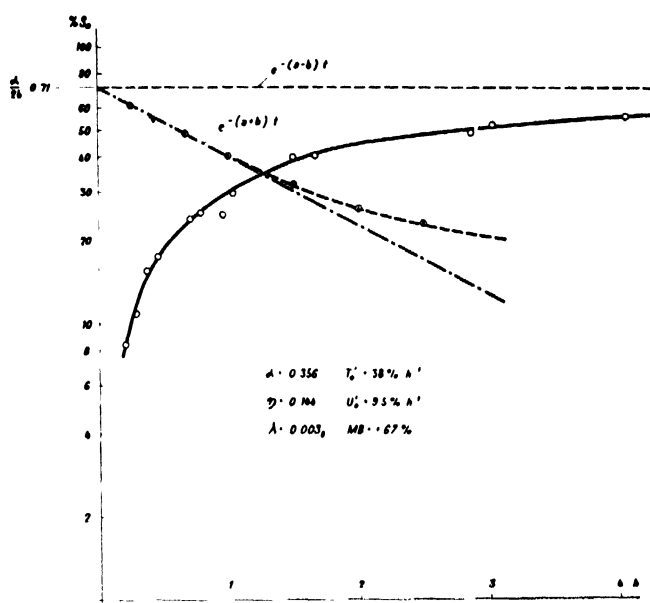


Figure 6. Grave's disease

ferred, making it possible to bring down the activity of injected I^{131} to some 10 to 20 μc .

The first counter, equipped with a *large collimator* measures, at a distance of 35 to 40 cm, the activity of the thyroid and neighboring territory, in a large 12×15 cm field. The activity of the blood in the perithyroid territory is subtracted from each reading.

In order to carry out this correction, it is assumed that the mean relative activity of the perithyroid territory bears, to the activity of the surface of the thigh, a certain ratio, k , which is constant for each subject. This ratio is determined with the help of the second counter, equipped with a *small collimator*, by making a few measurements, first on the thigh, at a given point, and then taking a few points around the thyroid, four to seven minutes after injection, as the activity of the gland still is not very high, but mixing of the tagged substance with the blood is already well underway. Each measure made with the large collimated counter (thyroid) is paired with a simultaneous measurement at the point chosen on the thigh with the small collimated counter.

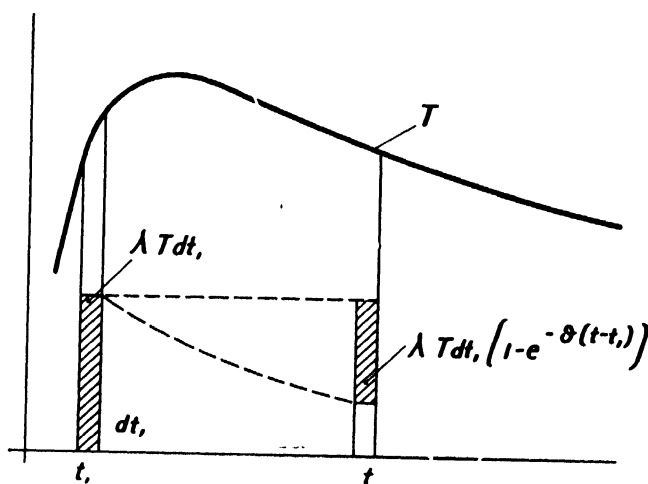


Figure 7. Plot of T vs t

Ratio k' of the relative measurements carried out with each of the counters in the relevant geometry is determined, once and for all, by filling a thin plexiglass cylinder, the dimensions of which are approximately those of the neck, with a solution of I^{131} . The two collimators are applied in turn, and the ratio of the two measurements gives k' .

From each measurement carried out on the thyroid, the simultaneous measurement made on the thigh is subtracted, following prior multiplication by kk' . The activity correction for the perithyroid territory thus supplies, for initial tangent T_0' , a correction which may vary from 30 to 100%.

In order correctly to determine T_0' in all cases, a first measurement should be made 20 minutes following the intravenous injection, and then forty-five minutes, and one hour and thirty minutes later. In cases of hypothyroidism, of euthyroidism, and of treated hyperthyroidism, it would be wise to carry out a fourth measurement 3 hours after injection.

In order to standardize the measurements made on the thigh with the counter, as a percentage of the injected solution (% of S_0) a weighed quantity of the solution to be injected is placed in a 50 cm³ glass container having approximately the shape of the thyroid, following which the amount is made up to 50 cm³. The activity of this fictitious thyroid is determined by placing it in a plexiglass cylinder full of water (scattering phantom), 15 cm in diameter and 3 mm thick, itself placed at the end of the collimator in the position of the patient's neck.

The values of the thyroid fixation rate of inorganic iodine, which we have determined with the help of T_0' , agree, for the normal and pathological areas, with those found in London by Rotblat and Marcus⁶ with the help of the renal excretion curve. By bringing in the notion of the iodine space (26% approximately of the total weight), one can compute the value of thyroid clearance which corresponds to thyroid fixation. The values thus transformed then correspond with those of Berson *et al.*² determined, in New York, during the first half hour following injection, for patients suffering from Graves's disease, and for the upper limit of the normal range. On the contrary, the agreement is not so good between the values found by these authors and ours, for the lower limit of the normal range. This difference may be attributed to an unduly hasty determination of the activity of the perithyroid territory.

Finally, taking a normal subject for whom $T_0' = 12\%$ an hour and an inorganic plasma iodine rate of 0.2 micrograms per 100 cm³, the daily influx of inorganic iodine to the thyroid is approximately 0.1 mg. Now this value is approximately equal to the amount of iodine, transformed into thyroxine, needed as the daily maintenance dosage in myxedema. The

daily influx of inorganic iodine to the thyroid is thus of the same order of magnitude as the daily outflow of organic iodine from the gland.

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A Critical and Statistical Study of the Functional Evaluation of the Thyroid by Means of Radioactive Iodine

By René Fauvert,* André Loverdo† and Lucien Hartmann,† France

Radioactive iodine can be used in order to follow quantitatively the various stages of thyroid activity. In practice, this reduces to measuring the concentration of inorganic iodine by the thyroid and the passage of hormone bound iodine into the blood.

TECHNIQUES

1. Various methods have been used in order to evaluate the concentration of radioactive iodine in the thyroid. Measurement of the urinary excretion of a given quantity of radioiodine following its introduction into the system was much used at first: in fact, it is known that the excretion of iodine by the urine and its fixation by the thyroid are, as it were, "competitive" processes, and that any variation in the rate of iodine fixation by the thyroid brings about an inversely proportional variation in that of the urinary excretion.

Unfortunately, measuring the urinary excretion of iodine as a test of thyroid activity offers a certain number of drawbacks, some of which are theoretical: e.g., the fairly substantial variations observed in the results and the possible interference due to renal insufficiency; while others are practical: e.g., the causes for errors inherent in the collection of the urine over 24 and 48-hour periods. A certain amount of I^{131} may be adsorbed on the walls of the flask used. These drawbacks rule out the use of urinary excretion measurements by the current method making use of large series of non-hospitalized subjects.

It is preferable to measure the concentration of I^{131} by the thyroid: this is a direct and immediate procedure which does not require the patient's active cooperation. Nevertheless, it can be exploited to the full only if it is carried out under optimal technical conditions which guarantee, not only the precision, but also the reproducibility of the measurements. These conditions are: (1) perfect geometry while a reading is being taken, by the exact and invariable positioning of the counting device, both vertically and laterally, with respect to the gland; (2) adequate shielding of the counting device against background radiation from the body, as a whole, particularly at

the beginning of the measurements; and (3) injection of a very accurately measured quantity of I^{131} .

In order to fulfill these conditions, we have designed special equipment consisting of a sliding table, on which the patient lies, making it possible to bring the subject's neck directly below a counting device made up of three Geiger-Mueller counters set up at equal intervals on an arc at the center of which is the patient's thyroid (see Figs. 2 and 3).

This equipment is provided with a mechanical and optical centering device, whereby the counters can be set precisely at a fixed height and opposite the two lobes and the isthmus of the thyroid respectively. This centering device makes use of electromagnetic components. In this fashion, the vertical and lateral geometry of the measurements is made accurate and constant, and the sensitivity of the readings is trebled by coupling the counters in parallel.

In addition, not only is each counter protected by an individual 3-cm thick lead shield, but, furthermore, there are two additional lead shields which can readily be moved by means of a set of counterpoises, in such a way as to isolate the thyroid and protect the detectors against stray radiation from the subject's head and body (see Figs. 2 and 3).

Finally, in order to accurately measure the amount of I^{131} injected into the patient's system, the amount selected (50 to 100 microcuries) is injected intravenously, following a reading of the radioactivity of

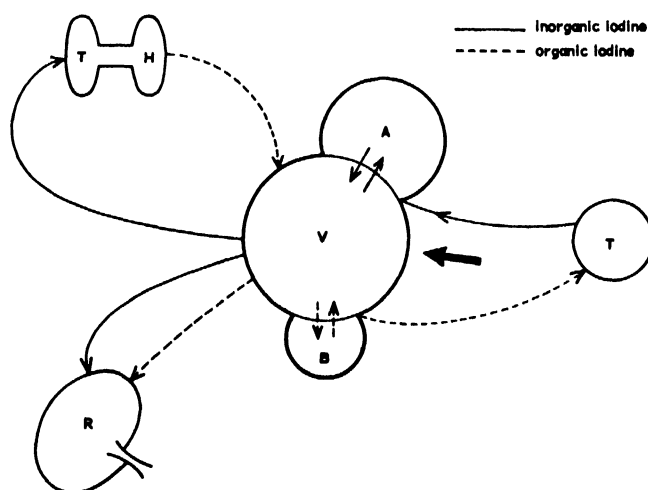


Figure 1. Iodine cycle. (V) vascular compartment; (V + A) inorganic iodine "space"; (V + B) organic iodine "space"; (TH) thyroid; (R) kidneys; (T) peripheral tissues

Original language: French.

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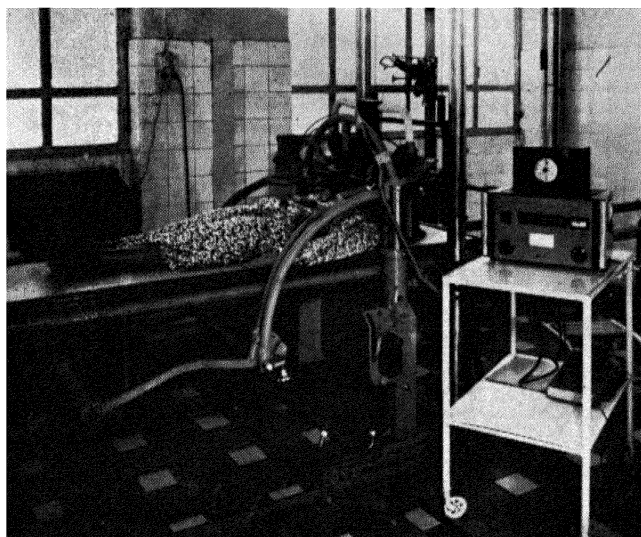


Figure 2 General arrangement of the thyroid concentration measuring apparatus used with I^{131} . Notice the sliding table on which the subject is stretched out, the three counting devices set on an arc of a circle protected by the two lead shields which isolate the neck from the head and body respectively. Closer up, the automatic counting scales made in the Isotope Department Laboratory of Beaujon Hospital

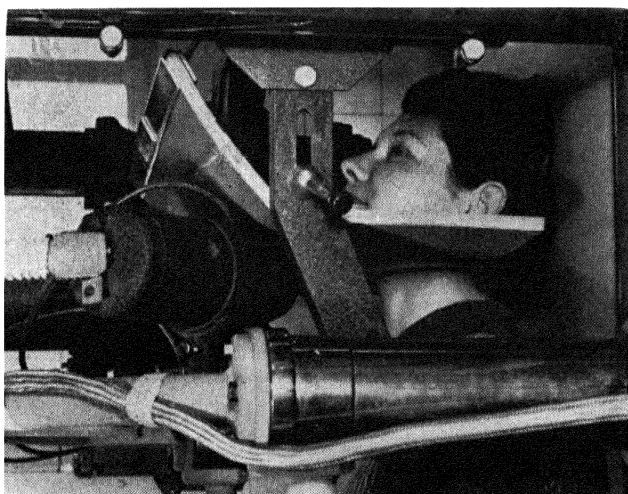


Figure 3. Close-up of the arrangement showing the counters, one of the lead shields, and the movable column which carries the counters

the filled syringe, previously embedded into a paraffine, where it is placed in the same measuring conditions as the thyroid will be. The residual radioactivity of the empty syringe (which may not be negligible) is subtracted from the initial figure.

Under such conditions, the measurements give results which are reproducible within 2% during the first hours, and within 5% 24 hours after the injection. The measurements are made in such a way as to give the largest possible number of points needed to draw a thyroid concentration curve: at 1 hour, 2 hours, 6 hours, 24 hours, and 48 hours if possible; and at the very least at 2 hours, 6 hours, and 24 hours.

It will be shown that it is totally inadequate to use only one point on the curve, particularly if this

point is taken, as an example by many American authors, 24 hours after the injection.

Figure 4 shows the various 48-hour thyroid fixation curves. With our technique, a normal subject fixes $20\% \pm 5$ of the injected dose within two hours, and $40\% \pm 10$ in 24 hours. The method can be made more sensitive by computing, as advised by Pochin, the ratio of concentration in the thyroid to that in the thigh or calf; this last concentration in an indifferent tissue is related to that in the plasma, conveniently giving a ratio (neck/tissue) which is a good approximation of thyroid clearance. Normally, with our technique, this ratio is somewhere between 10 and 15.

Figures 6 and 8 show the direct photographic recording of two thyroid and sural concentration curves, one pertaining to a euthyroid subject, the other to a patient suffering from hyperthyroidism.

2. The thyroid hormone blood level can be observed by following the passage of labeled protein-bound iodine to the plasma

Computation of the total plasma concentration of tagged iodine during the first 72 hours by iterative measurements first shows a progressive drop in that level due to combined thyroid fixation and urinary excretion.

In hyperthyroidism, the plasma level curve goes up again after a variable period of time, and this is due to the fact that hormone- (or protein-) bound iodine is being poured into the blood.

This can be shown in various ways: (a) indirectly, by a progressive decrease in the renal clearance of iodine (it will be all the lower as the protein-bound fraction of the iodine, which is not excreted by the kidneys, is larger (see Figs. 9 and 10), and (b) directly, by a more specific dosage of the protein-bound I^{131} found in the plasma (this is done by eliminating the non protein plasma iodine by dialysis—see Fig. 10).

American workers such as Scott, Reavis and White¹ suggested that the ratios of inorganic to protein-bound iodine be evaluated by investigating

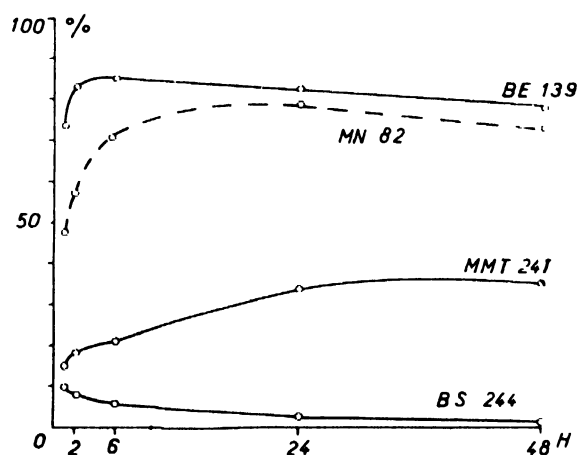


Figure 4. Thyroid fixation curves. (MMT 241) normal subject, (BE 139) gross hyperthyroidism, (BS 244) myxedema, (MN 84) simple goiter, avid for iodine

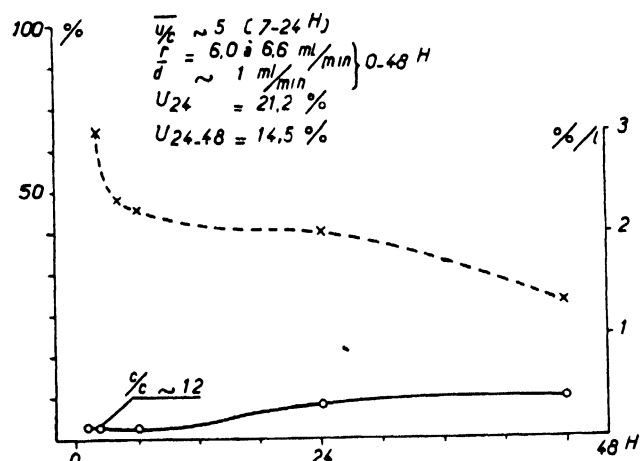


Figure 5. Curves showing thyroid fixation (solid line: left scale) and plasma level (dotted line: right scale) in myxedema with renal insufficiency. (C/C) neck / tissue ratio; (U/C) average ratio of total tagged iodine concentration in the urine to that found in the plasma; (r) renal clearance; ($\bar{d}$) average diuresis; (U) fraction excreted through the kidneys

the red cell plasma iodine ratio. It is a known fact, indeed, that the concentration of inorganic iodine in the red cells is approximately one half of that found in the plasma, while protein-bound iodine does not penetrate the cells. Under these conditions, Scott, Reavis and White showed that this normal ratio, which normally stands at 0.50 for the first hours then drops, all the more rapidly and all the more markedly, as thyroid hormone release is more rapid. Courier, Morel, Collonge and André² investigated this ratio experimentally, and showed it interesting.

We, for one, have found that, in 78 cases of hyperthyroidism, the ratio (red cell I^{131} /plasma I^{131}) at the end of 47 hours, was 72 times lower than

10 20 30 40 50 10 20 30 40

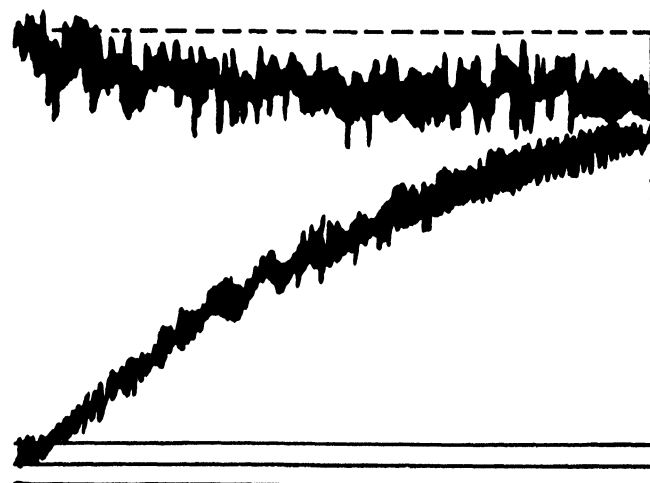


Figure 6. Variation of thyroid (lower curve) and calf (upper curve, inverted) activities in a normal subject during the first two hours following the administration, by the intravenous route, of a tracer dose of I^{131} . Note: the curves on Figs. 6 and 8 were obtained with two pulse integrating ratemeters and a photographic recorder, with two galvanometers. Three counters arranged on an arc of a circle around thyroid. One counter is directed to calf of the leg

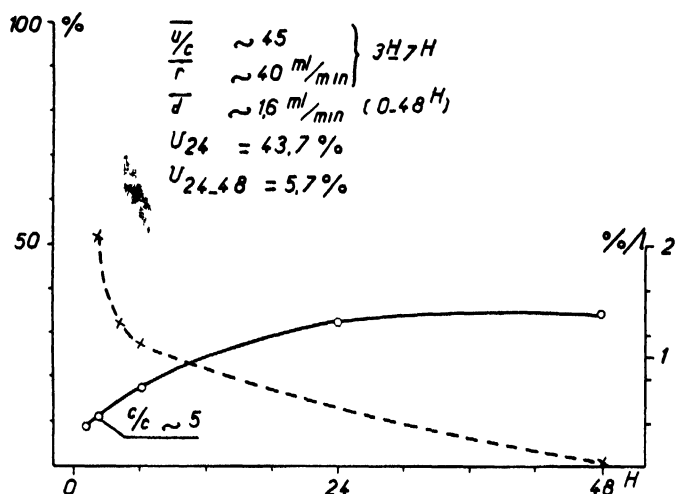


Figure 7. Thyroid fixation (solid line: left scale) and plasma level iodine in the urine and the plasma; (r) average renal clearance; ratio; ($\bar{u/c}$) average ratio between concentrations of total tagged iodine in the urine and the plasma; (r) average renal clearance; ($\bar{d}$) average diuresis; (U) fraction eliminated by the kidneys

normal. Similarly, in 69 cases of hyperthyroidism out of 75, the ratio was markedly smaller, after 48 hours, than after 2.

We have also taken, as an approximate measure of tagged protein-bound iodine, the difference between the plasma level and double the red cell level. Under these conditions, in 68 cases of hyperthyroidism, at the 48th hour, the plasma protein-iodine level evaluated this way is substantially above normal.

Figures 14 and 16 show the variations of the cell to plasma ratio in one normal subject and one hyperthyroid patient. Figure 15 shows these variations in a case of myxedema.

Before proceeding to review our results, there is an all too often neglected fact, bearing on the interpretation of the exploration of the thyroid by radioactive iodine which should be interpreted: the movements of iodine in the system can be evaluated quantitatively only as a function of the specific activity of radioactive iodine, which, in turn, can be computed only in the knowledge of the chemically determined total values of protein-bound iodine.

RESULTS

The results of the dosages made in fifteen hundred thyroid studies by means of I^{131} will now be described. In the first place, here are some illustrations showing typical curves of thyroid and plasma concentrations in euthyroidism (Fig. 7), in hyperthyroidism (Fig. 9) and in hypothyroidism (Fig. 5) respectively. In these three cases, the thyroid and plasma concentration curves agree. This, however, is not consistently true, and is precisely the most interesting feature in any comparative study of thyroid and plasma level curves, by making it possible to reveal functional dissociations which measurements of I^{131} levels in the thyroid would, of themselves, fail to objectivize. Such is the case, for instance, in plain goiter attended with a high iodine absorption

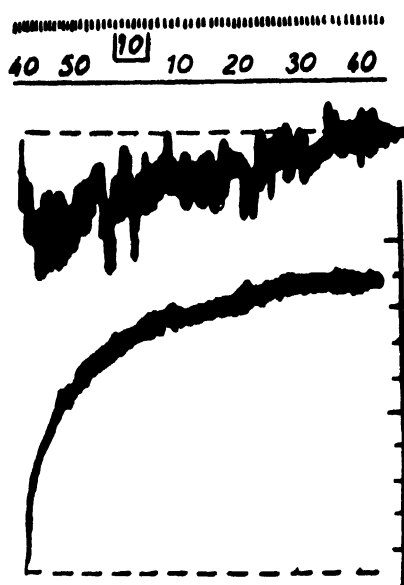


Figure 8. Variation of the thyroid (lower curve) and calf (upper curve, inverted) activities in a person suffering from extreme hyperthyroidism, during the first hour following administration, by the intravenous route, of a tracer dose of I^{131}

capacity in which hyperthyroidism is not paralleled by unduly high thyroid hormone plasma levels. Such, also, are the cases in which I^{131} evinces a therapeutic action in hyperthyroidism.

We now present the over-all results of fifteen hundred thyroid studies carried out with I^{131} . Figure 13 shows comparative results of thyroid fixation after 2 hours, of maximum fixation, of the neck/tissue ratio, and of basal metabolism, in cases of hyperthyroidism, hypothyroidism and plain goiter. It will be observed that the normal and pathological findings show practically no overlapping, as compared with the observations of a number of English language workers. This, we believe, is due to the greater precision of our measuring technique.

Figures 14, 15 and 16 also show curves giving a

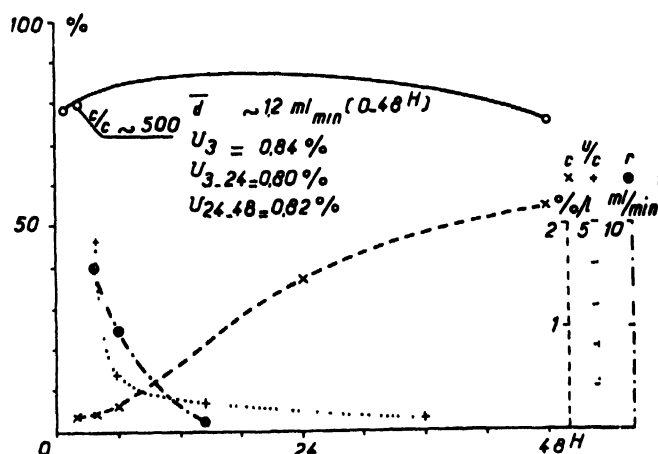


Figure 9. Thyroid fixation curves (solid line: left scale), plasma and urine levels, renal clearance (right scale), in a subject suffering from marked hyperthyroidism. (c/c) neck/tissue ratio; (c) plasma concentration; (u/c) ratio between the concentrations of total tagged iodine in the urine and plasma respectively; (r) renal clearance; ($\bar{d}$) average diuretics; (U) fraction eliminated by the kidneys

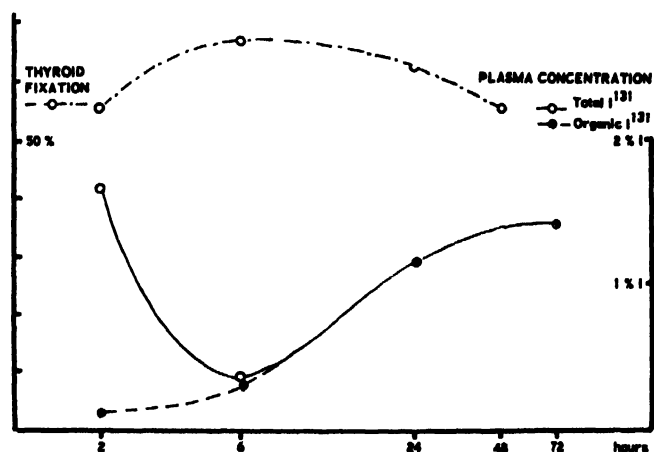


Figure 10. Hyperthyroidism. Mixed lines, thyroid fixation curve (left scale); solid line, plasma concentration curve (total I^{131}); dash-dot, plasma concentration curve (protein bound I^{131})

complete study of thyroid fixation, of the plasma and red cell levels, and of the variation in the red cell/plasma ratio, between the 30th minute and the 72nd hour, in a normal subject, a myxedema patient and one suffering from Graves's disease, all being under treatment.

Such are the currently used techniques for the functional exploration of the thyroid. It goes without saying that radioactive iodine lends itself to other research, such as the making of autoradiographs of thyroid sections, and a study of the distribution of the thyroid hormone in the plasma proteins.

Figure 18 shows an electrophoresis spectrum revealing the fixation of the tagged hormone by a "globulin alpha 1" fraction of the plasma. Figure 17 shows our instrumentation for the automatic analysis of the radioactivity of the various protein fractions by paper electrophoresis.

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APPENDIX

The complete mathematical analysis of the fate of tagged iodine administered during a functional exploration is very complex, in view of the many overlapping phenomena (see Fig. 1). However, as a first approximation, two fundamental stages may be distinguished: (1) the fixation of ionized labeled iodine by the thyroid, and (2) the secretion and utilization of the tagged thyroid hormone, which, in turn, takes place in two independent and distinct stages.

Utilization of Primary Tagged Inorganic Iodine

Let: A^* be the activity injected, i^* and u^* the quantities of tagged iodine fixed by the thyroid and excreted by the kidneys at a given instant τ , K and R the plasma clearances of the thyroid fixation and

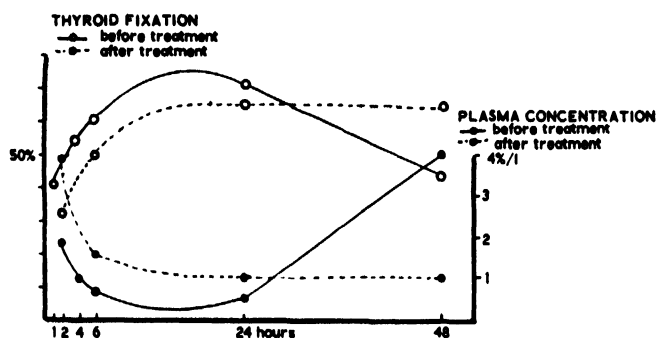


Figure 11. I^{131} in the treatment of Graves's disease. Before treatment: delayed "escape angles" (24-48 hours) in the thyroid fixation curve; very sharp ascending branch (24-48 hours) in the plasma concentration curve. Following treatment: no "escape angle" in the thyroid fixation curve; no new increase in the plasma concentration

renal excretion functions for the circulating inorganic iodine, c^* the plasma level of tagged (labeled) inorganic iodine, and v^* the tagged inorganic iodine space, symbolically defined as a function of its plasma level. Then

$$v^* = \frac{A^* - (t^* + u^*)}{c^*} \quad (1)$$

The variation in the plasma concentration (c^*) of tagged mineral iodine, as a function of the time τ , elapsed after the injection, is given by the equation:

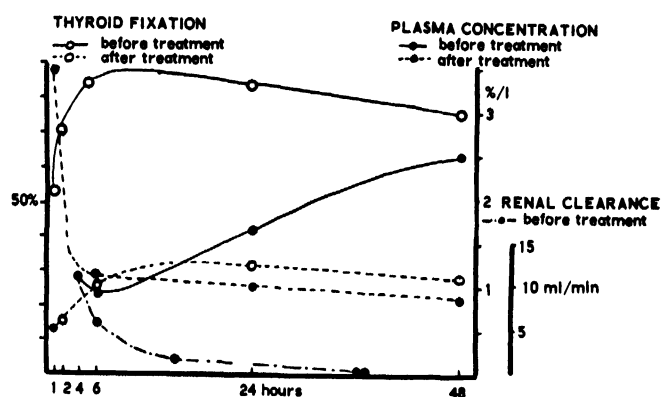


Figure 12. Hyperthyroidism treated by I^{131} . Before treatment: thyroid fixation is much increased with a sharp decline in plasma concentration curve, and a typical decrease in renal clearance. After treatment: normal thyroid fixation; no plasma concentration curve decline

$$\frac{d}{d\tau} (c^* v^*) = - (K + R) c^* \quad (2)$$

The amounts of activity fixed by the thyroid and excreted by the kidneys at instant τ thus are:

$$t^* = K \int_0^\tau c^* d\tau \quad (3)$$

$$u^* = R \int_0^\tau c^* d\tau \quad (4)$$

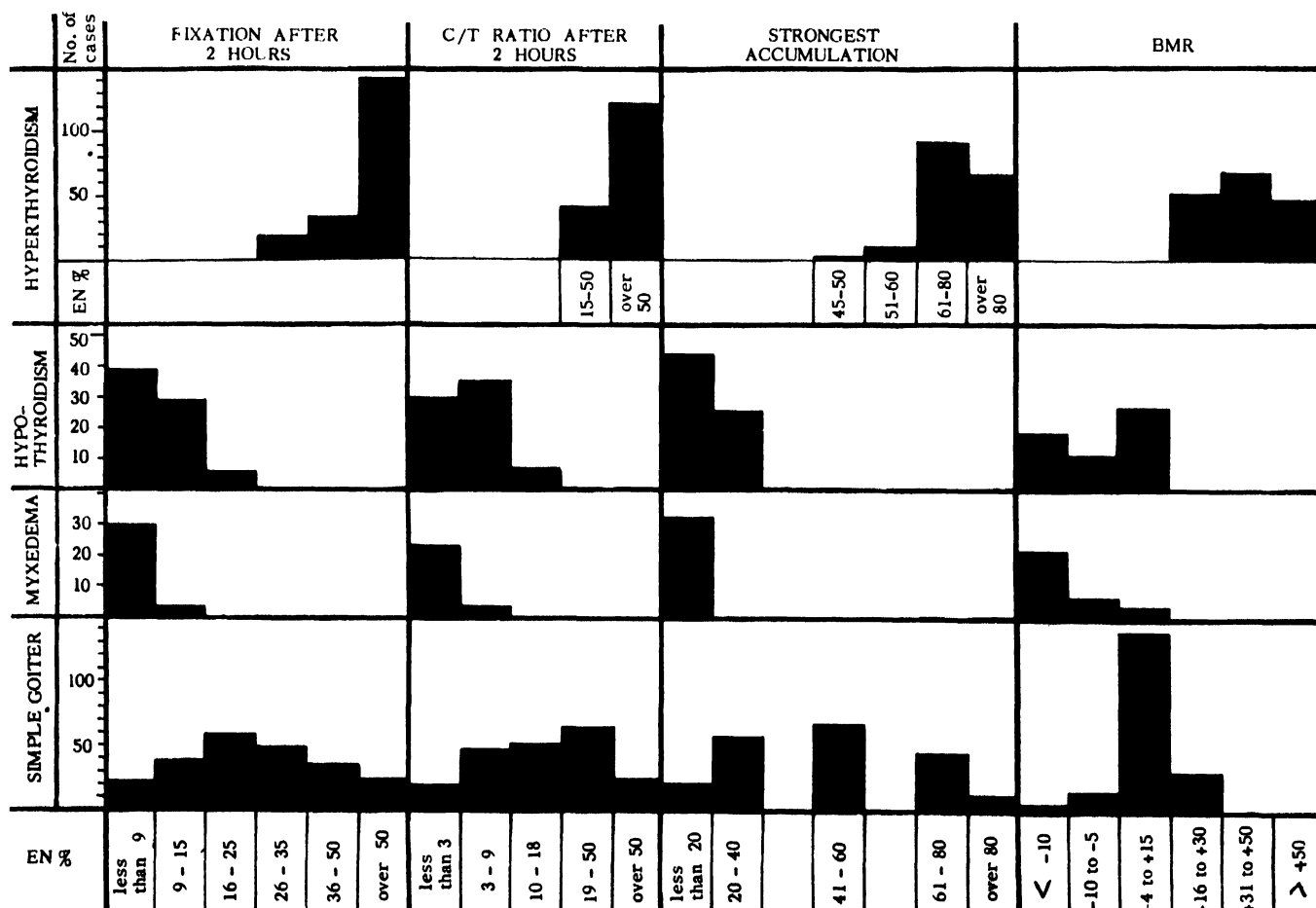


Figure 13. Statistical summary of the results of thyroid exploration with I^{131} bearing on four groups of patients: 189 cases of hyperthyroidism, 73 cases of simple hypothyroidism, 34 cases of severe myxedema in the adult, 215 cases of non-toxic goiter. We show, on

this table, for each group of patients: (1) thyroid concentration within 2 hr (normal: $20\% \pm 5$); (2) neck/tissue ratio after two hr (normal: 10 to 15); (3) maximum thyroid accumulation of I^{131} (normal: $40\% \pm 5$); (4) B M R

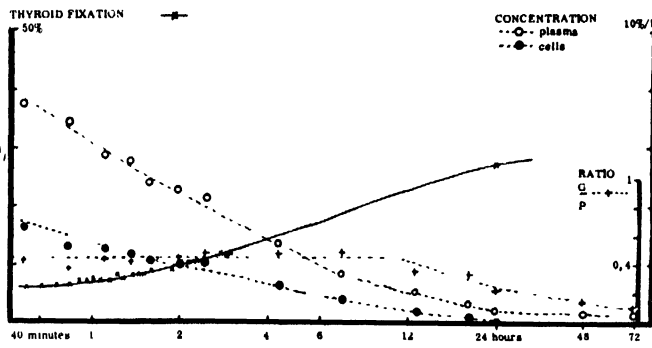


Figure 14. Thyroid fixation curves (solid line: left scale) plasma and cell concentration, changes in cell to plasma ratio (dotted line, right scales) in normal subject. If there is no apparent new increase in the concentration of the radioactive iodine, the belated decrease in the cell/plasma ratio, on an average 0.45 during the first hours, shows the apparition of tagged circulating hormone 12 hours later

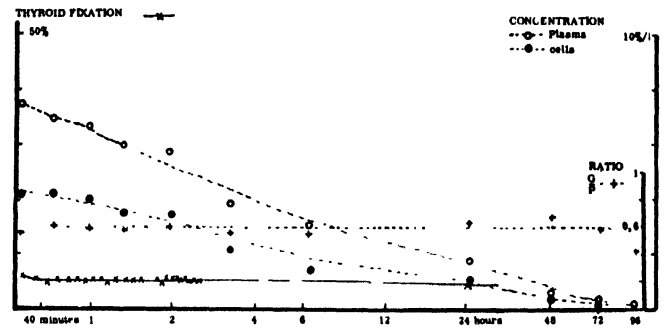


Figure 15. Curves showing thyroid fixation (solid line: left scale) plasma and cell concentration, changes in the cell/plasma ratio (dotted line: left scale) in hypothyroidism. The cell/plasma ratio remains constant and substantially equal to 0.6 for 72 hours: the secretion of tagged thyroid hormone thus remains negligible even three days after the administration of the tracer dose of I^{131}

Equations 1 to 4 state that tagged iodine, distributed in the extracellular spaces, is extracted from those by the thyroid and kidneys at rates defined by plasma clearances K and R , and proportionally to the plasma level c^* . These ratios are very general and do not imply any limiting postulate regarding the law expressing the dependency of the tagged inorganic iodine space on time.

It can be shown experimentally that space v^* changes very rapidly during the first hour (reaches 18 liters, on an average, in a normal 70 kg subject) and thereafter much more slowly (it stands at approximately 25 liters after six hours). Thus, equations 2, 3 and 4 can be made explicit, with a good approximation, by substituting a constant, V_0^* for τ^* :

$$c^* = \frac{A^*}{V_0^*} \exp \left\{ -\frac{1}{V_0^*} (K + R) \tau \right\} \quad (2a)$$

$$t^* = \frac{K}{K + R} A^* \left[1 - \exp \left\{ -\frac{1}{V_0^*} (K + R) \tau \right\} \right] \quad (3a)$$

$$u^* = \frac{K}{K + R} A^* \left[1 - \exp \left\{ -\frac{1}{V_0^*} (K + R) \tau \right\} \right] \quad (4a)$$

At the limit, the injected tagged iodine is distributed proportionally to K and R between t^* and u^* : this is confirmed in practice, with the one difference that this distribution applies roughly to only $\frac{9}{10}$ of the injected activity, (the balance allows for interference with the second phase of the iodine cycle).

By means of a simple measurement of the amount of radioactivity fixed at the level of the thyroid and

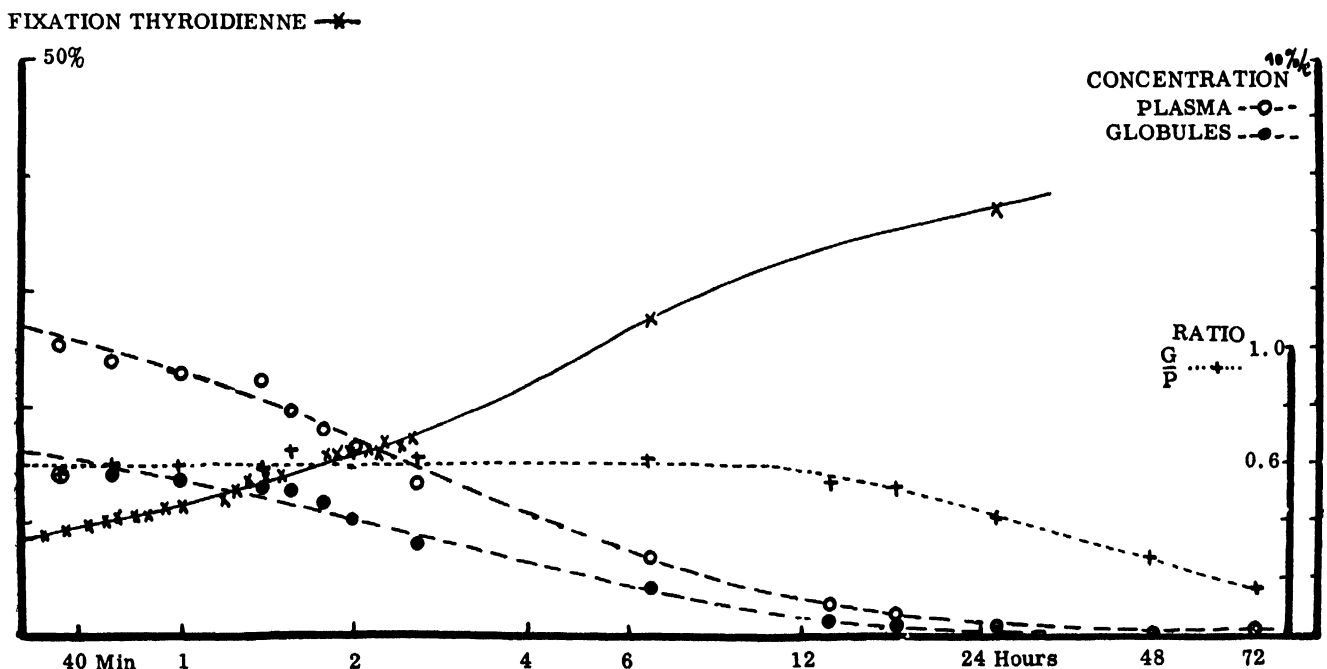


Figure 16. Curves showing thyroid fixation (solid line: left scale), plasma and cell fixation, changes in the cell/plasma ratio (dotted line: right scale) in I^{131} treated Graves's disease. The results of functional exploration are in complete agreement with those found in the normal subject (see Fig. 14)

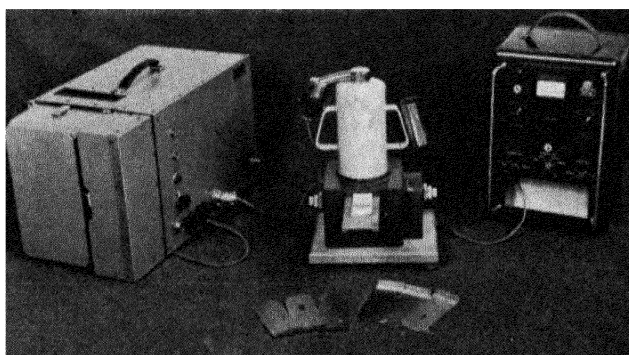


Figure 17. Automatic set-up such as used in the Laboratory of the Isotope Department of Beaujon Hospital for the Geiger counter exploration of the protein fractions separated by paper electrophoresis. The instrument shown at the center is the analyzer, in which the electrophoretic spectrum travels against the window of a Geiger counter of the "bell" type: the line observed at any given time is limited by a brass shutter chosen according to the fineness required and the radioactivity of the strip. The average radioactivity observed is measured by a three sensitivity impulse scaler on the right and recorded by a mirror galvanometer on a photographic film (left), the motion of which is synchronized with that of the electrophoretic spectrum moving before the Geiger counter

carried out, for instance, at the end of two hours (when the differentiation between the normal and increased K is best), it is possible to evaluate the thyroid clearance for the iodide in circulation, with sufficient accuracy for diagnostic purposes.

It would appear, at first sight, that a late measurement (at the end of 24 hours for example) of thyroid fixation, or of the activity recovered from the urine (u^* and t^* being complementary) would give equivalent results. Yet, a clinical test based on a single measurement of thyroid fixation (or renal excretion) after 24 hours, gives deceptive results whenever there is impaired renal function, not to mention interference with the hormone phase of the iodine cycle, which, in hyperthyroidism, already is considerable at the end of 24 hours.

Thus, the measure of fixation at the end of 2 hours remains at the base of any evaluation of the circulating iodide thyroid clearance. A late reading, taken after 24 hours, does remain very worth while, either as a confirmation, or mainly because it can draw attention to the existence of a renal insufficiency which may not have been detected. This, in some instances, will broaden the clinical importance of the radioactive iodine test.

While it is true that it is always possible, by a series of measures of t^* and c^* , for instance, to evaluate K with a better accuracy than that afforded by mere measures of thyroid fixation, the fact remains that the measures of radioactivity, of themselves, only give a *relative* notion of the avidity of the thyroid for the circulating iodides.

A very important question now arises: What are the ratios between K and the functional activity of the thyroid, which can be expressed by the quantity Θ of hormone-bound iodine secreted per unit of time?

Under conditions of physical equilibrium (an equilibrium which is practically not altered by adding a tracer in the dosages currently used), the total amount T of iodine present in the thyroid remains constant:

$$\frac{dT}{d\tau} = KC - \Theta = 0 \quad (5)$$

whence:

$$KC = \Theta \quad (5a)$$

C being the plasma level of the inorganic iodine.

Under these conditions, and provided the iodine level C be normal, there is a close correlation between K and Θ , which is at the base of the functional exploration of the thyroid by *in vivo* fixation measurements.

In reality, the quantitative evaluation of Θ remains closely bound to that of C , which is one of the most difficult physiological data to determine, due to the extremely low concentrations of circulating inorganic iodine (normally, a few γ per liter) which are accompanied, in addition, by relatively high concentrations of hormone-bound iodine (a few tens of gamma per liter, normally).

There is a method which is more direct, and at the same time, technically simpler, whereby an absolute value of the avidity of the thyroid for the circulating iodides can be computed, regardless of C .

Tagged iodine, as soon as it is injected into the system, makes it possible to trace the whole of the circulating iodides: the amounts of iodine fixed by the thyroid, t , and excreted by the kidneys, u , in time τ are:

$$t = KC\tau \quad (6)$$

$$u = RC\tau \quad (7)$$

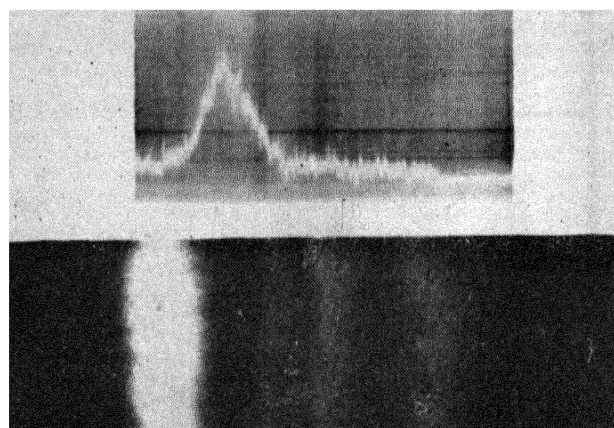


Figure 18. Radioelectrophoretogram of the serum in severe hyperthyroidism, taken 72 hours after the administration of a tracer dose of radioiodine-131. Below, the electrophoretic spectrum as revealed by bromophenol blue (left to right, in the order: albumins, alpha 2, beta and gamma globulins). Above, the relevant radioelectrophoretogram obtained with a set-up such as that shown on Fig. 17. The tagged circulating thyroid hormone is localized in the globulins of maximal electrophoretic mobility (alpha 1 globulin area)

It will be seen immediately, allowing for Equations 3 and 4, that it is possible quantitatively to evaluate t by two radioactivity measurements (t^* and u^*) and the chemical dosage of the total amount of inorganic iodine found in the urine at time τ :

$$t = \frac{t^*}{u^*} u \quad (8)$$

In order to make use of Equation 8, it is only necessary to assume that K and R remain constant through the duration of the test, which, in any case, must apply only to the very first hours following the administration of the tracer, in order to avoid interference with the second phase (hormone) of the iodine cycle.

As a matter of fact Equation 8 strictly holds good, regardless of the law according to which v^* varies as a function of time.

The measurement of t^* and u^* offers no technical difficulty. Figure 2 shows a typical setup for a measurement of t^* . Since u^* on the other hand is of the same order of magnitude, it can be measured very simply by external counting, with a standard container holding the whole of urine excreted during the test period and made up to a given level, then set up in a readily reproducible position, in the vicinity of one or several Geiger counters.

The situation is different when dealing with the chemical dosage of urinary inorganic iodine, which still raises difficult problems: the determination of u remains much easier than that of C , for the concentration of inorganic iodine in the urine is much higher than its plasma levels (normally, it exceeds 100 γ per liter) and the proportion of accompanying hormone-bound iodine is much smaller, very often negligible.

Metabolism of Tagged Hormone Bound Iodine

The equations bearing on the second phase of iodine metabolism (secretion and use of tagged thyroid hormones synthesized by the thyroid) are more complex: the tagged hormone secreted in the organic iodine space secondarily dissociates in the peripheral tissues into ionic-iodine, a part of which is again fixed by the thyroid.

The evolution of t^* —the thyroid radioactivity—and X^* , the radioactivity distributed in the "organic iodine" space, is determined approximately by a system of two linear differential equations of the first order with constant coefficients:

$$\frac{dt^*}{d\tau} = \rho b X^* - a t^* \quad (9)$$

$$\frac{dX^*}{d\tau} = a t^* - b X^* \quad (10)$$

in which ρ is the regeneration coefficient of tagged thyroid hormone, namely, the fraction of the secreted hormone bound iodine which is again fixed by the thyroid; $a = \Theta/T$, the fraction of thyroid iodine secreted per unit of time; and b , the proportion of extrathyroid hormone iodine which migrates from the organic iodine space per unit of time, namely Θ/X , X being the total amount of extra thyroid hormone-bound iodine.

The time origin is arbitrarily set to an instant on which the fixation of primary tagged ionized iodine is deemed finished, the second phase of the cycle of the injected tagged iodine not yet begun.

The variations of t^* and X^* as a function of time is difficult to interpret quantitatively in terms of the fundamental constant Θ : it depends, in particular, on the value of T , which varies a great deal from one individual to another. Broadly, t^* first decreases according to an exponential law, $\exp(-a\tau)$, a decrease which then slows down and normally tends to correspond to $\exp[-a(1-\rho)\tau]$. The initial "escape angle" observed at times in hyperthyroid cases then is all the more marked as a is larger, but gives only a loose quantitative indication of Θ while, reciprocally, the absence of a significant angle of escape does not necessarily indicate that there is no increase in the absolute amount of hormone-bound iodine secreted per unit of time by the thyroid.

As regards X^* , it increases until it reaches a maximum, and thereafter decreases according to a curve substantially parallel to t^* at the limit.

While a quantitative measurement of Θ is much more difficult, starting from Equations 9 and 10, than when starting from 5a, 6, 7 and 8, the measurement of the plasma concentration of tagged protein-bound iodine does have considerable clinical interest.

The early appearance of tagged hormone in the circulation in measurable amounts indicates the accelerated regeneration of the circulating hormone, and thus bears a close correlation to a high value of Θ : a two-branch plasma concentration curve, which first decreases rapidly (use of ionic-iodine), and then clearly goes up (appearance of tagged organic iodine) is typical of hyperthyroidism. By measuring the radioactivity of the plasma 48 hours, or better still 72 hours after the injection, and determining the cell to plasma ratio, one can clear up the few cases in which, for instance, a reduced value of C or a condition of iodine unbalance makes the application of Equation 5a uncertain in the interpretation of the fixation curves.

Diagnosis of Thyroid Function in 520 Patients with I^{131} : Diagnosis Possibilities in Patients Where the Iodine Metabolism Is Pharmacologically Disturbed

By V. H. Franco, L. Botelho, W. Clode, A. M. Baptista, M. A. Perez Fernandez and
M. L. Martins,* Portugal

Evidence of several years has shown that the radioiodine test is an accurate method for the differential diagnosis between euthyroid and hyperthyroid subjects. If the test is made in patients with the iodine metabolism not disturbed most of the proposed techniques are equally accurate. However, the presence of supplementary iodine from previous administrations of stable iodine either for diagnostic purposes or therapeutic ones, is considered to invalidate the radioiodine test whatsoever the method used. In our opinion this comes from the limitations of the radioiodine tests which did not exhaust the intrinsic possibilities of the method. In other words, the problem is considered rather unilaterally.

The iodine uptake curve of a thyrotoxic gland is different from a normal one because the increased metabolic activity leads to a higher accumulating power, which causes a greater percentage of plasma-
tic iodine to be absorbed.

The following are the bases of the diagnosis methods usually followed: (a) analysis of the initial part of the uptake curve with or without the evaluation of the plasma clearance rate;^{1,2,3,4} (b) thyroid uptake at a standard time after administration.^{5,6} However, the hyperthyroid gland not only absorbs a greater percentage of the plasma iodine but also uses it more rapidly due to an increasing rhythm of the phenomena which leads to the hormonal biosynthesis and hormonal excretion. There is an acceleration of the intra-glandular turnover of iodine. Accordingly, we think that the iodine uptake curve could show us the dynamic aspect of the gland function and consequently some data impossible to obtain from the other methods above mentioned.

This paper reports the results of the radioiodine uptake test in 520 patients selected according to the following conditions:

1. Patients from the Endocrinology Service of this Institute and consequently subjected to an uniform diagnostic criterion.

2. All of the patients were studied by other laboratory methods such as the basal metabolic rate and,

in certain cases, ballisto-cardiogram and the serum cholesterol analysis.

3. All the doubtful cases were followed during a sufficient period in order to observe the spontaneous disease evolution.

Our plan was to evaluate the possibilities of the radioiodine uptake techniques in patients with a definite clinical diagnosis.

METHOD

We have measured the uptake of radioiodine I^{131} given to a fasting patient at 2, 6 and 24 hours after the administration. Of course we have to limit the number of the uptake measurements in order to simplify matters. As we shall see later it would be convenient to have some point between the 6 and 24 hour measurements.

Measurements were carried out with a scintillation counter (thallium activated sodium iodide 1.2×1.2 cm) associated to a ratemeter with a single discriminator built in. The discriminator was set at 20 v which for a 136 v interdynode voltage of the photomultiplier EMI 5659, with an amplifier gain of 400, is sufficient to minimize the Compton scattering effects in the measurements conditions.

The activity to be administered was in a spherical bottle of 50 cm³ capacity inside a hole in the neck of a paraffin phantom (Fig. 1) at 40 cm from the scintillation head.

By measuring the counting rate with and without a lead shield 1 cm thick just big enough to cover the neck we can, by placing the patient after the doses administration at the same distance from the scintillation detector and making two measurements with and without the lead shield (Fig. 2), calculate the percentage of the doses in the gland at a given instant.

EXPERIMENTAL RESULTS

General

The frequency chart of Fig. 3 shows 341 cases of euthyroid uptakes obtained at 2, 6 and 24 hours after the radioiodine administration. Figure 4 shows 180 cases of hyperthyroidisms. From the study of these charts it can be concluded that:

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(a) The upper limit values for the euthyroidisms seem to be 30% at 2 hours, 40% at 6 hours and 50% at 24 hours.

(b) Endemic goiter shows in many cases values similar to the hyperthyroidism ones (18 cases, Table I).

(c) The only exceptions from these cases are 1% of the total (5 cases) euthyroid subjects (Fig. 3 and Table II) which show uptakes higher than 30% at the two hours measurement. On the contrary the number of the hyperthyroid patients with normal range uptakes at 2 hours (less than 30%) is 65 (13% of the total).

(d) With the 6 and 24 hour values, the number of diagnostic errors is almost the same for the euthyroid and hyperthyroid subjects, but then a progressive improvement is obtained when we displace towards the 24 hour measurement. The percentage error, if we take into consideration only the 2 hour uptake measurement, is 16.5% (86 cases), the 6 hour value is 15.2% (97 cases) and the 24 hour value 13.3% (69 cases). The 24 hour uptake measurement, which is one of the more popular tests, gives us similar results (accuracy 86.7%) to the ones obtained by other authors.⁵

(e) The greatest accuracy for hyperthyroid diagnosis can be obtained if we consider thyrotoxic the patients who have at least one uptake higher than the limits. The accuracy of this method is 87.5%. The percentage error is therefore 12.5% (65 cases): 31 hyperthyroid patients with all the uptake values within the normal range and 34 euthyroid subjects with high uptake values being 18 endemic goiters.

As we have seen, making the diagnosis with the three uptake values, the over-all accuracy is almost the same as if we only take the 24 hour uptake. This fact explains the preference given by many authors to the 24 hour uptake, since this greatly simplifies the test.

We must however point out that some of the errors do not correspond to the same individual patients: there are 9 hyperthyroid patients with the 24 hour uptake lower than 50% (Table III), but also there are 5 euthyroids with high values at 2 and 6 hours, but normal ones at 24 hours.

We will now see if these results can be improved by considering and interpreting the uptake curve.

Disturbances of the Iodine Metabolic Rhythm, in the Thyroid Gland

It is well known that the secretion rate of the hormonal iodine is increased in hyperthyroid patients or, what is the same, there is an acceleration of the intraglandular iodine turnover (Gross and Pitt-Rivers, 1952). This means that the maximum uptake comes earlier and the effective half-life of radioiodine is lessened.

We have confirmed that in a great number of cases, 36.1%, in hyperthyroid patients the uptake at 6 hours was higher than at 24 hours. That is, this time is quite within the excretion period.

In euthyroid patients on the contrary, 2 hours after the radioiodine administration we are still in the secretion period or at least near the maximum uptake point, that is, in the equilibrium moment between the two phenomena.

In 63.9% of the hyperthyroidism cases, however, curves were progressively increasing. This phenomenon can be explained by the fact that between the uptake measurement at 6 hours and the one at 24 hours there was not any measurement and a down inflexion near and before 24 hours cannot be detected.

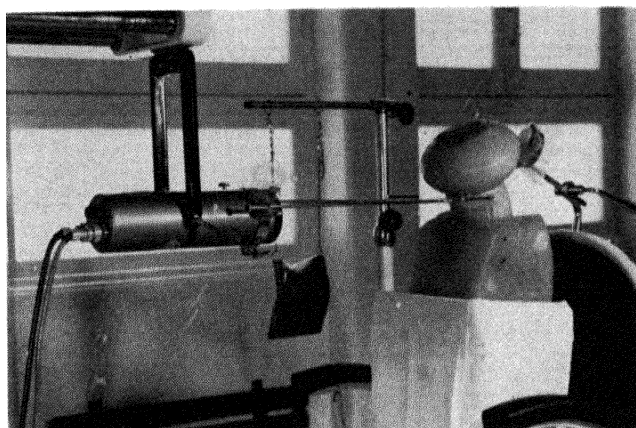


Figure 1. Paraffin phantom to reproduce scattering conditions. The ^{131}I solution is in a bottle inside the neck

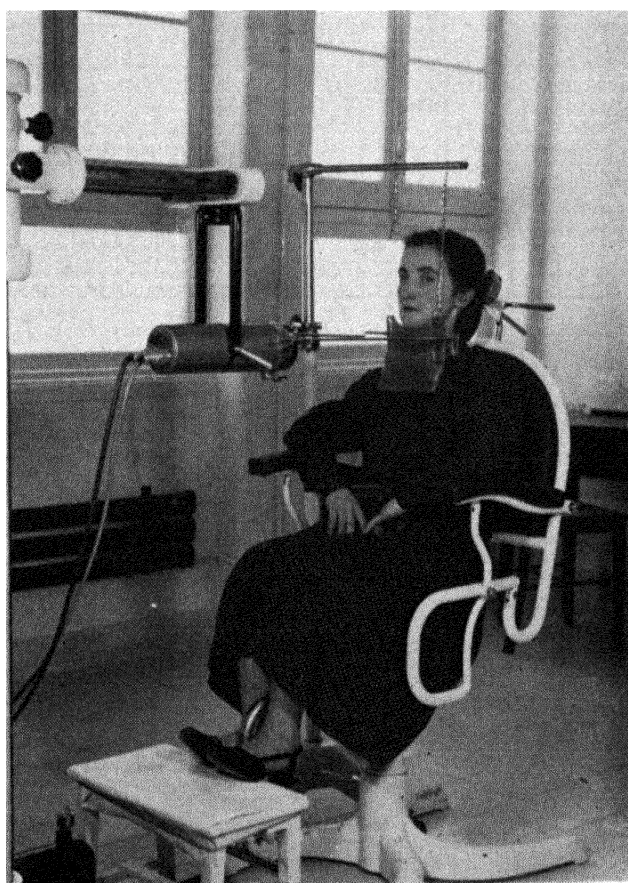


Figure 2. With two measurements (with and without the lead shield) and with two other similar measurements with the paraffin phantom (Fig. 1) the percentage of the administered activity in the gland can be calculated

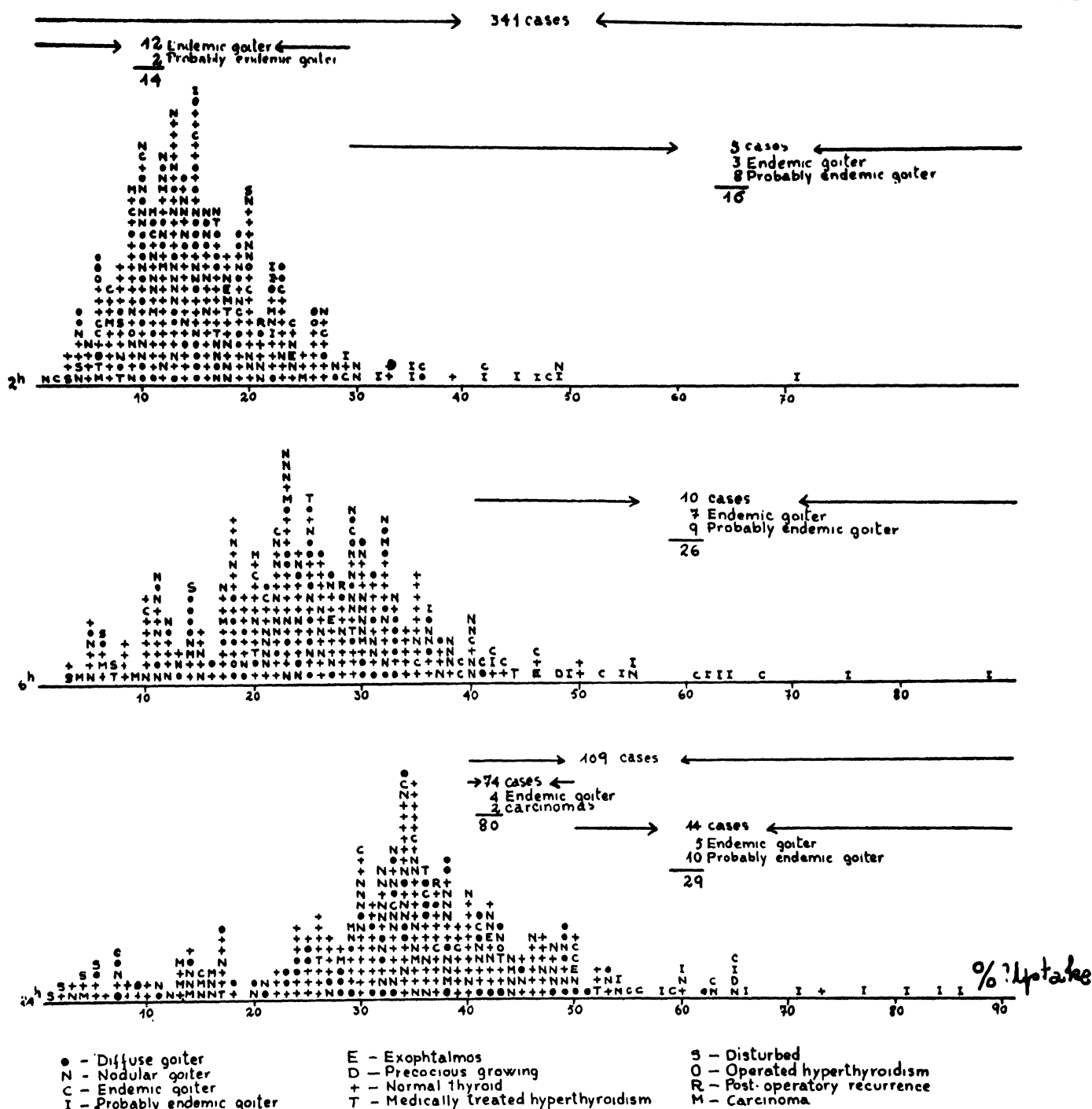


Figure 3. Frequency chart for euthyroid cases. Uptake in abscissae as percentage of the administered activity in the gland

In any case this very fact shows us that due to our timetable of measurements the severeness of the hyperthyroidism can be disclosed. In fact the more severe the disease the higher the 6 hour uptake in relation to the 24 hour uptake. On the other hand the mild cases give by this technique progressively increasing curves.

We think that we may reasonably aim at giving not only a diagnostic conclusion but also some idea of the seriousness of the disease.

Summing up, we find in thyrotoxic patients two types of curves: (a) progressively increasing curves, and (b) convex shaped curves. These two types ap-

peared with very high uptake values: in most of the cases with one, two or the three values above the limits mentioned before but sometimes (Table III, cases E and EC) there are curves with normal uptake values.

Hyperthyroidism with Normal Uptake

How can we explain that with the same method there exist thyrotoxic cases with curves of the same shape but with different uptake levels? Many authors believe that the thyroid gland absorbs only that quantity of iodine sufficient for the homonogenesis. That is, the dose absorbed by the thyroid varied inversely with the plasma concentration.⁷

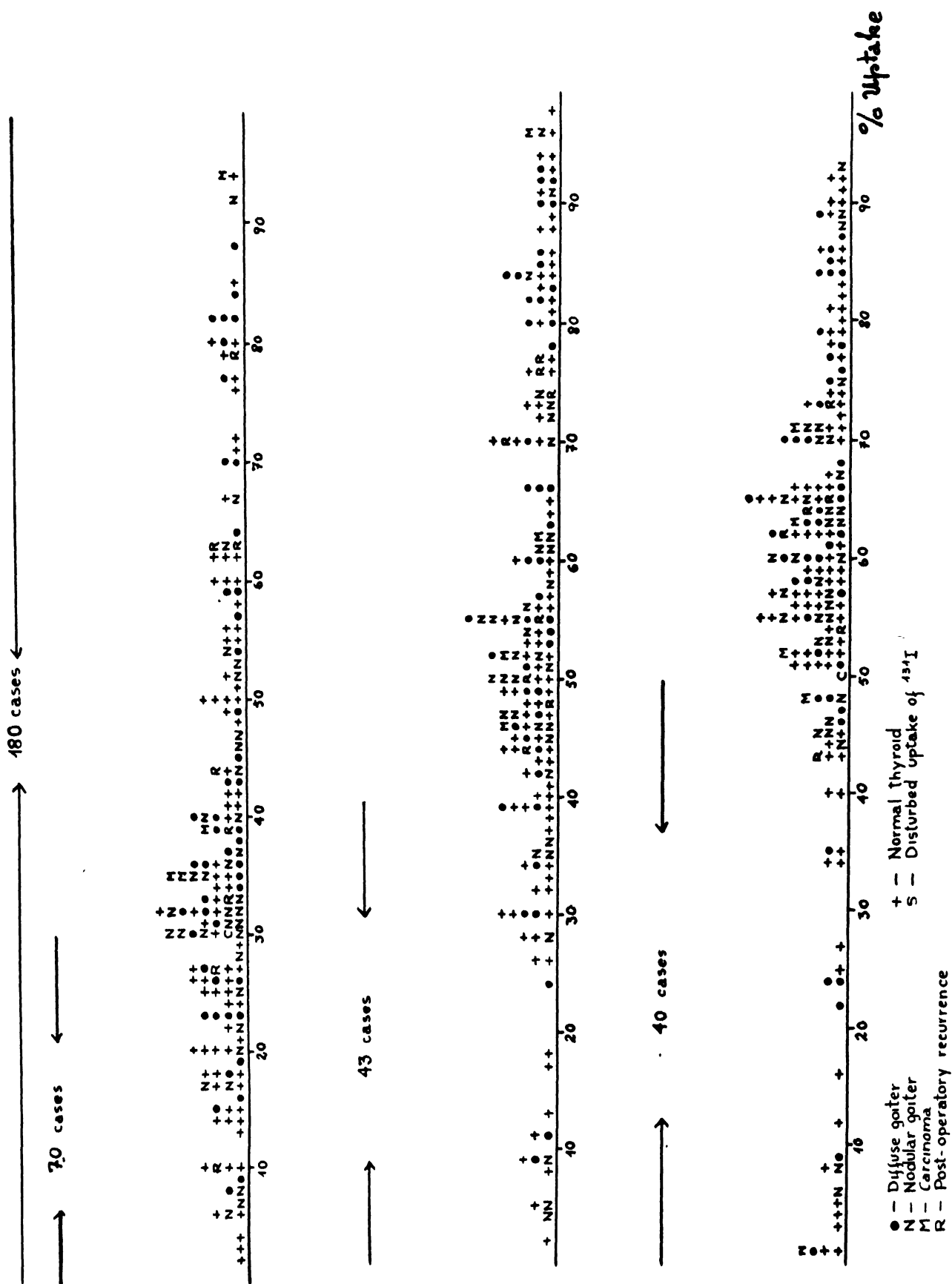


Figure 4. Frequency chart for hyperthyroid patients. Uptake in abscissae as percentage of the administered activity in the gland

Table I. Endemic Goiters

Obs. no.	Patient		Diagnosis	Uptake at		
	Name	Age		2 hr	6 hr	24 hr
59290	J.L.C.	24	Diffuse goiter	42	62	71
79390	M.T.A.S.	15	Diffuse goiter	35	55	65
95353	M.T.J.S.	42	Diffuse goiter op.	36	52	65
93833	J.S.N.	23	Nodular goiter op.	29	46	50
100786	A.P.A.	20	Diffuse goiter op.	48	61	41
102210	M.L.B.	15	Diffuse goiter op.	15	36	59
102911	J.C.	45	Diffuse goiter op.	27	43	56
104950	J.C.V.	17	Diffuse goiter	49	63	84
105037	M.L.S.	19	Diffuse goiter	22	40	54
106673	M.N.A.	18	Diffuse goiter	71	88	86
107106	E.E.G.	21	Diffuse goiter	22	42	58
107983	J.A.B.	19	Diffuse goiter	35	49	43
108832	A.G.J.	24	Diffuse goiter op.	42	67	63
109112	M.C.B.M.	44	Nodular goiter op.	24	42	55
110014	M.L.M.	24	Diffuse goiter op.	22	55	65
115314	M.O.G.M.	10	Nodular goiter	45	64	81
118423	M.A.M.L.	10	Nodular goiter	32	39	60
119068	M.C.S.	13	Diffuse goiter	49	75	77

Table II. Euthyroidism with High Uptake Curves

Obs. no.	Patient		Diagnosis	Uptake at		
	Name	Age		2 hr	6 hr	24 hr
54294	E.G.D.M.	46	Nodular goiter	49	55	63
74187	Z.P.	44	Diffuse goiter menop.	36	41	62
76587	R.C.G.	36	Graves des.	6	44	52
81072	F.N.C.	48	Bilat. exoph.	24	46	50
96272	J.N.L.G.	26	Psychoneurose	29	42	52
100135	M.A.R.L.	30	Cir. climat.	22	43	53
100212	M.A.M.C.G.	21	Psychoneurose	39	50	73
102300	J.M.P.	40	Obesity	23	46	50
102723	M.P.C.	45	Menopause	25	29	52
104044	C.G.L.S.R.	47	Nodular goiter	21	36	54
105687	A.J.B.G.	26	Nodular goiter	18	35	53
105800	C.M.G.	27	Psychoneurose	33	50	60
106116	A.B.	45	Nodular goiter	30	40	65
106579	M.C.A.	53	Nodular goiter	28	38	60
106758	M.L.F.S.C.	14	Precocious growing	33	48	65
115561	M.J.V.	56	Diffuse goiter	17	31	53

Our own evidence, in agreement with many authors, shows that the iodine accumulation function was not significantly altered by increasing appreciably the amount of carrier iodine. With iodide doses of the order of the tenths or hundredths of milligrams, this did not happen. More about this later.

On the contrary if a daily diet of a small quantity of stable iodine is administered during a long period, the accumulating function is lessened and the uptake curve progressively fall. Figures 5 to 9 show this effect in a thyrotoxic patient with a daily intake of 2 mg of supplementary iodine. We may notice that the uptake values after 27 days of the administration are in the range of the normal ones. These facts explain why sometimes there is overlapping of curves with different gland function degree. The two last curves (Figs. 8 and 9) show a very low level of uptake with a convex shaped curve not noticed in the beginning of the experiment (Figs. 5, 6 and 7). This particular shape appeared after increasing the

daily intake of iodine from 2 to 5 mg. We are now studying this problem.

The 18 cases of Table III (E and EC) and the 14 of the first group of Table IV present normal uptake values that correspond to thyrotoxic patients subjected to a previous iodine treatment or to diagnosis techniques with stable iodine organic containing dyes such as these used as contrast drugs.

To conclude, in 32 cases out of 180 with low uptake curves, diagnosis of hyperthyroidism is possible in 17 cases by the convex shape of the curve. It must be emphasized that the peculiar curve shape characteristic of hyperthyroidism as a rule in the low uptake curves series (Table IV) in 16 cases, only one of the curves was concave upwards. On the contrary, in 18 hyperthyroid cases with normal but not too low curves (Table III) only 4 (EC) show the convex shape.

These facts suggest that there is a correlation between the statistical results and the experimental case above mentioned in Figs. 5-9.

Table III. Hyperthyroidism*†

Diagnosis by the uptake at:				Percentage of the administered dose at: 2 hr, 6 hr and 24 hr				Diagnosis by the uptake at:				Percentage of the administered dose at: 2 hr, 6 hr and 24 hr				
Goiter*								Goiter*								
N	2 hr	6 hr	24 hr	47	72	+	67	—	E		20	30	44			
D	2 hr	6 hr	24 hr	36	55		62	N	—	6 hr	24 hr	25	40	57		
—		E		10	30		45	N	—	6 hr	24 hr	30	52	65		
N	2 hr	6 hr	24 hr	53	70	+	64	—	2 hr	6 hr	24 hr	50	64	77		
R	—	6 hr	24 hr	10	55	+	50	—	2 hr	6 hr	24 hr	63	76	+	73	
—	—	6 hr	24 hr	26	50		79	N	—	6 hr	24 hr	30	46	56		
N	—	6 hr	24 hr	17	61	=	63	N	—	—	24 hr	22	35	53		
D	2 hr	6 hr	24 hr	88	+	80	+	76	N	2 hr	6 hr	24 hr	52	74	=	75
M	2 hr	6 hr	24 hr	94		96	+	71	D	2 hr	6 hr	24 hr	33	45	62	
N		E		20	35		45	N	2 hr	6 hr	—	40	50	46		
	2 hr	6 hr	24 hr	35	55		65	—	2 hr	6 hr	24 hr	32	54	=	57	
	2 hr	6 hr	24 hr	32	50		60	—	—	—	24 hr	20	34	56		
—	2 hr	6 hr	24 hr	41	50		67	—	2 hr	6 hr	24 hr	87	95	+	87	
—	2 hr	6 hr	24 hr	41	55		72	—	—	—	24 hr	22	39	51		
—	2 hr	—	24 hr	35	40		55	N	2 hr	6 hr	24 hr	71	82	+	65	
—	2 hr	6 hr	24 hr	55	=	47	58	D	2 hr	6 hr	24 hr	37	60	66		
D	2 hr	6 hr	—	49	53	+	35	D	2 hr	6 hr	24 hr	43	66	+	64	
N		E		30	36		48	D	2 hr	6 hr	24 hr	38	80	+	66	
N	2 hr	6 hr	24 hr	92	96	+	88	R	—	6 hr	24 hr	27	48	62		
N	2 hr	6 hr	24 hr	36	62	=	63	D	2 hr	6 hr	24 hr	59	66	+	63	
—	2 hr	6 hr	24 hr	30	40		52	D	2 hr	6 hr	24 hr	57	82	=	84	
C	—	6 hr	—	30	42		50	—	2 hr	6 hr	24 hr	44	84	+	79	
D	2 hr	6 hr	—	39	54	+	47	—	2 hr	6 hr	24 hr	76	94	+	74	
R	2 hr	6 hr	24 hr	79	74	+	65	N	2 hr	6 hr	24 hr	34	48	=	51	
—		E		20	34		43	N	2 hr	6 hr	24 hr	46	73	+	70	
—	2 hr	6 hr	24 hr	60	84		92	N	2 hr	6 hr	24 hr	32	50	65		
N	2 hr	6 hr	—	56	57	+	45	D	2 hr	6 hr	24 hr	45	66	+	62	
—	2 hr	6 hr	24 hr	62	70	+	68	—	2 hr	6 hr	24 hr	79	91	+	78	
—		EC		21	39	+	25	N	2 hr	6 hr	24 hr	63	84	93		
—		E		18	32	=	35	—	2 hr	6 hr	24 hr	47	73	86		
—	—	6 hr	24 hr	18	49		70	—	2 hr	6 hr	24 hr	49	76	85		
D	—	6 hr	24 hr	26	47	=	51	—	—	6 hr	24 hr	24	44	57		
N		E		28	28	=	44	—	2 hr	6 hr	24 hr	34	51	71		
—	2 hr	6 hr	24 hr	60	77	=	80	—	2 hr	6 hr	24 hr	22	43	60		
N	2 hr	6 hr	24 hr	33	55	=	55	—	2 hr	6 hr	24 hr	35	53	66		
D	2 hr	6 hr	24 hr	82	92		63	D	—	6 hr	24 hr	27	42	60		
—	—	6 hr	24 hr	30	60	+	59	—	2 hr	6 hr	24 hr	32	52	=	57	
—	—	6 hr	24 hr	27	43		52	—	—	6 hr	24 hr	29	46	58		
N	—	6 hr	24 hr	30	45		55	C	2 hr	6 hr	24 hr	59	84	=	86	
—	—	—	24 hr	24	39		83	—	—	6 hr	24 hr	25	42	56		
R	2 hr	6 hr	24 hr	32	50	=	50	—	2 hr	6 hr	24 hr	79	98	+	90	
—	2 hr	6 hr	24 hr	49	56		65	—		EC		27	38	+	27	
D	—	6 hr	24 hr	23	49		55	D	2 hr	6 hr	24 hr	70	83	+	70	
D	2 hr	6 hr	24 hr	34	70		78	—	—	—	24 hr	15	30	55		
—	—	—	24 hr	14	32		51	N	2 hr	6 hr	24 hr	33	48	57		
D		E		23	30		48	R	2 hr	6 hr	—	39	44	+	43	
D	—	6 hr	24 hr	23	47		53	D	2 hr	6 hr	24 hr	54	90	+	76	
—	2 hr	6 hr	24 hr	55	72	+	56	D		E		23	34	46		
N	—	6 hr	24 hr	7	47		70	N	2 hr	6 hr	24 hr	35	55	=	58	
—		E		14	18		34	D	2 hr	6 hr	24 hr	32	51	58		
—	2 hr	—	—	60	28		40	D	2 hr	6 hr	24 hr	31	60	=	60	
D		E		16	30		48	—	—	6 hr	24 hr	17	42	56		
—	2 hr	6 hr	24 hr	42	52		62	D	2 hr	—	—	36	39	+	24	
N	2 hr	6 hr	24 hr	42	58		64	—		E		14	28	40		
D	2 hr	6 hr	24 hr	62	55	=	55	N	—	6 hr	24 hr	17	50	=	53	
—	2 hr	6 hr	24 hr	80	76	+	59	—	—	6 hr	24 hr	26	53	=	57	
—		E		20	33		43	—	2 hr	6 hr	24 hr	32	47	59		
—	2 hr	6 hr	24 hr	50	80	=	81	—		E		18	37	44		
D		EC		18	24		22	D	2 hr	6 hr	24 hr	70	92	+	84	
—	—	6 hr	24 hr	26	44		52	D	2 hr	6 hr	24 hr	82	84	+	73	
N	2 hr	6 hr	24 hr	37	50		55	C	2 hr	6 hr	24 hr	35	55	=	60	
—	2 hr	6 hr	24 hr	56	70	+	62	D	2 hr	6 hr	24 hr	40	57	70		
N	2 hr	6 hr	24 hr	67	91	+	89	—	2 hr	6 hr	24 hr	85	93	+	89	
—	2 hr	6 hr	24 hr	72	80	+	62	D	—	6 hr	24 hr	19	55	70		
D	—	6 hr	24 hr	30	94	+	73	—	2 hr	6 hr	24 hr	67	83	+	73	

(Continued on next page)

Down Inflexion After the 2 Hour Uptake

Our experience shows that all the curves obtained as we have above described, in which the maximum uptake value occurs at about 2 hours after administration, corresponded to patients with iodine metabolism deeply disturbed either due to a previous administration of thyouracil or anylin compounds or by a stable iodide excess intake, before or simultaneously with the radioiodine.

In the extreme situation which corresponds to recent administration of big doses of stable iodide, not easily absorbed, there is a relative inhibition of the hormonal synthesis. The absorption is maintained but most of the iodine remains in the gland as inorganic iodine. This fact observed by Childs *et al.*⁵ can be proved by the discharge with thiocyanates.

The uptake curves in this situation show the maximum at 2 hours but afterwards, there is a steady

decrease and the functional diagnosis cannot be made. However this condition is much less frequent in thyrotoxicosis. Table IV shows that only one case (Sat.) out of sixteen hyperthyroids shows this characteristic but four are noticeable in a group of euthyroids.

This is what we can expect since the renal clearance rate (about 0.10 hr⁻¹ according to our results) is not influenced by the inorganic plasma iodine level and tends to lower the iodine concentration.

Moreover, in thyrotoxicosis the increased metabolic activity with the related need of more iodine, leads to a shorter period of the enzyme system saturation. After this moment the convex shaped curves above described appeared.

In euthyroidism by identical reasoning the characteristic saturation curves are also modified but later on, as we have said, however, their shape is different, concave upwards and the minimum is about 6 hours after the administration. Table IV shows several examples of this type of curves.

Table III. Hyperthyroidism*† (continued)

Goiter*	Diagnosis by the uptake at:			Percentage of the administered dose at: 2 hr, 6 hr and 24 hr		
N	2 hr	6 hr	24 hr	54	61	71
—	—	6 hr	24 hr	27	44	66
D	2 hr	6 hr	24 hr	39	45	60
—	—	—	24 hr	10	26	52
R	2 hr	6 hr	24 hr	44	70	+
D	2 hr	6 hr	24 hr	80	90	+
R	2 hr	6 hr	24 hr	63	77	+
N	2 hr	6 hr	24 hr	40	52	71
D	2 hr	6 hr	24 hr	33	63	75
—	2 hr	6 hr	24 hr	58	85	=
D	2 hr	6 hr	24 hr	77	85	+
D	—	6 hr	24 hr	15	45	55
—	2 hr	6 hr	24 hr	80	88	=
—	—	6 hr	24 hr	25	65	+
D	2 hr	6 hr	24 hr	32	49	58
—	2 hr	6 hr	24 hr	43	86	+
—	2 hr	6 hr	24 hr	84	93	+
—	2 hr	6 hr	24 hr	50	70	+
—	2 hr	6 hr	24 hr	52	82	+
—	2 hr	6 hr	24 hr	48	60	=
D	2 hr	6 hr	24 hr	42	73	82
—	2 hr	—	24 hr	52	40	51
—	—	6 hr	24 hr	6	44	52
N	2 hr	6 hr	24 hr	32	55	60
—	2 hr	6 hr	24 hr	50	88	=
—	—	6 hr	24 hr	25	59	90
—	2 hr	6 hr	24 hr	32	48	65
D	2 hr	6 hr	24 hr	64	78	+
D	2 hr	6 hr	24 hr	40	46	61
M	2 hr	6 hr	24 hr	35	46	=
M	2 hr	6 hr	24 hr	35	52	63
M	2 hr	6 hr	—	39	62	+
D	—	EC	—	26	39	+
D	2 hr	6 hr	24 hr	82	82	77
—	2 hr	6 hr	24 hr	32	49	54

* N—nodular, D—diffuse, E—diagnosis error, EC—corrected error.

† Number of cases diagnosed by the uptake at: 2, 6 and 24 hr—97; 6 and 24 hr—30; 24 hr—8; 2 hr—2; 6 hr—1; 2 and 6 hr—6; and 2 and 24 hr—2.

This table must include the 14 special cases reported in Table IV.

CONCLUSIONS

By making three uptake measurements at 2, 6 and 24 hours after the oral administration of carrier-free I¹³¹ to fasting subjects with a definitive clinical diagnosis, it can be concluded:

1. Taking 30%, 40% and 50% as the upper normal limits the diagnosis is made when, at least, one of the uptake measurements is higher than the above mentioned, the accuracy is slightly improved (87.6% of the total number of cases) than with a single measurement at 24 hours (86.7%) (Figs. 3 and 4).

2. Results are considerably improved if we take into consideration the dynamic aspect of the thyroid function resulting from the iodine intra-glandular turnover. These data are given by the uptake curve with the 2, 6 and 24 hour measurements.

3. We have found that with most of the hyperthyroids with uptake curves within the normal range received supplementary stable iodide, before the carrier-free I¹³¹ test. In these conditions, and with our timetable measurements, we can observe the same two functional types of curves: a steadily increasing uptake curve and a convex shaped curve. The last one is interpreted as reflecting the increased metabolic iodine activity and therefore has diagnostic possibilities. The accuracy, taking into consideration these factors, can be increased up to 90.4%.

4. Diagnosis failed in 48 cases including: 1 hyperthyroidism with a relative biosynthetic blockade resulting from a recent iodide intake by therapeutic injections; 14 hyperthyroidism cases with curves of the ascending type within the normal range (Table III); 1 hyperthyroid (Case No. 2 Table IV) patient with a similar concave shaped curve characteristic of euthyroids patients with supplementary iodine intake (Table IV); 16 euthyroid subjects with high uptake curves (Table II). (In all these cases there were not observed the convex shaped type which, in

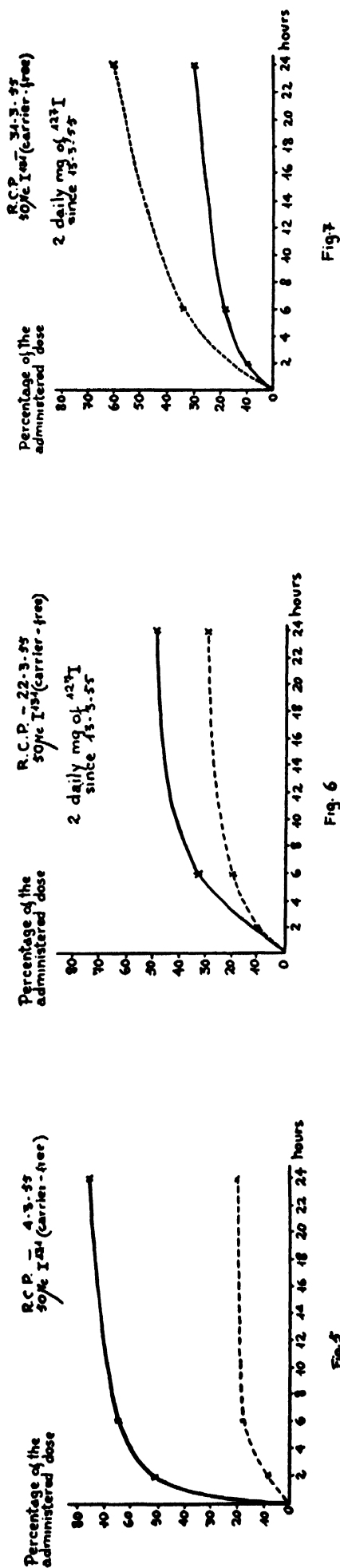


Fig. 5

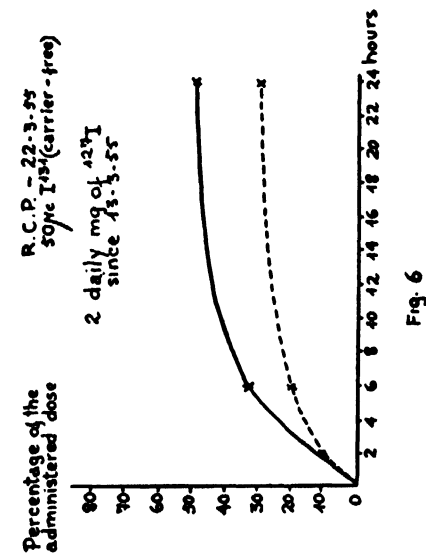


Fig. 6

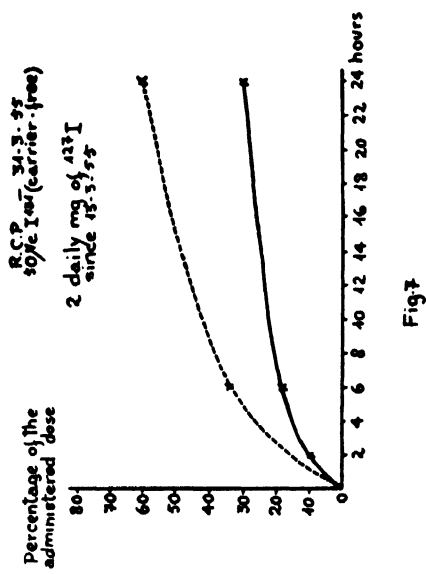


Fig. 7

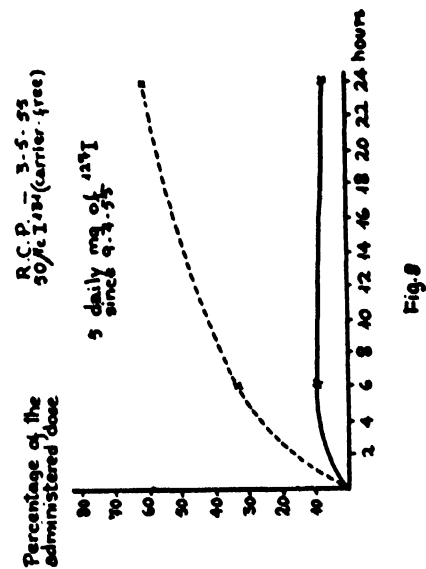


Fig. 8

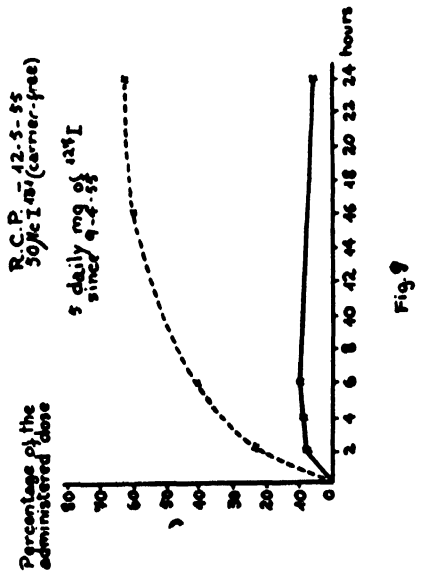


Fig. 9

Figures 5-9. Effect of a supplementary ration of iodine in the uptake of a hyperthyroid patient. Daily intake of 2 mg of sodium iodide during 27 days (Fig. 6 and Fig. 7) followed by an increased daily diet of 5 mg of sodium iodide (Fig. 8 and Fig. 9)

Table IV. Patients with Very Low Uptake Curves

Obs. no.	Patient		Diagnosis	Uptake at		
	Name	Age		2 hr	6 hr	24 hr
Hyperthyroidism						
44102	CCR	60	Hyper.	17.4	26.0	16.3
92582	RSB	40	Tox. nod. goiter	7.8	5.5	8.0 E
93287	CP	34	Hypert.	12.5	12.5	3.1
97337	CAS	53	Hyper.	1.5	1.5	1.2
100216	MASCS	28	Hyper.	3.3	4.6	1.0
100468	AV	60	Hyper.	6.3	7.8	5.3
101052	JCCM	62	Tox. nod. goiter	6.3	8.7	6.4
107443	ENOA	48	Tox. diff. goiter	8.1	11.0	8.5
109653	DNB	47	Tox. diff. goiter	9.2	9.2	0.9
				36.2	38.9	24.2—2nd test
122650	MJO	35	Hyper.	9.7	11.0	7.7
121283	MPJ	48	Tox. nod. goiter	6.5	4.3	1.3 Sat.
77477	ES	64	Hyper.	15.0	17.0	12.0
105800	AMG	29	Hyper.	4.1	8.8	3.7
				12.0	15.0	15.0—2nd test
Euthyroidism						
61336	MLA	44	Men. diabetes	9.0	8.0	10.0
66825	EFS	34	Psychoneurose	14.0	11.0	17.2
69754	CJ	28	Symp. nod. goiter	12.0	11.0	26.1
69838	RML	56	Symp. nod. goiter	3.0	4.0	9.0
95449	UJO	54	Symp. resc. goiter	3.9	2.8	7.7
96962	ONS	56	Diabetes	8.2	6.1	5.6 Sat.
97963	LRR	65	Thyr. casein	6.0	5.0	9.0
99499	CSML	43	Psychoneurose	11.2	10.8	17.5
102303	JGV	52	Simple goiter men.	6.2	6.2	9.0
102557	ALR	47	Menopause	4.9	3.2	1.4 Sat.
102847	MCLF	37	Obes. diabetes	7.0	5.0	2.0
116435	RPD	58	Thyr. carcinoma	8.6	7.2	2.3 Sat.
124390	BJTP	29	Diffuse goiter exo.	20.0	14.3	4.6 Sat.

our opinion, are related to the severeness of the disease); 18 endemic goiters (Table I) with normal thyroid function and high uptake curves. These last cases can be easily disclosed by the goiter existence in very young patients without any signs of thyrotoxicosis.

5. Taking into consideration all the above factors, we can make a correct diagnosis in 488 cases out of 520 patients (93.8%).

6. From the clinical point of view, our experience points out that the hyperthyroidism is a less frequent disease than was thought before the radioiodine test introduction, since this discloses quite easily the psychoneurosis and simple goiters both with a high basal metabolical rate.

SUMMARY

520 patients from the Endocrinology Service of this Institute subjected to a uniform diagnosis criterion were studied with radioiodine techniques. Uptake curves were studied by neck measurements at 2, 6, 24 and 48 hours after oral administration of I^{131} . Thyrotoxicosis was disclosed when the uptake was higher than 30% at 2 hours or 40% at 6 or 50% at 24 hours after administration.

The diagnosis proved accurate in 87% of the cases but the accuracy may be improved up to 94% if we took in consideration other clinical data.

(a) 18 cases of endemic goiter with very high uptakes could be disclosed in young patients with goiter without any signs of hyperthyroidism.

(b) 19 cases of pharmacological alteration of the iodine metabolism in hyperthyroid patients.

Most of these disturbances come from the use of mercapto-compounds and iodine. The first ones can easily be recognised by the 2 hour down inflection of the uptake curve, due to a biosynthetic blockade.

The iodine intake is very often masked when they are used, and it is accepted by other authors that, in these cases, the radioiodine test is worthless.

A sudden and high increase of plasma iodine also results on the 2 hour inflection by the same mechanism of the thiouracil.

A hyperiodemia steadily maintained gives a progressively lower uptake curve due to decrease of the accumulating function for iodide.

In thyrotoxicosis the uptake at 24 hours is lower than at 6 hours due to the acceleration of the iodine inner turnover.

In euthyroid patients there is inhibition of the thyroid hormone synthesis and the uptake curve is therefore different for the same reasons as above.

On the 26 cases studied in these conditions 13 were euthyroid and 13 thyrotoxic.

In 9 of the 13 euthyroids the uptake curve was concave upwards having minima at 6 hours. The

remaining 4, present hormonal synthesis inhibition due to the use of thiouracil compounds.

Of the 13 thyrotoxic 11 show the characteristic convex curves. The remaining 2 did not show this sign but there was some explanation for the different curves, which increases our confidence in the method.

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The Theory of Thyroid Function and Diagnosis of Its Conditions with Radioactive Iodine

By Julio Palacios,* Portugal

The thyroid can be compared to a factory which, using iodine and tyrosine as raw materials, produces a certain hormone, and, the latter being transported by the blood to the various organs, stimulates their activity in such a way that an excess of this hormone increases the functional rhythm, whereas a deficiency brings about a slowing down of the biological processes. The study of the thyroid function is a fascinating problem which subjects all the resources of contemporary science to a test.

The first step in the production of the thyroid hormone consists of the fixation of iodine. This element exists in the blood in the form of iodide, and is fixed by the thyroid at such a rapid rate that, if a few milligrams of stable iodine tagged with radioactive iodine are injected into a vein, less than a half hour is needed for approximately one half of it to be concentrated in the gland. The extraordinary speed with which the thyroid gland fixes the iodine would be out of the question, if it were not for the fact, which already drew the attention of the old anatomists, that it has a very abundant blood supply, brought by a number of large arteries, so that the volume of circulation through it is 4 or 5 times greater than it is in the kidneys.

The biochemical investigation of thyroid function made considerable progress as regards the various reactions which lead to the production of the hormone, but much remains to be investigated, and everything gives reason to believe that this is a very complicated process. For our purposes, it is enough to know that the iodine forms a certain organic compound called di-iodotyrosine, which is stored in the thyroid until two molecules are united, forming the thyroid hormone (thyroxine), and releasing alanine. These products are passed on to the blood, where they exert their action in the system.

The thyroid has the function of regulating the metabolism, and, in order to carry out its purpose, it must function in the fashion of some of those devices covered by a branch of technology called cybernetics. In so far as the consumption of thyroid hormone depends on outside conditions, such as cold or heat, and on the greater or lesser amount of work

which has to be carried out by each organ, it is necessary that there be a center charged with the gathering of information about the subject's needs at every moment, and with ordering the thyroid to let out a suitable amount of the hormone into the blood stream. Thanks to the work of Smith,¹ it is known that the role of a "command post", which receives the information and transmits the orders is fulfilled by a nerve center, the hypothalamus, which operates in conjunction with the anterior lobe of the pituitary. This gland produces a hormone, which was isolated in 1935 by Collip and Anderson,² which is transported by the blood, and has the property of stimulating the activity of the thyroid, so that it is called the thyroid stimulating hormone, and is designated by the initials T.S.H. Thanks to the work of Keating and his group,³ and Rawson,⁴ it is known that the first action of T.S.H. is the breakdown of di-iodotyrosine, which liberates the thyroid hormone. According to this theory, T.S.H. is a regulating device located at the thyroid outlet, thanks to which a greater or lesser amount of thyroid hormone comes out, in keeping with the needs of the moment.

HISTOLOGY OF THE THYROID

The histological study of the thyroid has contributed much to our knowledge of its function. It is enough, for our purposes, to know that there are many follicles in the thyroid (Fig. 1), which are in the nature of closed capsules, the walls of which are made up of cells. The conjugation of iodine with the thyroxine takes place in those cells, to form di-iodotyrosine, which passes inside the cavity, where it is stored until it is freed by the action of thyrotropine from the pituitary gland.

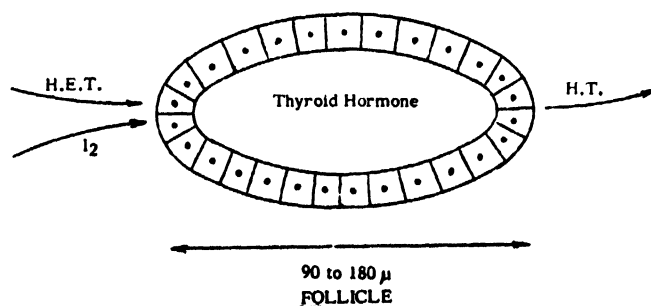


Figure 1. The cells which make up the walls of the follicles of the thyroid are those which elaborate di-iodotyrosine, which is stored inside the follicle

Original language: Spanish.

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Each follicle can be compared to a thyroid hormone factory, with its storage area, where it keeps the substances produced. We know, in addition, that the various follicles do not work continuously, rather that they alternate between periods of activity and inaction. Yet, not all the follicles go through the same phases simultaneously, but, rather, work, as it were, in some sort of shifts, in such a fashion that some are producing and storing the hormone, while the others rest.

SCHEMATIC OF THYROID FUNCTION

There is enough, in what has been stated above, to make up a model which substantially imitates the operation of the thyroid and which, according to the language in current use, we could term a "robot." It consists (Fig. 2) of a first tank *A*, in which the inorganic iodine from the blood is stored. Then comes the plant *F* in which, in addition to the iodine, thyroxine is received (there always is an excess of it), with a suitable catalyst to achieve the formation of di-iodotyrosine. This product is stored in tank *B* until, due to the arrival of a suitable enzyme it is disintegrated, after which the thyroid hormone goes out through *R*₂. This hormone is carried by the blood, and used by the various tissues, so that the iodine which it contains becomes free, and is again used by the thyroid, except for the part which is lost through the kidney.

What we now need are the regulating units—the "controls." We already have observed that, in the thyroid, there is an output regulation mechanism which, according to Keating and Rawson, is probably effected by the thyrotropine coming from the pituitary gland. This regulation would be sufficient for us to make up our artificial thyroid, provided we arrange

things in such a fashion that plant *F* always maintains storage system *B* full. However, if we try to imitate the real thyroid as faithfully as possible, we must put another regulator at the intake of the plant. What happens is that, due to the action of certain drugs (thiouracil and its derivatives), the hormone formation is interrupted, but not its ejection into the circulation, so that, as a consequence, after a certain time has elapsed, storage space *B* is empty, and we begin to note the effect of the thyroid hormone deficiency (hyperplasia of the thyroid, for instance). This indicates that, aside from output regulator *R*₂, it is necessary to have another regulator, *R*₁, independent of the one which regulates the operation of plant *F*.

From the books and publications we read, we were not able to determine what regulates hormone production. They all mention thyrotropine, but, if this substance were responsible for the regulation of the output, it could not also control the production of the hormone, since the action of thiouracil shows that we are dealing with independent processes.

In order to complete our model, we need a device which can take the iodides from the blood and store them in tank *A*. This could be achieved by placing, in *A*, a substance very avid for iodine, with the property of extracting it from the iodides, leaving it free for subsequent combination with thyroxine. Since the ratio of the iodine ingested to the food is much greater than that which is strictly necessary (except in the areas where goiter is endemic), tank *A* would always be saturated, so that the thyroid would not take, from the blood, more iodine than necessary for the production of the hormone. According to this theory, the amount of iodine which, under normal conditions and on an average, enters the thyroid per time unit, would have to be equal to that which is removed from the hormone. It appears to be the opinion of Keating that the expulsion of thyroid hormone is stimulated by thyrotropine, while its fixation would be due, more simply, to a lack of inorganic iodine in the thyroid.

Assuming Keating were right, it would be superfluous to have a regulator at the intake of the thyroid, and in this case we rest our views on an earlier piece of work,⁵ in which the equations which control iodine metabolism were drawn up. The experience acquired since then obliges us to give up this hypothesis, as will be seen below.

DIAGNOSIS OF THYROID DISORDERS WITH THE HELP OF RADIOACTIVE IODINE

Radioactive iodine is being used with great success in the diagnosis of the functional deviations of thyroid function. This method consists of supplying a patient with a given dose of some of the radioactive isotopes of iodine, for example I^{131} , and observing how the reactivity accumulated in this gland varies as a function of time. With this datum, and with a measurement of the activity excreted with urine, various in-

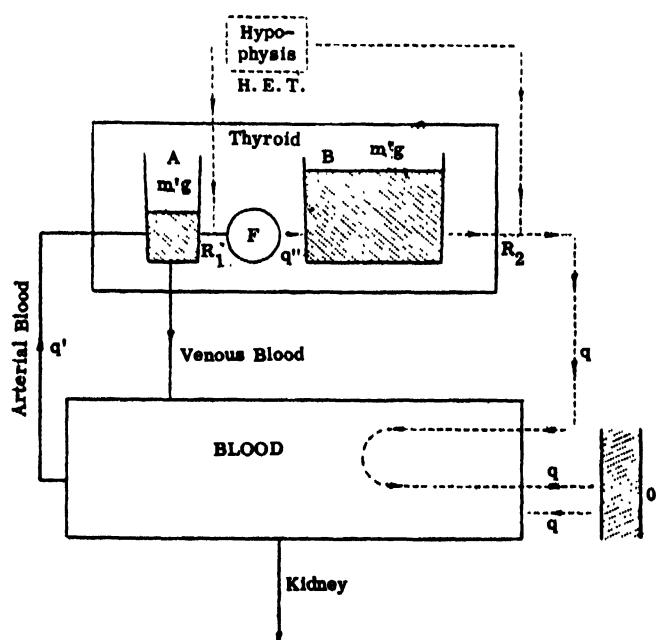


Figure 2. Schematic model of thyroid function: (A) inorganic iodine storage; (F) production of di-iodotyrosine; (B) di-iodotyrosine reserve; (*R*₁) regulation of the production of organic iodine; (*R*₂) regulation of the expulsion of thyroid hormone

dices are computed, which have no immediate physical significance, and with which, by an empirical method based on more or less favorable statistics, we can arrive at the diagnosis. This was done in the Portuguese Institute of Oncology, using a few modifications in the manner of carrying out measurements, which will be described by Dr. Baptista. Dr. Franco, for one, will give a critique of the results.

The empirical way in which the current methods of diagnosis are conducted, cannot be satisfactory to any physicist, and, therefore, we will investigate the problem of integrating the differential equations which describe the metabolism of iodine, which equations, with a few variations, already have been established by Oddie and Tubiana. After painstaking computations⁵ we succeeded in finding simple formulae which make it possible to compute the parameters which define the metabolism of iodine and, in particular, the activity of the thyroid, namely the quantity of iodine, q , which, on an average, is used per time unit to form the hormone.

It was to be hoped that the value of q would be the best criterion with which any abnormal function of the thyroid could be discovered. Having tested the new method in the Portuguese Institute of Oncology, we obtained the results which are shown on Fig. 3. We have taken, as the abscissa, the values of q in micrograms per hour, with intervals $\Delta q = 1 \mu\text{g/hr}$, and, as ordinates, the number of individuals whose thyroid has an activity comprised in the interval discussed.

As will be seen, the curve goes up rapidly from $q \mu\text{g/hr}$, passes through a maximum, and thereafter decreases slowly. Upon seeing this graph, we come to the conclusion that normal individuals use approximately 10 micrograms of iodine in the formation of thyroid hormone. (As stated by McGavack,⁶ the measurement of the activity of the thyroid is considered difficult, and not readily carried out. Aside from our measurements, we only found the results obtained by Stanley,⁷ according to which the thyroid of normal individuals consumes, on an average, some 300 micrograms of iodine per diem, a value which is in satisfactory agreement with that of the Portuguese Institute of Oncology.)

In order to measure activity q of the thyroid, we must add to the radioactive iodine a known quantity of stable iodine, which must be considerably greater than that contained in the daily ration, but not such that it might cause perturbations in the function of the gland. In our measurements, we used 1 milligram. It was to be foreseen that, under these conditions, there would be a considerable decrease in the radioactivity accumulated in the thyroid, since the specific activity of the iodine fixed by the gland is much less. However, upon drawing the statistics, and comparing the values of thyroid radioactivity, we get:

$$(d_g)_{max} = 42\% \text{ without any stable iodine}$$

$$(d_g)_{max} = 39\% \text{ with 1 mg of stable iodine}$$

This unexpected result clearly shows that the thyroid fixes iodine, not to compensate losses, but rather that it takes as much of it as it is offered and that there is a certain proportion between the amount of inorganic iodine, m_o , which exists in the thyroid gland and m_p' , contained in the intercellular plasma, a proportionality which is maintained up to a dosage of approximately 1 mg. This being the situation, and in so far as the daily iodine amount is doubtless greater than that which is strictly necessary, what then is the means of removal of the iodine which is not consumed to produce the hormone?

Giving the problem some thought, we came to the conclusion that there must be, in the thyroid, a substance which is capable of fixing the iodine until equilibrium be established, as regulated by the law of masses. The existence of such a substance can be ruled out for, from the measurements carried out by Stanley and Astwood,⁸ the ratio I_o/I_p between the concentrations of inorganic iodine in the thyroid and in the blood respectively, varies between 34 and 94 according to the individual, for normal subjects. However, work carried out by Wollman and Scow,⁹ and recently published, justifies our hypothesis. It explains the fixation of iodine by two mechanisms which lead to the same formula, namely:

Concentration of Iodine as an Adsorption Process

In this physical-chemical model, we assume that the thyroid contains an adsorbent, which forms a complex with the iodine. Let m_∞' be the adsorption capacity, namely the mass of inorganic iodine which would be adsorbed if all the points at which this substance can be found were occupied by iodine atoms. We assume that the adsorption process is regulated by the law of masses, or by its equivalent, the adsorption isotherm of Langmuir.¹⁰ According to this line of reasoning, the quantity of iodine which penetrates the thyroid per time unit, would be proportional to the number of spots available, or, which is the same thing, to the difference between the adsorption capacity m_∞' and the mass adsorbed m_o' . It must also be proportional to the concentration, I_p' , of the inorganic iodine in the blood.

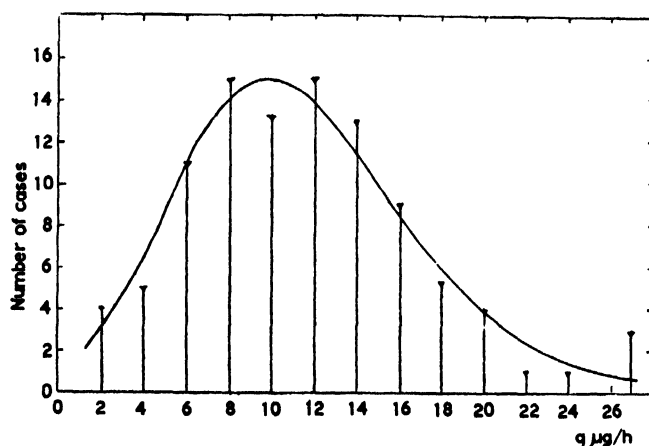


Figure 3. Statistical table of the activity of the thyroid

Accordingly it will be:

$$V_1 = k_1(m_{\infty}' - m_g')I_p' \quad (1)$$

Simultaneously, this substance will release, per time unit, a quantity of iodine proportional to the adsorbed mass:

$$V_2 = k_2 m_g' \quad (2)$$

and once the equilibrium is reached, it will have to be

$$k_1(m_{\infty}' - m_g')I_p' = k_2 m_g'$$

which indicates:

$$\frac{m_g'}{I_p'} = \frac{k_1 m_{\infty}'}{k_2 + k_1 I_p'} \quad (3)$$

The Concentration of Iodine as a Phenomenon of Transport through Membranes

In its most simple form, this model consists of assuming that the iodine fixed by the thyroid is found in cavities closed by a membrane, in which there is a substance, the molecules of which are capable of fixing iodine from the blood and transport it inside the cavity. A study of this model, with all its implications, was made by Karremann¹¹, who arrives at the same formula as Equation 3, which saves us the need for reproducing computations.

Equation 3 has been confirmed by Wollman and Scow in the work mentioned. We will show that, with its help, we account for the fact that the measurements of the thyroid activity, q , are somewhat impaired in cases of colloid goiter, and we will deduce from them a formula which must lead to correct values.

RETENTIVE POWER OF THE THYROID

Instead of concentration I_p' , shown in equation 3, it is convenient to use quantity m_p' , or inorganic iodine contained in the plasma, since it is easy to measure it using iodine tagged with one of its radioactive isotopes. If we represent the diffusion volume of the iodine in the extracellular plasma by V_p , we can write $I_p' = m_p'/V_p$, and Equation 3 will be converted to:

$$\frac{m_g'}{m_p'} = \frac{r}{1 + A m_p'} \quad (4)$$

in which we use the parameters

$$r = \frac{k_1}{k_2 V_p} m_{\infty}' \quad A = \frac{k_1}{k_2 V_p} \quad (5)$$

which depend on m_{∞}' and V_p , since k_1 and k_2 are constant. m_{∞}' , in turn, depends on the quantity of adsorbent contained in the thyroid.

It is clear from Equation 4 that the ratio m_g'/m_p' depends, in each individual, on the quantity of iodine m_p' which, at every moment, is present in the plasma, in such a way that, when m_p' tends toward ∞ , m_g' tends to m_{∞}' , since

$$m_g' \rightarrow \frac{r m_p'}{A m_p'} = \frac{r}{A} = m_{\infty}'$$

However, our experiments have shown that this ratio remains constant, even though 1 mg of iodine be incorporated in the blood plasma, a quantity much greater than that which it normally contains. This reveals that, as long as this amount is not exceeded, product $A m_p'$ is much smaller than the unit, and Equation 4 may be replaced by

$$\frac{m_g'}{m_p'} = r \quad (6)$$

in which we find only parameter r , which we can call the *power of retention or adsorption*.

This surmise is confirmed by the experiments of Freinkel and Ingbar¹² according to which the ratio m_g'/m_p' remains constant, even though m_p' varies between wide limits. It is necessary to reach concentrations of 150 to 250 mg of iodine per 100 cm³ of plasma before we begin to note a progressive decrease in this ratio.

FATE OF THE IODINE IN THE SYSTEM

The above already makes it possible to complete a model which reproduces the transformation suffered by iodine as regards the thyroid function. Let us take an individual whom we will assume to be euthyroid, who, due to an adequate diet every day, has a complete reserve, m_g' , of hormone iodine, which as is known,⁵ amounts to a few tenths of a milligram. When he is fasting, the quantities of inorganic iodine, m_{p0}' and m_{p0}' will be substantially less than the average value. In these conditions, we inject, by the intravenous route, a quantity of iodine M , which we will assume to be much greater than that found in the daily diet. This iodine will be carried by the circulation; part of it will come out through the kidney, and another part will reach the thyroid, where it will be adsorbed.

In the penetration of iodine in the thyroid we must distinguish two stages. In the first, the iodine is rapidly fixed, until it reaches adsorption equilibrium. In the second, the ratio $m_g'/m_p' = r$ is kept constant.

The duration of the first stage will depend on the quantity of iodine supplied, the rate of adsorption, the quantity of blood which reaches the thyroid per time unit, and the loss of iodine due to renal secretion. At any rate, the experiments made reveal that equilibrium is reached much more rapidly, in a few tenths of minutes, if we inject iodine in a vein.

In the second stage, the relationship between the inorganic iodine adsorbed by the thyroid and that found in the plasma remains constant. Since this last term decreases, due to losses through the kidney, the former will also have to decrease proportionally, and we may find two cases:

(a) If the dosage supplied is relatively small, that which escapes through the kidney will also be small as compared to the decrease suffered by the inorganic

iodine of the thyroid as a consequence of hormone formation, and, in order for this relationship to remain constant, it will be necessary that iodine continue penetrating the thyroid, although in much smaller doses than in the first stage.

(b) When the iodine amount exceeds a certain limit, the renal loss predominates over the iodine which comes to be fixed in the organic form, and the physical-chemical equilibrium between m_o' and m_p' requires that inorganic iodine pass from the thyroid to the blood. Thus, there will be a decrease in the iodine, which will take the form of a reduction in the radioactivity accumulated in the thyroid if one uses iodine tagged with one of its radioactive isotopes. (If, after having injected a large dose of stable iodine, radioactive iodine then is injected, the thyroid does not fix it. This commonly is expressed by saying that the thyroid is saturated. In reality, what happens is that the thyroid already is in the phase which we called the "decrease phase". It is not right to mention saturation, for as indicated by Equation 3, in order for m_o' to reach its limiting value m_∞ , the concentration I_p' of inorganic iodine in the plasma would have to be infinite.)

EQUATIONS USED TO DEPICT THE BEHAVIOR OF IODINE IN THE SYSTEM

Our model now being complete, we can write the equations which will give us, as a function of time, the quantities of iodine contained in the thyroid and in the extracellular plasma. This problem has been studied by Oddie, Tubiana and ourselves,⁵ but, as we already said, the equations were written under the assumption that, in keeping with the view of Keating, the thyroid took precisely the amount of iodine necessary for the production of hormones, which is only true when we take the average values for a day, and provided the customary dose of iodine be received. Now, with the help of the hypothesis of physical-chemical equilibrium, we are in a condition to set up the problem, and to solve it in greater detail.

First Stage, $m_o'/m_p' < r$

The mathematical study of this first phase is very complicated, if all the factors which influence the speed with which equilibrium is reached into account, such as the rate of diffusion of the iodine in the blood and in all the extracellular plasma, renal secretion, the parameters k_1 , k_2 , m' and I_p' which determine the fixation of iodine by the thyroid, and finally the output, q , which is used for the formation of the hormone and returns to the blood. However, in this stage the only elements of interest are the values acquired by m_p' and m_o' at the end of this period, after equilibrium has already been achieved, and this makes it possible to simplify the model, supposing that the quantity of iodine fixed by the thyroid in the time dt , is proportional to the mass m_p' of inorganic iodine contained in the plasma at the time con-

sidered. We know, of the renal secretion, that it is also proportional to m_p' , which gives the following equations:

$$\frac{dm_o'}{dt} = am_p' - q$$

$$\frac{dm_p'}{dt} = -(a + c)m_p' + q$$

in which c is the renal secretion coefficient, and a the fixation coefficient of the thyroid. For the reasons mentioned, this coefficient is not constant in the actual thyroid, but it decreases as we get closer to the first stage, so that the magnitude shown on this model will be accurate as a mean value, depending on the amount of iodine ingested.

The integration of the equations shown above leads to:

$$m_p' = Ce^{-(a+c)t} + \frac{q}{a+c}$$

$$m_o' = \frac{a}{a+c} C \left[1 - e^{-(a+c)t} \right] - \frac{a}{a+c} qt + m_{o0}'$$

The integration constants C and m_{o0}' depend on the quantities of iodine, m_{p0}' and $m_{o0}' = rm_{p0}'$, which exist at the moment of the injection. Since we assumed that the quantity injected M is much greater than that contained in the daily ration, we can admit that

$$m_{p0}' = M; m_{o0}' = 0 \quad \text{for } t = 0$$

from which:

$$m_p' = \left(M - \frac{q}{a+c} \right) e^{-(a+c)t} + \frac{q}{a+c} \quad (7)$$

$$m_o' = \frac{a}{a+c} \left(M - \frac{q}{a+c} \right) \left(1 - e^{-(a+c)t} \right) - \frac{c}{a+c} qt$$

or else, if M be sufficiently large:

$$m_p' = M e^{-(a+c)t} \quad (8)$$

$$m_o' = \frac{a}{a+c} M \left[1 - e^{-(a+c)t} \right] \quad (9)$$

The last moment, t_1 , of this first phase, is determined by the condition $m_o' = rm_p'$, or:

$$e^{(a+c)t_1} = 1 + r \cdot \frac{a+c}{a} \quad (10)$$

and the values of m_{p1}' and m_{o1}' will be obtained substituting the value of the exponential in Equations 8 and 9.

Second Stage

We will assume that, in this stage, the ratio $m_o'/m_p' = r$ is kept constant. The quantity of inor-

ganic iodine which comes out of the storage tank A , to be transformed into a hormone, is reincorporated into the blood by virtue of the disintegration of this hormone in the tissues. Therefore:

$$d(m_p' + m_g') = -cm_p' dt$$

or:

$$(1 + r) dm_p' = -cm_p' dt$$

from which:

$$\begin{aligned} m_{p1}' &= m_{p1}' e^{-\frac{a}{1+r} t'} \\ m_g' &= rm_p' \end{aligned} \quad (11)$$

with $t' = t - t_1$.

USE OF IODINE TAGGED WITH ONE OF ITS RADIOACTIVE ISOTOPES

In order to diagnose the functional abnormalities of the thyroid, it is of interest to know, above all, the quantity of inorganic iodine q which is transformed into iodine-containing hormone per time unit. In addition, it is necessary to determine retentive power r , because, as we will see, this value is useful for the diagnosis of goiter. We will show that by using iodine tagged with one of its radioactive isotopes, we can find, not only the values mentioned above, but also the renal secretion coefficient c and the captation power a of the thyroid.

Let it be assumed that, along with mass M of stable iodine, we administer a dose D_0 of radioactive iodine. This will first enter the storage tank A , and thereafter, being converted into hormone containing iodine, it will pass to B , where it will be mixed with what is to be found there, which amounts to a few tens of milligrams in normal individuals. From this, during the first 24 hours approximately, the specific radioactivity of the hormone iodine will be insignificant, so that the output q to the blood will not carry any appreciable radioactivity. It can be assumed, therefore, that during the first day there is no passage of hormone iodine in the radioactive form, from the thyroid to the blood.

In the first stage, the change suffered by radioactive iodine in the plasma will be given by:

$$dD_p = -(a + c) D_p' dt$$

from which:

$$D_p = D_0 e^{-(a+c)t} \quad (12)$$

The radioactivity of the thyroid will suffer, in the interval dt , an increase of:

$$dD_g = aD_p' dt = aD_0 e^{-(a+c)t} dt$$

and since $D_g = 0$ for $t = 0$ we can write:

$$D_g = \frac{a}{a+c} D_0 [1 - e^{-(a+c)t}]$$

Introducing the relative radioactivities, $d_g = D_g/D_0$ and $d_p = D_p/D_0$ we find:

$$d_p = e^{-(a+c)t} \quad (13)$$

$$d_g = \frac{a}{a+c} [1 - e^{-(a+c)t}]$$

and, at the end of this stage, we will have, by virtue of Equation 10

$$d_{p1} = \frac{1}{1 + r \frac{a+c}{a}} \quad (14)$$

In the second stage, the equation:

$$d(d_p + d_g) = -cd_p dt \quad (15)$$

also is verified.

In addition, total radioactivity d_g , of the iodine contained in the thyroid consists of the d_g' due to the inorganic iodine and d_g'' of the hormone iodine. The last mentioned is that transported by output q , the relative specific radioactivity of which is q/M so that it will be:

$$d_g'' = \frac{q}{M} t$$

and therefore:

$$dd_g = dd_g' + dd_g'' = dd_g' + \frac{q}{M} dt$$

Allowing for this, and remembering that, in this second stage, $dd_g' = rdd_p$, Equation 15 can be converted into:

$$(1 + r)dd_p + \frac{q}{M} dt = -cd_p dt$$

from which:

$$d_p = (d_{p1} + \frac{q}{cM}) e^{-\frac{a}{1+r} t'} - \frac{q}{cM} \quad (16)$$

$$d_g = rd_p + \frac{q}{M} t \quad (17)$$

MEASUREMENT OF THE PARAMETERS a , c , r , AND q

Measurement of radioactivity in the thyroid d_g and the plasma d_p , as well as that which is excreted with the urine, can be obtained easily by the method exposed in our earlier work.⁵ Representing the values of d_g and d_p as a function of time, we obtain various curves, the slopes of which are first very sharp, and then less steep. The changes in slope correspond to the end of the first stage, in other words they indicate that we already have reached adsorption equilibrium, and therefore Equation 17 gives, for the retentive power:

$$r = \frac{d_{g1}}{d_{p1}}$$

since the term qt_1/M is negligible if M is large enough. Since the values of d_g and d_p vary slowly when the second stage begins, it will not be necessary accurately to define instant t_1 , at which the change takes place.

From Equation 17, applied to a time t , at any stage of the second period, we can deduce the quantity of iodine which is converted into hormone iodine per time unit:

$$q = \frac{M}{t} (d_p - r d_p)$$

For sufficiently large values of t , the value of d_p is already so small that an error in measuring it does influence markedly the value of q .

In order to find the renal secretion coefficient, the radioactivity excreted with the urine, d_e , is measured in the time interval Δt , and one applies the formula

$$c = \frac{1}{\Delta t} \cdot \frac{d_e}{\bar{d}_p}$$

in which $\bar{d}_p$ is the average value of d_p in interval Δt . If one works on the urine recovered during the first stage,

$$d_p = \frac{1 + d_{p1}}{2}$$

can be taken as an adequate approximation.

It has already been stated that the captation coefficient a , is not a constant which is characteristic of the individual, since its measurement lacks importance for our purposes. However, we can find the value which corresponds to the dose applied and to the form of introduction in the organism, using Equation 13, which gives for $t = t_1$:

$$a = -\frac{1}{t_1} \ln d_{p1} - c$$

REGRESSION OF INORGANIC IODINE

When radioactive iodine is supplied with a relatively large amount of stabilized iodine, it is observed that a sudden increase in radioactivity in the thyroid is followed by a decrease, which is all the swifter as the quantity of iodine injected is greater. This fact is ascribed to an oversaturation of the thyroid, which accounts for the elimination of the excess iodine accumulated.

According to the theory we have developed, there can be no mention of saturation of the thyroid, but the phenomenon which we just described is satisfactorily accounted for by a decrease in the quantity of iodine as seen above. In fact, if dm be the mass of iodine which the thyroid takes from the blood in the time dt , the balance between the intake and outgo in the plasma leads to the equation:

$$dm_p' = q dt - cm_p' dt - dm$$

from which:

$$dm = q dt - cm_p' dt - dm_p'$$

and since Equation 11, which applies during the second stage gives:

$$dm_p' = \frac{c}{1+r} m_p' dt$$

the expression mentioned above can be converted into:

$$dm = (q - \frac{cr}{1+r} m_p') dt$$

There will be a reduction in the iodine from the thyroid to the blood during the second stage, in which $dm < 0$, namely:

$$m_p' = \frac{q}{c} \frac{1+r}{r}$$

consequently, the radioactivity of the thyroid will decrease. In the opposite case, d_p will continue increasing, but not so fast as in the first phase.

MINIMAL IODINE DOSE

According to Salter,¹³ the dose strictly necessary for the normal individual to live normally without consuming his hormone iodine reserve is 15 micrograms per diem. This quantity corresponds approximately to 300 micrograms of thyroxine, which is the figure which Means and Learman¹⁴ found to be necessary as a maintenance dose for the patients in myxedema.

Knowledge of the minimal ration will allow us to compute the maximum value of the captation coefficient a , since from Equations 1 and 2 it is clear that the quantity of iodine which passes from the blood to the thyroid per time unit is:

$$V_2 - V_1 = \frac{k_1}{V_p} (m_{\infty}' - m_g) m_p' - k_2 m_g'$$

and therefore, the captation coefficient will be:

$$a = \frac{k_1}{V_p} (m_{\infty}' - m_g') - k_2 \frac{m_g'}{m_p'} \quad (18)$$

This expression is maximal when m_g' is as small as possible, which would be the case, without any detriment for the health, if the individual habitually consumed said minimal dose.

Let us now see how it is possible to compute a , as a function of the iodine ration m , strictly necessary for the maintenance of health.

Let us suppose that an individual ingests his daily ration, divided into two equal doses, 12 hours apart. The minimum ration will be that with which, if the thyroid functions with its maximum iodine captation power, and immediately transforms it into hormone iodine, its reserve of the last mentioned substance is kept intact at the end of each cycle. Under those conditions, the quantity of inorganic iodine m_g' which exists in the thyroid would be practically nil, since it is transformed into hormone iodine as it comes in.

The value of m_p' will be given by Equation 7, but, to the dose m supplied it will be necessary to add the iodine m_{p0}' which still exists in the plasma, since it is currently not negligible as compared to m

$$m_p' = (m + m_{p0}' - \frac{q}{a+c}) e^{-(a+c)t} + \frac{q}{a+c}$$

On the other hand, in order for the reserve of hormone iodine to be kept intact we must have:

$$12q = a \int_0^{12} m_p' dt$$

Carrying out the integration, we get:

$$12q = \frac{a}{a+c} \left(m + m' - \frac{q}{a+c} \right) \times (1 - e^{-12(a+c)}) + \frac{q}{a+c}$$

from which:

$$m_{p0}' = \frac{q}{a+c} - \frac{12cq}{a(1 - e^{-12(a+c)})}$$

In addition, after a cycle of 12 hours it has to be $m_p' = m_{p0}'$, or:

$$m_{p0}' = \left(m + m_{p0}' - \frac{q}{a+c} \right) e^{-12(a+c)} + \frac{q}{a+c}$$

Eliminating m_{p0}' between those two last equations we obtain:

$$a = \frac{12cq}{m} \quad (19)$$

From the measurements carried out in the Portuguese Institute of Oncology we get for c and q the values:

$$c = 0.06 \text{ hr}^{-1}$$

$$q = 10 \text{ } \mu\text{g/hr}$$

and since, according to Salter, $m = 7.5 \text{ } \mu\text{g}$, we can get:

$$a = \frac{12 \times 0.06 \times 10}{7.5} = 0.96 \text{ hr}^{-1}$$

so that the thyroid has a fixation power for iodine some 16 times greater than the kidney.

This result leads to the belief that the fixation of iodine by the thyroid is controlled by the diffusion process of the iodine in the blood and by its transport to said gland, the rates of which would be much smaller than the captation rate calculated with Equation 18, which correspond to the case in which the whole of the blood would be simultaneously in contact with the adsorbent. We can use, in our computations, without any grave mistake, the value $a = 0.96 \text{ hr}^{-1}$ computed with Equation 19.

EXPERIMENTAL VERIFICATIONS

The many observations made for diagnostic purposes at the Instituto Português de Oncologia do not help to demonstrate our theory since the radioactive iodine is supplied by the oral route. It was necessary, therefore, to find some subjects who would lend themselves to such a test by receiving the iodine in intravenous injection. On the other hand, the precision achieved with the instrumentation used, while

it is adequate for routine measurements, leaves much to be desired for our purposes. Nevertheless, with the data obtained, we can state that, on the whole, everything takes place in agreement with our formulae.

Figures 4 and 5 refer to two series of measurements carried out in one and the same patient. In the first, the radioactive iodine ($25 \text{ } \mu\text{g}$) was accompanied by 0.5 mg of stable iodine, while in the second 3 mg of this last substance were added. The curves d_g and d_p represent respectively the activities in the thyroid and plasma calculated according to Equations 16 and 17, giving the following values to the parameters:

$$r = 0.24; \quad q = 7.2 \text{ } \mu\text{g/hr}; \quad c = 0.12 \text{ hr}^{-1}$$

with which, we see what happens when the mass M of stable iodine passes from 0.5 mg to 3.0 mg .

The values of d_{p1} were determined in each case so that the respective curves go through the point found experimentally at the end of 6 hours.

While, as stated, the precision of the measurements does not permit us to hope for perfect quantitative agreement, the most salient facts are clearly confirmed, as foreseen by our theory. First, there is a rapid increase in the activity in the thyroid, which is practically independent of the mass M of stable

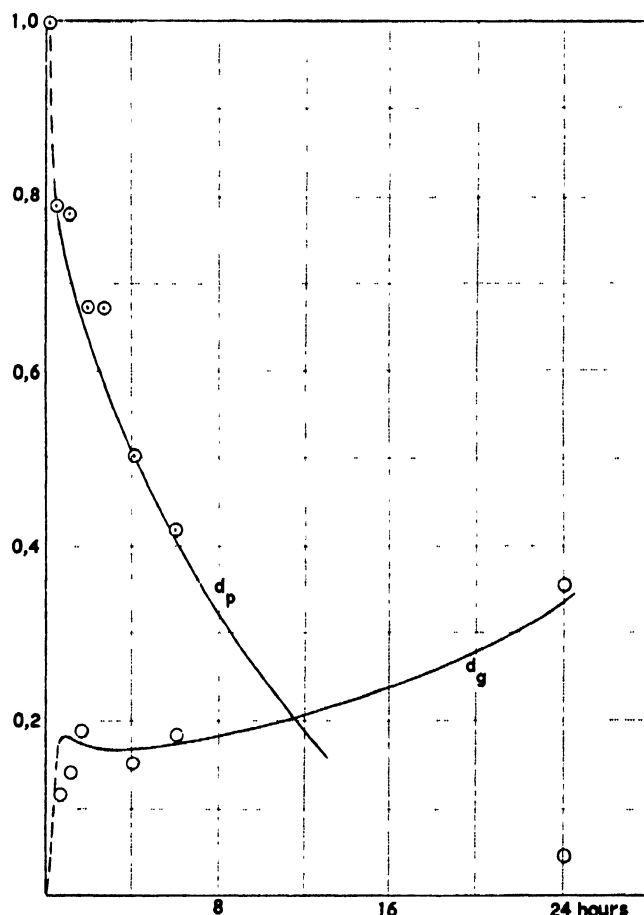


Figure 4. Measurements of the radioactivity of the thyroid, d_g , and of plasma d_p in a patient who was supposed to suffer from goiter. The radioactive iodine was accompanied by $M = 0.5 \text{ mg}$ of I^{127} . The curves were traced with the Equations 17 and 18 giving the parameters the values $r = 0.24$; $q = 7.2 \text{ } \mu\text{g/hr}$; $c = 0.12 \text{ hr}^{-1}$

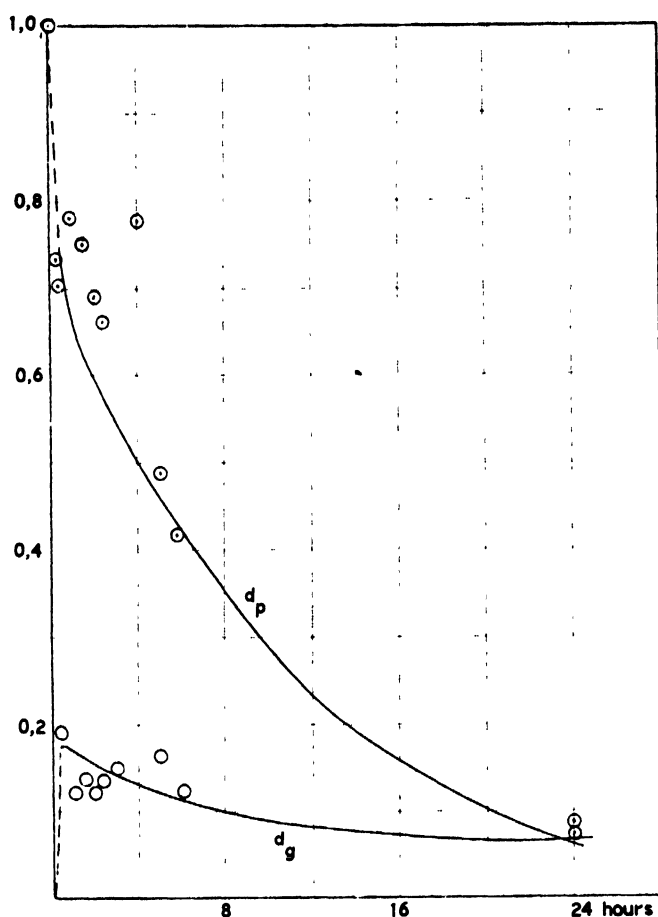


Figure 5. Repetition of the measurements with $M = 3$ mg. The curves have been drawn with the same values r , q , and c as in Fig. 4

iodine. On the other hand, the subsequent development of the curve is considerably under the influence of the value of M . If we make $M = 0.5$ mg we first observe a small decrease in the inorganic iodine from the thyroid to the blood, but then the curve increases until at the end of 24 hours

$$d_g = rd_p + d_g'' \simeq \frac{q}{M} t = 0.35$$

which coincides with the value found experimentally. With $M = 3.0$ mg, there is a very marked reduction in the inorganic iodine, but the production of hormone continues along the same lines, since the experimental value of d_g , at the end of 24 hours, coincides with the theoretical value:

$$d_g = rd_p + \frac{q}{M} t = 0.014 + 0.058 = 0.072$$

There is no reason therefore, to ascribe the rapid decrease in radioactivity of the thyroid to iodine saturation.

From the comparison of values obtained in healthy individuals, we can note that the elaboration of

hormone ($q = 7.2 \mu\text{g/hr}$) and urinary secretion ($c = 0.12 \text{ hr}^{-1}$) can be considered normal. On the other hand, the adsorbent power ($r = 0.24$) is exacerbated, which confirms the thought, suggested by clinical data that this patient had simple goiter.

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The Measurement of Thyroid Gland Uptake and Turnover of I^{131} in Man by External Counting: Its Use in Diagnosis and Treatment of Thyroid Disorders

By A. Stone Freedberg,* George S. Kurland* and Marvin Van Dilla,† USA

Considerable study has been devoted to the problem of direct measurement by external counting of the uptake and I^{131} content (in microcuries) of the thyroid gland following administration of this isotope in man. In order that this measurement yield the radioactivity of the gland in absolute units, the method must be able to take into account variations in size and location of the gland as well as variations in the amount of body tissue available for gamma-ray scattering and absorption from patient to patient. It is also important that there exist adequate sensitivity to small amounts ($<5-10 \mu c$) of I^{131} .

This kind of measurement would be easy to perform in the presence of certain simplifying conditions. Let us assume that we have available a counter whose efficiency is independent of quantum energy $h\nu$. If the absorbing material has a low effective atomic number and the gamma-ray source embedded in it has a quantum energy high enough so that Compton collisions are the only important mode of interaction, then the number of photons emitted by the gamma-ray source is conserved, although those photons suffering Compton collisions are degraded in energy. If enough counters of the above type are connected in parallel and arranged geometrically so as to completely surround the source plus absorber, then the counting rate will be independent of the amount of absorber present, the relative position of the source to absorber, and the position of source plus absorber inside the closed surface of counters. The only factor affecting the counting rate will be the absolute activity of the source.

The human body is composed mostly of low atomic number ($Z = 1-8$) elements; the skeleton (10 per cent of body weight) contains enough calcium ($Z = 15$) to account for about 25 per cent of its weight. The gamma rays of I^{131} are hard enough to have a small photoelectric absorption coefficient in body tissue and are too soft for pair production. Hence Compton collisions are the predominant mode of interaction.

Although there are no G-M counters completely

insensitive to quantum energy $h\nu$, those counters with heavy metal cathodes have fairly constant efficiencies in the region of quantum energies between 0.2 and 1.0 Mev.¹ Since it is quite impractical to attempt to surround a patient completely with counters, we have connected 4 G-M or scintillation (NaI-thallium activated crystals) counters in parallel and arranged them at equal intervals on the circumference of a horizontal circle of various radii 45-60 cm with the patient's neck at the center. The following theoretical considerations and experimental evidence show the degree to which this scheme approaches the ideal described above in freedom from the effects of variations in geometry and self-absorption.

ESTIMATION OF FREEDOM FROM GEOMETRY

With the counters arranged in this manner, geometrical relationships can be used to estimate the degree of freedom from geometrical effects gained. Davis² has done this for the case of a circle of small radius in connection with the assay of radium and has shown that the counter response is proportional to the solid angle subtended by the counter at the source position.

In Fig. 1, let A , B , C , and D represent the 4 counters and consider γ the counting rate with a small source at Q relative to the counting rate at the center; using the inverse square law, which may be expected to hold with this geometry, and neglecting scattering and absorption effects,

$$\gamma = \frac{R^2}{a^2} + \frac{2R^2}{b^2} + \frac{R^2}{c^2}$$

Using the identities

$$b^2 = r^2 + R^2$$

$$a = r + R$$

$$c = R - r$$

and letting $a = r/R$

$$\gamma = \frac{1}{4} \frac{1}{(1+a)^2} + \frac{2}{1+a^2} + \frac{1}{(1-a)^2}$$

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Table I. Variation in Expected Counting Rate with Positions of Source

A		B		C	
a	γ	a	γ	a	γ
0.1	1.01	0.1	1.01	0.1	0.99
0.2	1.05	0.2	1.04	0.2	0.96
0.3	1.12	0.3	1.08	0.3	0.92
0.4	1.25	0.4	1.13	0.4	0.86
				0.5	0.80

Column A of Table I shows values of γ for various a 's and indicates that with a 60 cm circle, the counting rate does not change by more than 5 per cent as one moves a small source from the center of the circle to a point $0.2 \times 60 = 12$ cm from center and toward one of the tubes.

If displacement of the source from the center at an angle of 45 degrees to the line AC (Fig. 1) is considered, one can deduce from the inverse square law that

$$2\gamma = \frac{1}{a + 1 + a\sqrt{2}} + \frac{1}{a^2 + 1 - a\sqrt{2}}$$

Column B shows the variation in counting rate with different positions of the source.

If vertical displacements of the source above and below center are considered, the inverse square law predicts that

$$\gamma = \frac{1}{1 + a^2}$$

from which is obtained Column C. Hence we see that for 60-cm circle of 4 counters, there is a volume (roughly spherical) at the center whose radius is about 12 cm and which is insensitive (to 5 per cent) to movement of a small source.

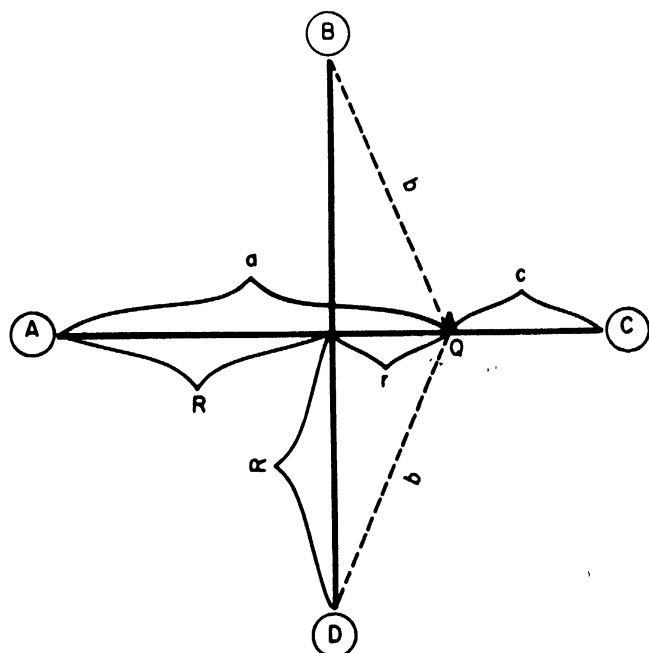


Figure 1. Geometric considerations in the four-tube setup

EXPERIMENTAL APPARATUS

Geiger-Mueller tubes with various cathodes have been utilized in our studies. In the following, the G-M tubes had 80-mesh platinum-screen cathodes 12.5 cm in length and 2.5 cm in diameter and were filled with a 10 per cent alcohol-argon mixture.

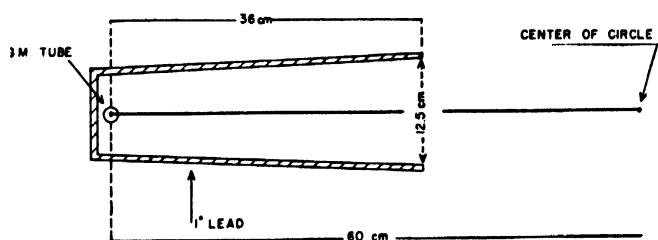


Figure 2. Scale drawing of lead shielding surrounding platinum screen cathode G-M tube

They were connected in parallel and arranged symmetrically on the periphery of a horizontal circle of radius 60 cm. The axis of each tube was also horizontal, and each tube was shielded with lead to reduce background and minimize the effect of circulating I^{131} , as shown in Fig. 2.

EXPERIMENTAL RESULTS

Experiments (Fig. 3) with a single unshielded tube show that the inverse square law holds within the limits of experimental accuracy for source-counter distances of between 30 and 90 cm. Both source and counter were 117 cm above the floor.

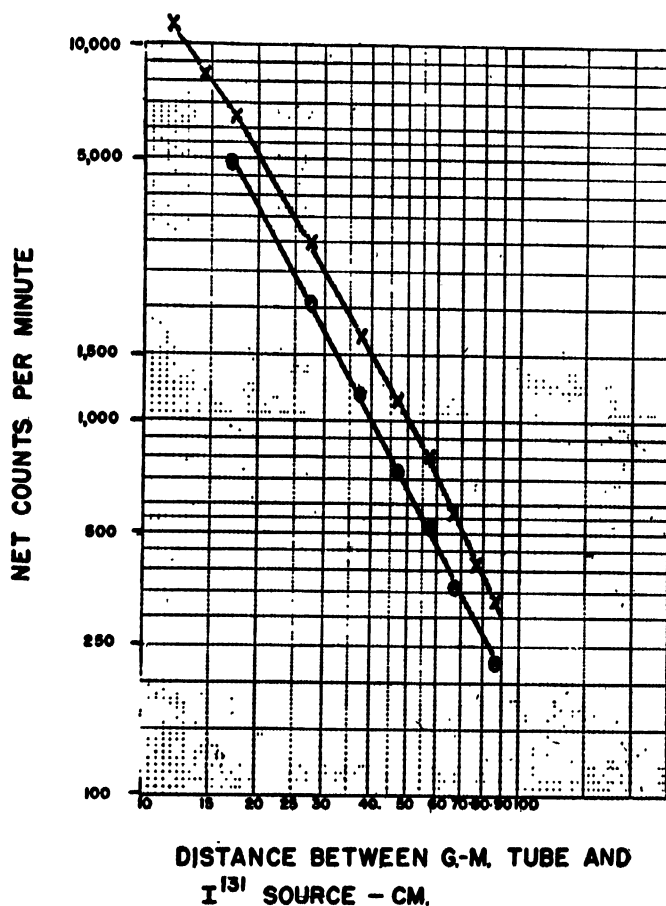


Figure 3. Single G-M tube geometry. Change in counting rate with horizontal displacement of source

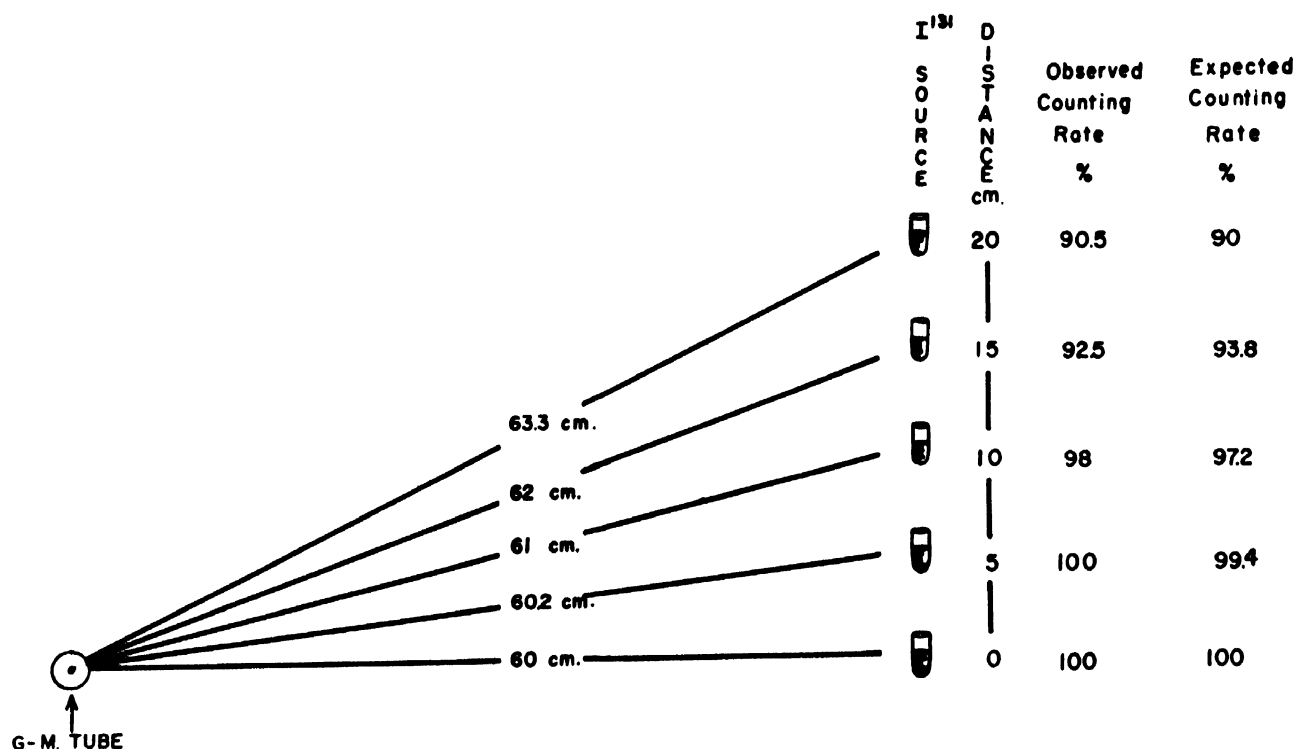


Figure 4. Single G-M tube geometry; change in counting rate with vertical displacement of source. Radiation measured with G-M tube and source 60 cm apart and 117 cm above floor taken to equal 100 per cent. The expected change in counting rate was calculated as described in the text

Similarly with vertical displacement of the source, the measured change in counting rate agrees with the expected change in counting rate (Fig. 4). When source and counter were 15 cm from the floor (Fig. 3, upper curve), higher counting rates and significant deviations from the inverse square law were observed.

With 4 tubes (each lead shielded as in Fig. 2) arranged in a horizontal circle of radius 60 cm, the variations in counting rate with movement of a small I^{131} source also closely agree with the values calculated above from the inverse square law. These results are shown in Fig. 5.

MOCK-UP EXPERIMENTS

Previous mock-up experiments have shown the degree of freedom from self-absorption and geometry effects obtained by utilizing 4 G-M tube circle, 58–60 cm radius.³ Five cm³ of I^{131} solution in a one gallon jug were placed in the center of such a circle. The net counting rate was 4500 counts per minute. The addition of 5, 95, 995, 1995, and 2995 cm³ of water resulted in a maximum decrease of 6.7 per cent in observed radiation.

To stimulate the normal structures around the thyroid gland, saline solution and meat (in quantities approximating those of the human neck) and bone (skull, vertebrae and ribs) were placed about I^{131} sources (18–50 μ c) in volumes of 5–350.0 cm³ in one or more volumetric flasks suspended in the center of the circle. When the I^{131} source was surrounded by muscle, bone, and saline solution, the maximum change in observed radiation was 5 per cent when compared to the unsurrounded source.⁴ With a con-

stant amount of radioactivity in volumes varying from 5–350 cm³ in 1–3 different shaped containers, no significant change in counting rate was observed. Movement of the I^{131} source from the center but within the free circle area (10-cm horizontal and 8-cm vertical) incurred no significant change in counting rate.

Experiments have been carried out with rubber models of the thyroid gland approximating the normal thyroid gland and an enlarged gland four times normal size. These were filled, respectively,

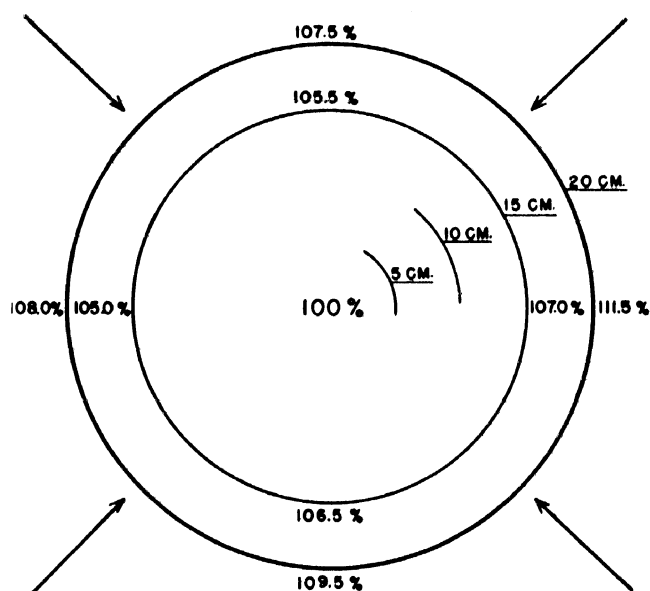


Figure 5. Change in measured radiation from a source of I^{131} at various horizontal distances from the center of the shielded four-tube circle, radius 60 cm. The arrows indicate the position of the four G-M tubes

with 15 and 50 cm³ of I¹³¹ solution (100 μ c) and strapped to the anterior neck over the position of the thyroid gland in 12 subjects. The radiation was measured and compared to the radiation from the model suspended alone in the circle center. The average change was a 5.0 per cent increase in counting rate (S.D., 3 per cent).⁵ Many additional mock-up experiments in human subjects confirmed these results, showing the effect from geometry, absorption and scatter to increase the counting rate 5 ± 3 per cent.

In another set of experiments, a Levine tube containing 45–80 microcuries of I¹³¹ sealed between two lead beads (1.5 inches apart) was passed into the esophagus of 4 subjects. Under fluoroscopic visualization, the I¹³¹ source was stationed opposite a marker previously placed on the skin over the thyroid gland. The subject was positioned so that the source would be in the approximate center of the circle. Counting rates from the tube source, both outside and within the subjects, were measured. With the I¹³¹ source in the subject, the counting rate was increased by approximately 8.5 per cent.

The above data confirms the theoretical considerations noted before. With the horizontal arrangement of four platinum-screen cathode Geiger-Mueller tubes placed in a circle and connected in a parallel electrical circuit, there is achieved both sensitivity to small quantities of I¹³¹ and virtual insensitivity to geometry, scatter and absorption effects. The sensitivity of the described apparatus is such that 4 μ c I¹³¹ doubles the background counting rate; approximately 50 net counts are obtained per microcurie I¹³¹. Other published data from these laboratories, however, show that the 60-cm radius circle is not necessary for human work and that a marked increase in sensitivity can be obtained by using a 45-cm circle.⁵ Thus for experiments in animals where the thyroid gland or other organs are small, considerable increase in sensitivity can be achieved by decreasing the size of the circle while still retaining an adequately large central "free circle."

We have utilized the platinum cathode G-M tube arranged as described above to measure urinary I¹³¹ excretion. The urinary I¹³¹ excretion was measured also by a gamma ray method using Marinelli type beakers.³ Excellent agreement was obtained between the two methods, showing virtual independence of the 4-tube method to urinary volumes varying from 500–3500 cm³. An arrangement similar to that described by us was utilized by Davis² to measure the radiation from radium samples; exact centering was not required and therefore excessive handling of the sample was avoided. Similar considerations apply to the measurement of urinary I¹³¹ excretion following large therapeutic doses in man.

The accuracy and simplicity of the 4-tube method is illustrated in Table II.

Each of 25 chicks (51 to 80 grams) was given approximately 138 microcuries I¹³¹ intraperitoneally. The excreta was collected in 3 periods during the

Table II. Recovery of Injected I¹³¹ in 25 Chicks

Number of chicks	25
Total I ¹³¹ injected (microcuries)	3460
I ¹³¹ injected per chick	138 $\pm$ 7.8 S.D.
Per cent of administered I ¹³¹ recovered	98.8
Pooled fecal and urinary I ¹³¹ excretion in 24 hours after injection	84.9%
Initial eight hours pooled	69.4%
Subsequent 16 hours pooled	13.9%
Pooled chick I ¹³¹ content 24 hours after injection	13.9%
Fecal and urinary I ¹³¹ excretion during period of 24 to 48 hours after injection	1.4%

next 48 hours. The radiation from the chicks and the excreta was measured by placing them in separate paper containers (1-pint ice cream boxes) at the center of the circle of counters. Table II shows virtually complete recovery of the injected I¹³¹. A similar balance experiment was carried out in guinea pigs. The urine and stools were collected separately. The stool specimens were placed in a pint paper container and the radiation measured in the center of the circle. The urine volume was not measured; the urine was collected in a beaker and the latter was placed in the center of the circle. The radiation from the whole guinea pig and the dissected thyroid gland was measured as described above for chicks. Of 52 μ c I¹³¹ injected per animal, 97% was accounted for.

In several balance experiments in man, thyroid radiation before and after subtotal thyroidectomy was measured by the 4-tube method. The radioactivity from the surgical specimen, sponge and dressings was similarly measured. Ninety-eight per cent of the preoperative neck counts were accounted for. Of the 98 per cent, approximately 3 per cent was present in the sponges and instruments. Utilizing this data and the urinary excretion, 95 per cent of the administered tracer dose was accounted for.

We have discussed elsewhere⁵ the characteristics of Geiger-Mueller tubes deemed necessary for application of this method. These included (1) availability, (2) comparable threshold voltages, (3) adequate plateaus, (4) equal sensitivity and (5) operating lives long enough for clinical purposes. The tubes utilized in these experiments are not generally available, and there was considerable difficulty in preparing and obtaining tubes with the requirements noted in (2), (3), and (4) above. We have, therefore, not utilized these tubes for measurements of therapeutic doses of I¹³¹ in man. Other published studies have shown the usefulness of a less-sensitive copper cathode Geiger-Mueller tube for the measurement of tracer and therapeutic quantities of I¹³¹ in man by the 4-tube method described here.⁵ In these studies, the necessity for determining in each patient the effects of geometry, absorption, and scatter were shown. Table III shows these effects with the various G-M tubes we have studied in the past 4 years.

Table III. Absorption and Scatter with Various 4-Tube Arrangements*

Cathode		Glass painted with steel	Bismuth	NaI-Th activated crystal scint.
Screen platinum	Copper			
+ 5%‡ (-1.5 to + 10)	+ 8% (0 to 15)	(a) - 20%† (0 to - 40)	+ 20 (+ 2 to + 38)	Varies with discriminator setting
		(b) - 25% (+ 5 to - 41)		
		(c) - 22% (+ 4 to - 40)		

* Change in counting rate of I^{131} standard strapped to front of patient's neck compared to same standard suspended in air in the center of 4-tube circle.

† Each of these series totals approximately 75 patients. More than 100 patients have been studied with each of the other arrangements listed in Table III.

‡ Average: figures in parentheses give range.

With scintillation counters utilizing sodium iodide (thallium activated) crystals, a substantial increase in sensitivity can be obtained. Details of the circuit and the setup which have been developed for this purpose at the Radioactivity Center, Department of Physics, Massachusetts Institute of Technology, will be published elsewhere by that group. Each Geiger-Mueller tube has been replaced by a scintillation counter consisting of a hermetically sealed sodium iodide crystal, an RCA 5819 photomultiplier tube and a single tube pre-amplifier; each channel is fed into an amplifier and a commercial scaler. While the background counting rate is somewhat higher than obtained with the 4 G-M arrangement, the net counting rate per microcurie of I^{131} is considerably higher, as shown in Table IV.

Using the idea of a factor of merit suggested by Thomas,⁶ it can be shown that a source may be measured at least 18 times as fast with the scintillation counters as with the Geiger-Mueller tubes. If the backgrounds are not known accurately, Thomas⁶ and Loevinger and Berman⁷ give formulae taking this into account.

In experiments with the scintillation array, it has been possible to measure the thyroid gland uptake utilizing a tracer dose of 15-25 microcuries. This dose is in contrast to the 50-150 microcuries with the various G-M tubes utilized in our studies. It is important, to emphasize, however, that these considerations do not permit neglect of the importance of the determination of the effects of geometry, absorption and scatter with the particular scintillation

set-up utilized. As pointed out above, similar considerations, however, apply in varying degrees to the Geiger-Mueller tubes we have utilized in our experiments.

THE THYROID GLAND UPTAKE IN MAN

The thyroid gland uptake was calculated by relating the radiation from the thyroid gland to the radiation from the standard, a 25 or 50 cm³ aliquot of the I^{131} stock solution from which the patient's dose was taken, using the formula:

Thyroid uptake in % =

$$\frac{\text{Net cpm thyroid} \times \text{"factor"} \times 100}{\text{Net cpm standard}}$$

$$\times \frac{\text{cm}^3 I^{131} \text{ sol. administered}}{\text{cm}^3 I^{131} \text{ sol. in standard}}$$

The "factor" was experimentally determined in each patient as described above.

Table V (A, B, and C) shows the excellent agreement obtained in comparisons of 24-hour thyroid gland uptake measured in various 4-tube arrangements. Part A shows that using the data obtained with the platinum cathode G-M tubes to equal 100 per cent, the average difference of the copper cathode data is -0.25 per cent and the range of the individual comparisons differed by -7.5 to +14.5 per cent, S.D. 3.1. Part B indicates that, using the scintillation counter data to equal 100 per cent, the average difference of the G-M tube data is -0.3 per cent. The range of the individual comparisons differed by +34 to -43 per cent. Forty-four of the 55 comparisons showed a difference of less than 10 per cent. The large differences were obtained with 24-hour thyroid uptakes lower than 10 per cent. In Part C, the results obtained by a single scintillation counter (one of the 4 used in the 4-channel scintillation array) 45 cm from the anterior surface of the neck show a significantly higher uptake. However, when the results were corrected by the factor obtained with the 4 scintillation counters, they were more nearly in agreement with the results obtained with the latter arrangement Table V,C,**).

The 24-Hour Thyroid Gland Uptake and Turnover of I^{131} in Man

Tables VI and VII show the thyroid gland uptake and turnover following a tracer dose of I^{131} in a group of over 500 patients studied with the 4-tube method, using copper cathode G-M tubes.⁸

Estimation of thyroid gland function was based on careful clinical evaluation and laboratory tests such as basal metabolic rate, cholesterol and, in some

Table IV. Net Counts per Minute per Microcurie I^{131} in Various 4-Channel Arrays

Circle radius	Scint. counter (bkgd = 360 cpm)	Cu cathode (bkgd = 270 cpm)	Pt cathode (bkgd = 200 cpm)	Bi cathode (bkgd = 140 cpm)	Glass wall coated with steel (bkgd = 120 cpm)
45 cm	370	20	90	60	13
60 cm	280	15	50		

Table V. Comparison of the Thyroid Gland Uptake Following Tracer Doses As Measured by Various 4-Tube Circles

A. Platinum vs. Copper Cathode

No. of comparisons	Thyroid gland uptake in %	
	Platinum cathode	Copper cathode
96	5.5-97	5.5-91

B. Scintillation Counters vs. Geiger-Mueller Tubes

No. of comparisons	Thyroid gland uptake in %	
	4 G-M tube-glass wall stainless steel coated cathode	4 scintillation counters—1" NaI-Th activated crystal
55	2-82	2-78

C. Single vs. 4 Scintillation Counters

No. of comparisons	Thyroid gland uptake in %		
	4 scintillation counters	1 scintillation counter (uncorrected)	1 scintillation counter
39	6-78	7.5-96.2*	6-74†

* These data showed an average difference of +22 per cent from the 4-tube results. Using the 4-tube results to equal 100 per cent, the range of individual comparisons differed by -13 to +50 per cent. Only 3 of the inter-comparisons showed uptake with the single tube lower than that obtained with the 4-tube set-up.

† These data, corrected as described in text for absorption and scatter, showed an average difference of -6% from the results with the 4 scintillation counters. Using the 4-tube results to equal 100 per cent, the range of individual comparisons differed by +15 to -24 per cent.

cases, serum protein bound I¹²⁷ and I¹³¹. Patients were classified into one of four major groups—euthyroid, hyperthyroid, hypothyroid, and miscellaneous. The latter group comprised 65 tracer studies which were considered to have been influenced significantly by low iodine diet, ingestion of iodine, organic iodine administration for X-ray examination, propylthiouracil, desiccated thyroid, cortisone or corticotrophin, recent thyroidectomy or I¹³¹ therapy and are not reported in detail here.

The data in Tables VI and VII indicate that I¹³¹ uptake studies are of value in the diagnosis of the state of thyroid function in a large number of cases. However, considerable overlap in uptake and effective half-life is noted between individual patients in the total euthyroid and hypothyroid groups and in the total euthyroid and hyperthyroid groups. Moreover, the possibility of modification of thyroid gland uptake by other factors has to be considered in every patient. These include those noted above and in Tables VI and VII and also others such as severe liver or renal failure⁹ which have been shown to be associated with a high 24-hour thyroid gland I¹³¹ uptake. It is thus clear that the tracer studies must be considered in the light of the clinical findings. Many additional thyroid I¹³¹ tests, including a 1-hour and 6-hour test, determination of thyroidal I¹³¹ clearance, urinary I¹³¹ clearance, rate of thyroidal secretion of organic I¹³¹, and determination of blood protein-bound iodine have all been proposed for the evaluation of thyroid function. In addition, Greer¹⁰ and Werner¹¹ have proposed the use of desiccated thyroid, or triiodothyronine to separate the high uptake of certain euthyroid subjects from that of hy-

Table VI. The Uptake of Tracer Doses of I¹³¹ in Various States of Thyroid Function

Group	No. of tracer studies	Mean uptake			t*		
		%	S.E.	S.D.	Normal control	Euthyroid goiter	Hypothyroid after I ¹³¹ therapy for hyperthyroidism
I. Euthyroid, total†	290	34.6	0.6	10.6	6.6		
A. Normal control	64‡	29.0	0.6	5.1	—		
B. Hypermetabolic	9	29.9	2.9	7.6	0.3		
C. Goiter§	71	37.1	1.3	10.6	5.8	—	
D. Post-thyroidectomy for hyperthyroidism	13	32.0	2.4	8.8	1.2		2.5
E. Post I ¹³¹ therapy for hyperthyroidism	62	39.5	1.5	12.1	5.5		—
F. Congestive heart failure	37	38.7	1.5	9.3	5.7		
II. Hyperthyroid, total	127	71.0	1.2	13.8	30.0	18.8	
A. Untreated							
1. Diffuse goiter	40‡	76.2	1.7	10.7	26.2	18.6	—
2. Nodular goiter	9	59.8	3.6	9.5			4.0
B. Persistent after I ¹³¹ therapy	33	70.0	2.5	13.3	17.1		2.2
III. Hypothyroid, total	43	11.9	1.0	6.3	14.3		
A. Post I ¹³¹ therapy for hyperthyroidism	18	13.3	2.0	8.3	7.5		9.7
B. All others	25	10.8	0.9	4.4			

* t = difference between the means/S.E. of the difference between the means. A value of 3.0 or more is considered significant.

† The difference between the total and the sum of the sub-

groups is made up of cases complicated by multiple factors.

‡ In the statistical analysis of this group n-2 was used, n in all others.

§ Nodular, 66 cases; diffuse, 5 cases.

Table VII. The Effective Half-Life of Tracer Doses of I^{131} in Various States of Thyroid Gland Function

Group	No. of tracer studies	Mean effective half-life			t^*		
		Days	S.E.	S.D.	Normal control	Hyperthyroid total	Hyperthyroid persistent after I^{131} therapy
I. Euthyroid, total†	191	6.4	0.1	1.4	4.4	6.0	
A. Normal control	47	7.1	0.1	0.8	—		
B. Hypermetabolic	5	7.6	—	—			
C. Goiter‡	34	7.0	0.1	0.8	0.6	8.8	
D. Post-thyroidectomy for hyperthyroidism	8	6.6	—	—			
E. Post I^{131} therapy for hyperthyroidism	51	4.4	0.2	1.1	13.5	5.5	0.4
F. Congestive heart failure	31	7.6	0.2	1.0	2.4		
II. Hyperthyroid, total	113	5.5	0.1	1.1	10.0	—	
A. Untreated							
1. Diffuse goiter	35	5.5	0.2	0.9	8.4		4.3
2. Nodular goiter	7	6.2	—	—			
B. Persistent after I^{131} therapy	31	4.5	0.2	1.0	12.4		—
III. Hypothyroid after I^{131} for hyperthyroidism	7	2.6	—	—			

* t = difference between the means/S.E. of the difference between the means. A value of 3.0 or more is considered significant.

† The difference between the total and the sum of the subgroups is made up of cases complicated by multiple factors.
‡ Nodular, 31 cases; diffuse, 3 cases.

perthyroidism. Insufficient experience has accumulated with each of these to define their clinical usefulness. It is our opinion that a multiphasic, integrated evaluation of several parameters of thyroid function in a large series of cases will be necessary to determine the most useful clinical tests.

THE THERAPEUTIC USE OF I^{131} IN HYPERTHYROIDISM

Clearly, radioiodine is an effective drug in the treatment of thyrotoxicosis.¹²⁻²⁰ However, many problems remain. Dosage is, at present, empiric, and agreement has not been reached as to the optimum schedule of treatment. We are continuing our investigation,²¹ exploring the possibility of treating thyrotoxicosis by the delivery to the thyroid gland of predetermined amounts of radiation with I^{131} . For this purpose, in addition to other factors, it is necessary that the method of measurement of thyroid uptake and turnover give reproducible and quantitative results unaffected by size of the thyroid gland or the location of I^{131} within the gland. Moreover, the uptake and turnover of the therapeutic dose has to be predictable on the basis of the data obtained following tracer doses.

Using the methods discussed above, an investigation of the predictability of uptake and turnover of the therapeutic dose from the tracer data has been carried out. Table VIII shows the excellent reproducibility of uptake and turnover (E.H.L.) following two or more tracer doses of I^{131} in 15 euthyroid subjects. Table VIII also shows uptake and turnover of tracer doses compared with the therapeutic doses in 55 thyrotoxic patients.

The therapeutic dose varied from 2 to 18 millicuries, averaging 7. For the entire group, the average difference in uptake of the therapeutic dose was only

4 per cent less than following the tracer dose, and the average difference in turnover, 1 per cent. Excellent agreement was obtained in a comparison of the individual therapeutic and tracer uptakes. In the few exceptions, the discrepancy was due to inadvertent use of iodides for therapeutic or diagnostic purposes.

Approximately 80 per cent of our cases showed excellent agreement in turnover. Twenty per cent showed a deviation of the therapeutic turnover compared to the tracer averaging approximately 10 per cent. Since the range in effective half-life observed in our studies was 1.5 to 8 days (aver. 5.5 days) or a biologic half-life of 1.8 days to infinity, it is apparent that any calculation of thyroid radiation using an average figure or ignoring the factor of thyroid turnover may have a significant error. The conclusion was drawn from this and subsequent data that the tracer dose can be utilized to estimate the uptake and turnover of a therapeutic dose.

Table VIII. Relationship of I^{131} Dose to Thyroid Uptake and Turnover

	No. of subjects	Size dose	Difference in 24-hour uptake %	Difference in E.H.L. %
Repeated tracers (2-6) in euthyroid subjects	10	100-150 μ c	— 1	— 3
Comparison of small vs. large tracer doses in euthyroids	5	20-110 μ c	0.8	—
Comparison of tracer and therapeutic doses in thyrotoxicosis	55	2-18 mc average 7.1	— 4	— 1.2

Using the information obtained from tracer data and estimating the size of the thyroid gland as noted below, we arrived at a therapeutic dose for each patient calculated to deliver an estimated 10,000 rep. Thyroid weight is estimated in our clinic by palpation by at least 5 observers, independently. The average is utilized in the calculations, the average difference of the extremes from the mean is ± 15 per cent. The normal thyroid gland is assumed to weigh 25 grams; the average weight of the normal thyroid gland obtained at autopsy is 23 grams (unpublished data).

The formula for delivered radiation is $\text{rep} = \text{dose (microcuries)} \times 24\text{-hour uptake (\%)} \times (\text{E.H.L.}/8) \times (\text{days}) \times 160 \div \text{wt of gland (gm)}$. The patients were observed 3 to 5 times during the first 10 days during which time measurements of thyroid uptake and turnover were made and then at 3 weekly intervals thereafter. The therapeutic result is evaluated not only by clinical examination but also by various laboratory tests including basal metabolic rate and, in many instances, blood protein-bound iodine and I^{131} studies.

Table IX shows the results of a single dose of I^{131} in a group of 90 patients followed at least one year. Fifty-eight or 64 per cent became euthyroid, 18 or 20 per cent were hypothyroid; 14 or 16 per cent, though improved, remained persistently thyrotoxic. This table also correlates the number of millicuries administered to these patients with the end result and the size of the thyroid gland separated into 3 groups, larger than 75 grams, 50–75 grams, and less than 50 grams. Although, as one might expect, the size of the dose is related to the size of the thyroid gland, there was overlap in dosage in the 3 gland groups. In the 50–75 gram gland group, the average number of millicuries administered in the group of patients who became hypothyroid after I^{131} is larger than in the patients becoming euthyroid or remaining persistently thyrotoxic; the difference, however, is probably not significant statistically. The percentage of patients remaining persistently thyrotoxic in the group with glands over 75 grams is larger than in the other two groups.

Table X shows the same data in terms of roentgens delivered.

The three patients in the large gland group, i.e., more than 75 grams, who became euthyroid received a distinctly larger amount of radiation than the patients in the same group who remained persistently thyrotoxic. In the middle gland group, the patients who became hypothyroid received a larger radiation on the average than the patients who became euthy-

Table IX. Relation Estimated Thyroid Size, mc I^{131} and Therapeutic Result*

Gland size (gm)	Euthyroid	Hypothyroid	Improved but persistent thyrotoxicosis
> 75	15.7 (13–17)		9.6 (6.5–15.6)
No. of patients	3		6
50–75	6.8 (2.7–9.5)	7.8 (4.7–18)	6.3 (3.3–8)
No. of patients	24	10	7
< 50	4.9 (2.7–9.6)	4.8 (3.2–8.7)	2.75
No. of patients	31	8	1
Total no. of patients	58	18	14
%	64	20	16

* Figures in parentheses are range (mc).

roid. As was noted above, however, there is overlapping. The extreme variations in estimated delivered radiation were in most instances related to an alteration in turnover of the therapeutic dose as compared to that anticipated on the basis of the tracer data. In a few instances, however, the uptake of the therapeutic dose was distinctly less due to the inadvertent administration to the patient of medications containing iodide or X-ray examinations utilizing organic iodide compounds. As was pointed out above, however, the number of such instances was small, and the average radiation dose of approximately 10,000 rep was obtained.

The high incidence of hypothyroidism in this series of patients is not adequately explained. The average number of millicuries of I^{131} used in the therapy of thyrotoxicosis in our clinic is not larger than that utilized in other clinics reporting distinctly smaller incidence of hypothyroidism. We were unable to correlate the high incidence of hypothyroidism with the type of gland, the level of thyrotoxicity or the age or sex of the patient. The incidence of hypothyroidism in the group of patients

Table X. Relation Thyroid Gland Size, rep and Result

Gland size (gm)	Euthyroid	Hypothyroid	Improved but persistent thyrotoxicosis
> 75	13000		8100 (5–11)*
No. of patients	3		6
50–75	8500 (4–13800)	9900 (6.3–19)	9400 (7.4–12.5)
No. of patients	24	10	7
< 50	9900 (4.5–15.5)	9800 (6.3–14)	10.7
No. of patients	31	8	1
Total no. of patients	58	18	14
%	64	20	16

* Figures in parentheses are range (rep).

‡ E.H.L. is Effective Half-Life or the number of days required for maximum neck thyroid counts, usually observed at 24 hours or before, to decrease by 50 per cent. The data is plotted on semi-logarithmic paper and the best straight line drawn.

§ rep including beta and gamma radiation estimated to be delivered by one microcurie of I^{131} per gram of tissue during the entire period of disintegration.

with small glands, i.e., less than 50 grams, is somewhat less than the incidence in the group with medium-sized glands; the differences are, however, not statistically significant. There is some indication that patients who have had their thyrotoxicosis for a short period of time are more likely to plummet into hypothyroidism or to develop myxedema after I^{131} than individuals who have had their thyrotoxicosis for a long period of time. Although some of our patients who developed hypothyroidism might have recovered spontaneously if thyroid medication had not been administered, we do not believe that the over-all final figures would be significantly altered since, in most instances, thyroid medication was not begun until symptoms or signs of marked hypothyroidism or myxedema were present. The final incidence of permanent hypothyroidism in our series is not known since all the patients have not been taken off thyroid extract. It should also be remembered that while most patients develop hypothyroidism within the first year after radioactive iodine a significant number of patients develop this complication after this period, just as they do after surgery. For example, we have observed one patient, euthyroid for 4 years after I^{131} who subsequently became hypothyroid.

The variation in response to I^{131} has been attributed, by some investigators, to differences in biologic sensitivity. This factor cannot be adequately assessed, however, until the known variables are examined in detail. We believe an important variable is the error in estimation of the size of the thyroid gland. The estimation of thyroid size may be particularly difficult in patients with previous subtotal or partial thyroidectomy or where the thyroid gland is located substernally. We have had the experience of treating a thyrotoxic patient with 2 doses of radioiodine based on an estimated weight of 35 grams and finding, at subsequent surgery, that 35 grams remained. After an initial therapeutic dose of I^{131} , the estimation of the weight of functioning tissue for the purpose of calculating subsequent I^{131} dosage is, in all probability, associated with a large error. More adequate methods to determine thyroid size should lead to more predictable results of I^{131} treatment.

SUMMARY AND CONCLUSIONS

1. Theoretical considerations and confirmatory experimental evidence are presented to show the degrees of freedom obtained from variation in geometry, self-absorption and scattering by an array of 4-G-M or scintillation (NaI-thallium-activated crystals) counters connected in parallel and arranged in a horizontal circle. The method offers quantitative accuracy, reduplicability, and simplicity in studies of the thyroid gland I^{131} uptake and turnover following tracer and therapeutic doses of I^{131} in man.

2. The average 24-hour thyroid gland uptake in euthyroid control subjects is 29 per cent (S.E. 0.6).

The uptake in patients with non-thyroidal hypermetabolism or angina pectoris or after surgical thyroidectomy for hyperthyroidism is not significantly different. Sex and age have not been demonstrated to significantly affect I^{131} metabolism as measured by 24-hour thyroid gland uptake and subsequent turnover.

3. Twenty-four-hour thyroid uptake in euthyroid patients with goiters, nodular or diffuse, patients with congestive heart failure or patients previously thyrotoxic who were euthyroid after I^{131} therapy was significantly higher than normal and averaged 37, 39 and 40 per cent, respectively.

4. The average 24-hour thyroid uptake in patients with hyperthyroidism is 71 per cent (S.E. 1.2). Patients with toxic nodular goiter have a significantly lower 24-hour thyroid gland uptake (average 60 per cent) than that observed in patients with diffusely enlarged toxic goiters (75 per cent).

5. The average 24-hour thyroid uptake in patients with hypothyroidism is 12 per cent. The uptake in some patients with spontaneous myxedema is lower, averaging 5 per cent.

6. The average thyroid gland effective half-life of I^{131} in euthyroid control subjects is 7.1 days (S.E. 0.1). The average turnover obtained in other groups of euthyroid patients with goiters, hypermetabolism of non-thyroid origin, or angina pectoris, or patients studied long after surgical thyroidectomy for hyperthyroidism is similar.

7. The average effective half-life of thyroid I^{131} in patients with hyperthyroidism is 5.5 days (S.E. 0.1) and is significantly faster than normal.

8. Many physiologic and pathologic factors influence thyroid I^{131} uptake and turnover. In addition to those noted above, hepatic and renal failure have been shown to be associated with high thyroid gland I^{131} uptake. Iodide deficient diets, e.g., the basic rice diet of Kempner, may result in a thyroid gland uptake in the range observed in hyperthyroid patients. On the other hand, many drugs, e.g., thyroid extract, iodides, inorganic and organic, thiourea derivatives, cortisone and corticotrophin lower the uptake to levels observed in hypothyroidism. A similar lowering of the ability of the thyroid gland to collect radioiodine is observed in euthyroid patients with thyroiditis and following the administration of 2 or more millicuries of I^{131} . Various considerations emphasize the importance of utilizing the tracer data in a diagnostic sense only in the light of the clinical findings.

9. Radioiodine, I^{131} , is an effective form of therapy in thyrotoxicosis or diffuse toxic goiter. Dosage is at present empiric, and many questions in regard to the optimum mode of I^{131} therapy, size of dose, single versus multiple doses, indications, use in nodular toxic goiter, and the place of stable iodide or goitrogens remain to be answered. Our studies demonstrate that the thyroid uptake and turnover (effective half-life) of a therapeutic dose of I^{131} can be determined

from the uptake and turnover following a tracer dose. We have concluded from these studies that the predictability of the delivered thyroid radiation of a therapeutic dose of I^{131} on the basis of the uptake and effective half-life following a tracer dose is sufficiently accurate to allow for calculated I^{131} dosage. Utilizing such information, we have investigated the effect of a single dose of I^{131} delivering an estimated 10,000 rep in 90 thyrotoxic patients. Sixty-four per cent became euthyroid, 20 per cent were rendered hypothyroid, and in 16 per cent there was significant improvement, but the patients remained persistently thyrotoxic. We are at present extending our studies to determine the effects of the delivery of an estimated 8000 rep to see whether the incidence of hypothyroidism can be significantly reduced without alteration in the incidence of persistent thyrotoxicosis.

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The Effect of Estradiol on Iodine Fixation in the Thyroid (Studied by Means of Radioactive Iodine I^{131})

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The nature of the reciprocal actions observed between the ovary and the thyroid gives rise to a good deal of controversy and remains somewhat obscure. From the clinical and experimental standpoint, it has been shown that there is a relationship between those two glands, the physio-pathological mechanism and nature of which, in particular, cannot very clearly be accounted for. According to some authors, castration gives rise to an increase in thyroid activity (Aron and Benoit, in the guinea pig), while, according to others, on the contrary, it sets the thyroid at rest (Gumbrecht and Loeser).

Thus, the administration of estrogens brings about, according to some authors, an inhibition in thyroid function, while, according to others it makes it hyperactive; finally, there are authors who hold that castration, as well as the administration of estrogens, has no effect on thyroid function. Most of these clinical and experimental controversies are due to the conditions under which the experimenters work, and particularly to the differences in the doses used. We now know that the effect of the hormones doubtless varies in some manner with the dose administered, so that, for instance, the estrogens, when administered in small doses stimulate thyroid function while, in the higher doses, they inhibit it. Parhon⁹ was among the first researchers who came out with the hypothesis of a thyro-ovarian antagonism. More recently, Milcou and Pitis⁶ demonstrated that the intermittent administration of folliculine in small doses brings about intense stimulation of the thyroid in aged and castrated rats and they consider it a rejuvenating hormone for the gland.

The possible avenues open for a study of the correlation between the ovary and the thyroid have been looked into more closely of recent times, thanks to the introduction of the synthetic antithyroids, of the natural and synthetic estrogens, and particularly thanks to the fact that it has been possible to obtain more sensitive indications and more accurate data on thyroid function with the assistance of radioactive iodine, I^{131} .

In the light of these data, we went back to the findings noted earlier at the level of the thyroid following the administration of high doses of estrogens for a long time, according to which we achieved a degree

of inhibition of the thyroid which went, in some cases, so far as to look like colloidal goiter.

The functional condition of the thyroid began to be appreciated better once radioactive iodine, I^{131} , became of current use, which made it possible to study the capacity of the thyroid to fix the iodine, following the administration of extracts and hormones. Money *et al.*⁸ observed an increase in the amount of radioactive iodine present in the rat thyroid, following treatment by estrogens such as progesterone and testosterone, and a decrease after estradiol and pregnenolane. Other authors, such as Paschkis and Cantorow¹⁰, studying the quantity of I^{131} present in rats, even 64 days after castration or the administration of estradiol benzoate for 1 to 4 weeks, observed no changes with respect to the controls. These authors come to the conclusion that the estrogens have no action on the thyroid.

The contradictory results obtained following the experiments mentioned above are due, indubitably, to the differences in the doses administered. This is why we find it interesting again to start our research on the action of the estrogens at the level of the thyroid, by studying thyroid function with the help of radioactive iodine. We obtained, in these experiments, a certain measure of inhibition of the thyroid activity, and thereafter studied the function of the thyroid and its capacity of fixation of radioactive iodine following the inhibiting action of estrogens administered in large doses and over a long period of time.

MATERIAL AND METHODS

The experiments were carried out on three groups of adult female rats, weighing, on an average, 130 to 160 gm.

1. The first group of six adult rats was treated with large doses of benzoic estradiol for 4 weeks (total dose 100,000 I.U. or 10 mg, for each animal). The activity of the thyroid body was investigated with the help of radioactive iodine I^{131} in a dosage of 2 μ c for each rat, which corresponds to 800 cpm with a Geiger counter.

2. The second group of 6 adult rats received a total dose of 500-1000 I.U. for each animal. The activity of the thyroid also was investigated with the help of I^{131} in a dose of two microcuries for each

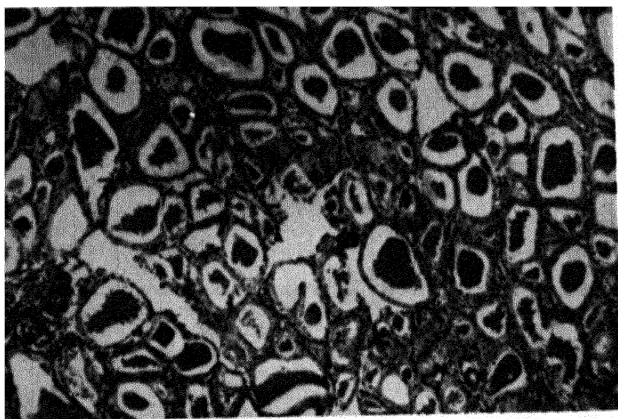


Figure 1. Thyroid, control rat. Azan $\times 8$

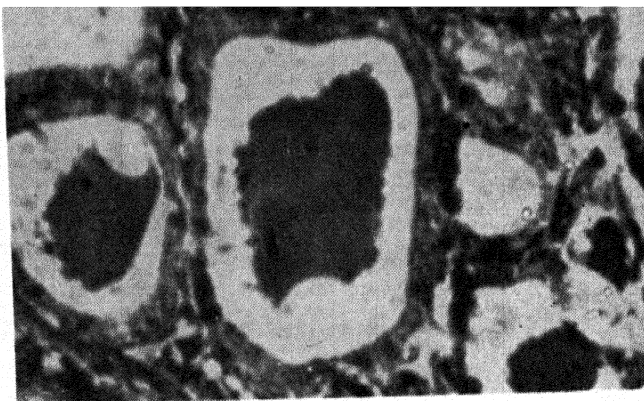


Figure 2. Thyroid, control rat. Azan. $\times 40$

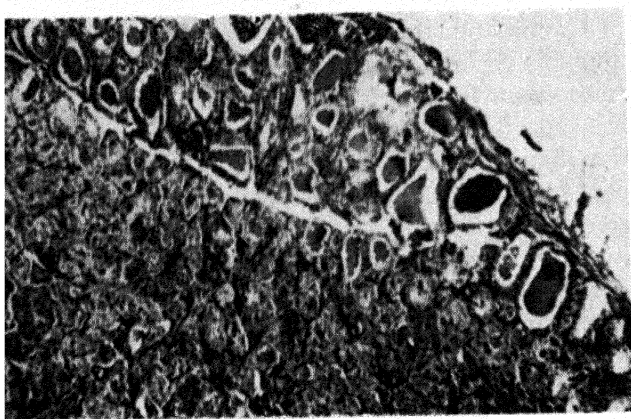


Figure 3. Thyroid, estradiol treated—small doses. Stimulation of thyroid function. Azan $\times 8$

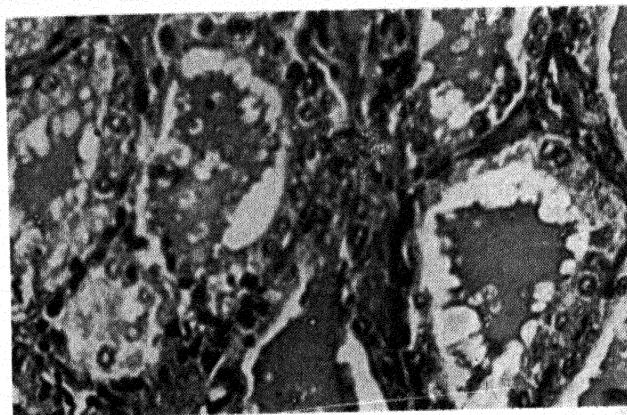


Figure 4. Thyroid, estradiol treated—small doses. Thyroid stimulation. Microfollicles with dilute colloid, and very many vacuoles showing the resorption. Azan $\times 8$

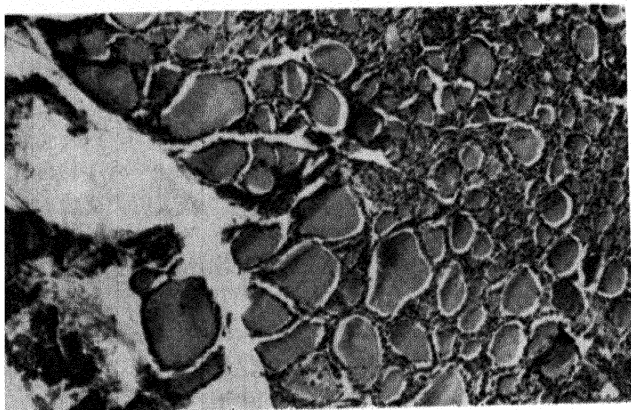


Figure 5. Thyroid, estradiol—large doses. Inhibition of thyroid function. Azan $\times 8$

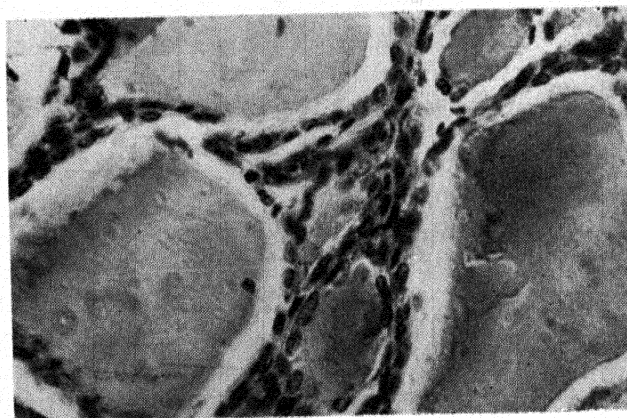


Figure 6. Thyroid, estradiol—large doses. Thyroid inhibition. Macrofollicles with dense colloid, homogeneous, no resorption vacuoles Azan $\times 40$



Figure 7. Thyroid, control rat. Golgi apparatus. Da Fano $\times$ immersion

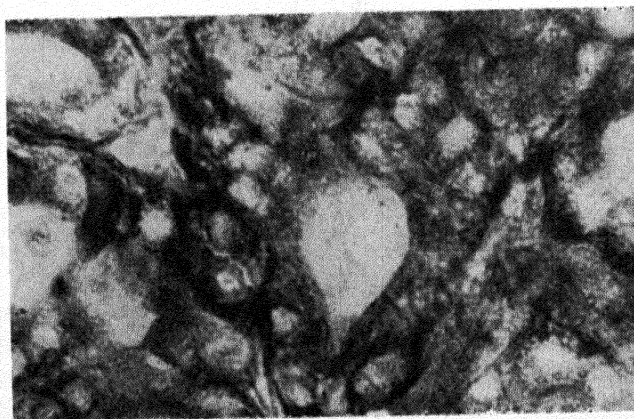


Figure 8. Thyroid, estradiol—large doses. Golgi apparatus. Da Fano $\times$ immersion

rat, which corresponds to 800 cpm on a Geiger counter.

3. The subjects of the third group of 4 adult female rats of the same weight, (used as controls) were treated with radioactive iodine only up to a total amount of 2 μ c.

Radioactivity was determined in the animals two and a half hours following the first injection, then 51 hours and 98 hours following it.

RESULTS

We noted that the fixation of radioactive iodine in the controls two and a half hours following injection was 323 cpm on an average with a retention of 40.4% of the injected radioactive iodine, while, in the animals treated by the estrogen in high doses, it was 38 cpm on an average, with a degree of retention of only 4.7% of the total quantity of iodine injected.

In the adult rats treated by small doses of estrogens (500–1000 I.U.), iodine fixation was, on an average, 380 cpm, with a retention of 50.2% of the iodine injected.

The degree of fixation of radioactive iodine increases and reaches, 51 hours following injection, 358 cpm and a retention rate of 51.2% in the controls, whereas, after high doses of estrogens, the count was only 71.2, with a retention rate of 10.2%. In the adult rats treated by small doses of estrogens, the rate of fixation increased to 420 cpm, with a retention of 60.2% of the total quantity of radioactive iodine injected.

This is the maximum of radioactive iodine fixation observed during the experiment; after this period, fixation of radioactive iodine decreases progressively, reaching, after 98 hours, 270 cpm, with a retention of 45% of the radioactive iodine in the controls, whereas, after high doses of estrogens, it falls to 30.4 cpm on an average, with a retention rate of 5.1%. In the animals treated by the estrogens in small doses, the fixation of radioactive iodine is reduced to 320 cpm, with a retention rate of 52.5%.

These findings show that thyroid fixation follows a slightly ascending curve, with maximum retention of radioactive iodine after 51 hours, maintaining, however, a slightly elevated level up to the end. These data are included in the appended graph (Fig. 9).

The rate of fixation of radioactive iodine is in full agreement with the microscopic findings. Thus, in rats treated by high doses of estrogens (first group), one finds, with respect to the controls (third group, Figs. 1 and 2) an aspect of thyroid function inhibition expressed by a predominance of the macrofollicles; the follicles are made up of cylindro-cubic cells with granular protoplasm, and they are full of homogeneous colloids, intensely eosinophilic, with very few resorption vacuoles, and occasionally desquamated cells. Everywhere, fairly marked vasodilatation is to be found. Cytological study also reveals cellular hypofunction (hypotrophic aspect, Fig. 8).

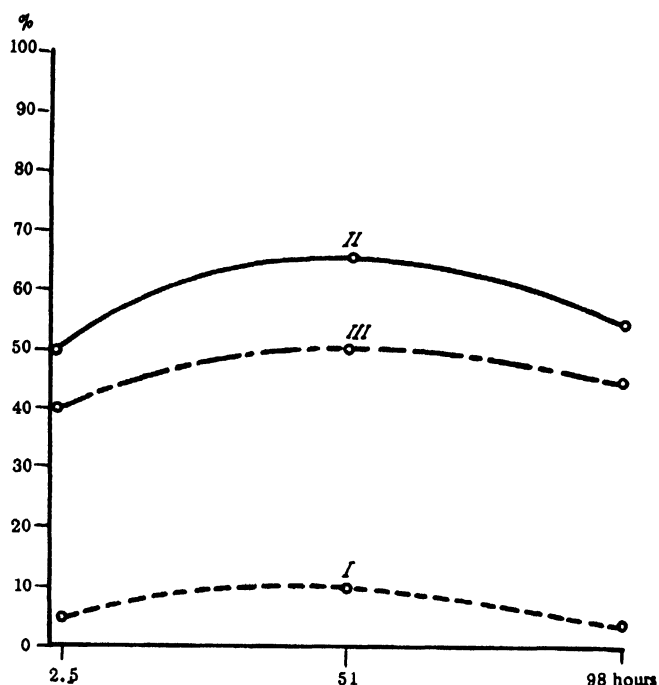


Figure 9. Fixation of radioactive I^{131} in the thyroid region after estradiol therapy: (I) estradiol, large doses; (II) estradiol, small doses; (III) estradiol, controls

On the contrary, in the rats treated with small doses of estrogens (second group) there is an aspect of histological stimulation which is expressed by a predominance of microfollicles and a reduction in the amount of colloid, which is diluted and full of resorption vacuoles (Figs. 3 and 4).

DISCUSSION OF THE RESULTS

The presentation of our results justifies the conclusion that the administration of high doses of estrogens brings about a marked reduction in the fixation of radioactive iodine, with a microscopic aspect of inhibition of the thyroid. This finding also is in contrast with the results obtained after the administration of small doses of estrogens (increase in the fixation of radioactive iodine and appearance of histological stimulation). Therefore, the action of the estrogens is exercised as a function of the doses administered. The results we obtained remind us of those of Gassner *et al.*⁴ who, following treatment on rats, for 8 to 20 weeks, by estrone⁸, in daily doses of 30 milligrams, observed a marked decrease in the thyroid iodine.

Money *et al.*⁸ experimenting on rats weighing 250 to 300 gm observed, following the administration of a total dose of 500 gamma of estrone, an increase in the radioactive iodine content I^{131} , whereas, in another group of animals treated by high doses of estradiol progressively administered, the opposite effect and a decrease in iodine fixation were found.

Opposite effects (namely, stimulation of the thyroid function) were obtained after administration of small doses of estrogen (Milcou and Pitis).

The administration of 50 γ of estrone increases the radioactive iodine content of the thyroid. On the contrary, Paschkis *et al.*¹⁰ following treatment of the

rats with 50 γ of estrone or 150 to 600 γ of benzoic estradiol, found that the fixation of radioactive iodine was the same as in the controls.

Another problem, which is the consequence of this work, and once was much discussed, is that of the mechanism of action of the estrogen at the level of the thyroid. Many authors (Desclin, Frank, Chamarro) claim that there can be no mention of a direct action of the estrogens on the thyroid, and that one is entitled to suppose that there is an indirect action through the anterior hypophysis, by the stimulation or slowing down of the secretion, and even the synthesis, of the thyrotropic hormone.

In hypophysectomized rats, the administration of estrogens does not accelerate the atrophy of the thyroid brought about by resection of the hypophysis, nor does it stimulate it.

Mahaux,⁵ experimenting on rabbits with estradiol benzoate, noted that the latter reduces the basal metabolism, and, at the same time, the heat-producing action of thyroxine. The author comes to the conclusion that the estrogens exercise their action through the diencephalo-hypophysis complex.

Milcou and Lupulescou,⁷ studying the action of the estrogens on decorticated guinea pigs, observed that the metaplastic conjunctivo-biliary action in the liver does not appear in decorticated animals.

Finally, such authors as Duplessis and Parot,¹¹ claim that the structural modifications of the thyroid are due to metabolic disorders following castration or the administration of estrogens, rather than to specific hormone action.

Our results confirm the hypothesis that high doses of estrogens inhibit thyroid function, and reduce the fixation of radioactive iodine in the thyroid, while small doses of estrogen stimulate thyroid function and increase the fixation of radioactive iodine in the thyroid.

SUMMARY

The authors made a study of the effect of the estrogens on the thyroid of rats with the help of radioactive iodine (I^{131}). They noted that the administration of large doses of estrogens, 100,000 I.U. (10 mg), for 4 weeks, to rats, to which one had in-

jected two microcuries of radioactive iodine (I^{131}) by the intraperitoneal route brings about inhibition of thyroid function with a massive decrease in the fixation of radioactive iodine in the thyroid.

The administration of small doses of estrogen for 4 weeks (500 to 1000 I.U.) to animals, the activity of the thyroid of which also was investigated with the help of I^{131} at a dosage of two microcuries for each rat, brings about stimulation of thyroid function, along with an increase in the fixation of radioactive iodine.

As a consequence, the effect of the estrogen on the thyroid is a function of the dose given.

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The Effect of Chlorphenothiazine (Chlorpromazine) on the Concentration of Radioiodine (I^{131}) in the Thyroid of the Albino Rat

By St. M. Milcou, I. Negoescu, N. Radian, Al. Bojinescu, C. Petrescu, Fl. Cocou and H. Stancou, Romania

The use of the radioactive isotopes in medicine, such as those of iodine, particularly of I^{131} , which shows greater stability than the others, made it possible to explore the function of the thyroid body more extensively, and brought greater precision to the study of the mechanism of thyroid secretion.²

In the course of our earlier research, we investigated the action of a phenothiazine (chlorpromazine, lacgatile or 4560 R.P.), in the rat, on the heat-generating effect of thyroxine. We followed the same effect in clinical hyperthyroidism. By constantly watching the mechanism of the action of this substance on the thyroid, we endeavored to look into the fixation capacity of radioactive iodine I^{131} by the gland, following prior administration of chlorpromazine, a neuroplegic and narcobiotic substance.

TECHNIQUE OF THE WORK

For our experimental animals, we used male albino rats weighing from 160 to 180 gm. Approximately 1 mg of chlorpromazine was administered to 5 animals by the parenteral route and 4 hours later, once the animals were in a state of semi-hibernation (sleepy, refusing to eat, offering no resistance when touched) 0.84 μ c of I^{131} was administered to each of them by intraperitoneal injection. The same amount of radioiodine was administered to 3 other controls, to which nothing was first administered which might influence iodine fixation capacity. The radioactivity of the animals was checked by means of a Geiger-Müller counter, 3 hours after injection. Thereafter, determinations were made after 24 hours, 3 days and 5 days.

It must be remembered that the administration of chlorpromazine was carried out every 12 hours during the first 48 hours, and thereafter every 24 hours for 5 days, which brought about continuous semi-hibernation lasting over the first 2 days. At the end of the 12-hour period, the animals were fed.

Since the determinations were carried out on living animals, it must be specified that the findings made after 3 hours and 24 hours respectively with a Geiger-Müller counter on other parts of the body of

the animal (belly, back, foot, etc.), showed a decrease in the count, which was more marked than that noted in the thyroid, and a little sharper than on the "base" curve. We interpreted the above as indicative of the existence of a certain quantity of I^{131} in the blood stream, as well as a consequence of the fact that we were close to a gland which contained a certain amount of radioactivity.

The number of counts separately recorded by the counter for each animal, was compared, for each determination, to the number of impulses given by a quantity equal to that injected, which was used as a standard.

RESULTS OBTAINED

In the group of controls, we obtained the following thyroid concentrations of I^{131} on an average: 39.9% after 3 hours, 29.2% after 24 hours, 24.7% after 3 days, 22.3% after 5 days. In the animals of the group which first received chlorpromazine, the accumulation of I^{131} in the thyroid was smaller than for the controls, which showed 23.9% after 3 hours, 25.9% after 24 hours, 11.7% after 3 days, and 9.5% after 5 days. Fourteen days after the injection of I^{131} , the controls still showed traces of radioactivity, since the iodine retained by the thyroid was calculated at 12.6%; whereas, for the animals which were in semi-hibernation, the Geiger-Müller counter, when applied to the thyroid, showed the same number of counts as it would at any point at all in the experimental chamber.

By following the two curves (Fig. 1) which represent the mean percentages of I^{131} fixed by the thyroids of the animals of the two experimental groups, we noted that, under the influence of chlorpromazine, a decrease in the absorption of radioactive iodine by the thyroid was registered. Iodine fixation was maximal during the first 24 hours. The two curves showing a decrease in the radioactivity of the gland form two lines which are nearly parallel.

DISCUSSION

When analyzing the results obtained, we note that, under the influence of chlorpromazine, there is a

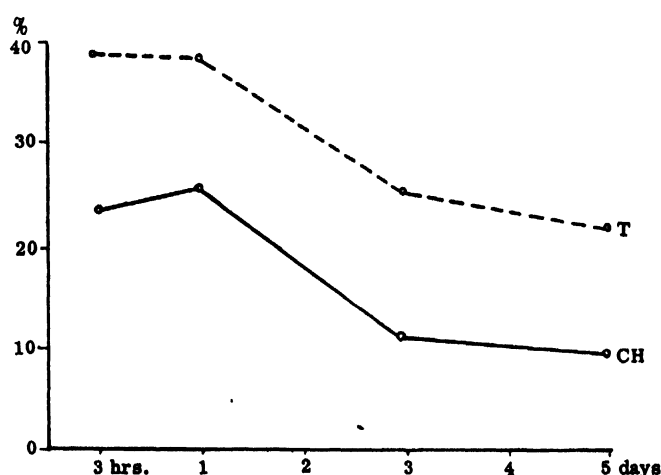


Figure 1. Variations, in per cent, in the fixation of I^{131} in the thyroid of the controls and of the subjects treated by chlorpromazine. T, control group; Ch, chlorpromazine group

decrease in the rate of thyroid fixation for radioiodine.

These results are in agreement with those found in our earlier experiments, when we noted a decrease in the thyroid function in a normal rat following injection of chlorpromazine, as well as in the experiments which showed us that this substance brings about a decrease in the heat-producing activity of the thyroxine administered by the parenteral route.

Chlorpromazine is part of the group of neuroplegic substances having a strong depressive action on the central nervous system, and particularly on the centers which control sleep, vomiting, thermogenesis, etc. It also has a clear influence on the tissues, reducing the activity induced in them by certain substances.³

A decrease in the rate of iodine fixation by the thyroid, following the administration of chlorpromazine, can be brought about by various methods. One must first allow for the effect on the nervous system,⁶ the inhibition of which depresses the thyroid;⁶ second, the effect on the vegetative ganglia associated with the thyroid.⁵

Recent research showed that chlorpromazine has a depressing action on the hypophysis. It can be stated, in this instance, that the substance acts on the neuro-diencephalo-hypophysis complex. It is known that the fixation of iodine by the thyroid depends to a great extent on the secretion of thyrotropic hormone. Thanks to chlorpromazine, we have been able to obtain a decrease in the thyroid fixation capacity of radioiodine.

The action of chlorpromazine on the vegetative ganglia contributes to this decrease, and they are related with the thyroid, through the diencephalo-hypophysis system, as well as by their direct action on the thyroid.

The fact that the two curves which represent the decrease in radioactivity of the thyroid in time are parallel, supports the hypothesis that the chlorpromazine acts on the iodine fixation capacity, rather than on its elimination from the system.

CONCLUSIONS

Following administration of chlorpromazine, the thyroid concentration of I^{131} is decreased. By a comparison with the concentration of I^{131} in the thyroid of the controls, this decrease is very significant. The difference in fixation between the controls and the treated animals is, percentage-wise, at the end of 3 hours, 24 hours, 3 and 5 days: 39.9–23.9%; 39.2–25.9%; 24.7–11.7%; 22.3–9.5%. The excretion time for I^{131} has not been modified.

The excretion of radioiodine is variable, both in the controls and in the treated animals treated by chlorpromazine.

The pharmacology of the phenothiazines shows us that these substances, of which chlorpromazine is one, have a depressive action on the central nervous system and on the peripheral system, and at the same time a narcobiotic action, which means that they slow down the vital functions of each and every cell. The reduction in the concentration capacity of the thyroid may also be due to a neuroplegic action as well as to the narcobiotic action, although the fundamental biochemical mechanism does not appear to be different in the nerve and thyroid cells. In a system dependent on a nervous control, the neuroplegic action is the one which appears to be dominant from the physiological standpoint. This research opens up interesting prospects in the treatment of hyperthyroidism.

SUMMARY

The authors made a study of the fixation capacity of I^{131} in the thyroid of male rats placed under the action of chlorpromazine. The quantity of I^{131} administered in the form of an intraperitoneal injection was $0.84 \mu\text{c}$ for each animal. Radioactivity, in the controls and treated animals, was noted with a Geiger-Müller counter. Following administration of chlorpromazine the thyroid concentration of I^{131} is reduced. With respect to the concentration of I^{131} in the thyroid of the controls, this decrease is very significant. The difference in the rate of fixation of the controls, in comparison with the other experimental animals was, in percentages, following 3 hours, 24 hours, 3 days and 5 days: 39.9–23.9%; 39.2–25.9%; 24.7–11.7%; 22.3–9.5%.

The time taken for the excretion of I^{131} did not suffer any modification. The excretion of radioiodine shows variations in the controls and treated animals, where chlorpromazine is used.

It is possible that a decrease in the thyroid concentration capacity with respect to I^{131} be due as much to the neuroplegic action as to the narcobiotic action, although the fundamental biochemical mechanism does not appear to be different in nerve and thyroid cells; in a system controlled by a neural mechanism, the neuroplegic action appears to dominate from the physiological standpoint.

This research offers interesting potentialities in the treatment of hyperthyroidism.

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Influence of the Dynamics of the Central Nervous System on the Rate of Iodine Fixation by the Thyroid

By St. Milcou, D. Belloiu, R. Holban, V. Sahleanu and Al. Bojinescu,* Romania

The problem of the nervous control of glandular activity is one of the most interesting questions in present day endocrinology. We have personally obtained (as the result of an earlier series of experiments carried out on rats) some results having specifically to do with the dynamics of the TSH (produced by the pituitary). We made use, for our work, not only of the BMR, of a study of the relative weight and histology of the hypophysis, thyroid, and other endocrine glands, but also, and particularly, of our evaluations of the TSH content of the pituitary, as determined by a very special method. This is the Voitkevici-Kabak method,¹ the principle of which is to measure the shortening of newt gut, following implantation of small pieces of pituitary or thyroid, the extent of this shortening being considered an index of metamorphogenic activity of implanted fragments.

In a paper published in 1953, we had endeavored to establish a correlation between the dynamics of the cerebral cortex and thyrotropic activity of the pituitary.² Cerebral excitation was achieved by administering benzedrine to the rats. Inhibition was obtained by means of intraperitoneal injections of chloralose. The duration of the experiments was 4 days. A third group of rats made up the controls.

Three other groups of rats were subjected to precisely the same conditions. In addition, each one of them underwent, on the first and last days of the experiment, an insulin test (namely an intramuscular injection of insulin: 0.2 to 0.4 I.U.).

The results of these experiments enabled us to reach the following conclusions:

(a) The thyrotropin content of the pituitary increases with the state of cerebral excitation.

(b) The insulin injection brings about a discharge of the thyrotropic hormone by the pituitary, the accumulation of which had been favored by benzedrine.

(c) Cortical inhibition with chloralose is not favorable to the production, or the accumulation, of thyrotropic hormone in the pituitary.

These results again stress the importance of brain control of the dynamics of the secretions of the anterior lobe of the pituitary.

We have also shown, on other occasions (1955), that partial bilateral decortication of the cerebral

hemispheres carried out on rats 20 days before the beginning of the experiments, while it modified the morphology of the thyroid to some extent, could delay the appearance of experimental goiter achieved following simultaneous administration of methylthiouracil. These results were interpreted as an alteration of regulation by the neurohypophysis leading to a modification in the overall reactivity of the pituitary-thyroid axis.

In order to complete, and more particularly to confirm, our earlier research, mentioned above, we resorted, in our current work, to another method, namely, to the fixation of I^{131} by the thyroid. Thus, we sought to determine the rate of iodine fixation by the thyroid tissue of rats, the central nervous system of which had first been subjected to various influences, by the administration of either benzedrine, chloralose, or phenobarbital.

Thyroid radioactivity was measured 24 hours after the intraperitoneal injection of 1 to 2.5 microcuries of I^{131} with a Geiger-Müller counter. The results were expressed as a number of counts per minute. The gross figures thus obtained underwent correction in order to allow for the weight of the animal and that of the thyroid tissue used for the determination. More accurately, the counts per minute are multiplied by the ratio of the weights of the respective animals to those of their thyroids.

EXPERIMENTS AND RESULTS

(a) We effected partial decortication, on young male rats, by the bilateral removal of a fraction of the convexity of the cerebral hemispheres. Twenty days after this procedure, we injected I^{131} (1 microcurie) by the intraperitoneal route, to 9 operated rats and to 4 controls. Twenty-four hours after this injection, all the animals were sacrificed in order to determine the radioactivity present in their thyroids and thus the rate of thyroid iodine fixation.

The results show that the rate of fixation of I^{131} by the thyroids of the decorticated rats is somewhat reduced: 60.8 counts per minute $\pm$ 2.8 S.E. (corrected figures) as against, for the controls, 72.8 $\pm$ 6.8 S.E. Nevertheless, these results are not statistically significant, so that we may conclude that the alteration in iodine fixation by the thyroid following decortication is negligible. It would thus seem that

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such effects of decortication on the development of experimental goiter as were obtained in our earlier experiments are due to a disturbance in the hypothalamic-hypophysis reactivity.

(b) In another series of experiments, we administered (per os) a daily dose of 1.5 mg of benzedrine, as central nervous system stimulant. Four of the rodents so treated received 2.5 microcuries of I^{131} intraperitoneally after the first day of treatment, while the other four rats received the same amount of I^{131} following three days of treatment. All the animals were sacrificed 24 hours after they had received the tracing dose of I^{131} , in order to make a determination of the radioactivity of their thyroid.

The results varied according to the duration of the benzedrine course. In the cases where the excitation, or stimulation, of the central nervous system was of short duration (1 day), thyroid fixation increased homogeneously: 102 ± 14 S.E.; while with respect to the controls it was 83 ± 14 S.E. On the other hand, when the period of excitation lasted longer (three days), the thyroid iodine fixation was more or less reduced: 42 ± 14 S.E. (observe that this is a non-homogeneous series).

The experiment was repeated by treating 6 female rats with the same dose of benzedrine for two days. The rate of thyroid fixation was again found to be increasing, at 37.6 ± 2.4 S.E., with respect to the 6 other female rats used as controls (12.5 ± 0.5 S.E.).

In conclusion, an excitation or stimulation lasting for an average period of time, may increase iodine fixation by the thyroid, which confirms the results of our earlier work.²

As regards the somewhat erratic results obtained with the animals which received benzedrine for a longer time, let us mention that I. B. Skebelskaia³ made similar findings, interpreting them as the result of a "hyperliminal" inhibition (namely, an inhibition due to an excessive stimulus, over and above a certain threshold).

(c) Finally, we gave our attentions to the issue of the extent to which the central nervous system sedatives can influence thyroid iodine fixation. In a first experiment, we have injected I^{131} to rats by the intraperitoneal route, during chloralose induced sleep.

The animals were subsequently sacrificed before they woke up, three hours following the injection of iodine, but we did not obtain any conclusive results.

In another experiment, we tried to find out whether prior sedation of the animals for a few days would influence the rate of iodine fixation. To this end, we administered 0.01 gm of chloralose *per diem* for four days to a group of male rats. On the last day of treatment, the animals received 2.5 microcuries of I^{131} at the same time as did the controls. All the animals were sacrificed after 24 hours, in order to determine how much iodine was fixed.

The results were 49.0 ± 15 S.E. in the animals treated, as against 82.7 ± 14 S.E. for the controls.

A similar experiment was carried out with the help of phenobarbital administered for 10 days. This time, the result was striking: thyroid fixation was 217 ± 48 S.E. per minute in the animals treated, as against 78 ± 8 S.E. for the controls.

We repeated the experiment on female rats in order to check on the results. At the same time, the amount of I^{131} administered was limited to 1 microcurie.

The results confirmed our first findings: 47.2 ± 5.8 S.E. in the animals treated, against 12.6 ± 0.5 S.E. in the controls.

We feel that our results confirm the hypothesis according to which inhibition of the central nervous system could influence iodine fixation by the thyroid. However, some reservation must be made as regards the action of the pharmacological agents, due to the possibility of their having a direct action on the thyroid. In fact, from this standpoint, phenobarbital may be considered an example of direct action on the thyroid, since we have already shown that an action similar to that of the synthetic antithyroids of the methyl-thiouracil type can be ascribed to phenobarbital.

On the whole, our present results agree with our earlier research, by means of which we had demonstrated that the very treatments which act on the central nervous system can bring about definite modifications in the thyrotropic hormone of the hypophysis.

SUMMARY

Following our research into the problem of the nervous control of glandular activity, particularly as regards the dynamics of the thyrotropic hormone of the pituitary, we endeavored to alter the capacity of I^{131} fixation by the thyroid depending on various influences capable of acting on the central nervous system, namely: (a) following partial decortication of the cerebral hemisphere; and following (b) the stimulation, (c) the inhibition, of the central nervous system, by first administering benzedrine, as well as chloralose and phenobarbital respectively.

Radioactivity of the thyroids was determined with the help of a Geiger-Müller counter, 24 hours after an intraperitoneal injection of I^{131} (1–2.5 microcuries).

The rate of fixation of I^{131} by the thyroid of rats, which, 20 hours before, had undergone partial bilateral decortication of the cerebral hemispheres, was reduced (60.8 ± 2.8 E.S., as against 72.8 ± 6.8 E.S. for the controls). Statistically, however, these results are not significant.

Our earlier research demonstrated that the same operation, while it modifies to some extent the morphology of the thyroid could also delay the appearance of experimental goiter, achieved following the administration of methyl-thiouracil.

Following treatment by benzedrine, we noted a small increase in the rate of I^{131} fixation ($102 \pm$

14 S.E. for a one day treatment as against 83 ± 14 S.E. for the controls), and, on the other hand, a reduction (42 ± 14 S.E.) after a three day course of treatment (this last result was interpreted by the theory of "hyperthreshold inhibition").

Following sedative treatment by chloralose, administered for four days in a dosage of 0.01 gm per day, the iodine fixation was reduced (49.0 ± 15 S.E., as against 82.7 ± 14 S.E. for the controls).

Following 10 days treatment by phenobarbital, thyroid fixation of I^{131} was plainly increased (217 ± 48 S.E.) with respect to the controls (78 ± 8 S.E.). The same results were obtained repeating the experiment with the females (47.2 ± 5.8 S.E. as against 12.6 ± 0.5 S.E. for the controls).

Our results generally confirm our earlier research, by which we had demonstrated that the same treatments (excitation and inhibition of the central nervous system) bring about clear modification in the dynamics of the thyreotropic pituitary hormone.

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The Use of I^{131} in a Study of Renal and Biliary Excretion of Thyroxine and Its Catabolism in the Tissues

By S. Cruchaud, B. R. Scazziga and A. Vannotti,* Switzerland

Thanks to I^{131} and the separation of the thyroid hormone and its derivatives by paper chromatography, it has been possible to make an accurate study of such mechanisms as the synthesis of thyroxine and its degradation in the various tissues. By using paper chromatography on butanol extracts of blood, urine and tissues, and by recording (with an automatic counter) the radioactivity of the I^{131} in the spots of the chromatograms (Lerch device), we were able, through a whole series of experiments, to separate and measure quantitatively the various iodine-containing organic compounds.

Our interest first concentrated on the problem of lipoid nephrosis, characterized by a clinical picture of hypothyroidism. The intrathyroid fixation curves of I^{131} and the conversion index (expression of the transformation of inorganic iodine into protein-bound iodine) revealed that, in nephrosis, we are faced with a hyperfixation of iodine by the thyroid. Since these results seem, at first sight, to be paradoxical, we made it a point to go deeper into the matter by tracing the urinary excretion of the iodine. By chromatography of the butanol extract of the urinary proteins, we were enabled to demonstrate the presence of thyroxine in the urine in an unequivocal fashion.

We may therefore¹ account for these findings in the following manner. The thyroxine produced by the thyroid cannot accumulate in blood plasma, for the carriers for this hormone (namely the proteins and particularly the albumins and α_1 globulin) show a considerably reduced level (hypoproteinemia of nephrosis). Peters and Man,² as well as Recant and Riggs,³ did observe a reduced protein bound iodine level during nephrosis. Thus, due to the proteinuria, a certain amount of thyroxine constantly is being lost with the urine. The increase in the intensity of iodine fixation at the level of the thyroid is the expression of an iodine deficiency which follows constant losses of iodine in the urine.

Having pursued our experiments further, we noted, with the help of the chromatography of butanol extracts of plasma, a distribution of the organic iodine compounds in subjects suffering from obstructive jaundice. Roche and his group showed, in

the bile, the existence of conjugates of thyroxine with glucuronic acid. These glucuronic conjugates were found again in the blood, when the common bile duct was ligated in laboratory animals.

In a patient suffering from obstructive jaundice, as well as in some cases of jaundice due to epidemic hepatitis, with considerable retention in the bowel, our work also enabled us to reveal, following an overload of I^{131} , the glucuro-conjugates of thyroxine. These substances never were found in normal subjects. However, the most interesting problem for the clinician, at the present time, is that of the catabolism of the thyroid hormone in the tissues. The findings bearing on the stimulation of metabolism by a derivative obtained by the removal of iodine from thyroxine, tri-iodothyronine, enabled us to work on the important problem of the mechanism of the peripheral use of the thyroid hormone.

Recently, Albright *et al.*⁴ reported a transformation of thyroxine into tri-iodothyronine following incubation of I^{131} -tagged and pure 1-thyroxine in the kidney. This showed that this removal of iodine, *in vivo*, takes place in the tissues. In his test, Albright noted that this transformation could be inhibited, either by an excess of iodides, or by adding cyanide.

We used similar techniques and incubated chromatographically pure radioactively tagged 1-thyroxine (0.05 γ) on 150 to 200 gm of kidney. We, thus, were able to confirm the findings of Albright and his group, to the effect that there is a clear transformation of thyroxine into tri-iodothyronine after 4 hours of incubation, and a more marked transformation after 8 hours of incubation.

By adding a synthetic thyroid inhibitor to tagged thyroxine (Thiomidil) during incubation, it was noted that the transformation of thyroxine into tri-iodothyronine in the renal parenchyma also was markedly inhibited. But iodine removal took place normally during incubation with estrogens (estradiol monophosphate in aqueous solution).

These experiments, which will be dealt with in full in another paper at some later date, show that the derivatives of thiourea, not only act as inhibitors of the mechanism of formation of thyroxine in the thyroid cell, but also act as inhibitors of the iodine removal from the thyroxine molecule to give tri-iodothyronine.

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SUMMARY

These few examples show the interest, for the clinician and to experimental medicine, of using the radioactive isotope I^{131} , with paper chromatography, for the separation of organic compounds of iodine when studying thyroid function.

We have seen how a clinical picture of hypothyroidism can develop following losses of thyroxine in the urine during nephrotic albuminuria, while, in cases of inadequate bile excretion, we note an accumulation of catabolites of thyroxine (glucuronic conjugates) in the blood.

Finally, the thiourca derivatives can not only slow down the synthesis of thyroxine, but also in-

hibit the mechanism of iodine removal which gives tri-iodothyronine in the peripheral areas, which would probably increase their inhibiting action on the function of the thyroid hormone.

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Separation and Determination of Iodized Amino Acids and of Thyroxine by Means of Anion Exchange Resins

By P. Blanquet,* France

The synthetic ion exchange resins, ever since they first came into use, have received a wide variety of applications. They first were used to solve such simple problems as the deionization of the water; more recently for fine analytical separations.¹⁻⁸

The anion exchangers, such as Dowex 1 & 2 (Dow Chemical Company, Midland, Mich.) are extremely interesting for all problems which have to do with the iodized amino acids, and therefore can be used in the biochemistry of the thyroid. Dowex 2 is selective for the following monovalent ions, in decreasing order: iodide > nitrate > bromide > nitrite > chloride, etc. In order to clarify matters, let it be indicated that the equilibrium constant of the resin is 8.7 for iodide, while it is only 1 for the chloride. Dowex 1 is endowed with characteristics which make it more attractive still.

Inorganic iodine is exchanged quantitatively and retained very strongly by the column (elution is practically impossible with the customary solvents) while on the contrary, organic iodide in the form of iodized amino acids or thyroxine is easily and quantitatively displaced by means of an acid eluant (HCl, for instance).

The separation of inorganic iodine from organic iodine thus is carried out immediately with the ion exchanger, and this fact is very important, since it is what makes it easy to purify the tagged amino acids by removing the remainder of sodium iodide which goes with them, and, on the other hand, it will be possible, in a biological medium, to separate the two categories of iodine.

Finally, by the passing of an acid eluting agent on the column, we carried out a conventional chromatographic operation which makes it possible to separate the iodized amino acids present in the mixture under study. (Dowex 2 had been used previously, in 1953, by Scott & Reilly,⁹ for clinical purposes, in order to separate the protein bound iodine from inorganic iodine in the plasma of patients).

EXPERIMENTAL METHOD

Before use, the Dowex 1 or 2 resin (200-400 mesh) is brought back to its sodium form by successive treatments, using 2*N* soda. Thereafter, it is

abundantly washed with distilled water, until the pH of the waters used for washing fall under 8 (this condition is very important). The substance to be analyzed is placed right at the top of the column, and once it has completely penetrated into it, a new wash, using distilled water, in the amount of 20 milliliters, is carried out. Acid elution then can begin immediately (we used solutions of HCl, 0.2*N* in the majority of cases, and an elution rate of 40 to 50 ml/hr). Coming out of the column, the eluant is recovered in fractions of 1 milliliter (fraction collector of the Autotechnicon design), in small cups placed on the collector tray. The cups are evaporated to dryness, and their radioactivity is measured (Saphymo, 1000 scale, Tracerlab tube TGC or scintillation counter P 20).

RESULTS

(a) *The column retains the whole of the inorganic iodine.* In order to verify this, we placed solutions of radioactive sodium iodide (NaI¹³¹), to which small quantities or ordinary NaI (carrier = a few milligrams) were added in some cases, on a great many Dowex 1 or 2 resins prepared according to the method described above. We then proceeded with the elution, using 0.1 or 0.2*N* HCl. The various fractions of the liquid used for the elution never evinced any greater reactivity than the counter background (we even used very active solutions of NaI¹³¹ on this column, namely 2 to 3 millicuries: the result of the elution always was negative). On the other hand, a portable counter, when taken to the top of the column, indicated considerable activity, which demonstrated that the iodide was being stored.

In addition to this, we treated a small quantity of resin in a Becher with a strongly active NaI solution. Following half an hour of contact, the resin was transferred to the chloroform. Only high temperature treatment (300°) of the resin makes it possible to displace the radioactive iodide from it.

(b) *Separation of iodine-containing amino acids from one another, and of thyroxine, is conveniently carried out by elution with HCl 0.1 or 0.2*N*.* We were led, in order to do so, to tag the following pure substances with I¹³¹: mono-iodotyrosine, di-iodotyrosine, thyroxine, tri-iodothyronine. By processing each of them separately in the Dowex 1 and 2 col-

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umns, we were able easily to separate the radioactive fractions of the eluants, and more specifically the receivers in which the substance under study had been collected. Thus, having real "reference spectra" (see below), we made mixtures of 2, 3 or 4 substances, in order to make a study of their separation. Before showing a few typical diagrams, obtained by means of various substances tagged with Dowex 1 or 2, we should like to clarify a few special points of the method which we used in order to tag each of the iodine-containing amino acids and the thyroxine with I^{131} .

Generally speaking, the methods used for a few years for tagging the various amino acids of the thyroid, and particularly thyroxine, by means of I^{131} were the following: adding iodine to casein,¹⁰ *in vivo* biosynthesis,^{11,12} adding iodine to di-iodothyronine under varied conditions,^{11,13,14,15,16} adding iodine to tri-iodothyronine,^{16b} exchange reaction between thyroxine and iodide,^{10,14,17,18} exchange reactions between thyroxine and iodine.¹⁹ These methods are of varying degrees of technical complexity and lead to obtaining tagged substances with a specific activity which also varies according to the technique followed. Driven by a desire to be simple, we adopted a method derived from that recommended by Chaikoff and his group,¹⁹ who used an exchange reaction between iodine metal and thyroxine or iodized amino acids. Radioactive sodium iodide is distilled in a completely sealed container (in a sulfuric acid medium in the presence of peroxide). The iodine released condenses in a vessel which contains the solution of the substance to be tagged (itself dissolved in a buffer adjusted to pH 4). Once this operation is completed, the solution is placed in an incubator at 55° for 6 hours. This distillation of the radioactive iodide is a fairly difficult operation and can be dangerous, when strong activities are used; in addition, there are comparatively large losses of substance on the walls of the glass container. Consequently, the yield is poor. In order to dispose of these difficulties, we modified the method as follows: the radioactive sodium iodide solution was placed in an ampoule of small capacity (10 ml maximum), to which 2 to 3 ml of a buffer made up of acetic acid = Na acetate, at a pH of 4, were added. The ampoule then is carefully sealed and placed for 15 minutes in a powerful ultrasonic field (Acoustic power 40 w, 900 kc). The vibration which takes place inside the ampoule releases the iodine from the sodium iodide, as can readily be verified by inserting a small quantity of natural NaI and a trace of starch into the ampoule before the ultrasonic treatment. A deep blue color will appear in the very first few minutes of irradiation. The ampoule, once removed from the flux of ultrasounds, then is opened at one end, and 1 ml of a titrated solution of mono-iodotyrosine, di-iodotyrosine, tri-iodothyronine or thyroxine is quickly injected with a capillary pipette (the last mentioned in solution in 50° alcohol). This technique

reduces soiling and radioactive contamination to the greatest possible extent.

The yield of these exchange reactions is very low, and differs according to the substances used. If we take as unity the yield of di-iodotyrosine (the tagging of which is simple since the mere incubation with I^{131} is enough, and for which the release of iodine from the sodium iodide is not necessary), the following table can be drawn up after the computations have been carried out:

Di-iodotyrosine	1
Mono-iodotyrosine	1/2.5 to 3 approximately
Tri-iodothyronine and thyroxine	1/20 approximately

The yield of the exchange reaction seems to us considerably less than that given by Taurog *et al.*,¹⁸ and the specific activities do not exceed 0.3 $\mu\text{C}/\mu\text{g}$ of thyroxine, while these authors give specific activities which reach up to 7 $\mu\text{C}/\mu\text{g}$ of thyroxine.

The method thus makes tagged products of low specific activity available. However, we found it most satisfactory for the preliminary calibration of our chromatograms. On the other hand, we can be sure that the tagged substance is absolutely free from NaI, by passing it again over Dowex 1 or 2. The similarity between the curves obtained following the first and second passages on the resin (Figs. 1 and 2, bearing on di-iodotyrosine) shows that this operation is superfluous, since the first operation took out the whole of the iodide.

The determination of the tagged substances is easily carried out with the help of specific activity standards known with accuracy, by simple comparison of the surface of the relevant peaks in each of the two cases. All the figures to which reference is made, show, on the abscissae, the number of milliliters of eluant (or, which is the same, the number of each capsule due to fractionation from the beginning, since each one contains 1 ml of liquid), and the radioactivities as ordinates (measuring time 0.60 minute). The tagged substances, whatever the acid used, always appear in the same order, starting from the beginning of elution: thyroxine, mono-iodotyrosine, di-iodotyrosine. The thyroxine always appears in the form of a very clear band, narrow and sharp, with a steep front; the mono-iodotyrosine is more spread out, finally the di-iodotyrosine extends over a broad space at the end of the diagram.

With Dowex 2, the top of each one of the peaks for elution with 0.2N HCl is found at:

30	approximately thyroxine (30 = 30 ml of eluant)
50	approximately mono-iodotyrosine
100-125	approximately di-iodotyrosine

With Dowex 1, still with 0.2N HCl:

10	thyroxine
25-30	mono-iodotyrosine
50	di-iodotyrosine

(the figures show the number of the capsules from the beginning of the elution).

Figures 1-10 show more effectively than any sort of comment that the separation of the various tagged substances is readily carried out. (As a matter of fact, we did not deem it useful to publish each one of the figures of the various substances considered separately, in order not to overload this paper.) Only the case of tri-iodothyronine lends itself to some comment. No mention was made of this substance with Dowex 2 resin. On the other hand, it will be seen for Dowex 1, on Fig. 9, that any substance with a very sharp band migrates behind the thyroxine, very close to the latter. In Fig. 10, we also note the presence of a second substance in back of the thy-

roxine. It is most likely that we are dealing with tri-iodothyronine, but we can not be categorical on this point. Given the small distance between the two peaks, the separation is haphazard, and this particular point is the object of our current investigations.

APPLICATION IN BIOLOGICAL CHEMISTRY—STUDY OF THYROID METABOLISM IN THE NORMAL RAT

Thyroid function is well known for the normal rat, and each of the substances which are found in the gland have been determined with care. For studies of that type, paper chromatography and its special application, namely radiochromatography, has received widespread use and clarified many points. Without any attempt at being complete in so vast a

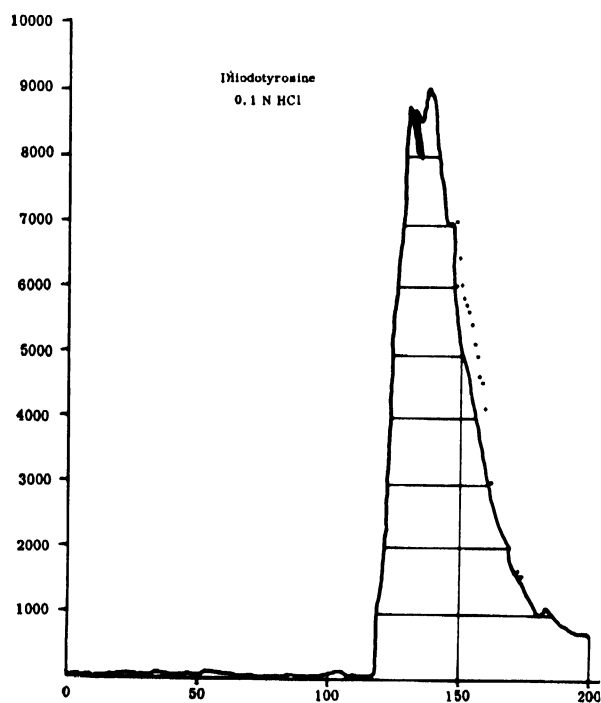


Figure 1. Di-iodotyrosine—first pass

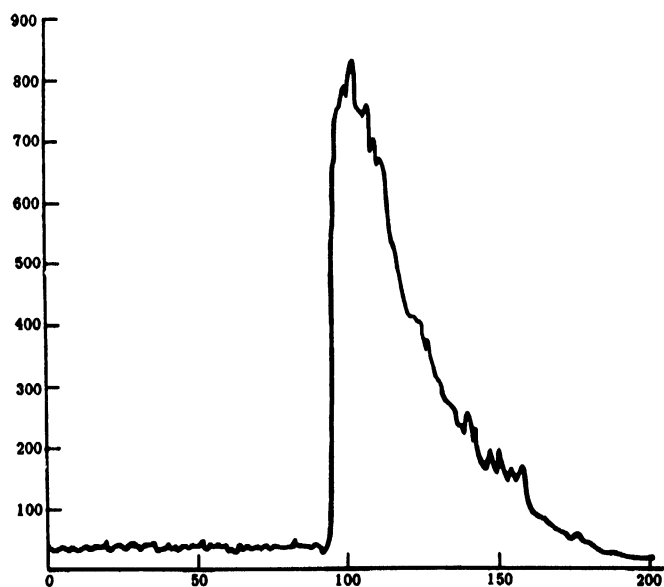


Figure 2. Di-iodotyrosine—second pass

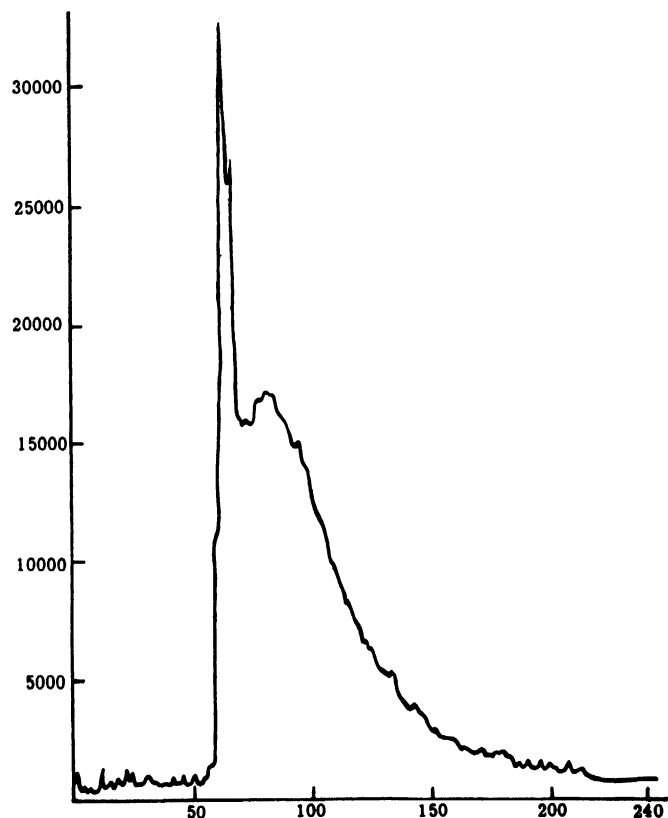


Figure 3. Thyroxine + mono-iodotyrosine—Dowex 2, eluant HCl 0.1N

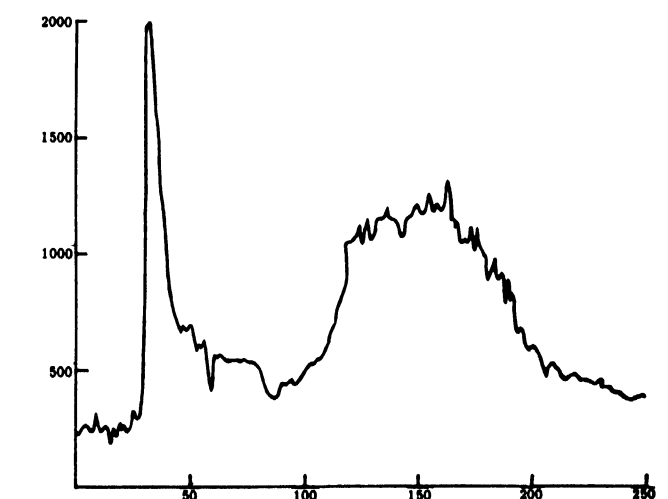


Figure 4. Thyroxine + di-iodotyrosine—Dowex 2, eluant HCl 0.2N

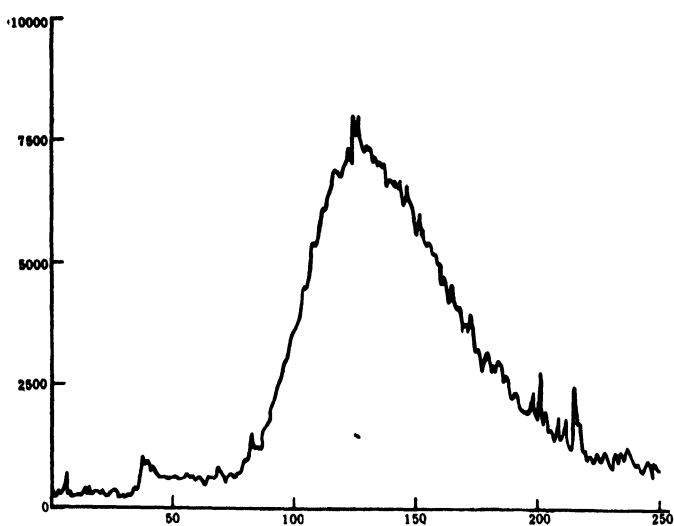


Figure 5. Di-iodotyrosine—Dowex 2, eluant HCl 0.2N

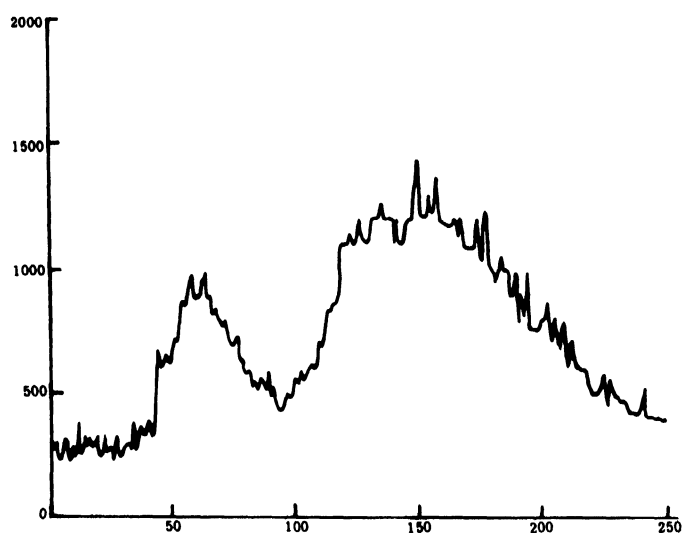


Figure 6. Mono-iodotyrosine + di-iodotyrosine—Dowex 2, eluant HCl 0.2N

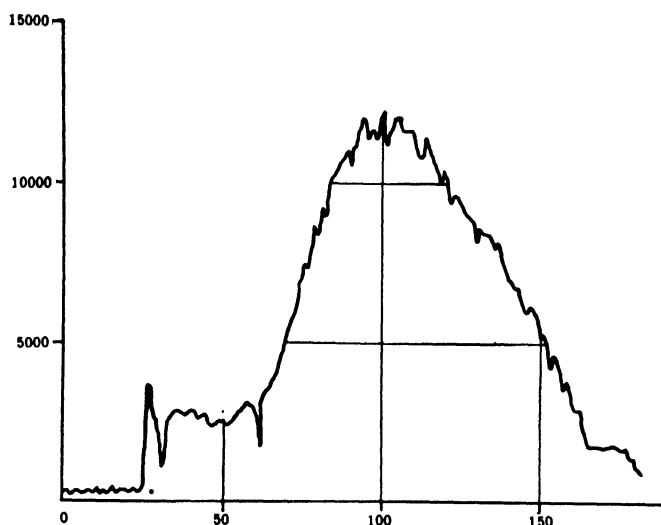


Figure 7. Mono-iodotyrosine + di-iodotyrosine + thyroxine—Dowex 2, eluant HCl 0.2N

domain, let us mention the work of Roche and his group in France, Gross and Pitt-Rivers in England, Leblond in Canada, and Taurog, Tong and Chaikoff in the U.S.A. All of this work has made an enormous contribution to the problem of thyroid metabolism.

Column chromatography has been much less used for studies of this type. Dobyns and Barry,²⁰ however, by using starch columns, obtained excellent separations and accurate estimates of each of the substances contained in the thyroid. The only drawbacks of the method used by these authors are to be found in the fact that it is necessary to change solvent during the operation, (elution of di-iodotyrosine by an acid solvent, while thyroxine and mono-iodotyrosine are eluted in the alkaline phase), and that, on the other hand, preparation of the starch column always is a somewhat delicate technique.

The study which follows has been undertaken merely to confirm, on biological material, the validity of the method of separation on anion exchange resins, as explained before. However, upon being tested, the method proved to work much faster than the interpretation of paper chromatographies by means of a Geiger counter²¹ or by autoradiography.²² Indeed, a complete separation can be obtained in about 5 hours, and the curve obtained immediately gives a spot check on the thyroid function at the time the experimental animal is sacrificed. Finally, allowing for the quantitative nature of the technique, it is possible easily to follow the turnover of each one of the tagged substances.

Experimental Method

Normal adult rats, Wistar strain, were given a uniform injection of a dose of 100 microcuries of radioactive sodium iodide, carrier free (intraperitoneal injection). At various times following injection, which range, in our experiments, from 30 minutes to 100 hours, the animals are sacrificed, the thyroid is taken out, finally crushed at a low temperature in a 9% NaCl solution, then hydrolyzed by means of trypsin at pH 8.4 and at 37°, for 36 hours. The hydrolysates obtained are directly poured on the column of Dowex 1 or 2. We thus eliminate extraction by butanol, and it is the hydrolysate, which has undergone no preliminary treatment, which is subjected to the anion exchange resins. The rest of the operation is conducted as before (washing, then elution with HCl 0.2N). The radioactivity peaks which correspond to each of the substances present in the hydrolysate are identified by the reference spectra obtained previously.

To carry out quantitative determination, the total activity of the hydrolysate (A) is measured before passing it over the column. B is the sum of the radioactivity found in each band, *i.e.*, $B = a + b + c$.

$$A - B = \text{mineral iodine}$$

a/A , b/A , c/A represent the proportions of iodine fixed as thyroxine, mono-iodotyrosine, di-iodotyrosine, etc.

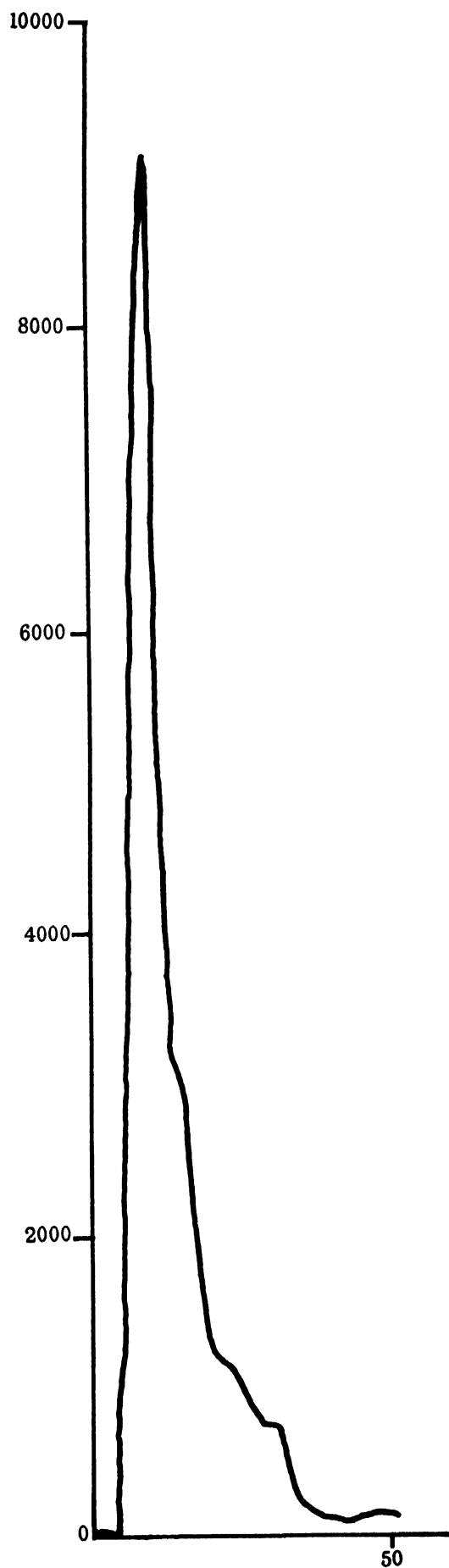


Figure 8. Thyroxine—Dowex I, eluant HCl 0.2N

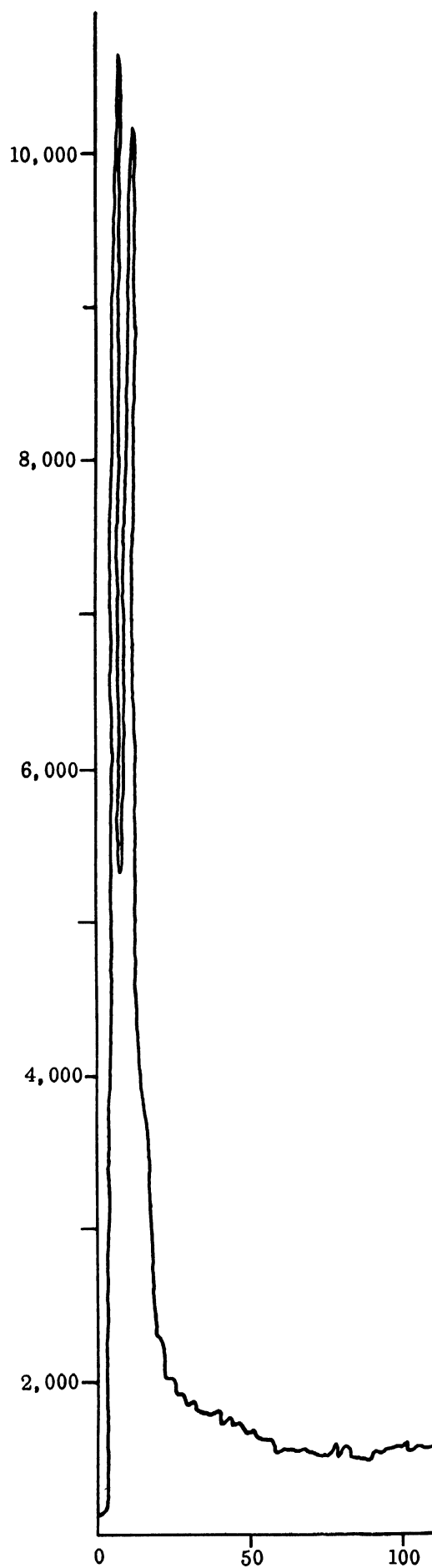


Figure 9. Thyroxine + tri-iodothyronine—Dowex I, eluant HCl 0.2N

RESULTS

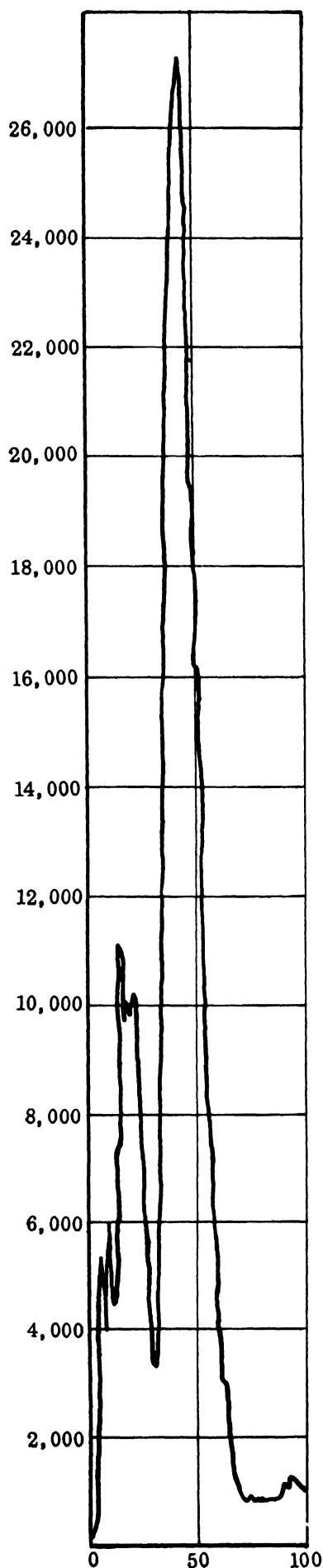


Figure 10. Thyroxine + tri-iodothyronine + mono-iodotyrosine + di-iodotyrosine—Dowex 1, eluant HCl 0.2N

Our investigations were carried out, as before, on Dowex 2 and Dowex 1 resins. Examination of Figs. 11, 12, 13, 14 (Dowex 2) shows that: mono-iodotyrosine appears first, since Fig. 11 shows the single band due to this compound. Di-iodotyrosine, at the same stage (0 hr 30 min), seems to be present only in a small quantity. At 4 hr 30 min, Fig. 12, we see a very marked peak on the side of the band corresponding to mono-iodotyrosine, this is thyroxine; on the other hand, the concentration of di-iodotyrosine increases. Figure 13 shows the 6 hr stage, the thyroxine appears as a sharp and acute band: the mono-iodotyrosine is in substantial amount, as well as di-iodotyrosine. Finally, at 21 hr, (Fig. 14), the situation is practically the same.

This series of figures shows very plainly the operation and constitution of the thyroxine band, which takes place within the first three hours following the injection of tagged iodine.

The results obtained with Dowex 2 are more interesting still. After barely one hour has elapsed, the curve on Fig. 15 shows a very fine band of thyroxine, and a substantial amount of mono-iodotyrosine, as well as a smaller quantity of di-iodotyrosine. At 4 hr 15 min, Fig. 16, the thyroxine peak is plainly exceeded by that of mono-iodotyrosine, the concentration of which increases considerably in comparison with the preceding stage. Consequently, its band becomes wide and spreads at the base while, for di-iodotyrosine, the situation is practically unchanged. After 6 hr, Fig. 17, the predominance of mono-iodotyrosine over thyroxine is confirmed and di-iodotyrosine increases perceptibly; let us mention an interesting fact, namely the splitting in two of the thyroxine peak. Is this due to tri-iodothyronine? After 11 hr, Fig. 18, it will be noted that the equilibrium is reversed, the top of the thyroxine band substantially exceeds that of mono-iodotyrosine, and the latter will never again become as predominant as it was over the hormone at the earlier stages, on the other hand, the concentration of di-iodotyrosine is multiplied by an important factor, and spreads out considerably. At 17 hr, Fig. 19, di-iodotyrosine again is in small quantities, but thyroxine evidences spectacular climbs. Between those two stages, 11 hr and 17 hr, the synthesis of thyroxine must be more active, and a considerable decrease in the quantity of di-iodotyrosine between Figs. 18 and 19 confirms that the hormone is formed by the coalescing of the two molecules of di-iodotyrosine. On Fig. 20, 44 hr after injection, the classic situation, which will be found with more or less modification in subsequent stages, is established. There is a very sharp peak for thyroxine, with small quantities of mono-iodotyrosine and di-iodotyrosine. After 80 hr (Fig. 21), this tendency is still more marked.

Summing up, mono-iodotyrosine is the first to appear (within the half hour which follows the injection or earlier); thyroxine follows it very closely,

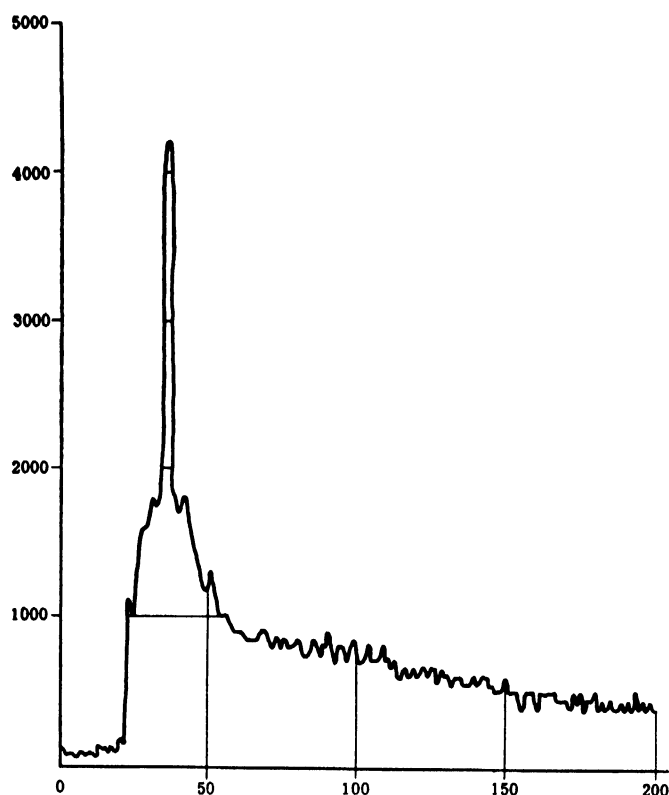


Figure 11. Rat sacrificed at 0 hr 30 min—Dowex 2, eluant HCl 0.2N

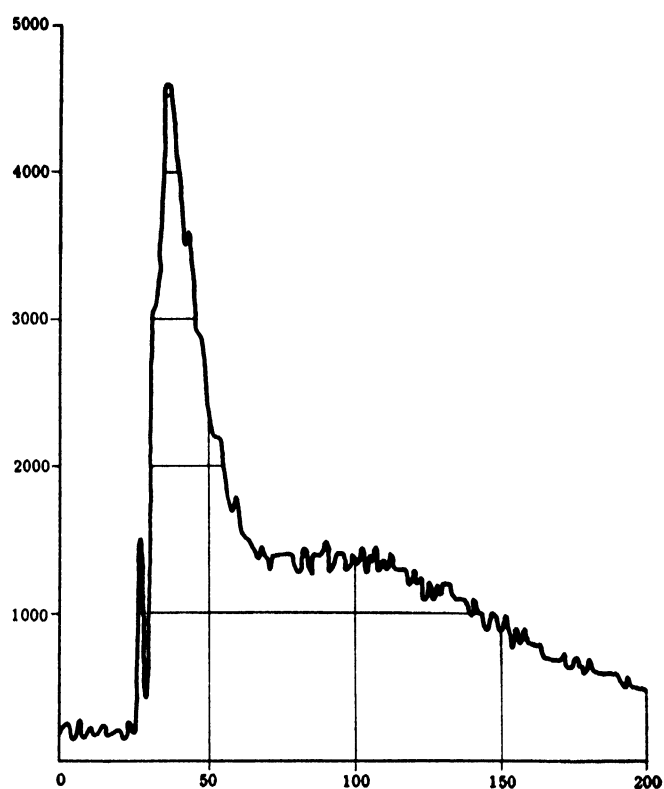


Figure 12. Rat sacrificed at 4 hr 30 min—Dowex 2, eluant HCl 0.2N

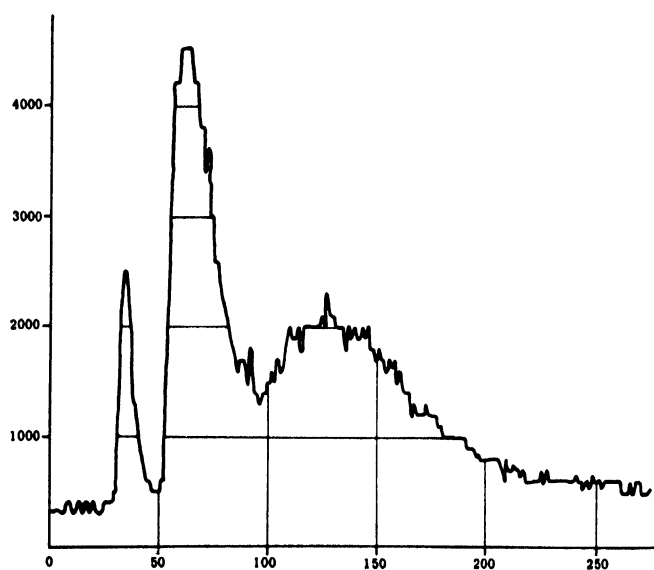


Figure 13. Rat sacrificed at 6 hr—Dowex 2, eluant HCl 0.2N

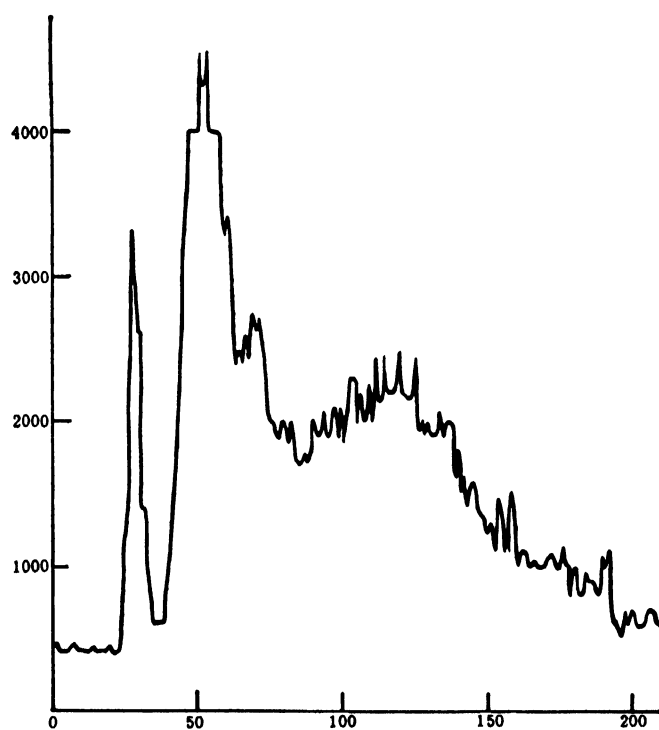


Figure 14. Rat sacrificed at 12 hr—Dowex 2, eluant HCl 0.2N

between 1 hr and 4 hr 30 min. While it is obvious that thyroxine is the result of the coupling of two molecules of di-iodotyrosine, a reaction which is maximal between 11 hr and 17 hr, mono-iodotyrosine appears in maximal concentration between 4 hr and 9 hr following injection and appears to play a less direct role, which is still obscure, in the synthesis of the hormone. This finding confirms the point of view of Roche and his group,²⁸ who believe that the

iodine fixation reaction progresses independently for the mono- and di-iodotyrosine.

Another type of experiment clearly shows this dynamic aspect of the intrathyroid metabolic phenomena (see Fig. 22). It consists of the reinjection of the second tracer dose of NaI into an animal a few hours before sacrifice. Thus, we carried out, in an animal which had already been subjected to the injection for 30 hr, a reinjection which was made

7 hr before its death. While the diagrams pertaining to rats sacrificed at approximately 40 hr were characterized by a broad band of thyroxine and a small quantity of mono- and di-iodotyrosine, we will note, on Fig. 22, a considerable increase in the mono-iodotyrosine which exceeds the top of the thyroxine peak, and a less marked increase in di-iodotyrosine, which proves that the substances appear in the following order $\rightarrow$ mono-iodotyrosine $\rightarrow$ di-iodotyrosine $\rightarrow$ thyroxine. However, this reinjection practice seems to be contraindicated as a general rule, for the superimposition of a second metabolic cycle over the first brings about, in the majority of cases, a considerable disturbance, and makes reading of the diagrams difficult. All the diagrams which have been published lend themselves to quantitative interpretation according to the rules discussed above (percentage of total radioactivity relative to each one of the substances: thyroxine, mono-iodotyrosine, di-iodotyrosine), but these tables of new figures would add nothing to the general scheme which we have just given.

From the quantitative standpoint, there is a peculiar point which deserves special attention. By subtracting the difference between total radioactivity of the hydrolysate, before passage on the column, and the sum of the radioactivities of each one of the fractions eluted by means of HCl 0.2N, we obtained the activity due to mineral iodine. For most of the experimental animals, we found figures on the order of 10% of total activity, and sometimes greater.

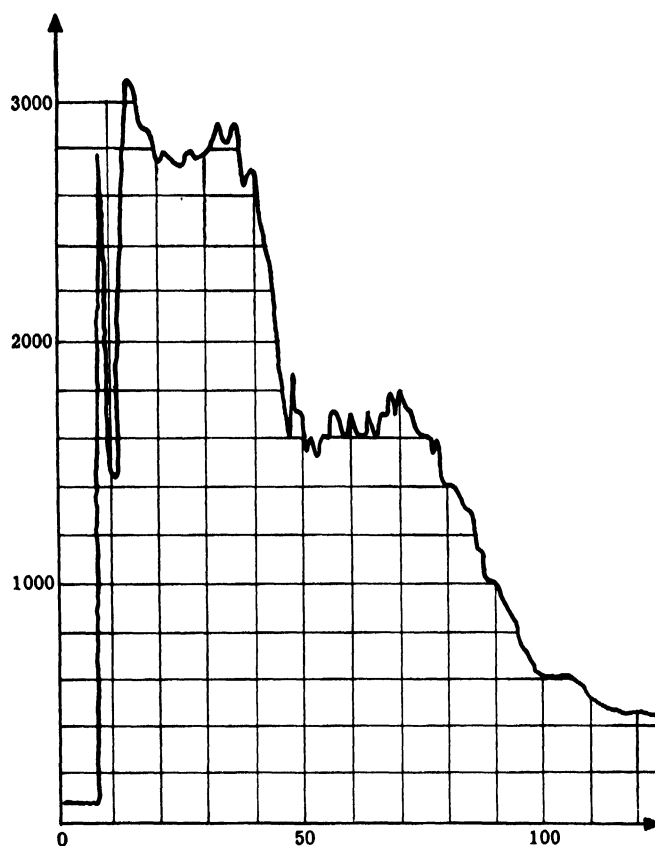


Figure 16. Rat sacrificed at 4 hr 15 min—Dowex 1, eluant HCl 0.2N

We find rates comparable to those given by Gutman and his group²⁴ for human thyroids (7 to 10%) and by Mann *et al.*²⁵ (3 to 18% for dog thyroids), but we are far from the low values given by Taurog *et al.*²⁶ who found less than 1% of the mineral iodine in the thyroid hydrolysates.

The selectivity of the Dowex 1 or Dowex 2 resins with respect to mineral iodine can not be doubted, and the hydrolysis of the thyroid by trypsin does not release iodine from the iodine-bearing amino acids, as would hydrolysis conducted with NaOH or Ba(OH)₂. Are we dealing with a large peptide or a large iodine-bearing protein molecule left on the column, which resisted hydrolysis? All these hypotheses are open to discussion. On the other hand, we know that we could obtain absolutely identical elution patterns when treating the hydrolysates by butanol at pH 1, then evaporating, and dissolving again in 2N ammonia, or when we were subjecting them to the column without prior treatment. We thus are certain that we are extracting the whole of organic iodine.

All these points are now being verified with great care but we believe that our method, such as we presented it, is one of those which involves a minimum of artefacts, and that the quantity of mineral iodine which is in the thyroid is not negligible, and does not represent as low a percentage as that given by Chaikoff and his group.²⁶ This viewpoint is confirmed on the other hand by unpublished original research bearing on the thyroid of chick embryos,²⁷ using the same technique. In the hydrolysates of

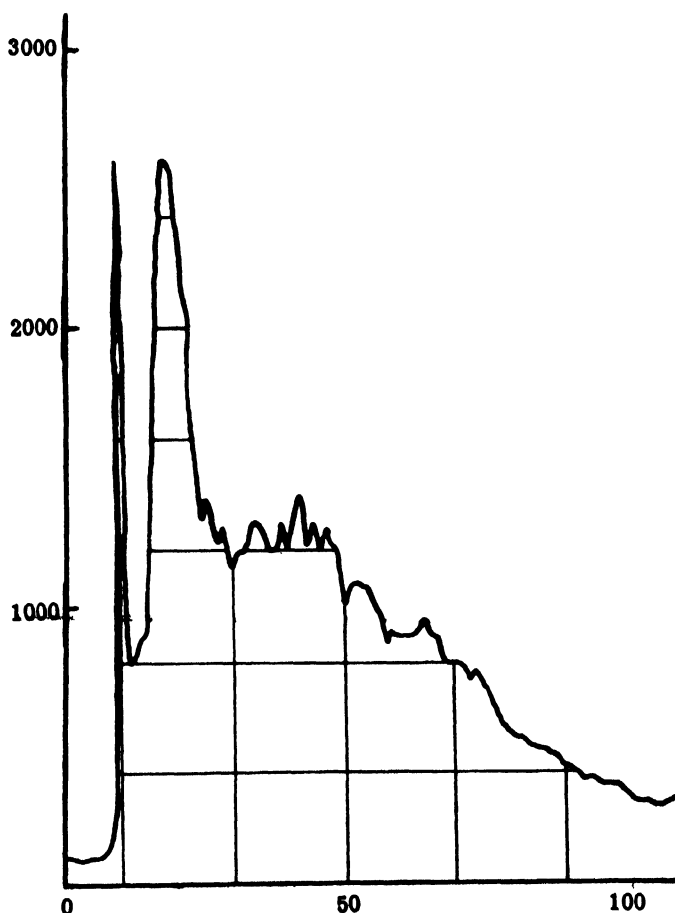


Figure 15. Rat sacrificed at 1 hr—Dowex 1, eluant HCl 0.2N

these thyroids, we find high percentages of mineral iodine (10 to 15%).

Note: Everything stated in this paper as regards the iodized amino acids and thyroxine, is valid only for tagged substances, and the quantities of inactive mono-iodotyrosine, di-iodotyrosine and thyroxine present in the gland when the animal is sacrificed are doubtless found in proportions very different from those determined for the tagged substances.

(Parallel to these studies, we are currently developing a technique which makes it possible quantitatively to determine the amount of thyroxine in the plasma. The latter is treated by acid butanol at pH 1, which quantitatively extracts the thyroxine.

Following evaporation of the butanol, under reduced pressure and temperature (30°), it is further treated with a buffer at pH 4, and the thyroxine is tagged, as indicated above, by means of strongly active NaI solutions, in order to avoid the low yield of the operation. The substance so tagged then is passed through a Dowex 1 or 2 column, and the curve obtained is interpreted from the quantitative standpoint).

SUMMARY

A method for separating and for estimating the iodized amino acids: mono-iodotyrosine, di-iodotyrosine and thyroxine, using the anion exchange resins Dowex 1 and 2, has been described.

In the course of the same operation, the separation of inorganic and organic iodine is easily carried out.

By making parallel use of injections of radioactive iodine and of the Dowex 1 and 2 resins, it is possible to separate and determine the various tagged substances contained in the thyroid hydrolysates, when the glands are removed at various stages of metabolism. As above, the blocking of the iodides at the top of the column from the very beginning of the operation makes it of additional value.

The simplicity and speed of the method, and its quantitative character, appear to make it unquestionably superior to paper chromatography.

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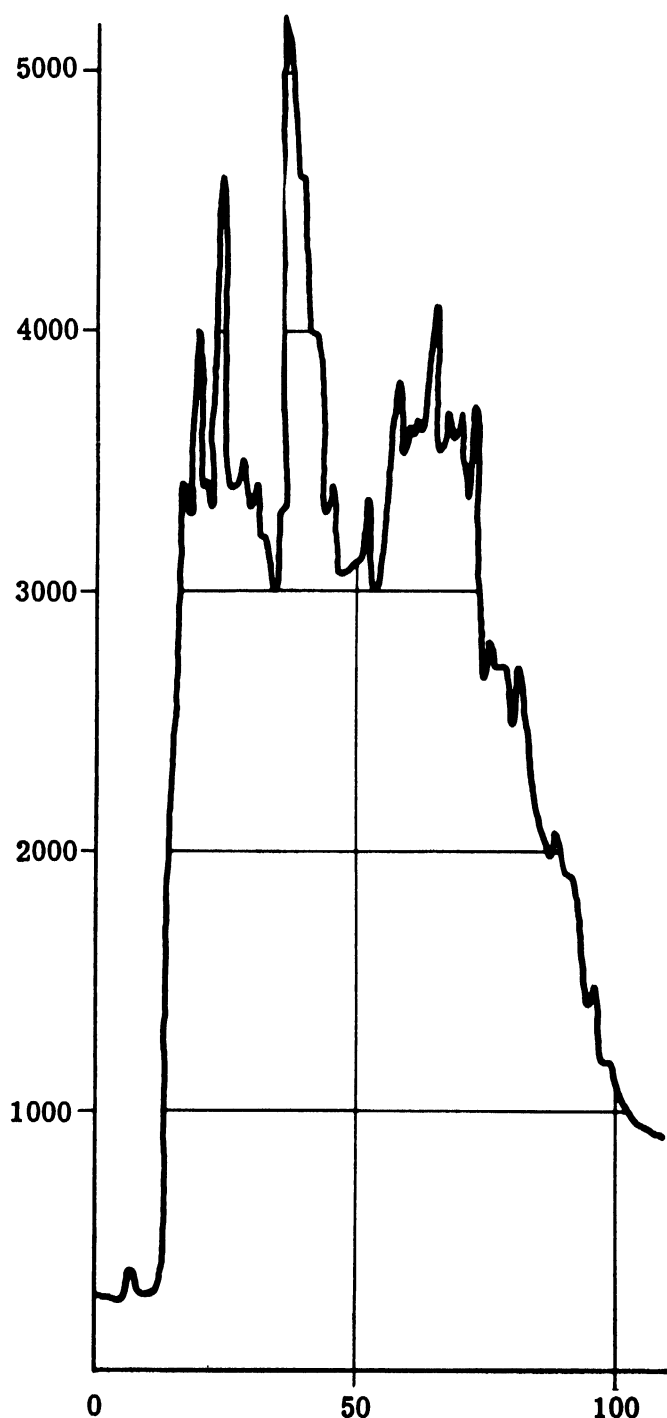


Figure 17. Rat sacrificed at 6 hr—Dowex 1, eluant HCl 0.2N

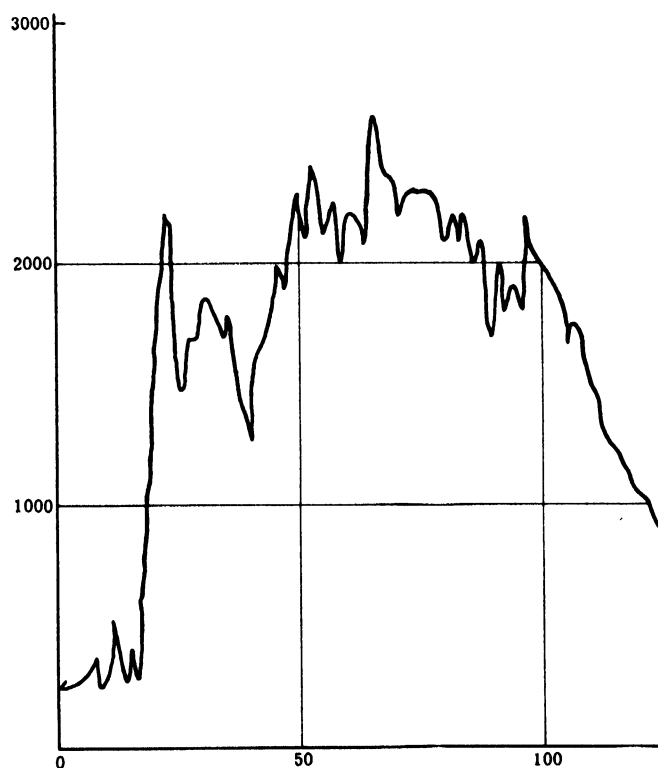


Figure 18. Rat sacrificed at 11 hr—Dowex 1, eluant HCl 0.2N

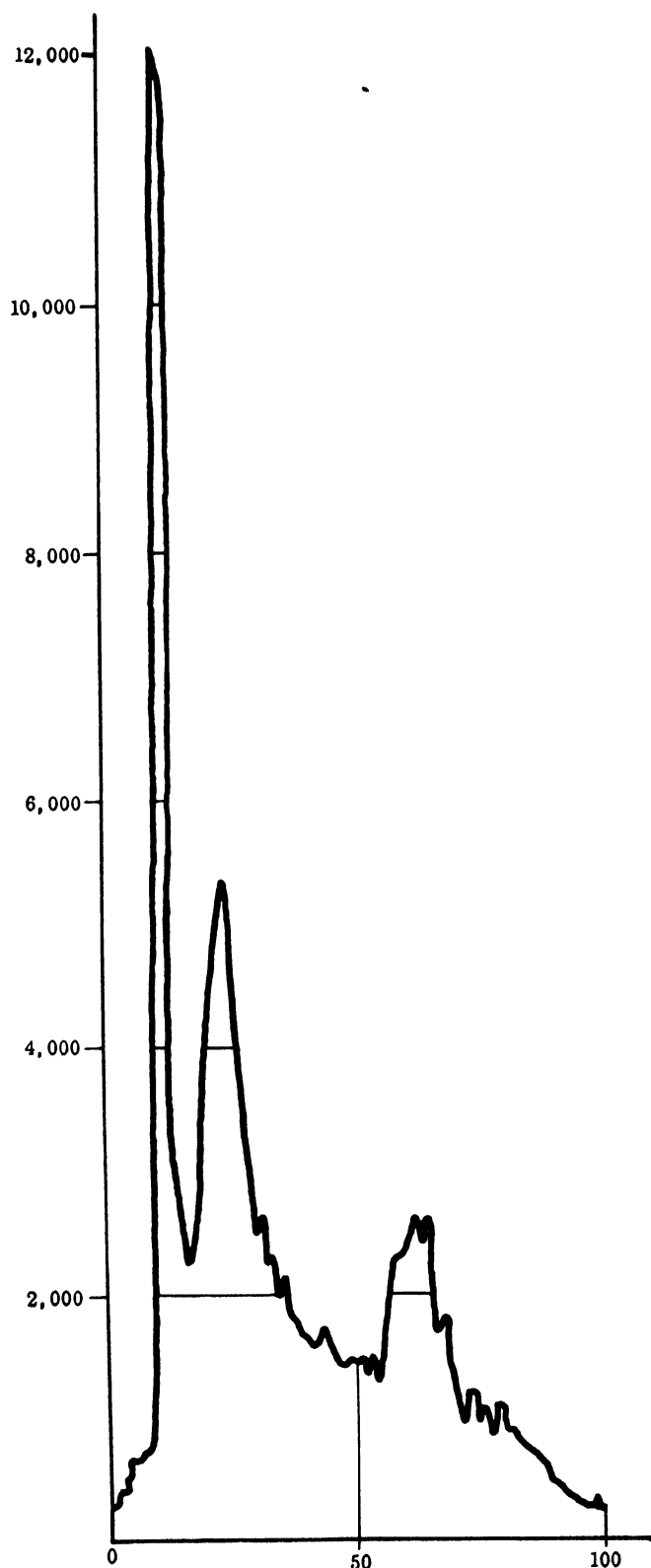


Figure 19. Rat sacrificed at 17 hr—Dowex I, eluant HCl 0.2N

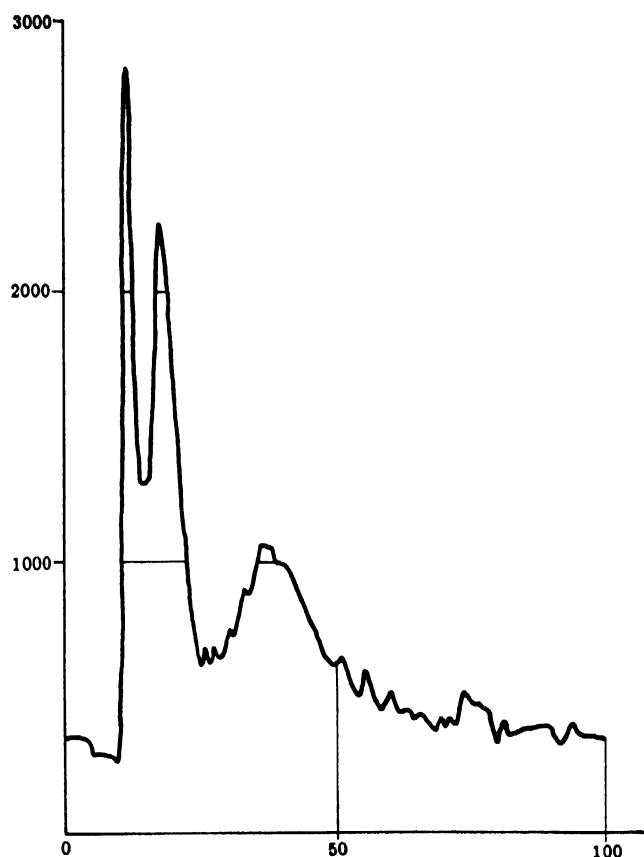


Figure 20. Rat sacrificed at 44 hr—Dowex I, eluant HCl 0.2N

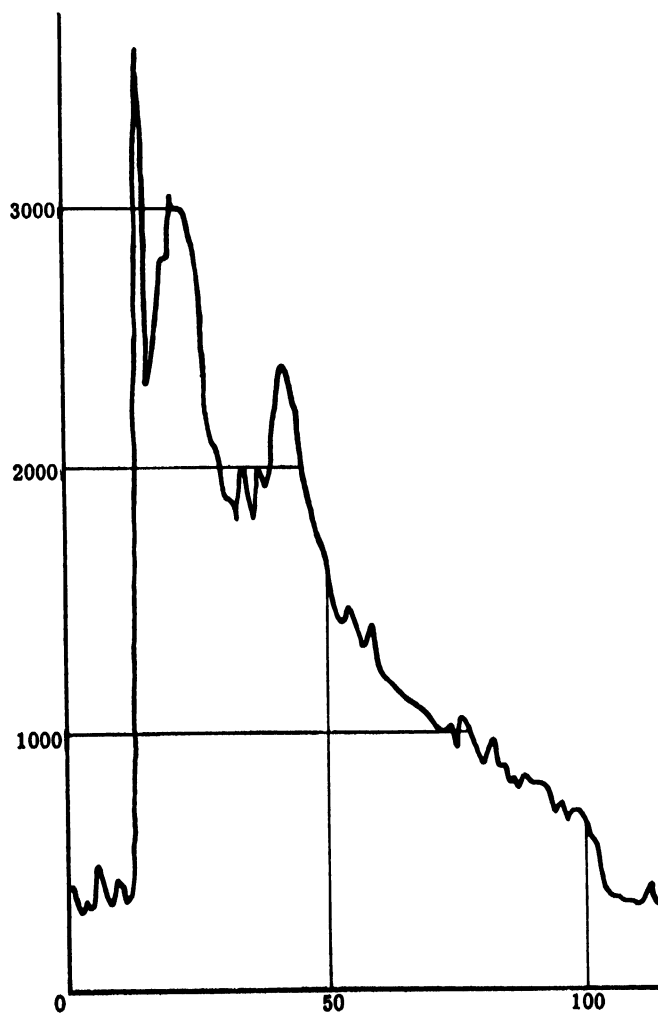


Figure 21. Rat sacrificed at 80 hr—Dowex I, eluant HCl 0.2N

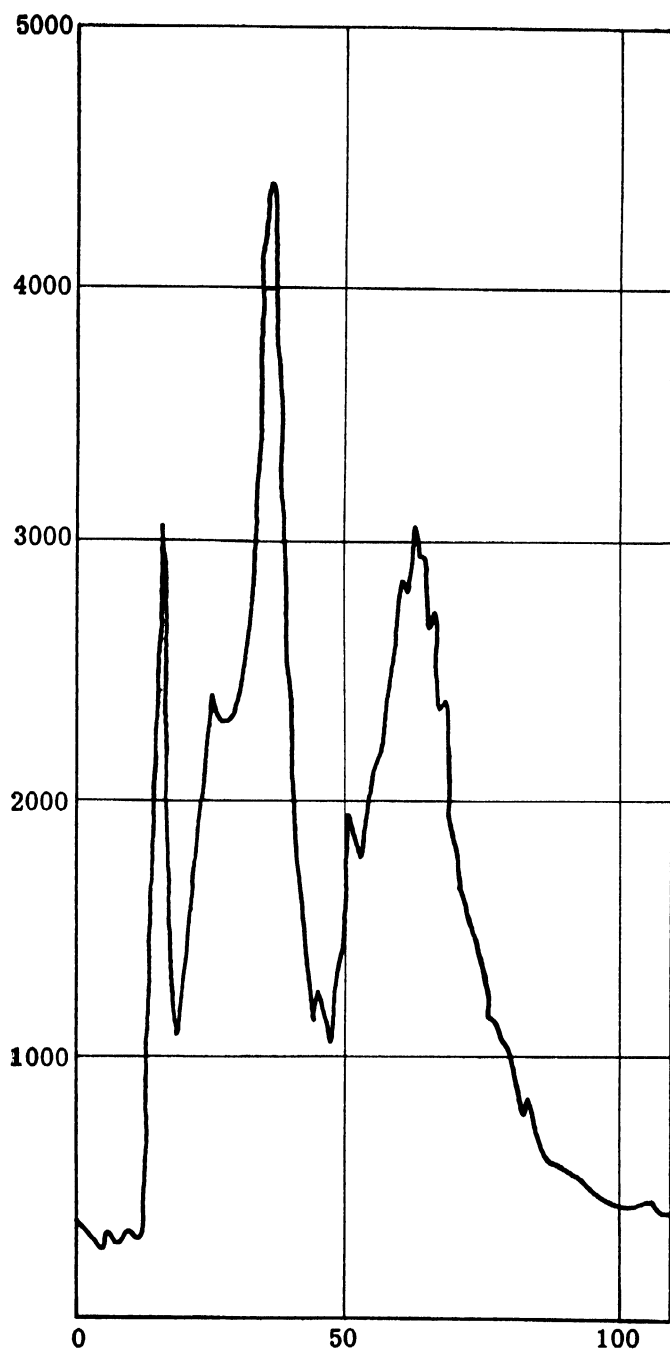


Figure 22. Effect of reinjection (see text)

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The Use of I^{131} Labelled Serum Albumin in the Study of Cardiac Output and Peripheral Vascular Flow

By W. J. MacIntyre, J. P. Storaasli, H. Krieger, W. H. Pritchard and H. L. Friedell,* USA

With the successful use of I^{131} tagged serum albumin in the measurement of plasma volumes, the possibility of the use of this material for the *in vivo* investigation of vascular flow appeared feasible. In addition to a slow rate of disappearance of this substance from circulating blood, the gamma radiation of I^{131} possesses sufficient energy to record such flow through a considerable depth of tissue.

It is this recording of the manner and rate in which the injected material is mixed and diffused by the circulating blood which is the basis for all such vascular flow investigations. The two studies to be described here include (1) the recording of arterial concentration following intravenous injection for cardiac output determination and (2) the recording of capillary mixing following intravenous injection for peripheral flow estimation.

CARDIAC OUTPUT DETERMINATION

The determination of cardiac output by the injection method is based on the measurement of the varying concentration of the injected material as it passes through the heart. If a small volume of highly concentrated material is injected into the inflow tract, the average dilution of the material coming out from the heart will be a direct indication of the volume passing through the heart and causing this dilution. The measurement of the change of concentration during a definite interval thus permits the output to be expressed as volume of flow per unit time.

While this method has been used earlier with various non-radioactive materials^{1,2} the applicability of radioactive isotopes is readily apparent. In 1950 Nylin and Celander³ reported the use of radiophosphorus tagged red cells to obtain such cardiac dilution curves. In both of these methods the varying dilution of the injected material was determined by serially sampling arterial blood. These samples were then quantitated chemically or by beta-particle assay. It is the main advantage of the I^{131} tagged serum albumin that this concentration may be determined by gamma-ray assay. A continuous sampling of concentration without sample preparation may then be made since the absorption in the blood or tissue is negligible. This has obviated the laborious system of withdrawal and assay of many samples and has

made accurate timing of the dilution curve more easily obtainable.

The loss of serum albumin from the vascular system is not more than about 3% for the first ten minutes following injection⁴ which is entirely satisfactory for the period of this measurement. Although the loss of red cells tagged with Cr^{51} or K^{42} is perhaps slower over this period, the advantage of serum is that this substance may be used directly, avoiding the incubation period required for the red cell methods.

The general experimental setup is shown in Fig. 1. Since the injected material suffers a large dilution quickly after injection, it is necessary to employ a detector of the highest sensitivity. Scintillation counters have been used throughout and at the present time a thallium-activated sodium iodide crystal of one and one-half-inch diameter and one inch thickness is used. This crystal was drilled longitudinally across the face at its midline and an open end aluminum cylinder sealed in so that the flow could actually be introduced through the crystal itself. For human studies a Cournand No. 18 arterial needle was inserted into either the brachial or femoral artery and adjusted within the arterial lumen so that a free, pulsatile flow existed when the stylette was removed. A stainless steel tubing of 2-mm bore was connected to the needle by plastic tubing and passed through the crystal. The blood was allowed to flow through this tubing at a rate of approximately 30 ml per minute. The dilution curve,

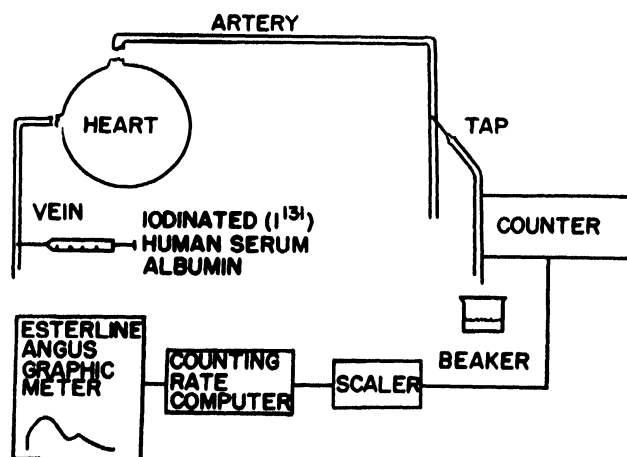


Figure 1. Experimental set-up for cardiac output determinations

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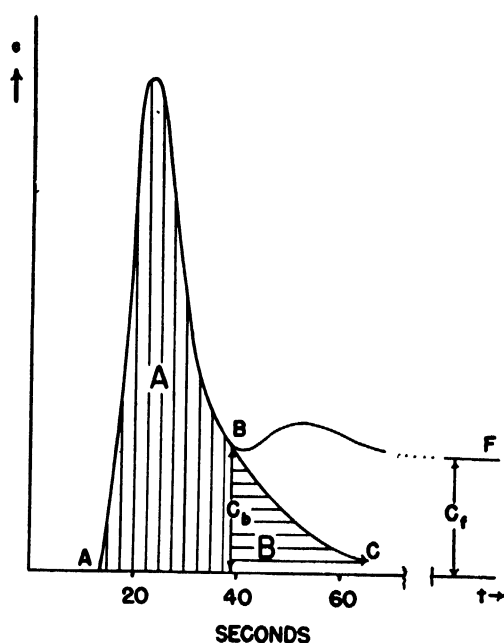


Figure 2. General shape of dilution curve. Actual recorded curve follows ABF. Extrapolated curve representing the first circulation follows ABC

or the change in concentration of the diluted material in the blood, was recorded by a scaler and counting rate computer combination and inscribed automatically by a direct writing recorder. A fast response time counting rate meter would have been satisfactory, but the scaler-computer combination was chosen so as to eliminate the memory effect of the rate meter and allow a lower counting rate and dose to be used. Originally about 150 microcuries of ¹³¹I tagged serum were given, but with the improvement in detector efficiency successful measurements have been made with as little as 25 microcuries.

The general form of the dilution curve obtained is shown in Fig. 2. This shows the characteristic rise as the bulk of the radioactivity passes the counter site followed by the second small recirculation curve. In order to correct for this recirculation effect, it is generally assumed that the concentration is decreasing exponentially and that the curve may be extrapolated on that basis from the point B.

Since the same total injected material, I , must come out of the heart as was injected into the inflow tract, this quantity of injected material must be equal to the product of the flow rate, F , multiplied by the concentration of the tagged material, c , in the blood integrated over the entire time of the primary circulation. This is represented by the equation:

$$I = \int_0^{\infty} F c dt \quad (1)$$

and if F , the flow rate or cardiac output is constant,

$$I = F \int_0^{\infty} c dt \text{ where } \int_0^{\infty} c dt \text{ represents the area}$$

under the primary circulation curve and the dotted extrapolation line. This area is equivalent to area A and area B as shown in Fig. 2. Since the injected

dose, I , is equivalent to the final dilution, C_f , multiplied by the blood volume, V , a convenient expression for the cardiac output is:

$$F = C_f V / (A + B) \quad (2)$$

On a study of dogs wherein a comparison was made between the values of cardiac output obtained by this method and that measured by an optically recording rotameter, the average deviation amounted to +9 per cent.⁵

In addition, measurements of cardiac output on patients have been made with simultaneous determinations of outputs by the Fick principle. The degree of deviation from the Fick values extended from -8.4% to +18.7% with an average of $\pm 8.3\%$.⁶ The amplitude of outputs in this study ranged from 2.8 liters/min to 11.4 liters/min. A typical curve obtained by this method is shown in Fig. 3.

While these studies all involve the isolation of arterial flow by cannulation or by arterial taps, this is not necessarily required by theory or method. As shown by Equation 2, all that is actually needed, in addition to the determination of blood volume, is a ratio of the final dilution of any arterial flow as compared to the measured area under the extrapolated dilution curve of this flow.

This has stimulated interest in attempts both in this laboratory and elsewhere^{7,8,9} to determine cardiac output by focussing a gamma detector externally on intact arterial flow, thus eliminating the need for the arterial tap. Two general difficulties have been found to this approach. The first requirement is that the final dilution must be recorded from the same flow site as the dilution curve. This means that the site must have a minimum of venous or capillary flow. For this reason the most convenient arterial flow site selected has been the large chambers or vessels of the heart itself. The second difficulty, which is the one found with the choice of the large chambers, is entailed in the correct extrapolation of the descending part of the curve. If the

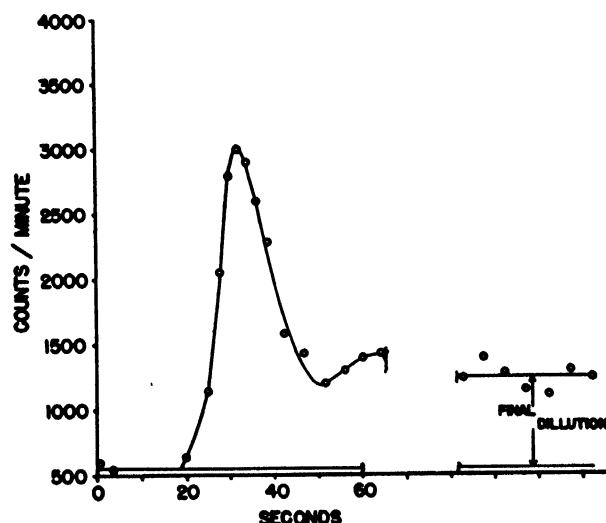


Figure 3. Typical dilution curve for human study. Cardiac output determined by dilution curve was 5.48 l/min and by Fick principle was 5.98 l/min. Blood volume was 6.11 l and cardiac index was 2.98

site selected has a number of clearances in different time phases this extrapolation may depart from an exponential decline and will be difficult to predict.

This effect has been minimized by focussing so that either the right or left heart can be predominant. By this system 29 successful correlation determinations have been obtained comparing the external focussing method with the arterial tap method and exhibiting an average deviation of approximately $\pm 9\%$. With patients in cardiac failure, outputs as low as 2.2 liters per minute have been successfully correlated by this procedure. As many as four output determinations have been made in succession on the same patient with a total of less than 150 microcuries of iodinated (I^{131}) albumin, so that the use of this method for repeated studies seems entirely feasible. While the arterial tap method has been used for routine clinical determinations of cardiac output in these laboratories since 1951, the external method is expected to supersede this because of the elimination of the arterial tap and the ease of repeated procedures.

PERIPHERAL VASCULAR INVESTIGATION

Although there are many clinical approaches to the evaluation of the circulation in peripheral vascular disease, most of these are indirect methods and do not involve the direct measurement of blood flow in the extremities. While attempts have been made to evaluate peripheral vascular circulation with radioisotopes previously, these have been mainly concerned with either (1) the intravenous injection of an electrolyte and observation of the subsequent build-up over a capillary bed^{10,11} or (2) the intramuscular injection of an electrolyte and observation of the subsequent disappearance from the capillary bed.^{12,13}

When an electrolyte is used for this type of measurement, the effect measured is due not only to blood flow but also from the diffusion rate of the electrolyte across the capillary membrane to or from the extra-vascular space. With this in mind it appeared advantageous to separate experimentally the actual vascular flow from the effects of diffusion through the capillary walls and the equilibrium of the material in electrolyte space. By utilizing I^{131} labelled albumin and the apparatus described for the cardiac output determination, it seemed possible to record over an extremity the rate and manner in which the tagged serum albumin and the normal serum albumin achieve equilibrium. There is a possible disadvantage of using a gamma emitter as the tagging substance since this will allow the blood in the larger vessels to contribute somewhat to the curve obtained. This objection has not been found to be serious with this method since the volume of blood in the arteries and veins is small compared to that of the capillary bed. In addition, the detector is applied to the surface so that the inverse square law becomes important and the contribution from the radioactivity from the deep vessels falls off

rapidly with distance. Thus the radioactivity measured arises primarily from the tagged albumin within the more superficial capillaries.

The general schema for this measurement was similar to that shown in Fig. 1. The scintillation counters were curved so that the sole of the foot or palm of the hand could be comfortably placed in close apposition to the crystal itself. Lead shielding was employed to prevent interference of radiation from the other extremities or sites than the one desired. The counting rate computer and scaler combination was again used but has no particular advantage in this case over conventional counting rate meters. The method used here is primarily a "comparative technique" wherein the patient's opposite extremity acts as a control for the extremity under investigation. Thus two identical counters were used for each measurement and independent characteristic build-up curves were recorded from both.

In Fig. 4 is shown a typical curve as recorded after injection into the antecubital vein of 75 to 130 microcuries of I^{131} labelled serum albumin in a volume of 1.5 ml or less. The latent period of the arm to foot circulation time (approximately twenty seconds) has been omitted from the figure. The next portion of the curve (A-B) shows the regular increase in detected radioactivity and is thus a measurement of the rate of mixing occurring in the vascular bed. It is primarily the rate at which the tagged plasma is mixed and brought into equilibrium with normal blood in the capillaries and **therefore represents the manner in which the capillary bed is cleared**. When complete mixing has occurred, the plateau B-C is reached with a final height dependent upon the volume of the vascular bed measured.

The exponentially rising curve of Fig. 4 may be approximated mathematically by considering the measurement of radioactivity, A , over the capillary bed as represented by the product of the volume of capillaries activated, V , (i.e., cleared of original blood and replaced with blood containing I^{131} labelled albumin) multiplied by the concentration, c , of

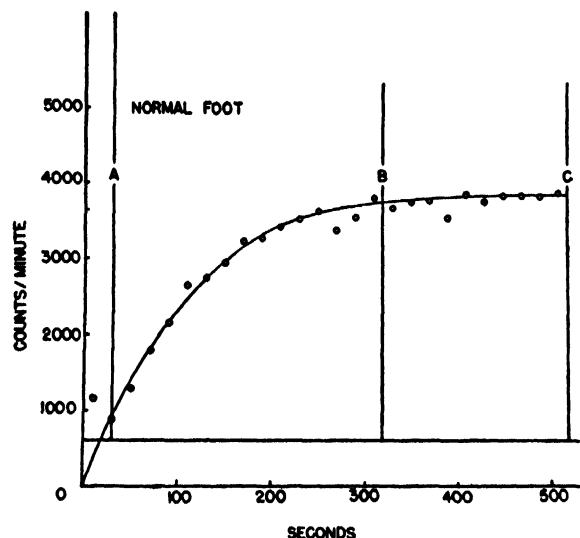


Figure 4. Typical equilibrium curve for peripheral vascular flow measurements

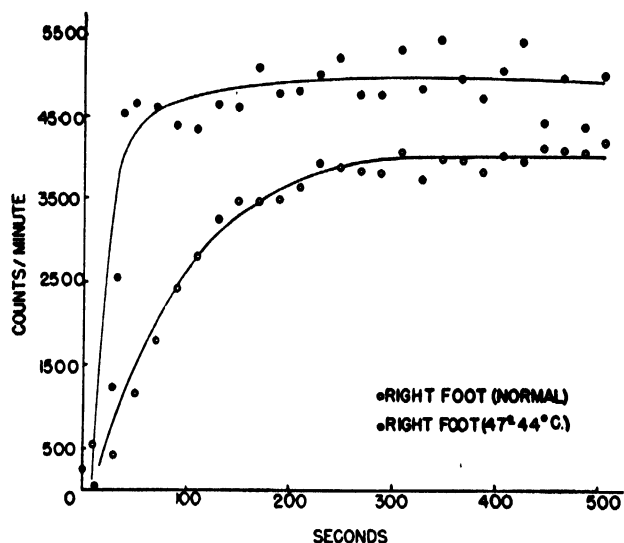


Figure 5. Effect of heat on peripheral vascular measurement. Note increased height of plateau and faster equilibrium time

the tagged albumin in the blood. Since the concentration of the tagged albumin in the large vessels reaches about 90% of its maximum dilution in about one minute, c can be treated as a constant; so that as a first approximation, the rate of change of radioactivity recorded by the detector is:

$$dA/dt = c dV/dt \quad (3)$$

This indicates that the uptake rate of radioactivity in the capillary bed is similar to the rate at which the volume of blood within the capillaries is activated or cleared. The rate of activation of the capillary volumes dV/dt , will now be directly proportional to the pressure, P , forcing the blood through the capillaries and inversely proportional to the total resistance, R , acting to oppose this flow. Since the capillaries offer many paths of activation or clearance, the blood will not necessarily follow the same paths each time and the activation rate must be proportional to the paths which are unactivated at that time. Thus it can be said, in addition, that the rate of activation will be proportional to the volume of blood within the capillaries as yet unactivated. This volume can be expressed as $(V_f - V)$, where V_f is the final volume activated.

This expression of proportionality may then be expressed as

$$dV/dt \propto (V_f - V) P/R, \text{ or } dV/dt = \frac{k (V_f - V) P/R}{k (V_f - V) P/R} \quad (4)$$

where k is a constant of proportionality.

This may be integrated to yield the equation:

$$V = V_f [1 - \exp(-k Pt/R)] \quad (5)$$

which describes the curve as exponentially rising with time.

Certain experiments utilizing heat, cold, and arterial and venous obstructions have been devised in order to study alterations in the clearance rate of the capillaries and in the volume of the vascular bed as suggested by this mathematical approximation. In Figs. 5 and 6 the effects of heat and cold on

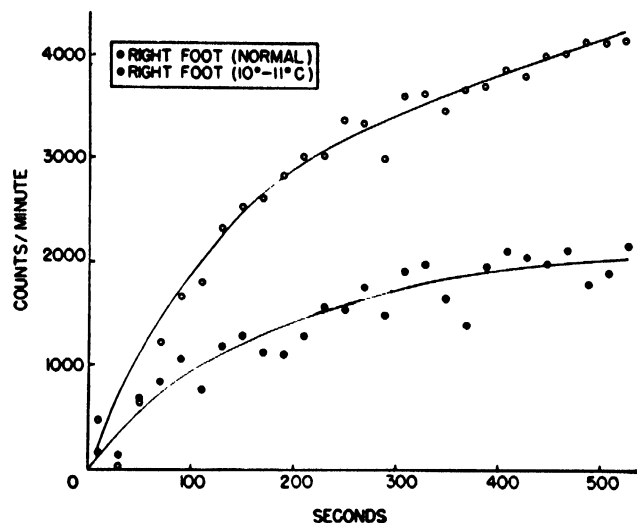


Figure 6. Effect of cold on peripheral vascular measurement. Note decreased height of plateau

peripheral vascular flow are graphically shown. As expected, the heat causes an increase in the rate of mixing due to the reduction in peripheral resistance which allows a more rapid clearing or flushing of the capillary bed. This appears to be due to dilatation of arterioles, capillaries or both. The increased volume for this dilatation is reflected by the higher plateau of the warm extremity. The opposite effects of increased peripheral resistance or vasoconstriction, slower mixing time, and lower plateau are seen in Fig. 6.

The effects of arterial and venous obstruction are shown in Fig. 7 where digital compression of the brachial artery shows a lag in the flow curve which is held until release. The "pooling" of blood in the vascular bed following venous obstruction is shown by the increased rise which again returns to normal upon release.

This test has had three main applications for clinical use.¹⁴ These include (1) evaluation of the status of the peripheral circulation in arteriosclerotic patients or others with peripheral vascular disease, (2) evaluation of the response of the peripheral cir-

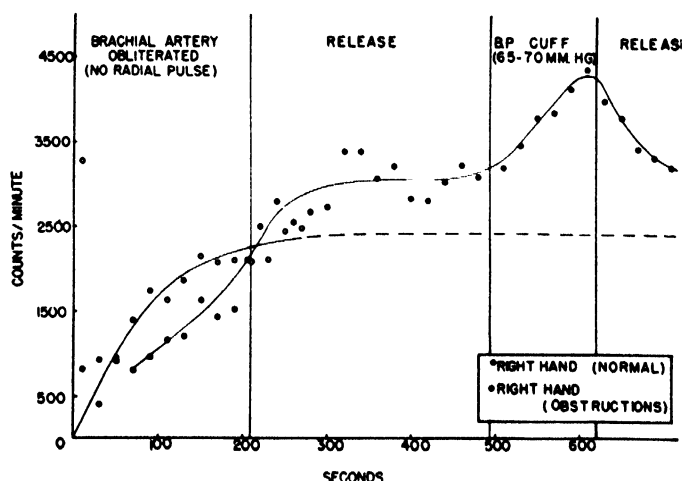


Figure 7. Effect of partial arterial obstruction and venous obstruction on peripheral vascular measurements

ulation to stimuli (such as observation of the effect of a paravertebral block before sympathectomy, as an aid to predicting possible benefits of the surgical procedures) and (3) objective evaluation of surgical procedures or other therapy.

At present this has been used as a "comparative technique" with no attempt made to quantitate the actual blood flow. Greater control of these measurements may very well, however, lead to a more exacting definition of the exponential factors and actual relation to blood flow.

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The Radioactive (I^{131} -Labeled) Rose Bengal Liver Uptake-Excretion Test. Its Use in the Evaluation of Hepatic Function

By George V. Taplin,* O. M. Meredith, Jr,* Harold Kade,* Joseph L. Westover,† Robert A. Hansen† and Leslie R. Bennett,† USA

This report is a summary of the present status of the Radioactive Rose Bengal Test for liver function which is being developed concurrently by two research groups at UCLA on a cooperative basis. In the initial report¹ the basic principles of the test, as developed in animal experiments, were described, together with modifications in technique required upon application of the test in human subjects. The test provides data on three important aspects of liver function - circulatory capacity, polygonal cell function and patency of the biliary passages. The Radioactive Rose Bengal Test has several advantages over non-radioactive dye tests for liver function. The test can be used safely in the presence of jaundice because of the minute amount and low toxicity of the dye. It has been shown to be many times more sensitive than the bromsultalein test in detecting liver damage in rabbits poisoned with chloroform and/or carbon tetrachloride. It helps differentiate primary hepatic cell dysfunction from obstructive biliary tract disease. It eliminates the necessity for repeated venipunctures for blood sampling.

These advantages are attained by labeling the Rose Bengal molecule, (the potassium salt of tetra-iodo-tetra-chlor-fluorescein) with I^{131} atoms which emit gamma rays. Following intravenous injection of the labeled dye the rates of liver uptake and excretion may be measured externally over the liver area with any sensitive gamma-ray scintillation counter and recording equipment.²

The purposes of this paper are (1) to describe some recent clinical studies which clarify the interpretation of the primary segment of the liver uptake records, (2) to present an analysis of the comparative liver uptake-excretion data from normal controls versus patients with known liver disease, (3) to present clinical data showing diagnostic value of an abbreviated modification of the original test and its special applicability and (4) to discuss further desirable or necessary improvements to develop the test into a standard procedure which could provide quantitative data on total liver circulation, polygonal cell function and biliary tract patency.

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MATERIAL AND EQUIPMENT

The Rose Bengal used (C.I. 779) is made radioactive by a procedure utilizing ion exchange reactions, giving a dye with high specific activity, i.e., about 1 millicurie/mg. The Radioactive Rose Bengal is supplied in a solution containing 10 mg of dye and 10 millicuries of I^{131} per milliliter. Samples used for test purposes are prepared to contain $5\mu\text{c/ml}$ by making suitable dilutions of the stock dye solution in normal saline or in non-radioactive Rose Bengal.

Gamma-ray activity was measured using a lead-collimated scintillation counter (NaI crystal) connected through a rate meter to an Esterline Angus Recorder (see Fig. 1).

TECHNIQUES FOR RADIOACTIVE ROSE BENGAL LIVER FUNCTION TESTS IN PATIENTS

For Liver Uptake and Excretion (60-90 Minute Test)

The lead-collimated counter is positioned on the right antero-lateral aspect of the chest over the upper margin of the liver, as determined by percussion and directed away from the gallbladder area (Fig. 1) with the sensitive element (NaI crystal) retracted 6 inches into the lead shield to provide collimation. Under such circumstances our equipment will register 8000 counts per minute from a one microcurie standard (antimony) button held at the end of the counter. The medium (10 second) time constant gives a tracing fairly free from random fluctuations.

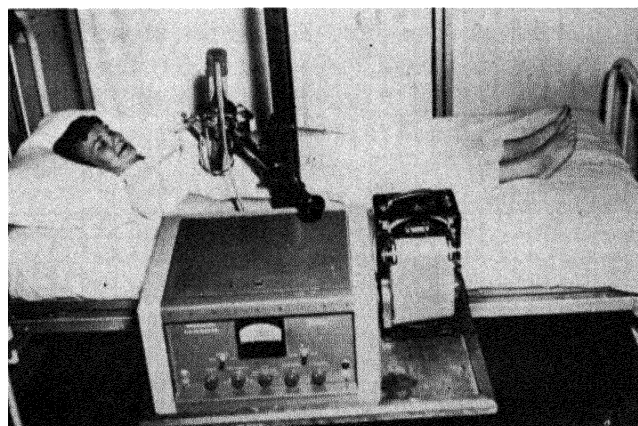


Figure 1. Equipment for performing the radiation measurements

The patient is instructed to remain in a reclining position for the duration of the test. Movement of the extremities is permissible since it is important only that no significant change occur in the position of the counter and the chest wall.

The dose of dye used varied between 0.2 and 5.0 mg, (depending on specific activity) but the amount of radioactivity was held constant at 5 microcuries. The test dose (volume 2-5 ml) is injected intravenously at a rate of approximately 0.1 ml per second. A satisfactory uptake and excretion pattern can be obtained in a normal individual in 60-90 minutes. More time is required in patients having severe impairment of liver function.

Abbreviated Liver Uptake Test

The rate of liver cell uptake of Radioactive Rose Bengal may be determined in 15 minutes. With this short test, it is not necessary to use a collimated counter, because interference from gamma-ray activity in the upper intestinal tract and gallbladder is not encountered during this time. Thus, a wide angle counter can be used and the improved geometric factors permit one to employ smaller amounts of radioactivity ($1-2 \mu\text{c } I^{131}$) than the $5.0 \mu\text{c}$ doses needed in the 60-90 minute test procedure. If recording equipment is not available, counts may be made 3, 10 and 15 minutes after injection. The slope of liver cell uptake may then be calculated.

RESULTS

Studies with IHSA

The initial leg of the Rose Bengal liver-uptake curve has been shown to indicate the total circulation capacity of the liver. Following intravenous injection of labeled Rose Bengal, there is a rapid rise in radioactivity over liver tissue, reaching a peak level in 1-2 minutes and then a slow steady rise for 10-30 minutes. By injecting I^{131} tagged Human Albumin and recording similar measurements over the liver there is an identical rapid uptake for 1-2 minutes followed by a plateau which remains constant for more than an hour. Since albumin remains in the circulation and is not selectively removed via the polygonal cells of the liver and then excreted in the bile as is the case with Rose Bengal, the height of the initial liver uptake segment of both curves appears to represent the amount of radioactivity in liver blood. Thus, it is evident that this leg of the

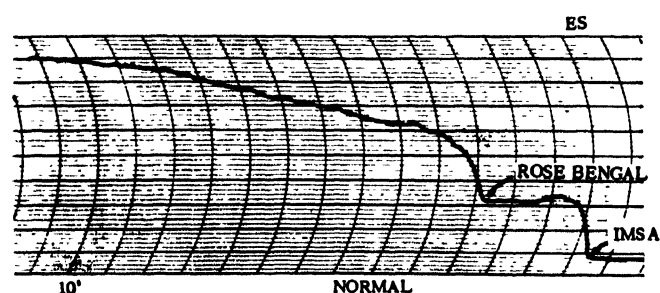


Figure 2. Liver uptake of I^{131} tagged human serum albumin compared with the uptake of radioactive Rose Bengal in a normal subject

Rose Bengal uptake curve is an index of total blood supply or vascular capacity of the liver (see Fig. 2).

60-90 Minute Test

Data presented in Table I were obtained by analysis of uptake-excretion records (1) of normal patients and of those having various forms of liver disease (for typical records, see Fig. 3). It is apparent that the values obtained in normal men and women fall within the same ranges as those found among a group of patients recently recovered from acute hepatitis. The values for liver uptake time in patients having cirrhosis are grossly lower than those among normals. The uptake times found in patients having acute and/or chronic hepatitis fall in an intermediate range. The maximum height of the uptake curve appears to have some relation to hepatic cell function and possibly to the number of functional polygonal cells; values being highest for normals, lowest for patients with cirrhosis and intermediate for patients with less severe impairment of liver function.

The excretion segment of the 60-90 minute test pattern is useful not only in evaluating biliary tract patency, but also liver cell function. Values for the slopes of these downward segments are high in normal individuals and are somewhat depressed in patients with acute and chronic hepatitis. In persons with advanced cirrhosis, it apparently requires longer than 90 minutes to reach a point where excretion rates exceed uptake from the blood; since by this time, no downward slope of the curve of radioactivity over liver tissue has yet been observed in any of the cases studied to date.

15-Minute Test

Results from the abbreviated liver-uptake test procedure in 40 individuals are shown in Fig. 4. The values for rate of liver uptake (arbitrary units) show significant differences between normal subjects (Group C) and patients suffering from advanced cirrhosis (Group A). Patients having mild to moderately severe liver impairment (Group B) have intermediate values for the rate of dye uptake in the liver. It is evident that this short test is capable of pro-

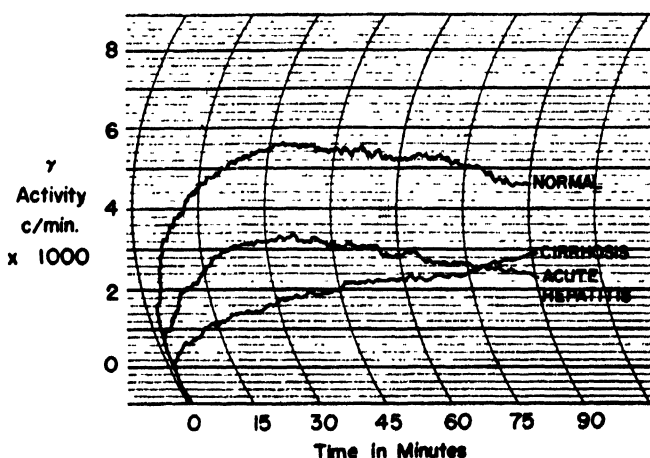


Figure 3. Typical radioactive Rose Bengal liver uptake-excretion patterns in human subjects

Table I. Radioactive (131) Rose Bengal Liver Uptake-Excretion Values from Patients with Liver Diseases and from Normal Individuals

Diagnosis	No. of patients	Uptake time ¹	Uptake slope ²	Maximum ³	Duration of max ¹	Excretion slope ⁴
Normal males	8	23.7 $\pm$ 5.1	12.00 $\pm$ 2.49	14.8 $\pm$ 2.0	17.0 $\pm$ 7.1	32.40 $\pm$ 8.40
Normal females	14	23.7 $\pm$ 4.5	11.01 $\pm$ 2.55	20.8 $\pm$ 2.6	15.7 $\pm$ 4.8	36.36 $\pm$ 7.26
Acute hepatitis	5	26.4 $\pm$ 6.9	8.87 $\pm$ 2.09	13.2 $\pm$ 4.0	24.2 $\pm$ 8.3	27.30 $\pm$ 14.76
Subsiding hepatitis	3	22.0 $\pm$ 5.0	9.56 $\pm$ 1.85	18.2 $\pm$ 2.8	23.6 $\pm$ 12.3	22.86 $\pm$ 12.60
Chronic recurring hepatitis	3	25.0 $\pm$ 2.2	8.11 $\pm$ 1.04	13.6 $\pm$ 1.2	18.2 $\pm$ 3.8	29.46 $\pm$ 7.02
Recovered hepatitis	6	19.7 $\pm$ 4.8	11.07 $\pm$ 1.62	20.0 $\pm$ 1.8	17.5 $\pm$ 5.2	32.16 $\pm$ 6.84
Laenec's ⁵ cirrhosis	4	37.2 $\pm$ 4.6	3.88 $\pm$ 0.40	7.4 $\pm$ 2.2	39 +	0

¹ Uptake time and duration of maximum in minutes.

² Uptake slope = % uptake/min of gamma-ray activity remaining in circulation.

³ Maximum = amplitude of gamma-ray activity as per cent of total injection dose.

⁴ Excretion slope = % excretion/hour of maximum liver gamma-ray activity.

⁵ Maximum maintained throughout 90 minute duration of test—no excretion observed.

viding important information regarding polygonal cell function. Similar analysis of the initial segment of the liver uptake curve (maximum height in first 3 minutes) has shown that those values provide an index of total blood capacity of the liver. Therefore, this short test may be developed into a useful clinical procedure for estimating liver blood capacity and hepatic cell function in individual patients or as a simple procedure adaptable to the liver survey of large groups of patients (e.g. as a pre- and post-employment test for workers exposed to liver toxins).

DISCUSSION

At its present state of development the Radioactive Rose Bengal Liver Function Test can provide qualitative data useful in the clinical evaluation of liver function. The initial segment of the uptake leg of the curve has been shown to be an index of total vascular capacity of the organ. The height of this segment is found to be greatly reduced in patients with cirrhosis and moderately depressed in acute forms of hepatitis. These findings agree with known changes in liver tissue vascularity in these diseases.

The rate of liver cell uptake of the dye appears to give a good estimate of polygonal cell function, since the slope of this curve is found to be reduced in proportion to the degree of liver damage from hepatitis and/or cirrhosis and in experimental liver injury from carbon tetrachloride poisoning in rabbits.¹

The maximum height of the uptake curve appears to have a relation to the number of functional hepatic cells, as judged from both laboratory studies in rabbits suffering from carbon tetrachloride poisoning and from clinical data from patients with various forms of liver disease. The interpretation of a prolonged plateau following the maximum uptake is

more complicated. A long plateau may indicate impaired excretory function of the cells, partial obstruction of the biliary passages, vascular congestion and edema of the liver parenchyma or combinations of all these factors. If the uptake slope and the maximum value are depressed, delayed excretion may be related wholly or partially to primary liver cell destruction or injury. However, if the uptake rate and maximum value are normal, retarded excretion is probably indicative of reduced patency of the biliary passages.

In the presence of acute complete extra-hepatic obstructive jaundice, this test should show a characteristic uptake-excretion curve with normal circulatory and uptake segments and a normal height

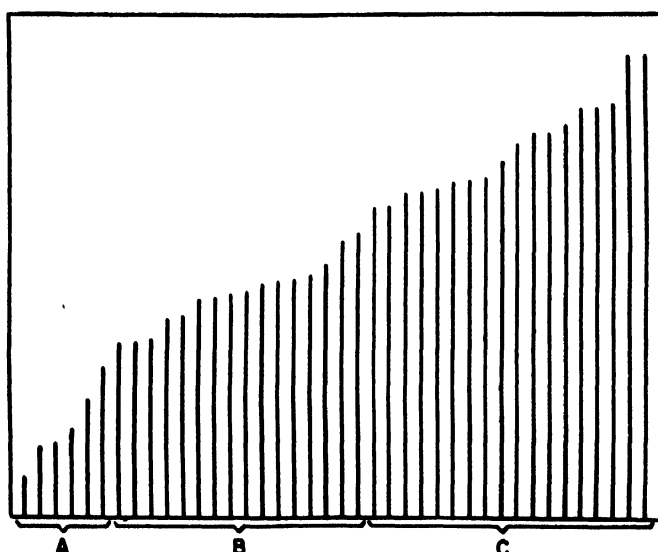


Figure 4. Relative values (arbitrary units) for liver uptake of 131 I-tagged Rose Bengal in normal subjects (group C) and patients with mild to moderately severe liver impairment (group B) and in patients with advanced cirrhosis (group A)

at the peak; however, the curve should then remain at a constant plateau for at least several hours. Furthermore, the level of dye in the blood should be barely detectable or zero. Persistence of the same level of activity over the liver for 24 hours after injection should be almost pathognomonic for acute common bile duct obstruction. A low vascular segment combined with slow uptake, a low maximum level and a greatly delayed excretion rate is characteristic of cirrhosis.

After evaluating the clinical results with both techniques employed to date, namely the 60–90 minute and 10–15 minute tests, it is apparent that further important information could be obtained by making similar measurements of radioactivity over the liver at extended time intervals. For example, from an analysis of the excretory segments of curves from normal patients, it is apparent that the liver should be entirely free of dye by four to six hours post-injection. By similar extrapolation from curves obtained from patients with acute and/or chronic hepatitis, the excretion rate is decreased, so that if measurements were made over the liver of such individuals around 6 hours following injection, definite levels of gamma-ray activity would indicate abnormal liver or biliary tract function. Moreover, if blood levels were found to be negligible and liver levels high, these findings would indicate acute obstructive disease, whereas, if both blood and liver levels were high, primary impairment of liver cell function would be indicated.

From extrapolation of similar curves, obtained from patients suffering from advanced cirrhosis, it is apparent that if measurements of blood and liver gamma-ray activity were repeated at about 6 and 24 hours, further valuable data could be obtained. For example, in patients with severely impaired liver function, one would expect to find high blood and elevated liver levels at 6 hours and persistence of the high liver values even out to 24 hours. Thus, it is evident that these additional measurements would be useful in evaluating impairment of liver function. Furthermore, the differentiation between acute intra- and extra-hepatic jaundice might be made with greater certainty by performing repeated measurements over the liver 24-hours and by also determining the rate of blood clearance simultaneously.

For others who wish to employ these blood clearance and liver uptake tests with Radioactive Rose Bengal, the authors offer the following general suggestions:

1. The tests should be performed first in a series of normal subjects to establish a set of control values, with the equipment being used.
2. Strict attention should be given to standardizing the test dose of radioactivity (about 5 μ c), whereas the dose of dye may be varied between 0.2 and 5.0 mg.
3. For radioactivity measurements over the liver area

(a) A wide angle counter is satisfactory for the short test (10–15 minutes).

(b) A well-collimated counter is necessary for the long test because after 15 minutes of liver uptake, radioactivity from dye entering the gallbladder and upper gastrointestinal tract may complicate the measurements.

4. For repeated measurements after increasing time intervals, meticulous attention must be given to factors of geometry in placement of the counter over the same area of liver.

Finally, it is emphasized that at this stage of development, the radioactivity values obtained are relative and have not been expressed quantitatively in respect to the dose of Radioactive Rose Bengal employed.

SUMMARY

The present status of the Radioactive Rose Bengal Test for liver function has been presented. The magnitude of the initial leg of the uptake curve of radioactivity over the liver has been shown to provide an index of total circulatory capacity of the organ. The rate of dye uptake measured over the liver between 3 and 10–15 minutes following intravenous injection of the dye gives valuable information concerning polygonal cell function. The slope of the curve following maximum liver uptake gives further information on excretory ability as well as patency of the biliary passages. In considering further modifications and additions to the test procedures, it is apparent that if measurements of radioactivity over the liver were repeated at about six and twenty-four hours post-injection, such data would be useful in making more accurate estimates of hepatic cell dysfunction. Finally, techniques are discussed by which Radioactive Rose Bengal Blood Clearance and Liver Uptake-Excretion Tests may be employed as a valuable aid in differentiating obstructive versus intrahepatic jaundice.

ACKNOWLEDGEMENTS

The authors are grateful for technical assistance given by Patricia Peel, Rhoda Devick, and Lenora Anderson. Likewise, we wish to express our appreciation to Dr. Franz Bauer, Chief of the Radiosotope Section, for his cooperation and for use of his equipment during the clinical studies; to Drs. Benedict Cassen and Leo Kaplan for repeated consultation and valuable advice; and to Dr. Donalee Tabern of Abbott Laboratories who provided the tagged Rose Bengal on an experimental basis from his Department of Radioactive Pharmaceuticals.

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Thyroid Radioiodine Uptake Calibration

By Marshall Brucer, James Eldridge and Jack Trombka,* USA

For a number of years it has been realized that radioiodine uptake measurements, as performed by many different techniques over the world, are highly variable. Interest in attempting to unify these methods has been widespread.¹ When *in vivo* studies are made there is a necessity for a host of unknown correction factors for absorption, scatter, body background, geometry, and instrument sensitivity. In order to calibrate the results of many laboratories, it is desirable to have a full-sized phantom closely duplicating the upper half of the human torso complete with appropriate radioactive organs. Such an expensive construction will not be warranted if the model is frequently refilled with a short-lived material such as iodine-131. Further, the calibration of repeated fillings will be open to question. This can be avoided if the gamma rays of iodine-131 can be imitated sufficiently closely by the gamma rays from a mixture of longer-lived radioactive isotopes. Permanent phantoms can then be constructed for regular calibrations of equipment and methods in any one institution and for the intercomparison of different experimental techniques throughout the world.

Such phantoms have been devised and tested. They are filled with a mixture of barium-133 (9.5-year half-life) and cesium-137 (33-year half-life) in suitable proportions so that their combined gamma-ray spectrum closely resembles that of iodine-131. The mixture is called mock-iodine.

When sources of iodine and mock-iodine are examined with a scintillation spectrometer the distribution of pulse heights can be plotted as a spectral curve. With good equipment the positions of the peaks on the curve show a linear correspondence with the energies of the gamma rays emitted by the source, but the heights of the peaks are affected by scattering and by partial absorption in the crystal detector. Also, Compton scattering may cause new peaks to appear in the spectrum. If, however, a constant geometrical arrangement is maintained, comparative spectra may be run with sources of different materials. Spectra of this type, obtained with a 20-channel scintillation spectrometer and a 1.5 inch diameter sodium iodide (TI) crystal, are shown for point sources of iodine-131, barium-133, and cesium-137, in Fig. 1.

GAMMA-RAY SPECTRA OF MOCK-IODINE MIXTURES

From theoretical considerations of the known energy spectrum of cesium-137 and the presumed energy spectrum of barium-133,² it appears that under conditions of broad beam geometry the iodine-131 spectrum can be closely matched. The unconverted 637 and 721 kev gamma rays of iodine-131 can be simulated by the unconverted 661 kev rays of cesium-137. The 284 and 364 kev iodine-131 gamma rays can be simulated by the 357 kev line known to be given off by barium-133. It is apparent from pulse height analyses that barium-133 also gives off a weaker line at about 280 kev.

In the middle energy ranges the weak 163 kev iodine-131 line and four presumed barium-133 lines will be masked in the Compton scatter peak of all three isotopes. The 80 kev line of iodine-131 is matched by the 85 kev line of barium-133; but barium-133 yields an 85 kev line in about 75 per cent of disintegrations while the iodine-131 line is present in only about two per cent of disintegrations.

In iodine-131 radiation, there is an X-ray from xenon following conversion that is mainly *K* radiation at 30 to 34 kev. The barium-133 isotope yields X-rays from cesium following *K*-electron capture and also converted gamma rays, their energies at 31 to 36 kev. The cesium-137 isotope through its barium daughter also yields X-rays following conversion that are mainly *K* radiation from 32 to 37 kev. The correct proportions of a mixture of barium-133 and cesium-137 should match the spectrum of iodine-131 except that there will be too much very low energy radiation. It appears from the decay schemes that the correct iodine-131 matching proportions should be at about eight parts of barium-133 to one part of cesium-137. The differential pulse height spectra of two extremes of mixture proportions is compared with iodine-131 in Fig. 2.

Since there is too much low energy radiation emerging from the barium-cesium mixture, a better approximation to an iodine-131 gamma-ray source is obtainable by using a suitable filter to reduce the intensities of the 85 kev gamma rays and of the X-rays derived from the higher energies. The filter will surround the barium-cesium mixture so that in practical use "broad beam" conditions of absorption will be approached. Furthermore, it is of advantage

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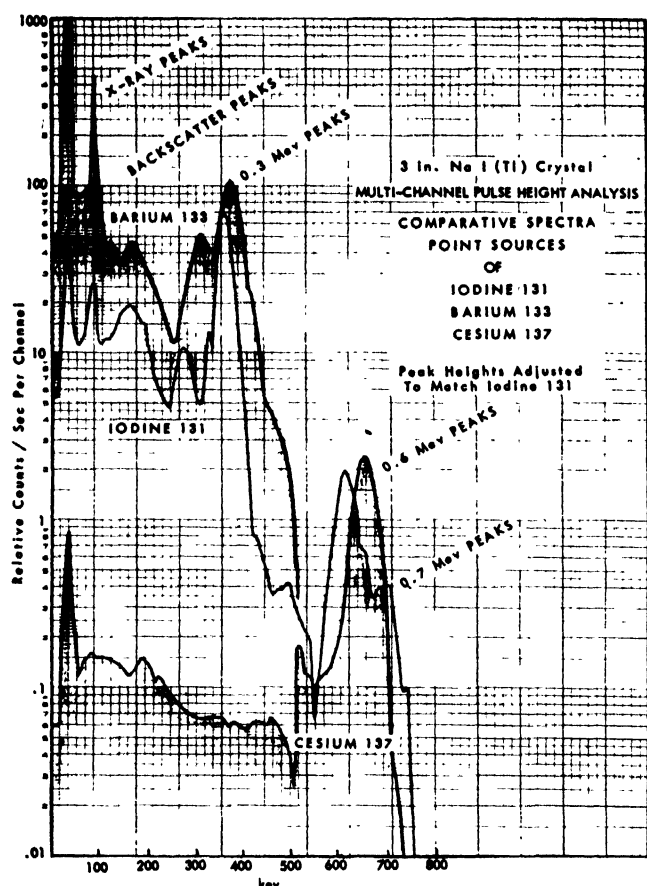


Figure 1. Differential pulse height spectra of Iodine-131, barium-133, and cesium-137

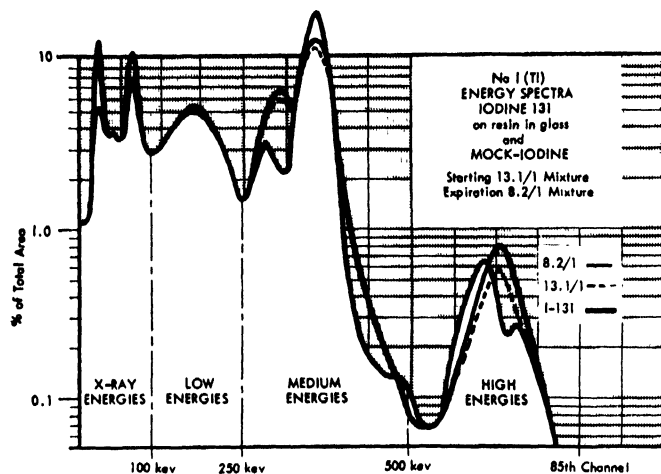


Figure 2. Differential pulse height spectrum of two extremes of barium-133-cesium-137 mixture proportions compared with Iodine-131

to choose a filter material having its K absorption edge just below 85 keV because the absorption coefficient is then relatively high for radiation of that energy. The effect on the differential spectrum of various thicknesses of tin is shown in Fig. 3. Of many elements meeting this requirement babbitt metal (a tin, antimony, copper alloy) has a proper absorption and is mechanically satisfactory.

The linear absorption coefficients in babbitt metal, excluding the attenuation caused by scattering, have been calculated for the several gamma-ray energies of interest from the data tabulated by White⁸. From these data it appears that about 1 mm of babbitt metal

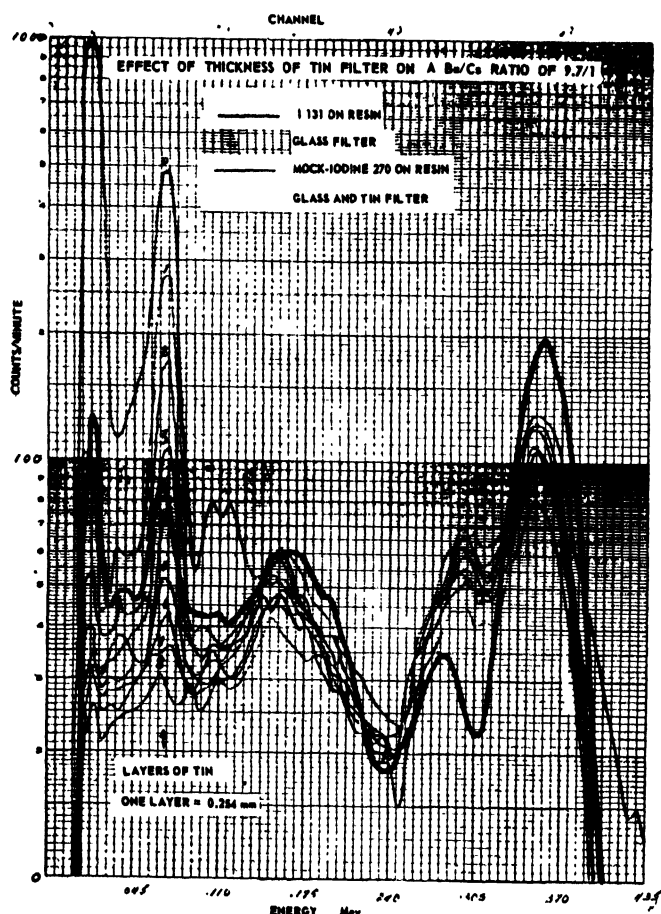


Figure 3. Differential spectrum of mock-Iodine shielded with various thicknesses of tin

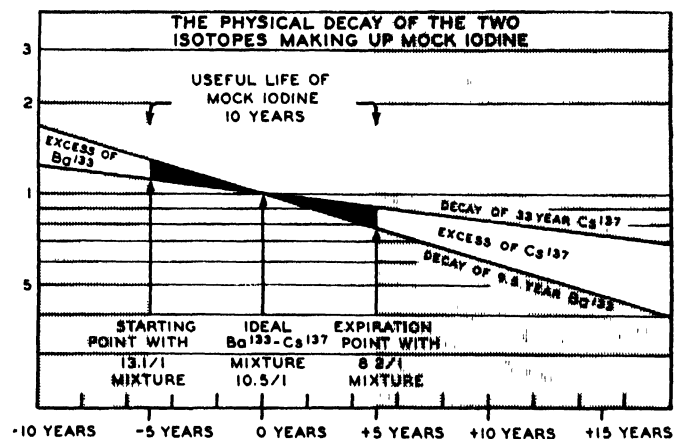


Figure 4. The decay of Ba^{133} and Cs^{137} normalized at an "ideal" mixture ratio of 10.5 to 1

is essential. The filter will, of course, remove substantially all of the characteristic cesium and barium X-rays produced in the mock-Iodine source; whereas very few xenon X-rays are attenuated by the glass screening of an Iodine-131 source. Although it is theoretically possible to select a better filter material, there are several reasons why not much can be done to equalize these lowest energy rays. First, the shape, size, and wall thickness of the Iodine-131 source markedly affects the amount of low energy radiation emerging from the container; second, to add X-rays of about the correct energy to the mock-Iodine source, one would need to find a suitable long-lived

isotope of atomic number about 55 that decays by *K* electron capture without the emission of higher energy gamma rays. Moreover, such a material would need to be placed outside of the babbitt filter surrounding the barium-cesium mixture; third, the better filter materials are mechanically and economically unsatisfactory.

COMPARATIVE TESTS ON IODINE-131 AND MOCK-IODINE SOURCES

For the preparation of trial source mixtures, barium-133 was obtained as barium nitrate and cesium-137 as cesium chloride, both with stable carrier material present. The 9.5-year barium-133 is the daughter of an isomer with a 39-hour half-life. It was proved by spectrometry that the shorter-lived isomer had decayed to a negligible proportion of the barium-133 activity. Some samples of cesium-137 have a contaminant of cesium-134, but this was shown by spectrometry to be in negligible proportions.

The three radioisotopes, barium-133, cesium-137, and iodine-131 were assayed in a high pressure ionization chamber to find their absolute strength in millicuries based on the assumed energy distributions. Since the decay scheme of barium-133 is unknown, a barium calibration kit has been prepared that defines the barium-133 millicurie based upon these assumptions. This arbitrarily defined millicurie has been used for all later sample preparations.

Mixtures of barium and cesium were then prepared and enclosed in a babbitt container for comparison with iodine-131. The walls of the cylindrical containers were made 0.82 mm thick, because the oblique path of some of the radiation leaving the source in the direction of the detector makes the effective thickness greater than the actual thickness. Moreover, there will be some self-absorption in the source material itself since the isotopes were uniformly distributed on an ion exchange resin in a 2 cm³ volume.

In order to compare the ratios of the various peaks of energy, a series of spectra were run on different mixtures under identical conditions. These spectra were normalized to the total area under the differential curve of an 85-channel spectrum from 12 kev to 760 kev. The increment of area of each channel was determined as its percentage of the total area under the entire spectral curve. Distribution of areas under this curve is shown in Table I for a point source of iodine-131 and is compared with iodine-131 on resin in a plastic and in a glass container.

In Table II the energy distribution of iodine-131 is compared with various barium-cesium mixtures. The match of the proportions of low energies, Compton scatter, and medium and high energies with iodine-131 is an excellent one when these broad areas of energy are considered. It is felt that this is the essential feature in the contemplated use of mock-iodine. On close inspection, however, with very

Table I. The Distribution of Energy in Iodine-131 Spectra*

	Low energy peaks (12 to 115 kev), %	Compton scatter peak (115 to 250 kev), %	Medium energy peaks (250 to 550 kev), %	High energy peak (550 to 760 kev), %
Iodine-131 (point source on Pliofilm)	41.2	18.6	38.8	1.4
Iodine-131 (on 2cm ³ resin in plastic container)	39.1	19.7	38.9	2.3
Iodine-131 (on 2cm ³ resin in glass container)	38.9	20.6	39.1	1.4

* The proportionate area under a differential pulse height analysis curve; 1½ inch NaI(Tl) scintillation crystal; normalized to 85th channel (760 kev) = 100%.

Table II. The Distribution of Energy, Iodine-131 Compared with Mock-Iodine Spectra*

Sources on 2 cm ³ resin in glass container	Low energy peaks (12 to 115 kev), %	Compton scatter peak (115 to 250 kev), %	Medium energy peaks (250 to 550 kev), %	High energy peak (550 to 760 kev), %
Iodine-131	38.9	20.6	39.1	1.4
15.3/1 mock-iodine mixture	37.0	19.0	42.9	1.1
12/1 mock-iodine mixture	36.3	19.3	43.0	1.4
10.5/1 mock-iodine mixture	38.8	19.0	40.6	1.6
9.3/1 mock-iodine mixture	37.1	19.4	41.7	1.8
8.2/1 mock-iodine mixture	40.2	18.1	39.8	1.9

* The proportionate area under a differential pulse height analysis curve; 1½ inch NaI(Tl) scintillation crystal; normalized to 85th channel (760 kev) = 100%.

Table III. Intensity Measurements of an Iodine-131 Source Compared with a Similar Mock-Iodine Source under Different Conditions of Measurement (Iodine-131 in 4 cm³ water in glass container; mock-iodine on 4 cm³ resin in tin container)

Source arrangement	Detector	Ratio I^{131}/mI
In air with about 4π geometry	Hi-pressure ionization chamber	1.378
In air 30 cm from crystal	Scintillation counter 1.5-inch diameter crystal	1.274
In air 22 cm from crystal	Scintillation counter 0.5-inch diameter crystal	1.291
In water in center of beaker 13 cm diameter		1.362
In water in 13 cm diameter beaker, 6 mm from side adjacent to detector		1.345
In air 28 cm from counter	Geiger counter, Victoreen type	1.452
In water in center of beaker 13 cm diameter		1.340
Mean value		$1.349 \pm 4.3\%$

Table IV. Intensity Measurements of an Iodine-131 Source Compared with a Similar Mock-Iodine Source under Different Conditions of Measurement

(Iodine-131 on 2 cm³ resin in glass container; mock-iodine on 2 cm³ resin in babbitt container)If mock-iodine were identical with iodine-131 then each column of figures would give the same readings $\pm$ about four per cent. Since a different iodine-131 standard was used for each barium-133-cesium-137 mixture there is no relationship between the columns.

Type of detector	Scatterma conditions	Source surrounded by	Ratio, mock-iodine/iodine-131, for Ba ¹³³ /Cs ¹³⁷ mixtures of:					
			27	28	29	30	31	32
1½ inch NaI(Tl) crystal. Poor collimation with lead	On concrete floor	Air	5.19	2.62	1.96	1.76	2.17	1.62
	against wall	Wax	5.30	2.48	1.80	1.64	2.00	1.53
	On wood	Air	5.00	2.54	1.77	1.73	2.13	1.69
	against wood	Wax	5.15	2.50	1.88	1.67	2.08	1.55
	On lead	Air	5.10	2.50	2.00	1.63	2.06	1.50
	against lead	Wax	5.16	2.65	1.80	1.72	2.17	1.63
Geiger-Muller glass wall tube. Good collimation with lead	Floor	Air	4.50	2.66	2.00	1.69	2.31	1.50
		Wax	6.00	2.56	1.80	1.54	2.06	1.48
	Wood	Air	4.06	2.40	1.81	1.54	2.10	1.36
		Wax	6.00	2.53	1.80	1.54	2.14	1.45
	Lead	Air	4.80	2.23	1.60	1.60	2.24	1.52
		Wax	6.00	2.58	1.74	1.66	2.28	1.53
½ inch CaWO ₄ crystal. Very good collimation with tungsten	In air at 15 cm	Air	5.60	2.60	2.00	1.60	2.12	1.48
		Wax	5.50	2.67	1.89	1.62	2.01	1.50
	In air at 30 cm	Air	5.30	2.65	1.90	1.64	2.21	1.52
		Wax	5.40	2.55	1.82	1.63	2.31	1.55
	In air at 60 cm	Air	5.66	2.50	2.00	1.65	2.01	1.60
		Wax	5.54	2.56	2.00	1.58	2.11	1.62
In well of hi-pressure ion chamber	In air at 120 cm	Air	5.23	2.58	1.78	1.65	2.11	1.58
		Wax	5.80	2.60	1.80	1.60	2.12	1.52
Mean $\pm$ coefficient of variation			5.30 $\pm$ 9.0%	2.54 $\pm$ 3.9%	1.80 $\pm$ 6.8%	1.63 $\pm$ 3.3%	2.14 $\pm$ 3.2%	1.54 $\pm$ 4.8%

narrow areas of energy, the filtered mock-iodine is seen to have too little intensity under the 32 kev peak but a balancing excess under the 85 kev peak. Also, in the middle energy range there is too great a 280 kev intensity but a balancing deficiency at 360 kev in mock-iodine. The Compton scatter peaks match almost exactly. The high energy peak matches in intensity but it is shifted upward from 640 to 660 kev. From the normalized spectra of many mixtures, a barium-133 to cesium-137 ratio of 10.5 to 1 appears to approach most closely an "ideal" mixture.

TIME RELATIONSHIPS AND DECAY OF MOCK-IODINE

There is an effective distortion of the "ideal" matching mixture with the decay of the two isotopes since these decay at different rates. Figure 4 shows the mixture's two decay rates normalized at an "ideal" mixture ratio of 10.5 to 1. If the isotopes were mixed in a barium-cesium ratio at 15.3 to 1, then it would have an excess of barium-133, but in five years would decay to the ideal mixture. Five years later it would decay to a ratio of 8.3 to 1 and thereafter there would be an ever increasing excess of cesium-137. The time scale and related proportions are shown in the figure with an indication of the useful life of mock-iodine.

EXTERNAL COUNTING COMPARISONS OF IODINE AND MOCK-IODINE

As a more practical test of the agreement of mock-iodine with iodine under different gamma-ray count-

ing conditions, various matching sources were prepared with the same over-all dimensions. These sources have been measured in several ways as shown in Table III. In the tests illustrated in Table III the barium-cesium ratio was 10.5 to 1. In other tests shown in Table IV, with barium-cesium ratios from 15.7/1 to 6.2/1, similar results were obtained. Thus the proportion of cesium-137 can vary rather widely without causing much change in the observed ratio of strengths when the source is measured in air and in a phantom at various distances.

The differences that do appear in the tables should be expected because the mock-iodine spectrum does not duplicate exactly the iodine spectrum in the very low energy region. Also, Geiger and scintillation counters do not have the same efficiency of detection at different energies. It is felt that some, but not all, of the differences can be overcome by more careful matching and control of the filter material. Such experiments are now under way.

CONSTRUCTION OF EXTENDED SOURCES WITH ION EXCHANGE RESIN

Since the primary reason for developing a long-lived iodine-131 substitute is to construct phantoms that simulate the geometry of clinical situations, it was necessary to find a way of dispersing the mock-iodine in a uniform manner in various strengths through large and small sources of variable shapes. The dispersion medium must have a soft-tissue-like effective atomic number; its density should be close

to unity; for practical reasons it must be noncorrosive, and for safety reasons it must bind the long-lived isotopes tightly. Many materials were discarded that did not meet one or another of the necessary criteria. An ion exchange resin "Dowex-50"⁴ was found to be an ideal dispersion medium.

"Dowex-50" is a cation exchange resin consisting of a polymerized hydrocarbon containing substituent sulfonic acid groups ($-\text{SO}_3\text{H}$). The hydrocarbon chain is cross linked to form a 3-dimensional network. The large hydrocarbon molecules are combined and the final product is prepared in the form of small spheres that can be separated into size groups by sieving. Resin particles in the size group 50 to 100 mesh were found to be suitable for mock-iodine sources.

The sulfonic acid group is easily dissociated so that hydrogen ions are available for exchange with any other cation in contact with the resin. The exchange capacity (the quantity of exchangeable hydrogen ions) of "Dowex-50" is about 5 milliequivalents per gram of dry resin. Because of this exchange capacity, there is a limit to the amount of radioactivity that can be placed on the resin since the barium-133 is not supplied as a carrier-free isotope. The particular samples of barium used so far average about 42 milligrams of barium per milliliter of solution with an activity of about 10 microcuries per milliliter. Thus, there is an upper activity limit of about 85 microcuries per gram of dry resin with the materials now available.

Extended sources of active materials are made by passing the isotope in solution through the resin in a column at a flow of about 5 to 10 milliliters per minute. The column is of sufficient length so that there is considerable excess resin. The cations in the radioactive solution quantitatively replace the ionized hydrogen in the top of the column. As long as the exchange capacity is not exceeded and the flow rate is sufficiently slow, all of the cation will be retained in the resin column.

Mixed activity resin can be obtained by mechanically mixing the proper quantities of resin that have been labeled with the respective isotopes; i.e., the barium-cesium mixed resin is made by combining and mixing a barium-133 labeled resin with a cesium-137 labeled resin in the proper proportions. Another way of obtaining the mixed resin is to mix the barium-133 and cesium-137 in solution in the proper proportion and then to pass the mixed solution through a column of resin. After drying, the resin is mixed to give a uniform dispersion of the two isotopes.

Under intense radiation the resins will tend to decompose. This is a problem that is encountered in the separation of intensely radioactive fission products by means of the ion exchange process. In order to test this phenomenon, a small amount of "Dowex-50" was exposed to one million roentgens of gamma radiation from a cobalt-60 source. Immediately fol-



Figure 5. Mannequin and "total dose (100 per cent) standards"

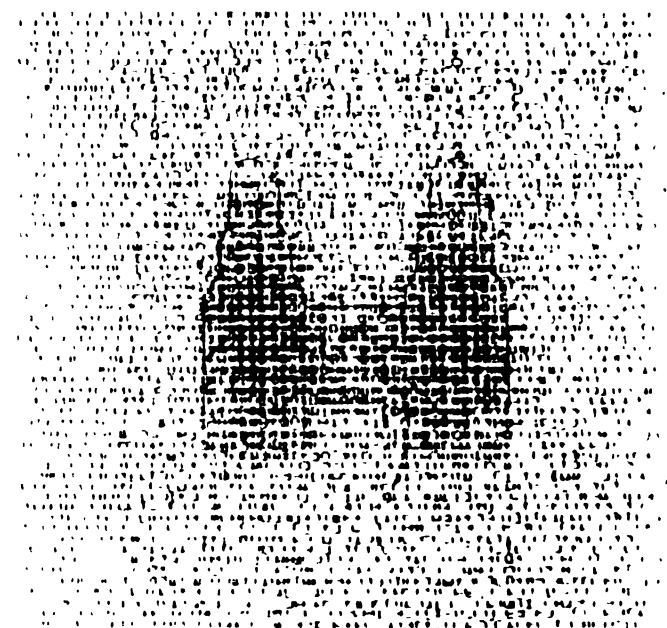


Figure 6. Scintiscan record of the thyroid phantom filled with mock-iodine

lowing the exposure there was no qualitative difference in the exchange capacity. The resin used in this study is sufficiently wet that the radioactive isotope will eventually find its way from a decomposed to an active center in some other area unless there is complete destruction of the resin. Complete destruction is not probable with the microcurie levels used in mock-iodine.

"Dowex-50" is practically insoluble and is not decomposed by ordinary solvents. Water will not remove the isotope from the resin unless the active resin remains in contact with the water for an extended period of time. The mixtures thus far prepared have been rinsed several times with water and no detectable activity has been found in the rinse water. It is necessary to have a concentrated solution of a cation in contact with the resin in order to remove the isotopes. Thus the labeled resin appears to be a safe and convenient way of handling long-lived barium and cesium mixtures.

PLANS FOR THE USE OF MOCK-IODINE IN AN UPTAKE CALIBRATION SURVEY

After the long-lived substitute was found to represent iodine-131 reasonably well, a set of phantoms was constructed incorporating discrete sources to represent the thyroid gland or other iodine-131 containing metastatic lesions, together with a weaker material to represent the body background and other organs.

The phantom is the upper torso of a plastic store-mannequin complete with head and detachable upper halves of each arm (shown in Fig. 5). The plastic shell, which is 3 to 4 mm thick, and its contained resin are a sufficient filter for the excess of low energy radiation so that the containers for the thyroid and other internal sources can be made of aluminum or plastic. Figure 6 shows a scintiscan record of the thyroid phantom filled with mock-iodine.

The mock thyroid gland is filled with a sufficient number of millicuries of mock-iodine to simulate the conditions found in either the hypothyroid, hyperthyroid, or euthyroid patient 24 hours after the patient has been given a "100 per cent" dose of iodine. A body background is accomplished by filling the phantom with labeled resin at an activity similar to that of the iodine-131 circulating in the human body 24 hours after the administration of the dose.

Because many investigators use the thigh for an estimate of body background, a thigh section is included with the mannequin. Another thickness of the body is available in the detachable arms.

Most investigators measuring thyroid uptake use a measurement of the total dose as a "100 per cent standard" against which their other measurements are calibrated. Glass beakers, paper cups, and test tubes are most commonly used as containers for this standard. It is impractical to match all of the geometries of all of the "standards" that are used in thyroid uptake measurements, but a compromise se-

lection has been made in a series of eight "total dose (100 per cent) standards" shown in Fig. 5. The containers for these total dose standards are made of 0.82 mm thickness babbitt metal. A calibration kit that will allow for the uniform calibration of the many methods of measuring thyroid uptake has been designed and will be distributed with the mannequin kits. A "primary standard" of mock-iodine measured as iodine has been calibrated by the National Bureau of Standards.

A survey and calibration of the methods of measuring radioiodine thyroid uptake methods are now in process in the United States. Six sets of mannequins, representing two unknown uptakes in mock patients with hypothyroidism, hyperthyroidism, and euthyroidism, are circulating through the country. Physicians will be invited to measure the uptake in these mock patients. They will be asked to use their own methods of clinically measuring thyroid uptake. A different set of phantoms has been devised for physicists. The results of this survey will be one of the subjects for the third advanced course in the medical use of radioisotopes to be held in Oak Ridge early in 1956.

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Recent Advances in Methods for the Study of Red Cell Mass and Red Cell Production and Destruction

By N. I. Berlin and John H. Lawrence,* USA

With the development of the cyclotron and nuclear reactors, it has become possible to produce radioactive isotopes of elements associated with normal metabolic processes. As a result it has become possible to follow the metabolism of a given element or substance by the introduction of a labeled isotope into the body. Recent great improvements in the methods for measurement of radioactive isotopes, particularly the developments in scintillation counters, have made it possible to carry out tracer studies in laboratories or hospitals with relative ease. For most of these studies it is not necessary to have expensive or complex equipment.

In the evaluation of erythropoiesis, one must know the total volume of circulating red cells, the rate of production of red cells, and the life span of red cells. Knowing these three values, one can then determine whether an individual is normal, polycythemic, or anemic, and also whether an increase or decrease in the total circulating red-cell volume is due to a change in the life span of red cells or in the rate of production of red cells, or to both.

METHODS

Blood Volume

The availability of radioactive isotopes of iron, phosphorus, chromium, potassium, and thorium makes it possible to measure the total circulating red-cell volume with accuracy and ease. The isotopes phosphorus-32,¹ chromium-51,² potassium-42,³ and thorium-C⁴ may be incorporated into the red cell *in vitro*. This permits the labeling of an individual's own red cells. Iron, on the other hand, must first be administered to a donor, whose red cells are subsequently injected into the individual whose blood volume is to be determined. Although the radioiron method would be the ideal method for determining blood volume, it has not found wide usefulness in clinical work because of the necessity for maintaining a pool of donors of various blood groups and the need for cross-matching, blood typing, and the various serological studies that are required before transfusion. Furthermore, the dose of radioiron to be administered to the donor is so large that the radiation to the bone marrow approaches tolerance

values. In experimental animals, however—particularly in the rat, where inbreeding results in a uniform strain of animals in which cross-matching is no problem—the iron method has been of great value in the determination of blood volume.

For the P³², Cr⁵¹, and K⁴² methods in which red cells are labeled *in vitro*, it is necessary to remove only a small quantity of blood (10 to 15 cm³) from the individual and to “incubate” these cells with the isotope at 37°C with constant mixing. In general, more than 50% of the isotope will have been incorporated into the red cells at the end of one hour. The red cells are then washed to remove the excess, unincorporated isotope and are re-suspended in either saline or plasma. The reconstituted whole blood containing the labeled red cells is then administered to the patient, and after adequate time for mixing, a sample is withdrawn. An aliquot of the blood injected into the patient is kept and the total radioactivity administered is determined from it. Thus, knowing the total radioactivity administered and the radioactivity per milliliter of whole blood withdrawn from the patient, one may calculate the blood volume. The total red-cell volume is determined from the blood volumes and the peripheral hematocrit corrected for trapped plasma. Actually, this method is precise. The plasma volume can be calculated from the total red-cell volume and the hematocrit. Although there has been some objection to this method of calculation of the plasma volume (because several studies indicate that the hematocrit of the large-vessel blood may not be the same as that in the smaller vessels), we do not consider this to be a significant factor and believe that the plasma volume can be calculated with a high degree of precision from total red-cell volume and venous hematocrit.⁵

Of P³², Cr⁵¹, and K⁴², the isotope of choice for the determination of blood volume is Cr⁵¹. It is easily measured in a well-type scintillation counter; the rate of elution of Cr⁵¹ from the red cell is relatively slow compared with that of P³², and the physical half-life is long compared with K⁴². While many valuable studies have been made using P³², most workers have now adopted the Cr⁵¹ method. The usefulness of K⁴² is limited by its short half-life (12.4 hours), which means that the worker must have access to a facility for its production. The ThC

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method has been explored by Hevesy and Nylin, and should prove valuable. It has not been used extensively in other laboratories although it does offer some promise for greater ease of labeling red cells than K^{42} , Cr^{51} , and P^{32} . The radiation dose involved in the use of ThC remains to be established. The rapid physical decay of the other isotopes presents no radiation hazard to the individual receiving the isotope.

The results obtained with these methods show that for normal males (71 subjects): average total blood volume, 69.0 cm³/kg of body weight; average plasma volume, 38.7 cm³/kg; average total red-cell volume, 29.9 cm³/kg. For sixteen normal females studied in this laboratory: average total blood volume, 64.4 cm³/kg of body weight; average plasma volume, 37.0 cm³/kg; average total red-cell volume, 27.0 cm³/kg. (This was the first study of the blood volume of the normal female by isotopic methods.)

Subsequently, Wadsworth reported, for a series of eight normal women: average total blood volume, 66.5 cm³/kg; average plasma volume, 43.1 cm³/kg (by the Evans-Blue method); average total red-cell volume, 23.4 cm³/kg (by the P^{32} method). The value for the total red-cell volume in the males, 30 cm³/kg, appears to be well confirmed.

Red-Cell Production

At the present there is only one method for the direct determination of the rate of red-cell production, and that is the measurement of the rate of disappearance of radioactive iron from the plasma and subsequent uptake of radioiron in the red cells.⁹ An analysis of the rate of disappearance of radioiron from the plasma indicates that approximately 80% to 90% of the iron is removed by a first-order process. That is, for each given time period a constant fraction of the radioiron remaining in the plasma is cleared, largely by the bone marrow and to some extent by the liver, spleen, and other tissues. From the rate of disappearance of radioiron from the plasma, the turnover of iron in the plasma can be calculated as follows:

$$\frac{(0.693) \times (\mu\text{g Fe/ml in plasma})}{\times (\text{Plasma volume in ml}) \times (24 \text{ hours})} = \text{Plasma radioiron half-time in hours}$$

The plasma iron concentration is determined by the method of Kitzes *et al.*¹⁰ The plasma volume is determined from the blood volume by use of labeled cells, as described above. After the determination of the rate of removal of radioactive iron from the plasma, a study is made of the uptake of iron in the red cells. At approximately 7 to 10 days, most of the iron that is going to appear in the red cells has been cleared from the plasma to the bone marrow and has been incorporated into hemoglobin, and the mature red cells containing the radioiron are in the peripheral blood. Thus, the percentage of iron that passes through the plasma, ultimately to reach the peripheral blood in red cells, may be determined at

approximately 7 to 10 days following the original injection of the isotope.

This now completes the data required for determination of the rate of production of red cells. In general it is found that approximately 0.26 milligram of iron is utilized for red-cell production per kilogram per day in a normal male. Much higher values are found in polycythemia. In leukemia these values are usually normal or increased to two to three times normal, whereas in certain anemias the values may be normal, increased, or (in case of failure to produce red cells) as low as one-fifth to one-third normal.⁹

The use of scintillation counters placed over the spleen, liver, and sacrum—the latter representing bone marrow—makes it possible to determine the time course of the distribution of radioiron in these tissues.¹¹ (Fig. 1). This shows that there is a rapid clearance of iron by the bone marrow and a later release of the iron from the marrow to the peripheral red cells. Some iron reaches the liver and the spleen and thereafter is slowly removed from these tissues. It is this slow release from the spleen and liver that accounts for the slight rise of radioiron in the peripheral blood after 7 to 10 days.

Red-Cell Life Span

Prior to the introduction of isotopes into the study of red-cell physiology, it was possible to measure the life span of the red cell by the Ashby Differential Agglutination technique.¹² To obtain valuable data by use of the Ashby technique, one must administer a large volume of blood (approximately 500 cm³ from a suitable donor. This method has usually been applied by the transfusion of normal red cells to an individual with a disease state, and yields information regarding only the fate of normal cells in an abnormal environment. Some studies have been carried out by transfusing cells from a donor having sickle-cell anemia to a normal individual, and in this experiment the survival of sickle cells in a normal environment could be observed.

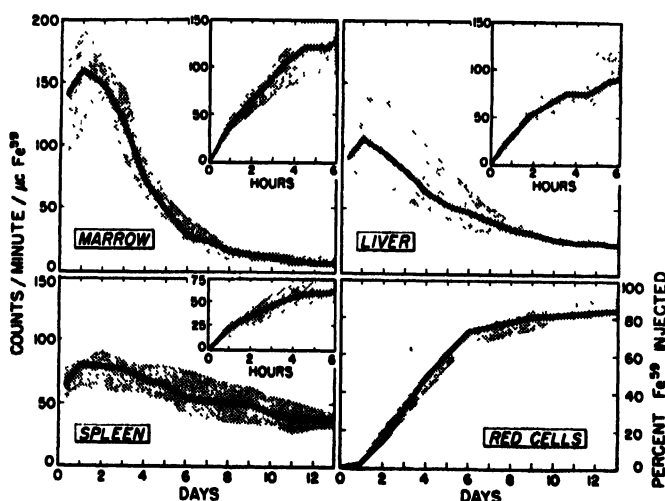


Figure 1. Uptake of Fe^{59} by red cells, bone marrow, liver, and spleen plotted against time in normals. Shaded area indicates range

The Ashby method, however, does not permit the determination of the life span of the red cells in its own environment.

With the demonstration by Shemin and Rittenberg¹³ that the nitrogen atom of glycine is specifically used for the four nitrogen atoms of hemin and the preparation of N¹⁵-labeled glycine, it became possible to study the life span of the red cell in its own environment. This study demonstrated that the normal life span of the red cell was approximately 120 days, and valuable information on the red-cell life in sickle-cell anemia and in pernicious anemia was also obtained. In our laboratory C¹⁴-labeled glycine was introduced for such studies¹⁴ because it has been shown that the alpha-carbon of glycine atom is the source of 8 of the 34 carbon atoms of glycine,¹⁵ and N¹⁵ is much more difficult to measure.

Some indication of red-cell life span may also be obtained by following the activity of radioiron in the peripheral blood. This method is less satisfactory than the C¹⁴ method, however, since 70% to 90% of red-cell iron liberated at the end of the life span of a red cell is reutilized for the formation of new red cells. As the red cells reach the ends of their life spans, the radioiron is released and reutilized, and there is no sharp drop in the curve of Fe⁵⁹ hemoglobin activity. This disadvantage can be overcome in experimental animals by loading the animal with iron after the red cells are labeled.¹⁶ Thus, when the red-cell iron is released, it is released into a large pool of iron, and the specific activity becomes considerably lower, so that when it is reutilized in red-cell formation it is of a much lower specific activity than that originally present in the first labeled cells. A sharp drop in the curve for the Fe⁵⁹ hemoglobin specific activity is noted, and the red-cell life can be determined.

Recently, Cr⁵¹ has been introduced as the labeling agent for study of the life span of the red blood cells. With the demonstration that the rate of elution of Cr⁵¹ from the labeled red cells *in vivo* is very slow, it has now become possible to obtain a measure of the red-cell life span by following the *in vivo* rate of disappearance of Cr⁵¹ following the labeling of the cells; these studies do not give an absolute value for the red-cell life span, but they may be used to compare a red-cell life span in a given state with that of a normal.¹⁷⁻¹⁹ It should be emphasized that this is a valuable method for clinical determination of alterations in the life span of the red blood cell, but that the values obtained—even with the present methods of analysis—are not comparable with the actual value of red-cell life span, and must be compared with the normal as determined with this method. Various methods of analysis have been proposed for interpretation of the curves of the graphs obtained. At the present time, however, we feel that these have not been completely substantiated.

In comparing the various methods for study of red-cell life, the Ashby method might be criticized

because normal cells are placed in a pathologic environment or pathologic red cells in a normal environment, and red cells are never studied in their normal environment. In most normal individuals, the Ashby C¹⁴, and Fe⁵⁹ methods give comparable results. We have seen one instance, however, in which the values by the Ashby technique indicated a longer red-cell life than those techniques using the C¹⁴ and Fe⁵⁹ methods. There was another case in which the Ashby technique showed a normal red-cell life, but the C¹⁴ and Fe⁵⁹ results showed a shortened red-cell life. The conclusion might be drawn from these findings that the individual's own cells were qualitatively poor, while the destructive mechanisms were normal, and therefore the normal donor cells survived their normal life span.

DISCUSSION

With the development of methods for determining total red-cell volume the rate of production of red cells, and the life span of the red cell, it has become possible to assay the erythropoietic state. Anemia or polycythemia can only arise from some change in the rate of production or in the life span of the red cell. This may even apply to bleeding states. Although the rate of production of red cells could conceivably be unaffected, the life span of the cells would be shortened in the bleeding state in the sense that cells did not survive their normal life span. The resulting anemia would then be due to a decrease in the red-cell life span.

The methods described have been applied simultaneously in the study of various hematopoietic disorders, such as polycythemia, leukemia, aplastic anemia, hemolytic anemia, and hypersplenism.

Polycythemia

With P³²-labeled red cells it can be shown that there is an elevation of the blood volume in polycythemia (Fig. 2).²⁷ This figure clearly indicates that the hematocrit cannot be used to predict the total red-cell volume, although in general, as the hematocrit increases the total red-cell volume increases. In 32 patients with a hematocrit above 55, the total red-cell volume was elevated in all but two, ranging from 38.8 to 93.9 cm³/kg of body weight. The plasma volume was low in 22 patients, in the lower range of normal in 8, and high normal in 2. Thus, in polycythemia vera in relapse with an elevated hematocrit, there is usually an increased total red-cell volume and a low plasma volume, the latter being below normal range in approximately two thirds of the cases in this series. In patients with hematocrits less than 55, the total red-cell volume may be elevated and the plasma volume tends to be higher than in the group with hematocrits above 55. In general then, in polycythemia vera the total red-cell volume is elevated, and the plasma volume is low.

In polycythemia vera it has been clearly demonstrated that there is a considerable increase—in some

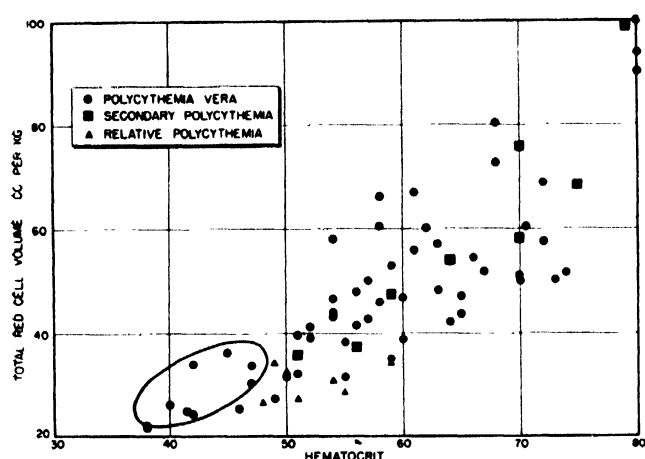


Figure 2. The blood volume in polycythemia. The ellipse represents area for normal values

patients as much as 10 times normal—in the quantity of iron incorporated into hemoglobin per day. The *in vivo* iron studies show that this increase is due to hyperactivity of the bone marrow. In most patients with polycythemia vera the liver and spleen are not significant sites of red-cell formation, although occasional instances of splenic erythropoiesis are observed. Studies with N^{15} of the life span of the red cell indicated a normal life span.²⁰ Data from the C^{14} studies,²¹ however, can best be explained by postulating the presence of two populations of red cells, one having a normal life span and the other a shortened red-cell life.

This hypothesis fits well with the iron data, which show that there must be a considerable increase in the amount of iron released from red cells per day, and this can be explained only by supposing that either all the cells have a shortened life span or that some cells have a short cell life.

The blood volume changes in polycythemia secondary to congenital heart disease, acquired pulmonary disease, or residence at high altitude are the same as those in polycythemia vera, and for this reason primary and secondary polycythemia cannot be distinguished on the basis of such studies.

In individuals with polycythemia secondary to either congenital heart disease, acquired pulmonary disease, or residence at high altitude, it is found that there is an increase in the rate of utilization of radioactive iron for the production of red cells.²² However, the increase is directly proportional to the increase in total red-cell volume. Studies of individuals having a polycythemia secondary to residence at high altitude show a normal red-cell life span.²³ When sea-level dwellers are taken to high altitude there is an immediate and rapid increase in the rate of utilization of iron for red-cell formation.²² However, their red cells have been found to have a normal life span during the period of acclimatization.²⁴ Therefore, the mechanism for the development of polycythemia at high altitude is entirely confined to an increase in the rate of production of red cells and does not involve an alteration of the life span of

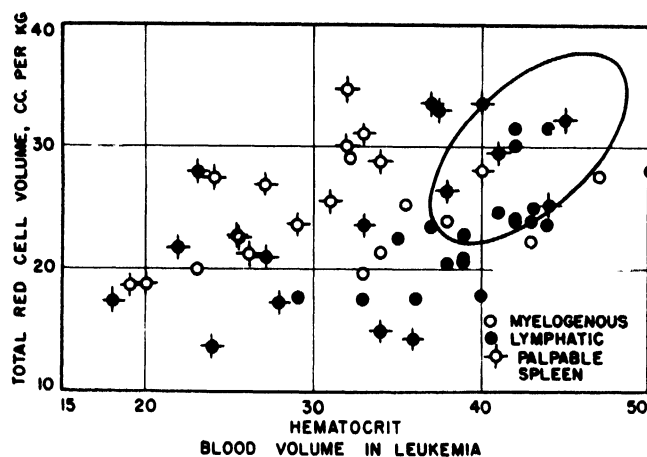


Figure 3. The blood volume in chronic leukemia. The ellipse represents area for normal values

the red cell. When individuals with polycythemia secondary to residence at high altitude are taken to sea level, there is a remarkable decrease in the rate of production of red cells.²² The increased urobilinogen excretion shortly after arriving at sea level might be evidence that there is premature destruction of red cells under these conditions. Studies of the life span of the red cell by the C^{14} method are now in progress to determine whether there is actually a shortening of cell life following descent from high altitude.

Leukemia

In leukemia, studies with P^{32} labeled red cells show again that the hematocrit is of little value for the prediction of total red-cell volumes (Fig. 3). This is particularly true of patients with chronic myelogenous leukemia with splenomegaly. Most of the patients with chronic myelogenous leukemia studied had total red-cell volumes within the normal range, but their hematocrits were less than normal owing to large plasma volumes. In chronic lymphatic leukemia there appeared to be less relation between splenomegaly and large plasma volumes, and in addition there was a greater incidence of true anemia as shown by the low total red-cell volumes.⁶

Until recently the anemia associated with leukemia has been attributed (Fig. 3) to a crowding out of the erythropoietic elements of the bone marrow by leukemia tissue, and it has been assumed that the anemia resulted from a failure to produce red cells. This has not been borne out by studies of chronic myelogenous and lymphatic leukemia with radioiron and C^{14} -labeled glycine. Patients with chronic leukemia have been shown to produce a normal, and in many instances a greater than normal, number of red cells a day.

In all patients with chronic myelogenous leukemia studied by *in vivo* iron methods, there has been evidence of production of red cells in the spleen and possibly some destruction in the spleen.²⁵

This does not occur in most patients with chronic lymphatic leukemia.

C^{14} red-cell life span studies in myelogenous leukemia show that the red-cell life span is shortened but is finite (as compared to the pattern of random destruction as seen in a hemolytic process).²⁶ This was true of all patients studied. In chronic lymphatic leukemia, however, the life span is either normal or shortened, but if it is shortened, the pattern is one of random destruction of red cells. In myelogenous leukemia there is a relatively uniform red-cell life span of about 75 to 90 days. In lymphatic leukemia, when shortening of red-cell life was observed, the average life span of the cells was approximately 20 to 30 days.²⁸

Aplastic Anemia

Studies of one patient with aplastic anemia (F.W.) showed that the rate of production of red cells as indicated by the rate of disappearance of radioiron from the plasma was considerably decreased. The *in vivo* iron studies showed a normal pattern (Fig. 4). The red-cell life span was found to be 100 days, which is a low normal, or at most a slightly shortened life span (Fig. 5). Thus, in this individual we were able to demonstrate that the anemia, or decreased total red-cell volume, could be attributed to a failure of the bone marrow to produce an adequate number of red cells. The red cells that were produced were apparently almost normal qualitatively.

Hemolytic Anemia

The following case history illustrates one diagnostic problem studied by these methods.

A 23-year-old male (Patient C.O.) was referred for diagnostic studies in August 1953. He had experienced several episodes of jaundice, and three years previously a diagnosis of hemolytic anemia of unknown etiology had been made. His initial red-cell count was 2.55 million, with 9.1 grams of hemoglobin. The reticulocyte count was 5.4%. A bone-marrow biopsy showed hyperplasia of the hematopoietic tissue, with replacement of the adipose tissue. There appeared to be a considerable increase in the

number of cells of the erythroid series. The Coombs test was negative. Serum bilirubin was elevated, and the icteric index was 26 units. Total red-cell volume was 20.9 cm³/kg of body weight; plasma volume, 48.9 cm³/kg.

Radioiron studies on this patient, who was anemic by red-cell volume standards, showed a rapid turnover of radioiron and an increase in the rate of production of red cells (Fig. 6). *In vivo* iron studies showed a rapid rise in the counting rate over the spleen, which was interpreted as indicating an early destruction of red cells. Also, it should be noted that only 30% of the iron could be detected in the red cells at any one time (as compared with 70% to 90% of the injected activity in normals). This would occur if there were a fast cycling of the iron through red cells and back into the bone marrow and spleen.

A splenectomy was performed, and a normal *in vivo* iron pattern was observed (Fig. 7), with 80% to 90% of the injected activity noted in red cells at 7 to 10 days. The repeat studies now showed the plasma iron turnover to be decreased to approximately half its former rate, and the rate of incorporation of iron into red cells was also decreased, although it remained faster than normal. *In vivo* iron studies showed no secondary spleen curve as before, and the marrow curve was of more normal pattern. Thus, in this individual we have been able to demonstrate a remarkable change in the turnover of radioiron following a splenectomy.

Hypersplenism

Recently we have had an opportunity to study a number of individuals with a syndrome that we call the short-red-cell-life syndrome. All these patients had splenomegaly, and their bone marrows were histologically abnormal, generally showing extensive areas of myelofibrosis with little if any hematopoietic tissue remaining. These patients show a rapid disappearance of iron from the plasma, and all had adequate or greater than adequate iron turnover for red-cell formation. The *in vivo* iron turnover patterns showed an early, marked rise in the counting rate over the spleen, with maintenance of this counting rate over the spleen, indicating early de-

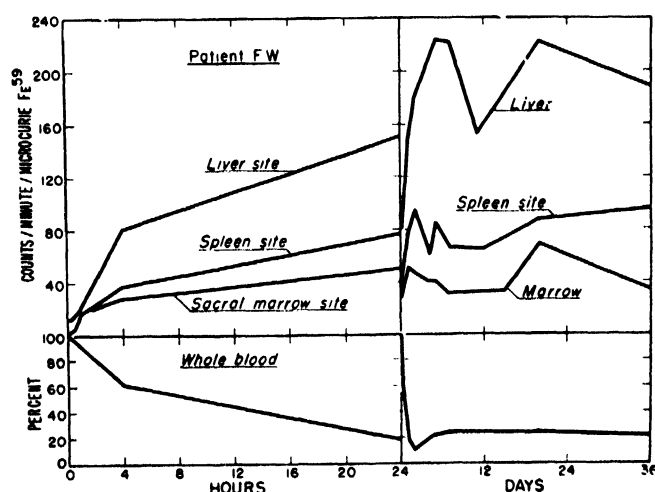


Figure 4. Uptake of Fe^{59} in red cells and by bone marrow, liver, and spleen with time in Patient F. W.

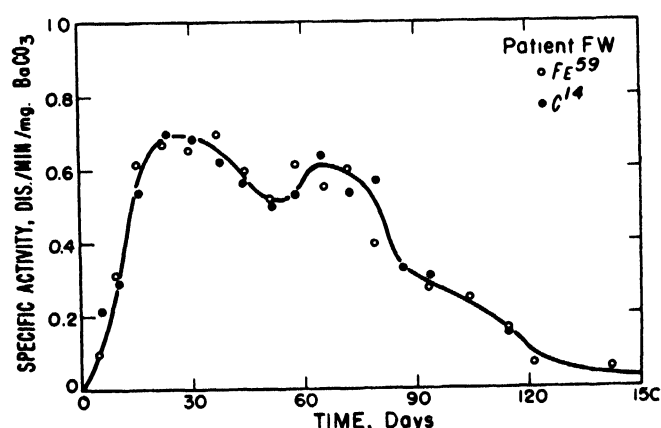


Figure 5. The life span of the red cell in Patient F. W., as measured with Fe^{59} and C^{14}

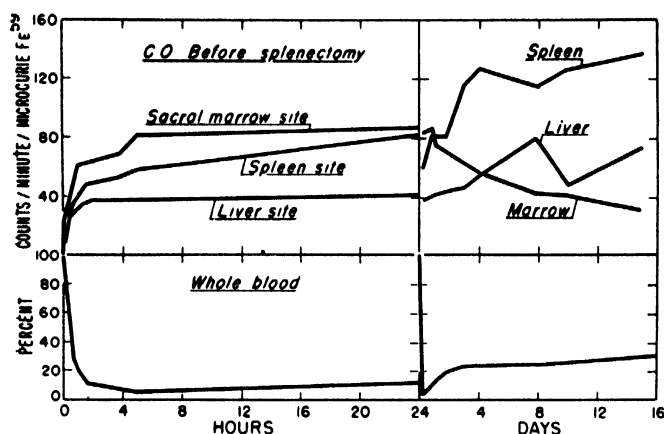


Figure 6. Uptake of Fe^{59} in red cells and by bone marrow, liver, and spleen in Patient C. O. before splenectomy

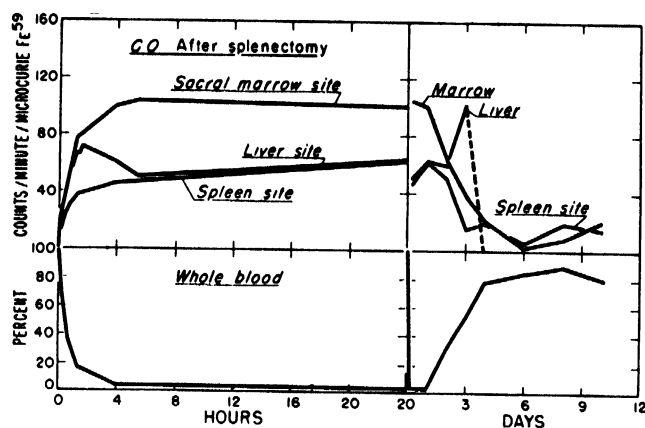


Figure 7. Uptake of Fe^{59} in red cells and by bone marrow, liver, and spleen in Patient C. O. after splenectomy

struction of red cells. The C^{14} red-cell life span studies showed random destruction of the red cells, with a red-cell life of 20 to 30 days. In one patient (M.B.) a normal red-cell life span was observed following administration of adrenal cortical hormones (Fig. 8). In another patient (A.F., Figs. 9-11), the red-cell life span returned to normal following a splenectomy.

THE ERYTHROPOIETIC STIMULUS

The fundamental stimuli to red-cell production is discussed in a comprehensive review by Grant and Root.²⁸ They consider that there are only three ways in which the total red-cell volume can be increased. These are: (1) reduction in oxygen tension resulting in a secondary polycythemia, such as is seen in experimental animals subjected to low oxygen tension,²⁹ in high altitude dwellers,³⁰ and in patients with certain types of congenital heart disease and chronic fibrosis of the lung; (2) administration of cobalt; and (3) the presence of a humoral erythropoietic factor elaborated by the pituitary.

Studies in this laboratory have shown that in polycythemia secondary to a reduced oxygen tension, whether this be a reduced oxygen tension in the atmosphere or an anoxia resulting from pulmonary of heart disease, the rate of red-cell formation is increased²² but the red-cell life span is unchanged,³¹ and there is a true increase in total circulating red-cell volume.³⁰ We have also shown that a true polycythemia can be produced in the rat by the chronic administration of cobalt in adequate doses,³² that the resulting polycythemia is due to an increased rate of red-cell production,³³ and that cobalt does not influence the life span of the red cell.³⁴ These two erythropoietic stimuli—reduction in oxygen tension and administration of cobalt—are probably not the usual mechanisms for control of total red-cell volume. It is possible that in man and in the experimental animal a hormone elaborated by the pituitary is necessary for the regulation of erythropoiesis. Evidence for this is based upon studies of the changes in blood volume following hypophysectomy.³⁵ As a result of this demonstration that a true anemia did

occur following hypophysectomy, studies were initiated by this laboratory and the Institute of Experimental Biology to determine which of the hormonal factors elaborated by the pituitary known at the present time could be responsible. The erythropoietic effect is apparently not mediated through any of the other endocrine glands.³⁶ A sheep pituitary extract has been prepared,³⁷ and careful studies have revealed that injection of this substance repairs the anemia of the new born rat,^{38,39} repairs the anemia

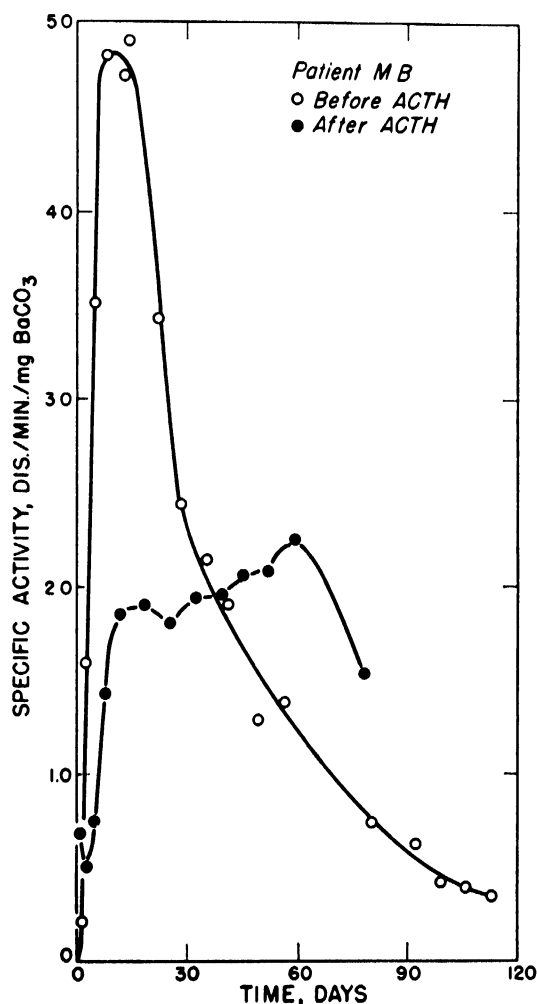


Figure 8. The life span of the red cell in Patient M. B. before (open circles) and after (black dots) treatment with ACTH

of hypophysectomy in the presence or absence of pituitary target organs;⁴⁰ produces a polycythemia in normal and hypophysectomized rats;^{38,41} and apparently is not identical with ACTH acting through the adrenal.^{36,40,41} Attempts to isolate a pure extract of the active factor are now in progress.

The three methods for studying erythropoiesis outlined previously—measurements of the total red-cell volume using P^{32} -labeled cells, the rate of red-cell production using Fe^{59} and the life span of the

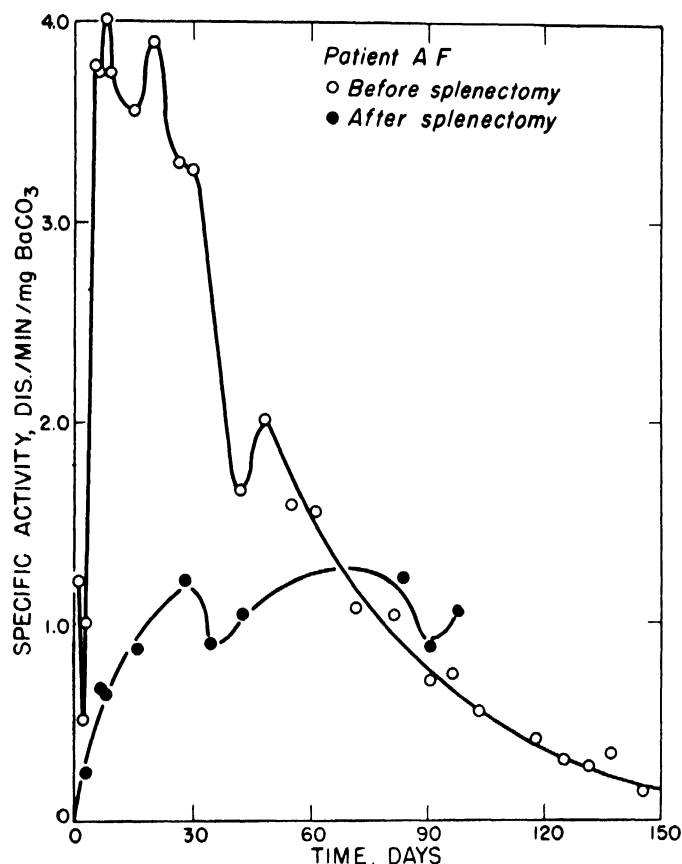


Figure 9. The life span of the red cell in Patient A. F.

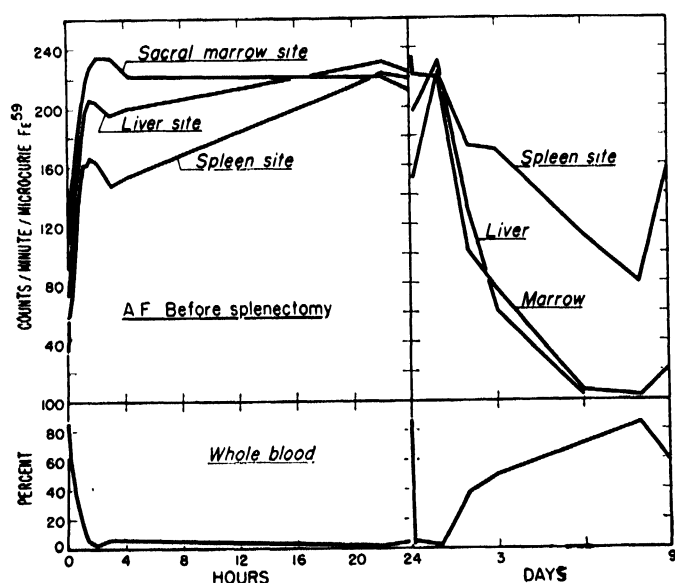


Figure 10. Uptake of Fe^{59} in red cells and by bone marrow, liver, and spleen in Patient A. F. before splenectomy

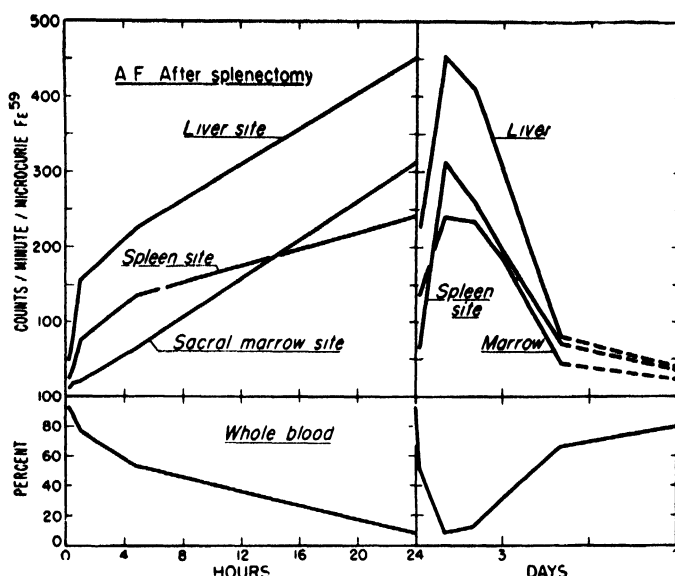


Figure 11. Uptake of Fe^{59} in red cells and by bone marrow, liver, and spleen in Patient A. F. after splenectomy

red cell by the C^{14} -labeled glycine method—have all served to delineate the nature of the changes observed in animals that become polycythemic. In cobalt-induced polycythemia and polycythemia secondary to anoxia, these methods have shown that there is an increased total red-cell volume and that this increase is due to an increase in the rate of formation of red cells and not due to a change in the life span of the red cells. The total red-cell volumes of experimental rats in the studies of hormonal control of erythropoiesis are usually measured by the Fe^{59} -labeled red-cell method. As mentioned in the discussion of methods, Fe^{59} is of particular value in studying the blood volume of animals of a uniform strain.

CONCLUSION

In conclusion, then, it may be stated that these three methods—namely, blood-volume determination, radioiron studies, and the measurement of the life span of the red cell—permit an adequate description of the pathogenesis of anemia or polycythemia. They have been of great value in selecting patients with unusual anemias that may or may not be related to hypersplenism when splenectomy is under consideration.

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The Absorption of Radioiron-Labeled Foods and Inorganic Iron by Normal, Iron-Deficient, and Hemochromatotic Subjects

By R. B. Chodos, J. F. Ross, L. Apt, M. Pollycove and J. Halkett,* USA

Although iron is one of the earth's most ubiquitous elements, its entrance into the human body appears to be accomplished only with difficulty. It is therefore not too surprising that iron deficiency is one of the commonest nutritional deficiencies afflicting the human race.

Fundamental studies utilizing chemical and balance techniques have contributed significant information regarding iron absorption.¹⁻³ Using radioiron Hahn,^{4,5} Dubach and Moore,⁶⁻⁸ and others have studied various aspects of this problem in both experimental animals and human subjects.

Many of the conclusions drawn from these studies with radioiron were based on the assumption that the per cent of an administered dose of iron which appeared in hemoglobin represented the quantity of iron that had been absorbed. Moore has pointed out that in certain subjects a significant quantity of iron may be absorbed and deposited in body stores in addition to that incorporated into hemoglobin.^{6,7} His conclusions were based on the administration of a known quantity of radioiron per month and the quantitative evaluation of the per cent recovered in hemoglobin and in stools. The unaccounted for per cent was presumed also to have been absorbed.

The development of more sensitive counting equipment has made it possible to measure readily the radioiron in blood and stool material. It has been the object of the present study to evaluate iron absorption in subjects with normal, diminished, and increased iron stores as represented by normal subjects, and by patients with iron deficiency or hemochromatosis, respectively. Gamma-counting techniques have been used exclusively in studies carried out under a variety of regulated conditions utilizing inorganic radioiron or radioiron-labeled foods.

The subjects utilized and the type of iron absorption study completed are summarized in Table I.

METHODS

Red cell indices were determined using certified Bureau of Standards equipment. Serum iron was

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measured by the method of Kitzes.⁹ The total, red cell, and plasma volumes were determined by a modified radiophosphorus tagged red cell method.¹⁰

Tracer doses of radioiron were administered by mouth to the fasting subject either as inorganic ferrous or ferric chloride or as a labeled food. Ascorbic acid in 1.0 gram tablets was usually administered concomitantly with doses of ferrous chloride and non-radioactive carrier iron in quantities ranging from 4 to 80 mg of iron was added to the tracer dose of Fe⁵⁹. The iron-59 labeled food products included chicken eggs and vegetables. These doses were given with toast and coffee, and with a few exceptions, no specific reducing substance such as ascorbic acid was given with the labeled food.

After the administration of either inorganic or food iron, stools were quantitatively collected until less than 1 per cent of the oral tracer dose could be recovered in a 24 or more hour collection. Aliquots of blood were taken at intervals of 2 to 5 days until a plateau of constant activity was obtained. Stool, blood and appropriate standards of inorganic and food iron in 25 ml aliquots were prepared in screw cap vials. The gamma radiation was quantitatively measured by Geiger-Mueller counting tubes. Counts were recorded on an appropriate scaler.

Experiments designed to evaluate the accuracy of the counting methods revealed that 96 to 100 per cent of Fe⁵⁹ added to a fecal collection were recovered by the counting techniques employed. The almost quantitative total recovery of iron in feces and hemoglobin in iron-deficient subjects would appear to establish the validity of the balance method used.

Table I. Subjects and Studies

Group	No.	Iron-59		As labeled food	Total no. of studies
		As FeCl ₃	or FeCl ₂		
Normal subjects	32	25	2	20	47
Iron deficient	14	13	1	5	19
Hemochromatosis	10	14	1	3	18
Miscellaneous	11	8	4	0	12
	—	—	—	—	—
Total	67	60	8	28	96

RESULTS

Absorption of Iron by Normal Subjects

There is a considerable difference between the absorption data obtained with food and inorganic iron. One to 12 per cent of the inorganic iron was absorbed and incorporated into hemoglobin representing the absorption of from 1 to 4.5 mg of iron. If the unaccounted for portion was also absorbed and deposited in body stores, an even larger quantity of iron may have been absorbed.

In striking contrast, markedly less Fe^{59} in labeled food was absorbed and utilized. If only the per cent incorporated into hemoglobin was definitely absorbed, 0.3 mg or less of the 4.0 to 8.0 mg of iron administered was absorbed. The difference in absorption of food and inorganic iron is even more apparent when the absorption of comparable quantities of inorganic and food iron is evaluated. The quantity of inorganic iron absorbed and used was 2 to 100 times the absorption of food iron. When radioiron ferrous chloride was given to four subjects along with non-labeled scrambled eggs, a considerably smaller per cent was absorbed and used than was absorbed when quantitatively similar amounts of inorganic iron were administered alone. The absorption of inorganic iron under these conditions was 1 to 10 times the amount of food iron absorbed.

The results of these studies are summarized in Figs. 1, 2 and 3. (In all figures, designation +++ above bar indicates subject received ferric chloride; ++ or no designation indicates subject received ferrous chloride.)

Absorption of Iron by Iron-Deficient Subjects

A large per cent of iron was absorbed and incorporated into hemoglobin when Fe^{59} was administered as ferrous chloride to iron-deficient subjects. With three exceptions the amount directly appearing in hemoglobin exceeded 21 per cent and usually was more than 40 per cent (Fig. 4).

Considerably less iron was absorbed in studies using iron-59 labeled food. Iron-deficient subjects

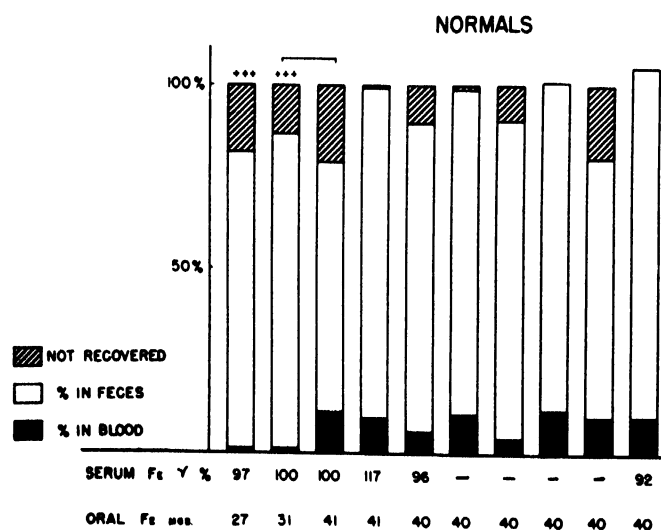


Figure 1. Recovery of ferrous and ferric chloride in normal subjects

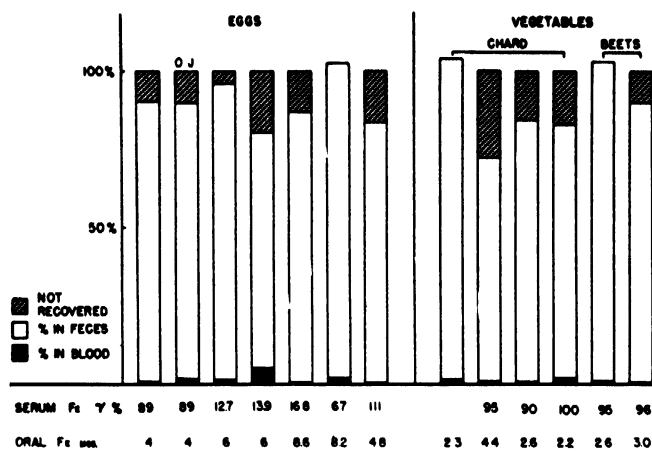


Figure 2. Recovery of egg and vegetable iron in normal subjects

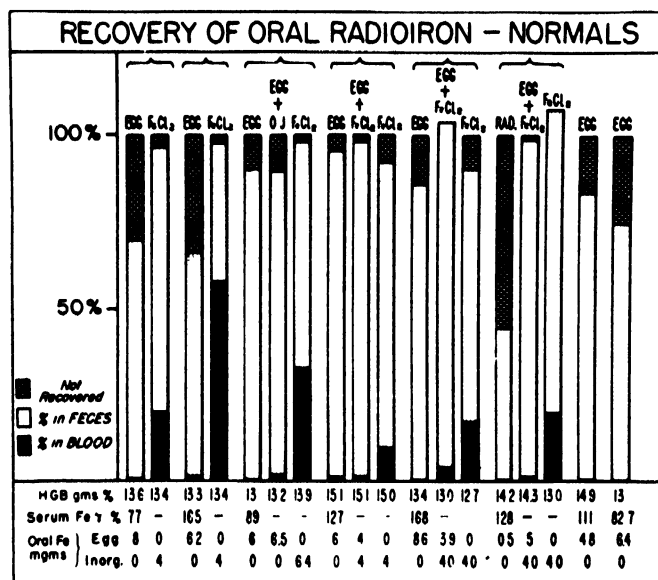


Figure 3. Effect of egg upon recovery of inorganic iron in normal subjects

absorbed 10.3, 20.5, and 0.6 per cent of iron in Fe^{59} -labeled chicken eggs and 3.6 and 30 per cent of Fe^{59} -labeled vegetables. However, none of these patients absorbed and utilized much more than 1.0 mg of iron, even if the unaccounted for portion not recovered in the hemoglobin or feces is considered to have been absorbed. Multiple studies on two iron-deficient subjects to evaluate directly the comparative absorption of inorganic iron and Fe^{59} -labeled food illustrates the greater absorption that occurs with ferrous chloride (Fig. 5). The iron in Fe^{59} -labeled eggs and chard was absorbed to a particularly small extent in a patient who had had a subtotal gastrectomy. In striking contrast, a comparable quantity of Fe^{59} as ferrous chloride was readily absorbed and incorporated into hemoglobin.

Absorption of Iron by Subjects with Excess Iron

It is evident that in patients with well established, untreated hemochromatosis of long duration, neither inorganic ferrous chloride nor food iron is absorbed to any greater extent than in normal subjects. From 0.5 to 11 per cent of Fe^{59} as ferrous chloride and

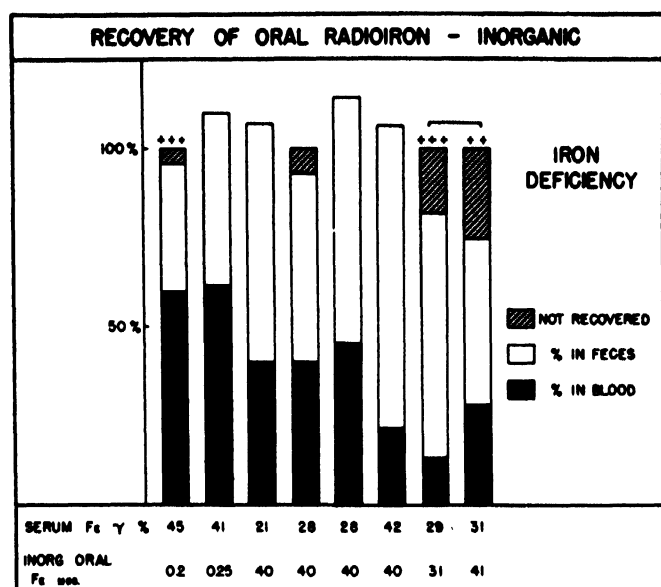


Figure 4. Recovery of ferrous or ferric chloride in iron-deficient patients

1.0 per cent of Fe^{59} in labeled beets was absorbed and used. Most of the remaining was recovered in feces (Fig. 6). Multiple studies were made in one subject (Fig. 7). Prevenesection absorption studies with ferrous chloride approximated the results obtained in normal patients. After 13 gm of iron were removed over an 18 month period, food iron was absorbed much as in iron deficient subjects. Inorganic iron was absorbed to an even greater extent even after his serum iron had risen to normal levels. A similar great absorption and use of inorganic iron was observed in a second patient after venesection.

DISCUSSION

There is evidence that in the absence of bleeding the daily loss of iron from the human body does not exceed 1 mg.^{1-3,11} In addition careful balance studies by McCance and Widdowson have demonstrated that iron in feces is almost entirely unabsorbed dietary iron.¹ This observation has been confirmed

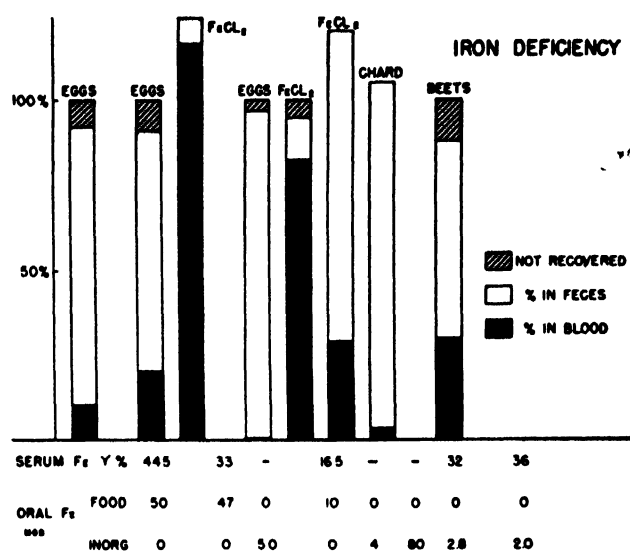


Figure 5. Comparative recovery of food and inorganic iron in iron-deficient patients

in man by the failure to demonstrate any increased excretion of iron in feces following its intravenous administration. The body iron stores would therefore appear to be determined by the quantity of iron absorbed.

Our data illustrate that normal subjects, iron-deficient patients, and patients with hemochromatosis absorb inorganic iron far more readily than food iron. It should be emphasized, however, that the inorganic iron was usually administered under conditions that were optimal for absorption. Thus ferrous iron solution was given to a fasting subject along with a reducing agent. Interfering chemical or physical substances were excluded. The addition of egg to the dose materially reduced its absorption.

The per cent of inorganic radioiron absorbed and incorporated into hemoglobin by normal subjects suggests that iron in excess of the daily loss of 1 mg may be absorbed under these experimental conditions. These results agree with those reported by

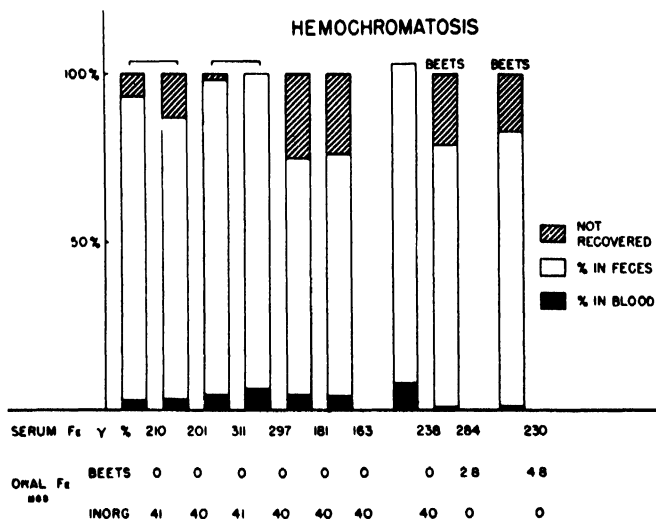


Figure 6. Recovery of food and inorganic iron in hemochromatosis

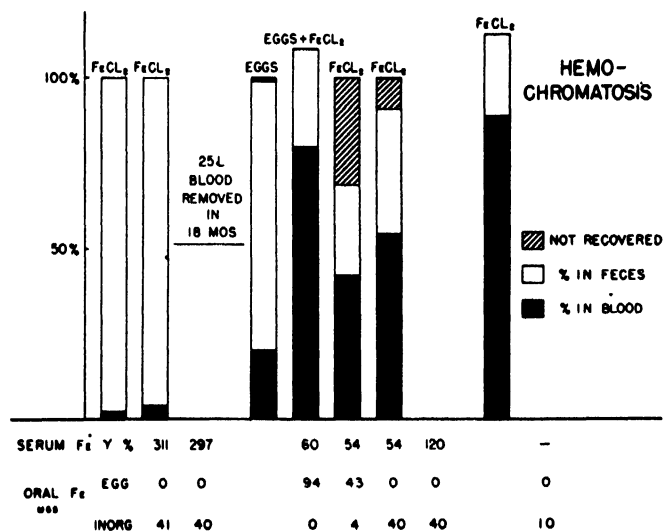


Figure 7. Effect of venesection upon recovery of food and inorganic iron in hemochromatosis

Dubach and Moore.⁷ Our recovery data also lend support to the suggestion of Dubach, *et al.* that normal subjects may absorb significantly more iron than appears to be utilized in hemoglobin formation.⁷

In contrast, normal subjects absorbed much less iron after a single feeding of radioiron-labeled eggs or vegetables. These observations were obtained under physiological conditions more closely approximating the ingestion of a meal containing 5 mg of iron. This limited absorption of food iron suggests that the daily adult requirement of 12 to 15 mg of dietary iron as recommended by the National Research Council¹² may be just sufficient to maintain the body iron stores in the absence of blood loss.

Our data indicate that patients with iron deficiency will usually absorb significantly more food iron than will normal subjects. However, on a quantitative basis the amount absorbed was not much greater than the usual daily loss in the absence of bleeding. Moore actually found that only 2 of 11 iron-deficient subjects absorbed more food iron than did his normal group,⁸ although the co-administration of a reducing substance such as ascorbic acid increased the quantity of iron absorbed. Iron as ferrous chloride was of course readily absorbed in large quantities by iron-deficient patients.

In view of these observations it is obvious that it would be difficult for an iron-deficient patient to replenish his stores by diet alone. Supplementary medicinal iron should be given to patients who undergo chronic blood loss, including that related to menses. Even with an average menstrual blood loss of 35-70 ml per month, the average diet will not quite supply the required replacement iron. Repeated blood donations may require supplementary medicinal iron,⁸ as may frequent pregnancies, prolonged lactation, and the growth of children.¹¹ Finally, it is possible that individuals on a very deficient diet or with an absorptive defect may develop iron deficiency on a nutritional basis. Under these circumstances supplementary iron therapy is indicated either as a therapeutic or a preventive measure. Similarly iron supplementation as a tool of preventive medicine is indicated in areas of the world where a large segment of the population may subsist on a marginal diet.

A completely different situation exists in hemochromatosis. Although our studies indicate that neither food nor inorganic iron is absorbed in well established hemochromatosis to any greater extent than in normal subjects, it is apparent that excess iron must be absorbed during the developmental phase of this disease. The increased absorption of both food and inorganic iron by our patients after an extensive venesection program would support this conclusion. Peterson also found absorption increased in a patient who had been venesected.¹³ Alper,¹⁴ and Bothwell, *et al.*¹⁵ have also shown that iron absorption is increased in patients with hemochromatosis studied at a younger age, presumably before their stores were saturated.

These studies suggest that the therapy of hemochromatosis should consist of a continuing venesection program designed to remove iron and to prevent its reaccumulation. Restriction of dietary iron should be attempted although this is admittedly difficult to accomplish. By such a combined program it may be possible to treat or to prevent the development of manifestations of hemochromatosis such as cirrhosis, diabetes, or other endocrine dysfunctions. By studying the siblings and children of patients with hemochromatosis by the techniques used in this study, it would appear possible to discover the presence of this disorder at an earlier time in its course when preventive venesection may be quite effective. In addition further information concerning the pathophysiology of this disorder might be obtained.

SUMMARY

1. Normal subjects and patients with iron deficiency or hemochromatosis all absorb iron in food less readily than inorganic iron.
2. The recommended daily dietary allowance of 12 to 15 mg of iron would appear to be barely sufficient for the maintenance of iron stores by normal subjects.
3. Supplementary medicinal inorganic iron should be administered to replenish depleted iron stores due to blood loss, a marginal diet, or an absorptive defect. Iron supplements should be given also to meet the increased demand of pregnancy, lactation, or growth.
4. Iron stores will be effectively decreased in hemochromatosis by intensive venesection. These stores will reaccumulate unless the venesection program is continued.

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The Use of Radioactive-Tagged Red Cells and Platelets to Study the Phenomenon of Sequestration

By W. H. Crosby,* USA

The word sequestration is used to designate a temporary removal from the active circulation of a portion of the blood, either whole blood or one or more of its parts. The classical example of this phenomenon is the so-called reservoir function of the spleen. The sequestering spleen is said to act as a private blood bank for red cells, and in time of stress injects these cells into the blood stream. There is good evidence that the spleen of the dog possesses this ability, but the normal human spleen apparently does not.^{1,2}

The massive spleen in certain human hematologic diseases is also capable of sequestering red cells. In this case the phenomenon of sequestration has been demonstrated by using radioisotopes to tag the red cells.³ When the tagged cells are transfused into a normal circulation they mix rapidly and completely with all of the cells in the blood stream. This is demonstrated by taking samples of venous blood at short intervals to follow dilution of the isotope. The dilution is reflected in the diminishing number of radioactive counts in the successive samples until equilibrium is reached after three to five minutes of mixing.

In cases of massive splenomegaly there is rapid initial mixing but equilibrium is not reached at the end of this period. There occurs a prolonged period of further diminishing isotope activity in the serial samples indicating that the tagged red cells are gradually mixing with a portion of the blood that is moving sluggishly through some part of the circulatory system. Following splenectomy, this sluggish component of the mixing time disappears.

A similar pattern has been observed in severely shocked dogs.⁴ The prolonged mixing time of isotope-labelled red cells injected in these animals has been said to be due to stagnation of the circulation or to the appearance in the circulation of previously sequestered red cells.

These are examples of what is meant by sequestration. The purpose of this report is to describe the ways in which radioactive isotopes are being used in our work to study the phenomenon of sequestration in several pathologic conditions.

MATERIALS AND METHODS

Studies using radioactive platelets were carried out in dogs using a donor (Dog A) and a recipient (Dog B). Two mc of P^{32} ($NaH_2P^{32}O_4$ Abbott) were injected into Dog A. Two weeks later, Dog A was bled by cardiac puncture, and 500 ml of blood were drawn into a plastic bag containing acid-citrate-dextrose (ACD) solution. Simultaneously 500 ml of blood were taken from Dog B. Dog B was then transfused immediately with the blood from Dog A containing radioactive platelets. One-half hour later, using siliconized equipment and a 2-syringe technique, 15 ml of blood were taken from Dog B. The platelets were separated by differential centrifugation and washed once using platelet-free plasma from the blood drawn earlier from Dog B. After washing the platelets were suspended in this plasma, and they were counted in a hemocytometer chamber using a phase-contrast microscope. A portion of the platelet suspension was placed in a planchet and the P^{32} radioactivity was measured in a Geiger-Müller counter with a window thickness of 2.2 mg/cm² (Tracerlab). Leukocyte, erythrocyte and platelet counts, using standard methods, were also performed on the dog's whole blood. Dog B, anesthetized with pentothal, was now placed in ice until his body temperature fell to 18°C and his platelet count fell from 204,000 to 58,000. He was then rewarmed. When his platelet count had risen, a second 15 ml sample of blood was drawn and the procedure of counting described above was repeated.

Measurements of circulating red cell volume were carried out by injection of autogenous red cells tagged with Cr^{51} using Read's modification of the method of Sterling and Gray.^{5,6} The isotope was used as sodium chromate ($Na_2Cr^{51}O_4$, Abbott), and the dose in humans was approximately 25 μ c. ACD solution was used as the anticoagulant. Red cell mixing time was established by frequent serial sampling of the circulating blood after the tagged cells were injected. Complete mixing was said to have occurred when no further dilution of the isotope was apparent. Circulating plasma volume was measured by injection of albumin tagged with I^{131} using Field's modification of the method of Fine and Seligman.^{7,8} Plasma mixing time was established as for red cells. Radioactivity of the Cr^{51} and I^{131} tagged samples was measured in a well scintillation counter (Nuclear,

* Walter Reed Medical Center. Including work by E. Adelson, J. W. Hollingsworth, W. A. O'Brien, R. Ogborn and T. Villalobos.

Chicago), and the counting was performed for a length of time sufficient to reduce the counting error below 3 per cent.

External counting of radioactivity in the tissues of live animals was performed with a scintillation counter, mounted vertically on a movable side arm. The animals were immobilized on a table, and duplicate 1 or 2 minute counts were made over each site with the detection head of the counter 2 cm from the animal's skin.

PROCEDURES AND RESULTS

Can Extravasated Blood Cells Be Regarded as Sequestered

The leukocytes of the body probably spend more time out of the blood than they do in it, and most of their activity takes place in the tissues.⁹ They may be considered as passengers rather than inhabitants of the blood, entering and leaving the circulation as required. In a similar fashion a small portion of the plasma moves in and out of the blood stream: the existence of an extravascular plasma space is well recognized. It is a matter of definition whether or not one considers the temporary extravasation of leukocytes or plasma as a sort of sequestration. Extravasated red cells, however, are definitely out of place. Experiments were planned to determine whether or not extravasated red cells are sequestered, that is to say whether they are removed permanently or only temporarily from the circulation.

Autogenous rabbit red cells were tagged with Cr⁵¹ and injected into several extravascular areas: subcutaneous, intramuscular, intrapleural, intrathecal and intraperitoneal. The decay of radioactivity in the areas of injection was followed by surface scintillation counting. The appearance of tagged red cells in the circulation was also looked for. After intraperitoneal injection, most of the tagged red cells appeared rapidly in the peripheral blood, and to a lesser extent this was true of cells injected into the pleural cavity. At the other sites of injection, however, the radioactivity diminished over a period of several days but only a few tagged cells appeared in the circulation. (Table I). This is interpreted to mean that red cells when they are extravasated into tissues are gradually hemolyzed, probably by phagocytosis, at the site of extravasation. This is not regarded as sequestration, which we consider to be a temporary state.

Table I. The Fate of Extravascular Red Cells

Site of injection of Cr ⁵¹ tagged RBC (4 to 6 rabbits in each group)	Half-life of radio activity at site of injection (days)	Tagged RBC appearing in circulation (% of those injected)
Intraperitoneal	1.5	55-90
Intrapleural	6	20-35
Subcutaneous	1	5
Intramuscular	4	5

Epidemic Hemorrhagic Fever

On about the seventh day of illness in this disease, patients who are severely affected may go into shock unassociated with obvious gross blood loss. The plasma volume is greatly diminished due to extravasation through damaged small vessels, and the computed red cell mass also tends to diminish. It was suggested that the reduced circulating red cell volume was probably the result of pooling or trapping of erythrocytes in dilated capillaries.¹⁰ To test this hypothesis, normal compatible red cells were labelled with Cr⁵¹ and injected into twenty-five patients during the hypotensive phase of epidemic hemorrhagic fever.

It was found that mixing of the injected erythrocytes was rapid and complete within five minutes. (Fig. 1) Although observations were continued for several hours or days, there was no evidence of a sluggishly moving component of the circulation. It is postulated that the diminished red cell mass in epidemic hemorrhagic fever is due to actual loss of erythrocytes, either by hemolysis or by extravasation through the damaged endothelium of small vessels.

Post Traumatic Shock

Following severe mechanical injury it has been found that massive transfusions of whole blood are often needed to maintain hemodynamic stability during resuscitation and surgery. The size of a required transfusion may be far in excess of the patient's blood volume, even in the absence of a comparable amount of external or intra-abdominal hemorrhage.¹¹ Because these patients are often severely shocked it has been suggested that much of the blood was "stagnating" or "pooling" in widely dilated areas of the circulatory system and that it was necessary to fill these "pools" before the central, rapidly moving portion of the circulation could be effective and normotensive.

To test this proposition, studies were carried out on sixteen severely injured patients just at the time that massive transfusions had been completed. Red cells tagged with Cr⁵¹ were injected intravenously. The mixing time was determined and the total red cell mass was computed. (Fig. 2) Two distinctive patterns were observed:

1. In spite of massive transfusion and adequate hemostasis, the hematocrit was normal or low, the red cell mass was normal or smaller than normal and the mixing time of the tagged red cells was prompt and complete.

2. The hematocrit was high and the red cell mass was found to be normal or greatly expanded. The patients were in severe shock and responded poorly. They usually died. The mixing time of tagged cells showed clearly two components, one moving with normal rapidity and one that was sluggish. At autopsy in cases where the blood volume was known to have been expanded by transfusion the skeletal muscles, liver and lungs of the patients were greatly filled with blood.

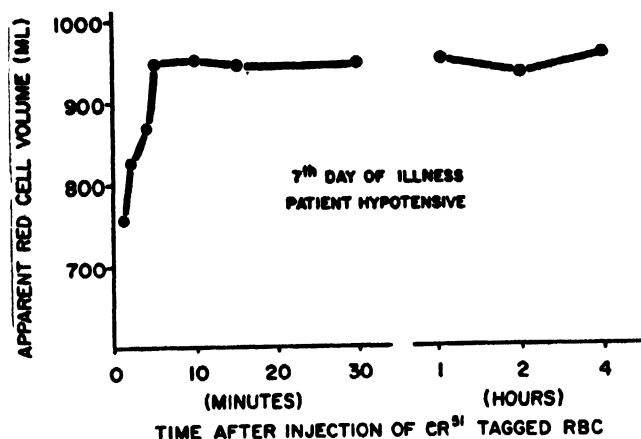


Figure 1. Red cell mixing time in severe epidemic hemorrhagic fever. The red cell mass was small (13 ml per kg of body weight) but there was no evidence of sequestered red cells

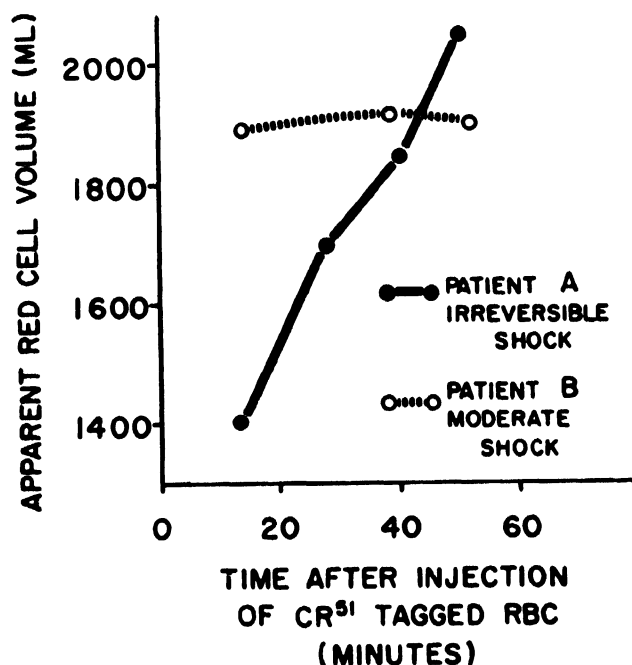


Figure 2. Red cell mixing time in severe injury. Patient A died three hours later; examined post mortem, there was no gross evidence of congestion. However, the tagged cell study suggested that there was rapid mixing with 1400 ml of circulating red cells and slower mixing with another 700 ml

It was only in this second group of patients that evidence of pooling or sequestration was found. In the first group, the discrepancy between the volume of blood transfused and the amount measured in the circulation was often of considerable magnitude, but the "missing" red cells did not reappear. These patients frequently became progressively anemic and required subsequent transfusion (Table II).

We have encountered the same sort of discrepancy in mechanically injured animals treated with transfusion. The circulatory red cell mass was unaccountably diminished. But when Cr^{51} -tagged red cells were transfused into such animals, surface scintillation counting revealed the presence of excessive radioactivity over a wide area adjacent to the site of injury; and autopsy demonstrated extensive extravasation of red cells in these areas. This suggests that in severe mechanical injury there may be

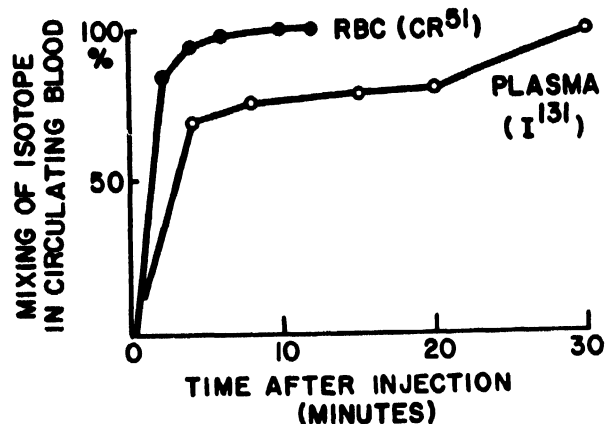


Figure 3. Dilution of injected isotopes in severe hypothermia. These studies were not done in the same animal. There is immediate, complete mixing of tagged red cells but mixing of the tagged protein shows prompt mixing with only 70 per cent of the plasma volume

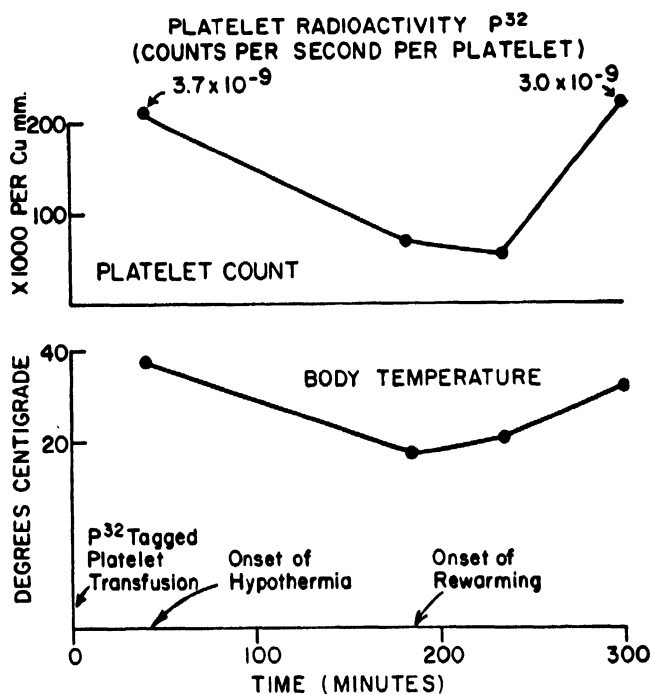


Figure 4. Sequestration of platelets in artificial hypothermia

serious injury to the vascular endothelium in areas extending well beyond the evident trauma. The vascular injury may permit extravasation of hitherto unsuspected proportions which evidently requires massive transfusions of whole blood to compensate for it.

Artificial Hypothermia

When anesthetized dogs are placed in ice water to reduce the body temperature to 20°C , certain striking and consistent changes occur in the components of the arterial blood.¹² The hematocrit becomes greatly increased, and the leukocytes and platelets are diminished almost to zero. When the dog is rewarmed, the leukocyte and platelet counts quickly recover to normal and the hematocrit gradually declines.

Further investigation has revealed that these alterations are, to some extent, a result of sequestration.

Table II. Unaccountable Red Cell Loss in Severe Injury

<i>Patient R. S. Traumatic amputation of thighs</i> (Blood group A, transfused with group O blood)			
Red cell volume before injury (presumed)	2000 ml		
Red cells in transfusion (10 liters)	4000		
Total to be accounted for		6000	
Red cell loss at time of injury	1200		
Loss at surgery	600		
Loss by anti-A hemolysis	800		
Loss of nonviable transfused red cells (15%)	600		
Red cell volume after surgery (Hct 37)	1300		
Total accounted for		4500	
Discrepancy		1500	
Equivalent to 4 liters of bank blood			

The changes in hematocrit were studied by means of albumin tagged with I^{131} and red cells tagged with Cr^{51} . It was found that the red cell mass did not greatly change during the experiment. The rise in hematocrit was due to reduction of the circulating plasma volume. It was also found that mixing of the tagged red cells was rapid and complete, but the plasma mixing time was appreciably delayed (Fig. 3). Whether this was due to sequestration or extravascular circulation of the plasma has not been established.

Platelets tagged with P^{32} were transfused into a dog before he was made hypothermic. A sample of blood was obtained, the platelets were washed, re-suspended, counted and their radioactivity was determined to be 3.7×10^{-9} counts per second per platelet. At this time the platelet count in the peripheral blood was 204,000 per mm^3 . The dog's body temperature was now reduced to $18^\circ C$ and his platelet count fell to 58,000. He was rewarmed, the platelet count rose to 218,000 and a second sample of blood was taken. The platelets were separated, their radioactivity was measured and found to be 3.0×10^{-9} counts per second per platelet. (Fig. 4) This appears to be an unequivocal example of platelet sequestration. During refrigeration most of the

platelets abruptly disappeared from the circulation. When the count returned to normal after warming, the amount of radioactivity in the platelets was substantially the same as it had been before. The platelets that had disappeared were those that reappeared. The site of their hibernation has not yet been found.

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Tissue Specific Antibodies as Carriers of Radioactive Materials for the Treatment of Cancer

By W. F. Bale and I. L. Spar,* USA

Radioactive isotopes will be much more useful in the radiation therapy of cancer when means are developed for concentrating them in tumor tissues as specifically as inorganic radioiodine will concentrate in the human thyroid following intravenous injection. A possible tool in accomplishing this specific radiation effect may be a radioactive isotope attached to an antibody that will be preferentially bound to some antigenic component associated with the tumor. This antigen might be some tumor tissue component, or some part of a virus or bacteria preferentially infecting the tumor. Besides carrying radioactive isotopes, such localizing antibodies may possibly be used as carrier of boron-10 or uranium-235 to permit subsequent effective slow neutron therapy.

In the first approach to this problem Pressman, Korngold, and their associates^{1,2} achieved substantial success in preparing I^{131} labeled antibodies against rat kidney that would localize in the rat kidney following intravenous injection. They have also achieved some success in preparing localizing antibodies against certain transplantable rat and mouse tumors as well as other normal rat organs.³ These researches have not as yet led to specificity of I^{131} localization that, if transferred to humans, would be useful in cancer therapy.

Research carried out at Rochester^{4,5} indicates that relative failure to achieve more specific I^{131} localization by these methods in certain rat tissues and tumors is due to two facts. One, many antigenic components of rat tissue are *in vivo* in locations where circulating antibodies do not have access to them. Two, important cross reactions occur between antibodies prepared against specific rat tissues and other rat tissues and organs acting as antigens; that is, antibodies against specific rat tissues also seem to be generalized anti-rat antibodies.

It is generally supposed that not all immunologically important elements of an antibody-like nature are free in the blood. Antibodies fixed to skin and other tissues may be important defense agents in nature.⁶ Also important immunological elements may be a part of the formed entities of the blood. There

is evidence that the extremely subtle immunological distinctions that lead to rejection of homotransplants of skin and other organs may be of this nature, rather than free in the gamma globulin fraction of the blood.⁷ It is conceivable that these bound antibodies or antibody-like substances can be isolated and made to serve as carriers of therapeutic radioactivity.

It has also seemed possible that animals immunized against organs of another species might produce antibodies that would react with their own tissues.^{8,9,10} For example, rabbits immunized against rat kidney might produce antibodies that would react with the rabbit's own kidneys and be bound there. Remaining in circulation would be only species-specific antibodies with no tendency to react with organs of the host animal. Reported here are the results of experiments based on this latter concept and designed to isolate from the kidney of such immunized rabbits anti-kidney antibodies that would relocate in kidney after intravenous injection. A portion of these results are reported earlier in abstract form.⁹

EXPERIMENTAL

Kidneys from rabbits immunized against rat kidney by standard techniques⁴ were removed and the organs homogenized in borate buffer and washed to remove soluble proteins on the assumption that anti-kidney antibodies would remain bound to insoluble kidney proteins. This insoluble residue was stirred in pH 9 borate buffer at 60°C for 15 minutes on the further assumption that this treatment would break antigen-antibody bonds and release the antibodies into solution. The eluted protein obtained by this treatment was labeled with I^{131} at a level of 200 microcuries per milligram protein. It was then either directly injected into animals or further "purified" by *in vitro* absorption onto rabbit kidney homogenate followed by a similar 60°C elution.

The open bars of Fig. 1 show the average distribution in six rabbits of I^{131} three days after the intravenous injection of a "purified" anti-kidney preparation of this type. Each animal received a different and separately made preparation. Different immunized rabbits provided the starting kidney material for each preparation; the preparation procedures were similar but not identical. A similar

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pattern of localization was found for each animal; the average per cent of I^{131} per gram kidney cortex was 0.805 with a standard deviation of 0.45. However, some preparations showed much more specific kidney localization than others. The best preparation in terms of specificity of kidney localization showed 1.64 per cent of the dose per gram kidney cortex, a value fifteen times greater than in the highest competing tissue, the spleen. Values of I^{131} per gram tissue at seven days after injection showed that I^{131} localization, after correction for radioactive decay,

decreased only a few per cent from the three-day values.

The solid black bars of Fig. 1 show average I^{131} distributions following injection of similar preparations, but with kidneys of normal, non-immunized rabbits as the source of eluted material. These materials also have a kidney localizing tendency, probably but not certainly less than for the preparations derived from immunized kidney.

Figure 2 shows averaged results when these two types of preparations are injected intravenously into rats. Ten rats were injected with six preparations of material derived from immunized rabbits, four rats with material derived from non-immunized rabbits. A greater kidney localizing tendency is shown by the preparation derived from immunized rabbit kidney. This preparation also localizes in the rat small intestine, particularly the duodenum. The fraction from normal rabbit kidney does not localize here.

The rat intestine localizing component was largely separated from the component that localizes in rat kidney by the following *in vivo* isolation and purification procedure. A rat was injected intravenously with an unpurified I^{131} labeled basic eluate of immunized rabbit kidney. Three days later the small intestine and kidneys were removed from this animal, homogenized, washed, and the washed residues extracted for 15 minutes at 60°C with pH 9 borate buffer. A portion of the resulting intestine eluate was injected intravenously into one rat, the kidney eluate into two rats. The resulting I^{131} distribution

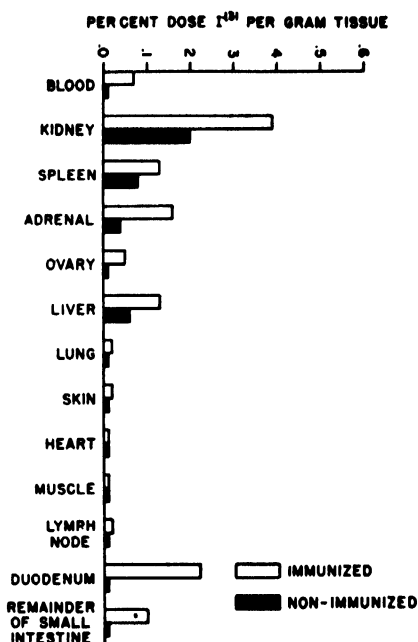


Figure 1. Distribution of I^{131} in organs of perfused rabbits three days after injection of I^{131} -labeled and "purified" basic eluates of kidneys from rabbits immunized against rat kidney or from normal rabbits. Data normalized to 300 gram animals

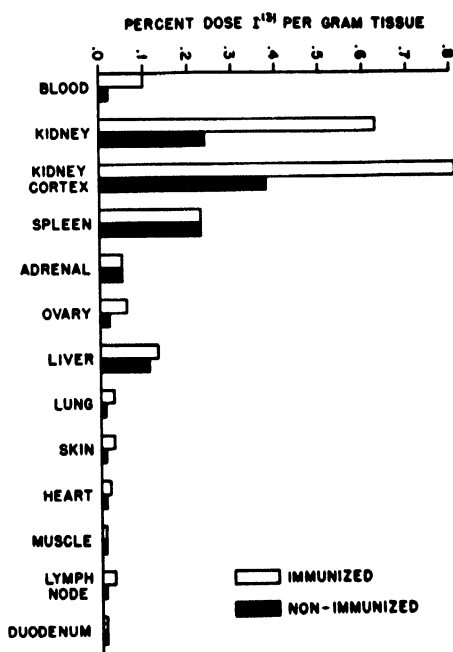


Figure 2. Distribution of I^{131} in organs of perfused rats three days after injection of I^{131} -labeled and "purified" basic eluates of kidneys from rabbits immunized against rat kidney or from normal rabbits. Data normalized to 300 gram animals

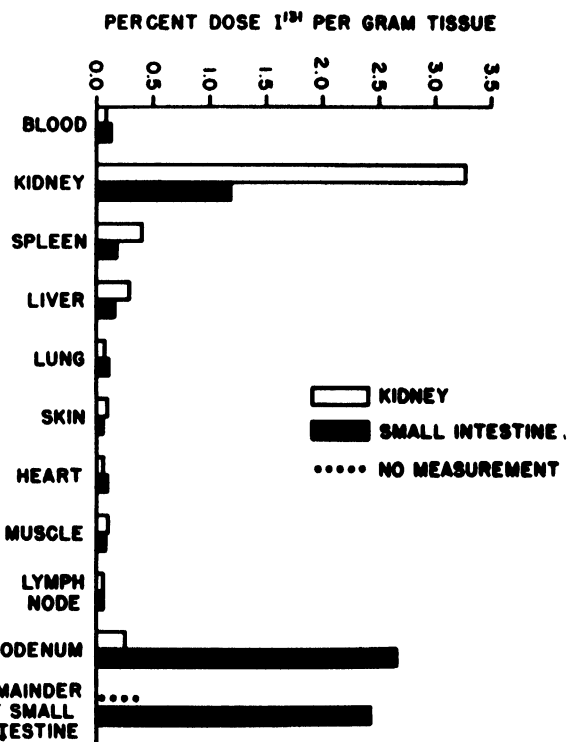


Figure 3. Distribution of I^{131} in organs of perfused rats three days after injection of basic eluate of small intestine or kidney. The eluted organs were removed from a rat that had been injected with I^{131} -labeled basic eluate of kidneys obtained from rabbits immunized against rat kidney. Data normalized to 300 gram animals

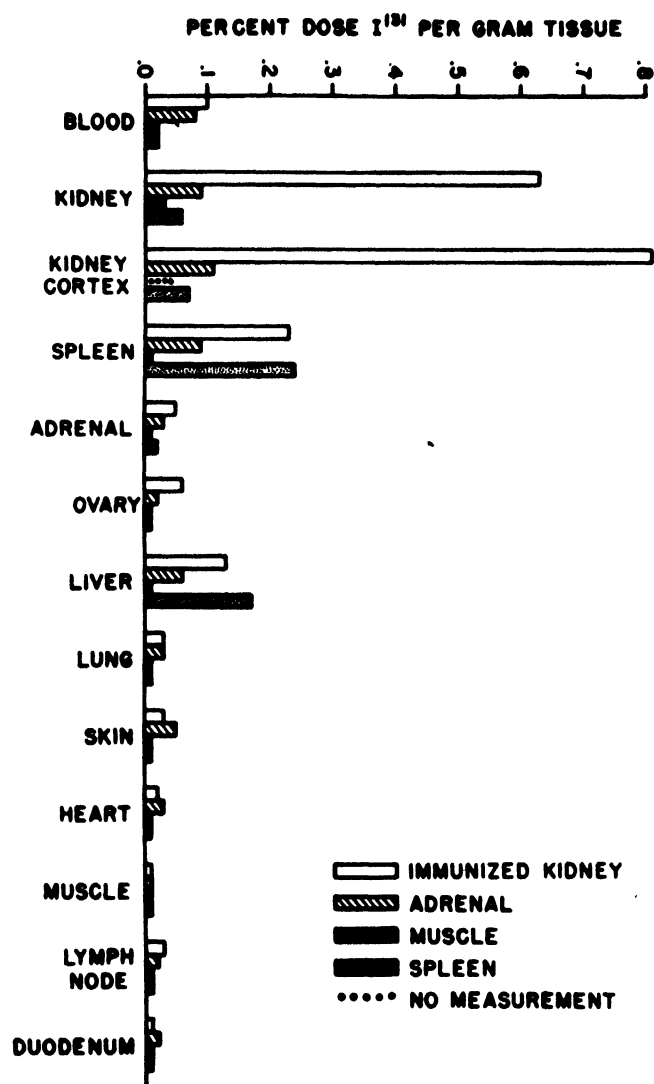


Figure 4. Distribution of I^{131} in organs of perfused rabbits three days after injection of I^{131} -labeled and "purified" basic eluates of normal rabbit organs or kidneys from rabbits immunized against rat kidney. Data normalized to 300 gram animals

following perfusion is shown in Fig. 3. The kidney eluate figures are the average of closely similar values for two rats. The material obtained from small intestine relocates quite strongly in this organ, with some activity present in the kidney. I^{131} values for stomach and large intestine were very low. The kidney fraction returns very specifically to the kidney.

As controls for the preceding series of experiments with iodinated material derived from the kidneys of normal rabbits and from rabbits immunized against rat kidney, similar experiments were carried out with eluates of spleen, adrenal, and striated muscle tissue from normal rabbits. These eluates made from the washed normal organ residues at 60°C were iodinated, the iodinated material "purified" by absorption and 60°C elution from an homogenate of the same organ, and the eluate injected intravenously into normal rabbits and rats. Average results of rabbit injection experiments are shown in Fig. 4, contrasted with the results found after the injection of the "purified" preparation of immunized rabbit kidney (the same data shown in Fig. 1). In general,

these other preparations showed low kidney localization in both rabbits and rats. The preparation made from normal spleen possibly localizes to some extent in spleen.

Besides heat at pH 9, we have also made experiments using mild acidity, pH 3.2 for 15 minutes at room temperature, as a means of eluting a soluble fraction from washed kidney homogenates, and for elution in subsequent absorption-elution "purification" procedures. Figure 5 shows the average high degree of kidney localization of radioactivity following the intravenous injection of such purified preparations into rabbits. As much as 10 per cent of the injected radioactivity is found in the kidneys of the recipient animals three days later. Five such preparations were made in all, two from normal kidneys, three from the kidneys of rabbits immunized against rat kidney. It seems to make little difference whether the rabbit kidney was derived from normal or immunized animals. These fractions were also injected intravenously into rats to study *in vivo* localization of radioactivity. The pattern obtained was similar to that seen in rabbits (Fig. 5), although the per cent uptake in the organs was about one-third as much. In this case the fraction from immunized rabbits showed no tendency to localize in rat small intestine.

Correlated studies have been made using I^{131} labeled rabbit gamma globulin containing antibodies against rat Walker carcinoma 256. In Fig. 6 are shown the I^{131} distributions 3 and 7 days after the

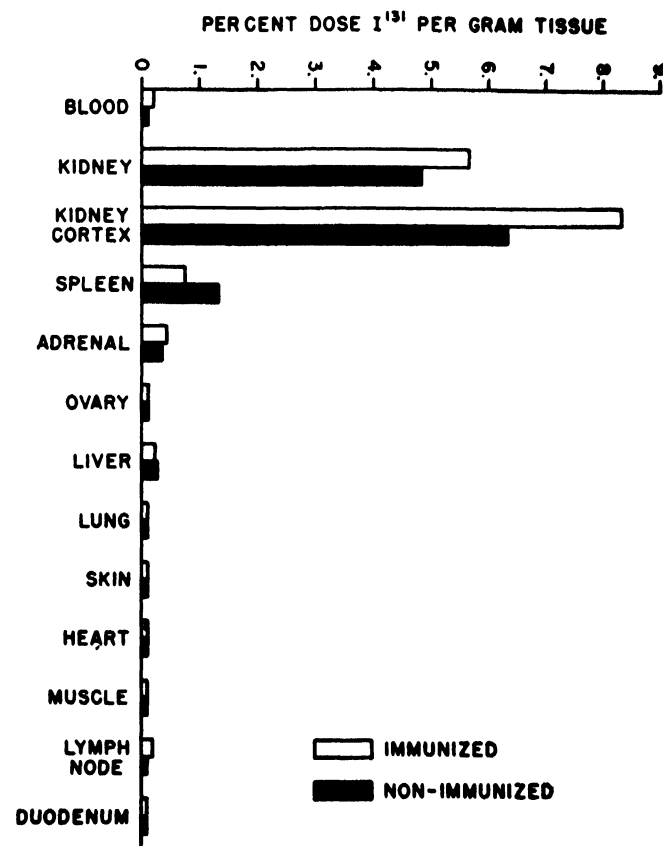


Figure 5. Distribution of I^{131} in organs of perfused rabbits three days after injection of I^{131} -labeled and "purified" acid eluates of kidneys from rabbits immunized against rat kidney or from normal rabbits. Data normalized to 300 gram animals

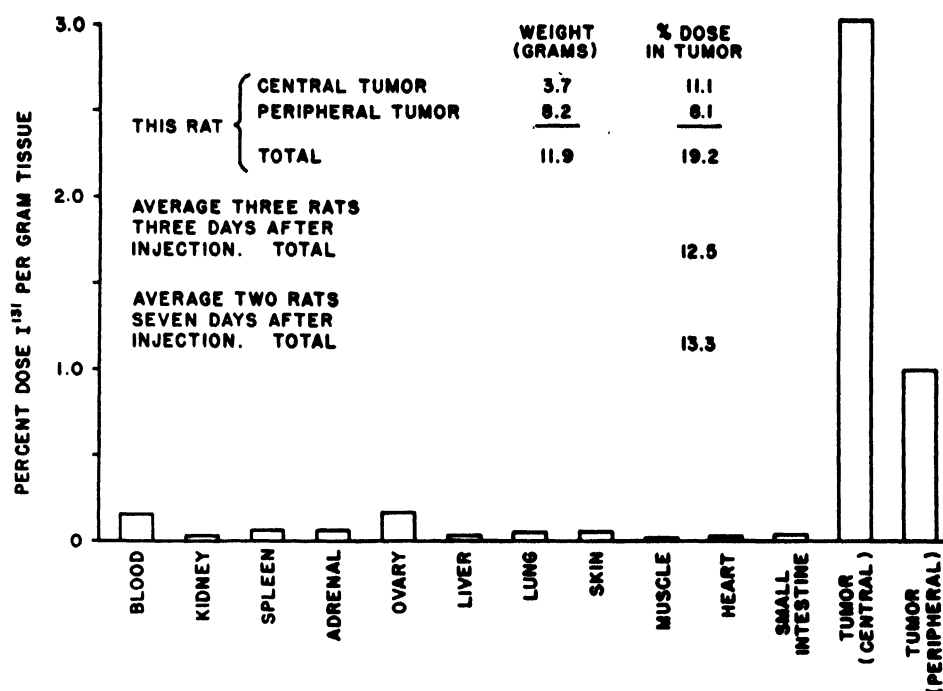


Figure 6. Distribution of ^{131}I in a perfused rat carrying Walker Carcinoma 256 three days after injection directly into the tumor of an ^{131}I -labeled rabbit gamma globulin fraction containing antibodies against this tumor. Data normalized to a 300 gram animal. The table shows other data on ^{131}I retention in tumors from similar experiments

injection of such a preparation directly into the growing tumor. The labeled antibodies remain predominantly bound in that site for days. It seems probable from associated experiments on antigen saturation that the extent of diffusion before binding of these initially soluble antibodies can be controlled by the amount of unlabeled antibody contained in the injected preparation. Because of these properties antibodies of this type coupled with radioiodine may possibly have advantages over radiogold for interstitial therapeutic use in human subjects.

DISCUSSION

The experimental data reported here demonstrate that one can obtain and label with ^{131}I fractions of rabbit kidney that show very marked kidney localizing properties when injected intravenously into rabbits and rats. Animals injected with these preparations do not show the high ^{131}I concentrations in adrenal, ovary, and spleen found when labeled gamma globulin preparations from the serum of rabbits immunized against rat kidney are injected intravenously into rats.⁴ The rat intestine localizing fraction isolated is presumably of an antibody nature, or at least its production mediated by an immunological response, since it was extractable only from the kidneys of rabbits immunized against rat kidney. Conceivably the kidney localizing materials may not be wholly of a strictly antibody-like nature, but instead samples of a larger group such as the complementary substances postulated by Tyler.¹¹ We are working to decide whether they are immunological in nature, and whether this type reaction is limited to rabbit kidney, or whether it can be generalized to other organs and species, including man.

SUMMARY

Rabbits were immunized against rat kidney. Eluates were prepared from washed kidney residues of these rabbits, and from the kidneys of normal rabbits, and labeled with ^{131}I . Such eluates, injected intravenously into rats and rabbits, localized preferentially in the kidneys of the recipient animals. With one type of elution and purification procedure over 10 per cent of the injected ^{131}I was found in the kidneys of recipient rabbits three days after injection. Localization in kidney was greater for fractions prepared from immunized animals, but this difference is not yet demonstrated to be statistically significant.

The labeled kidney eluates of immunized rabbits, but not from normal control rabbits, localized in rat small intestine as well as kidney. It was possible to separate the small intestine localizing fraction from the kidney localizing fraction.

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A Photographic Method for Determining the Localization and Distribution of a Radioactive Substance in the System

By C. Kellershohn* and P. Pellerin,† France

The ability *in vivo* to determine the localization and distribution of a radioactive substance is of considerable interest in the fields of medicine and physiology. Let us mention a few examples:

In the case of the thyroid, which selectively fixes I^{131} , this ability makes it possible to obtain a picture of the functional topography of the gland, in addition to determining its shape and volume.

In the case of a local injection of a radioisotope for therapeutic purposes, it makes it possible to find out whether the dose is suitably distributed in order to give the desired result.

Finally, when it is feasible to "tag" a compound, which is selectively taken up by tumor tissue, a very useful method of cancer diagnosis is provided.

A method currently in use consists of manually using a suitably collimated Geiger or scintillation counter, in such a manner as to draw a "map" of radioactive distribution.¹⁻³ This operation, which is lengthy and painstaking, is difficult to use in practice. Some automatic scanning devices using scintillation counters have been made, which give a picture of radioactive distribution.⁴⁻⁶ They are complicated, costly and difficult to handle.

There is yet another method which eliminates all complicated electronic devices by using, as a radiation receiver, a photographic plate or film. Two techniques have been used to that end:

(a) A picture is obtained by means of a gamma-ray camera, in which the "lens" is a very small diameter hole in the lead wall (gamma-ray pinhole camera).⁷⁻⁹ The value of this method is limited by the poor geometry of such a camera, in which only a very small fraction of the quanta emitted by the source is used for image formation. It would seem that, despite the reinforcement achieved by means of a thallium-activated sodium iodide crystal, it is impossible, with an exposure time sufficiently short to be usable, to obtain an image of a radioactive source having an activity smaller than 1 millicurie per cm^3 . Needless to say, such a technique is not applicable to the amount of radioisotopes used for diagnostic investigation.

(b) One could also use, along with Skanse and Johansson,¹⁰ a lead grid, each opening of which isolates a bundle of gamma rays emitted by the elementary volume of the source which is "seen" by it. These authors used, in addition to the grid, a reinforcing device made up of a large thallium-activated sodium iodide crystal, 5 mm thick. In this fashion, they obtained pictures of radioactive sources, the activity of which, although it had to remain too large, came closer to those compatible with the needs of diagnostic investigation. Nevertheless, the images obtained were of poor quality, due to scattering of the photons caused by the thickness of the reinforcing crystal.

We endeavored to develop a photographic method which also makes use of a lead grid, coupled with a group of such reinforcing devices as to give an image of sufficient definition with an adequately short exposure time and a radioactive concentration low enough to lend itself to biological uses.

PRINCIPLE OF THE METHOD

The lead grid, made up of a regular network of cylindrical channels, is located in front of a radioactive source, and an X-ray film is placed against the surface of the grid, on the opposite side from the source. The gamma rays emitted by the source darken the film in line with each channel. If the thickness of the grid, and thus the collimation, is adequate, the degree of darkening, in front of each channel, depends only on the activity present in the elementary volume of the source geometrically "seen" by the channel.

Thus, a regular pattern of circular spots is obtained on the film, which, taken as a whole, makes up an image of the projection of the source on the plane of the emulsion.

According to Mayneord,¹¹ the high speed radiological film and the high voltage reinforcing screen used with it must receive approximately 5 mr of X-rays produced by a potential drop of about 100 kv, in order to obtain a darkening of unit photographic density. Now, according to Mayneord and Sinclair,¹² a point source of 1 mc of I^{131} delivers, at a distance of 1 cm, 2.25 r/hr. From these figures, it can be computed that, with a grid 20 mm thick, the cylindrical channels of which are 2 mm in diameter, and a homogeneous radioactive source 2-cm thick having

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a radioactivity of $10 \mu\text{c}$ per cm^3 , unit darkening is achieved with a 5-hr exposure time.

It was shown experimentally, with a "Kodak Regulix" film and a reinforcing screen of the "sinegran ultra" type, that the exposure time needed to achieve unit darkening took about 15 hours. This difference may be due to the fact that the average radiation of I^{131} has an energy of approximately 400 kv, rather than 100 kv.

In so far as such an exposure time is not usable *in vivo*, we endeavored to reduce it by sensitizing the radioactive films by means of mercury vapor in a perfectly sealed space, at 24°C and 50% relative humidity, for 4 hours. This multiplies the sensitivity of the radiological film by a factor of about 4. Thus we have been able, with a 15 mm thick grid, the channels of which were 2 mm in diameter, to obtain, with a 3-hr exposure, acceptable pictures of the thyroids of Graves' disease patients, which had fixed $400 \mu\text{c}$ of I^{131} .¹³⁻¹⁵

However, the fixation of $400 \mu\text{c}$ by the thyroid still remained much higher than acceptable tracer doses, and an exposure time of 3 hours was too long for practical use, so that we endeavored to increase the sensitivity of the system by substituting a thallium activated sodium iodide crystal for the reinforcing screen. Instead of using a single large crystal outside the grid, as did Skanse and Johansson, we set a small NaI(Tl) crystal in each channel, making it possible to obtain a picture of adequate definition.¹⁶ The experiment showed, that, with a prismatic crystal of NaI(Tl) , 6 mm long, at the end of an 18 mm long channel, 2 mm in diameter, against which the X-ray film is located, the sensitivity is multiplied by a factor of some 30 over that achieved by means of the "sinegran-ultra" reinforcing screen.

DETAILS OF THE EXPERIMENT

Since the cutting of a great number of small crystals was a lengthy, painstaking and difficult operation, we tried out a liquid scintillator, in the form of a 5 gm/litre solution of paraterphenyl in xylene. With

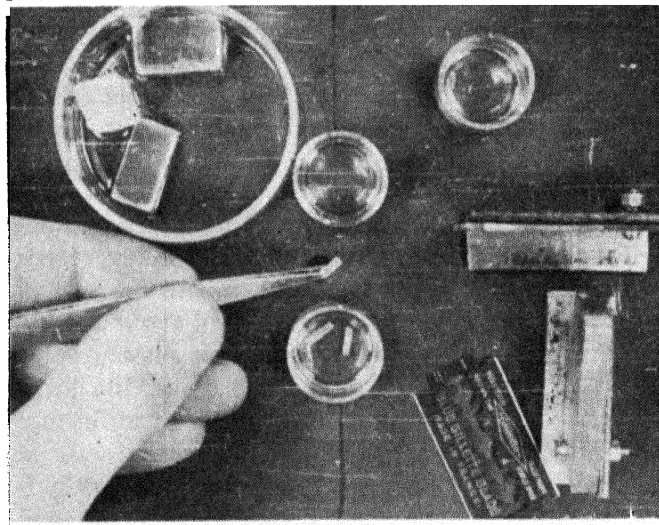


Figure 1. NaI crystals

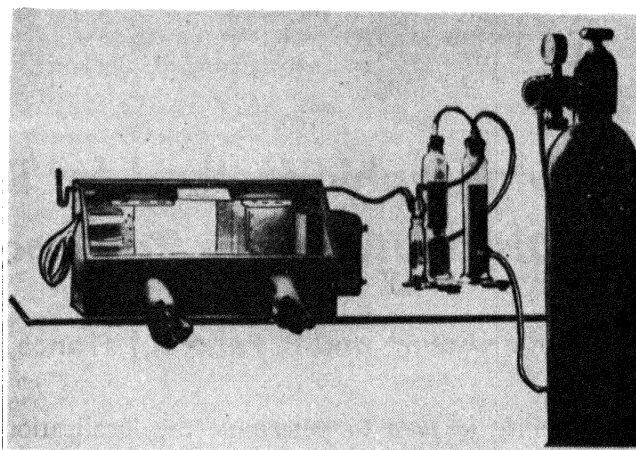


Figure 2. Preparation of crystals under dry nitrogen pressure

channels 18 mm long and 2 mm in diameter, we failed to obtain much better results than those given by the reinforcing screen, even where we used a one cm thickness of such a scintillator. This could be accounted for by the relatively low efficiency of liquid scintillators with respect to gamma rays and their small density.

The efficiency of calcium tungstate crystals is excellent but, in view of the fact that they do not crystallize in the cubic system, they do not allow of cleavage in the simple form of small, rectangular, prismatic crystals. On the other hand, the making of a great number of identical small crystals of such a material by a mechanical method does not appear worthy of practical consideration.

Finally, we resorted to sodium iodide which, although it is hygroscopic, crystallizes in the cubic system and readily lends itself to right angle cleavage. Thus, we prepared NaI(Tl) crystals in the form of rectangular parallelepipeds, the square sections of which could be inscribed in a circle 2 mm in diameter. Cutting was by cleavage to the required dimensions, by means of carefully-made metal templates (Fig. 1). This operation was carried out in a perfectly tight dry box, in a nitrogen atmosphere dehydrated by phosphorus pentoxide (Fig. 2). The crystals used in making up a grid all come from one single crystal and are made as homogeneous as possible. By means of a small test grid, it is possible to test photographically the crystals in groups of 30 on a large, flat, homogeneous, radioactive source. All crystals, which, for a variety of reasons, (e.g. differences in the dimensions when cutting, lack of homogeneity, etc.) fail to give a darkened spot identical, or close enough to identical, to that given by the crystal which has been set permanently as a standard, are rejected. Thus, with enough patience, it is possible to obtain enough crystals which give under similar conditions, identical photographic results, and can make up the grid. This matter of the homogeneity and photographic efficiency of the crystals, which evidently is fundamental to achieving an adequate picture, is one of the greatest difficulties encountered in the making of such a grid.

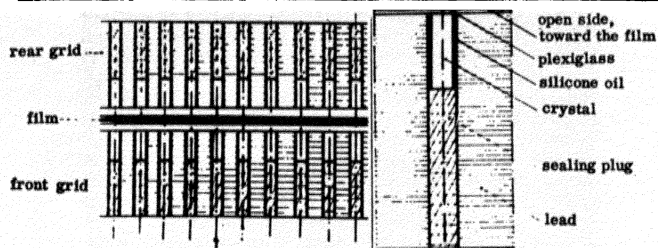


Figure 3. Positioning of X-ray film

This led us to make two small experimental grids, 18 mm thick, the lattice of which consisted of 15 rows of 15 holes each, thus 225 holes in all, with each hole at the top of a 5 mm square. Each channel had a diameter of 2 mm. The two 10% antimony lead grids were drilled together. X-ray film (Kodak Regulix) was placed between the two grids and separated from each channel by a plexiglass plate 0.8 mm thick, sealed against the lead surface with araldite (Fig. 3).

The canals are filled up with dehydrated silicone oil, dehydration being achieved by heating under high vacuum. This silicone oil ensures optical contact with the plexiglass on the side of the crystal which is turned toward the film, as well as the keeping of the crystals. Each crystal is kept in position in its channel by means of a plug. Finally, the two sides of the grid which face away from the film are plugged by a sheet of aluminium sealed with araldite against the lead. Figure 4 shows a photograph of the side of one of the two grids, on which the crystals can be seen in their channels.

Experience shows that this dual grid device, which was suggested by its similarity to a reinforcing screen, affords a sensitivity gain of 50%. Let it be noted, finally, that the 2 mm diameter has been taken for the channels, since it is small enough to give a hole density compatible with a suitable picture, yet large enough to cut the crystals used without undue difficulty.

RESULTS

Figure 5 shows a 40 cm³ "glass thyroid," which contains 400 microcuries of I¹³¹, namely 10 micro-

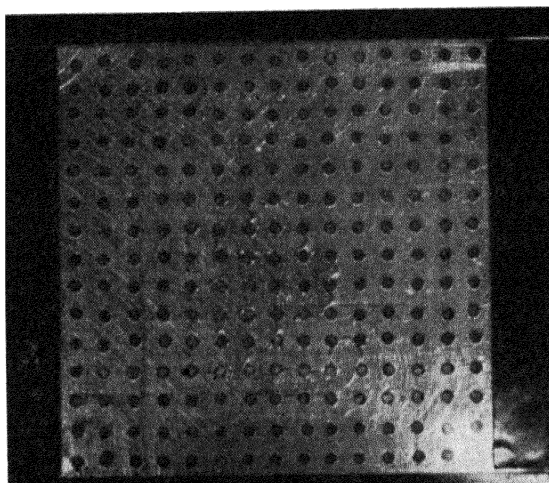


Figure 4. Side of one grid, showing crystals in channels

curies per cm³, and the picture obtained with a 4-minute exposure on mercury vapor sensitized Kodak Regulix film, the part closest to the source being 2 cm from the grid.

Figure 6 shows the picture obtained where the object is a radioactive ring made up of an I¹³¹ solution arranged in the space located between two concentric crystallizers having diameters of 6 and 4 cm respectively. Concentration is 10 microcuries per cm³ and the thickness of the solution 1 cm. Exposure time is 15 minutes, with non-sensitized film. It can be seen, in both cases, that the picture is a satisfactory image of the object.

Figure 7 shows the picture obtained of the thyroid in a woman patient 24 hours after the oral administration of 500 microcuries of I¹³¹, the fixation rate then being 50%. The exposure time was ten minutes, with mercury vapor sensitized film. The figure also shows the part of the gland extracted in a subtotal thyroidectomy, as well as a "map" of the thyroid obtained with directional Geiger counter. It will be noted that the gammagraph obtained gives a good picture of the two lobes of the thyroid, and, particularly, that it brings out the presence of a stricture in the middle of the right lobe.

Figure 8 shows the apparatus used in order to obtain a gammagraph in man and the exact photographic superimposition of this over the relevant anatomical region. Figure 9 shows the result of this superimposition.

Let us add that it is possible, using two double grids set in mutually perpendicular planes, to obtain two gammagraphs, the one frontal and the other sagittal, of the thyroid in these two planes, affording a possibility of computing the volume of the gland with a certain measure of accuracy.

CONCLUSION

The method just developed makes it possible photographically to represent the plane projection of a gamma-ray emitting radioactive collection *in vivo*. It requires neither a counting tube, nor any complex electronic equipment, nor a delicate mobile scanning mechanical device. Finally, the picture is obtained with a relatively short exposure, which is entirely acceptable practically, with activity concentrations having an order of magnitude of the type used for diagnostic investigation.

Nevertheless, two factors limit the value of the pictures obtained with this first experimental grid:

(a) In the first place, the collimation achieved by the thickness of 18 mm adopted in the case of the rays emitted by I¹³¹ is still imperfect. The sensitivity of the device is such that crystals located outside the projection of the source on the grid give spots of appreciable photographic density, thus reducing the sharpness with which the picture can be seen.

(b) In the second place, the relatively broad spacing of the channels on this experimental grid reduces the sharpness of the pictures.

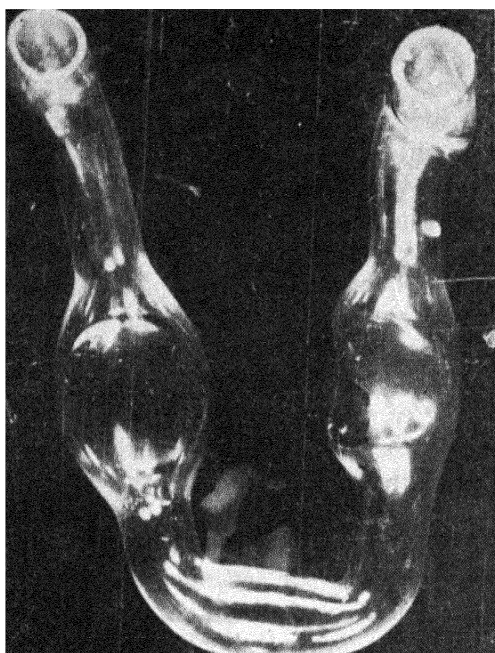


Figure 5. Glass "thyroid"

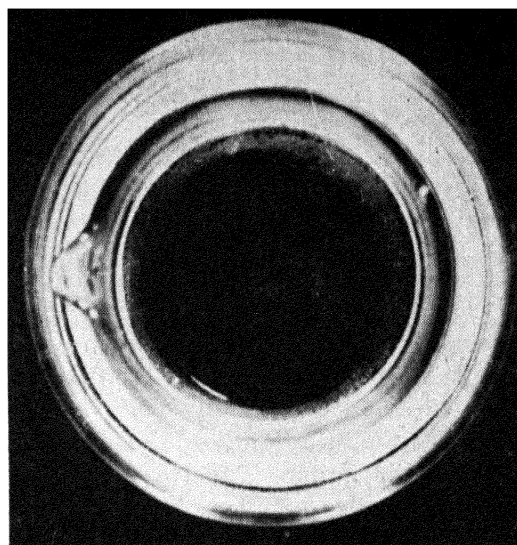


Figure 6. Radioactive ring

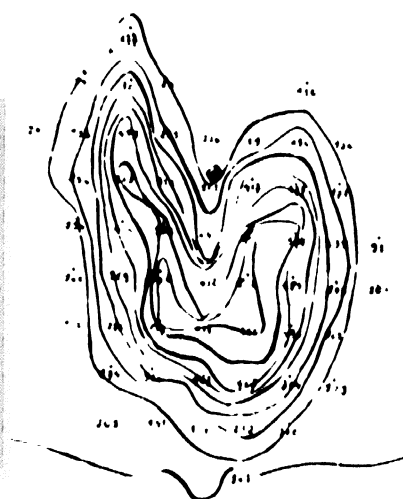
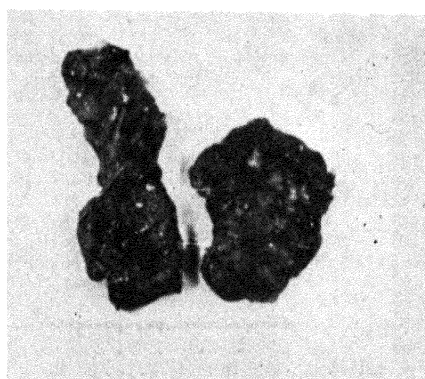
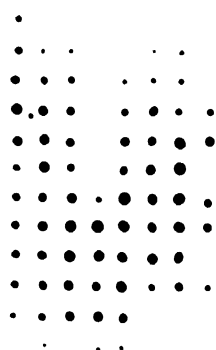
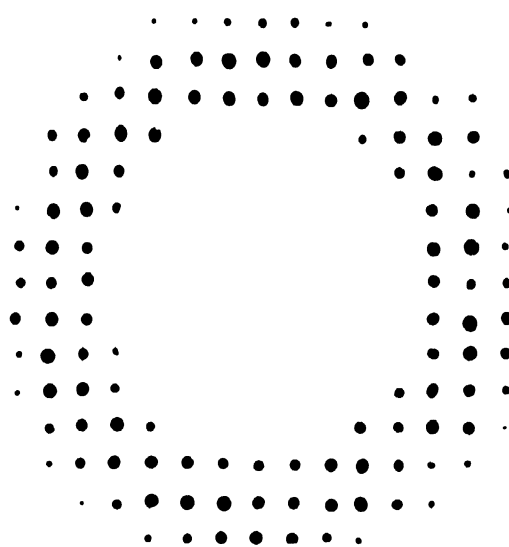
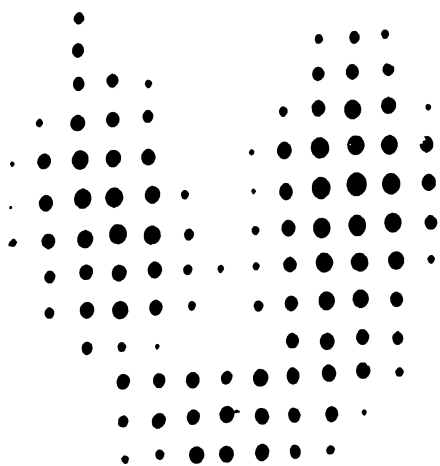


Figure 7. Picture of human thyroid

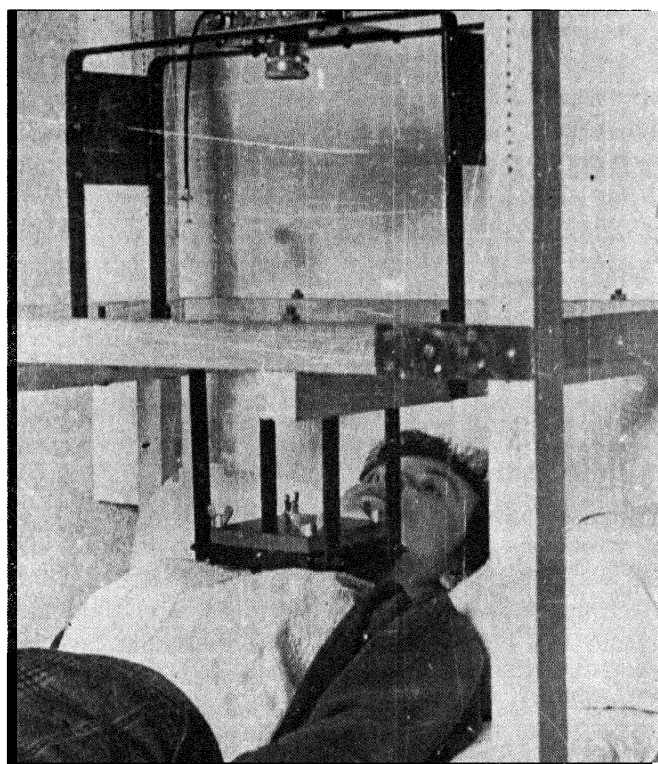


Figure 8. Gammagram apparatus

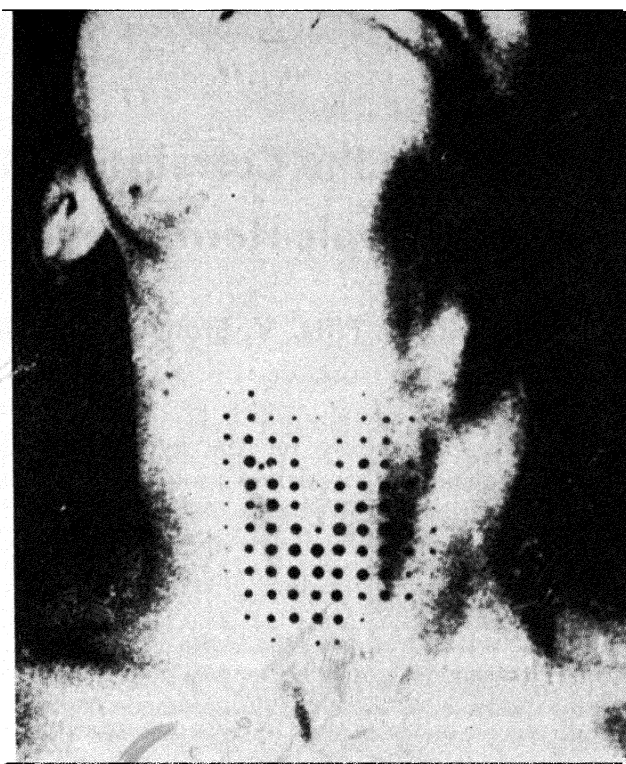


Figure 9. Gammagram made in Fig. 8

We propose to achieve, by improving the production technique used to make the crystals, a thicker and larger grid affording better collimation, with a much tighter channel lattice. We think we will compensate for the loss of sensitivity due to the increase in collimation, (needed if the lattice is tighter), by increasing the light collection factor used with the crystals. The order of magnitude of the latter, in the case where polished surface crystals, as in our experimental grid, are placed in a lead wall container and immersed in silicone oil, is difficult to compute. We believe we will succeed in increasing it by making a wall, for lodging the crystals, having a higher scattering factor for the light quanta of NaI(Tl) fluorescence.

Finally, the application of the method which we used so far has to do only with the ideal case of the investigation of thyroid function by means of I^{131} . We propose to apply it to some other types of investigation in man with the same isotope, as well as to other gamma-emitting isotopes used in medicine and physiology.

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Research into the Correlation Between the Thyrotropic and Corticotropic Hormones

By C. I. Parhon, M. Pitis, V. Stanescu, V. Ionescu and S. Segal, Romania

For the endocrine glands, which produce several hormones, and particularly for the hypophysis, a series of interglandular correlation problems appear. One of those refers to the gland's ability to secrete a group of hormones over a given period during which, at the same time, it is stimulated to secrete another group of them.

Selye¹ has introduced the hypothesis of a balance between the various tropic hormones of the hypophysis on the basis of the observations made on animals subjected to various forms of stress. In these animals he observed an increase in the secretion of corticotropic hormones and, at the same time, a decrease in the secretion of the thyrotropic and gonadotropic hormones.¹

However, the hypothesis of a balance between the tropic hormones of the hypophysis has not been verified in each and every case. Under the influence of cold, the thyrotropic and corticotropic hormones have been observed to increase simultaneously. Following the administration of insulin, Milcou and Pitis² obtained, in the rat, a histological picture of stimulation of the adrenals and thyroid, caused by the administration of certain doses at given moments. Ferrari and his group³ observed that the rate of thyroid fixation of I^{131} increases in hypoglycemic shock, in the guinea pig, but is not changed in the rat.

One of the surest ways of specifically bringing about a production of tropic hormones of the hypophysis is partially to remove the peripheral gland considered. In this fashion, one unleashes a mechanism of compensatory hypertrophy in which the mediation of the tropic hormones of the hypophysis is well proven.

We used this method to bring about secretion of TSH by the hypophysis, as well as that of the corticotropic hormone, by carrying out a hemithyroidectomy and unilateral adrenalectomy, and we explored thyrotropic activity by evaluating the degree of fixation of I^{131} in the remaining thyroid lobe.

METHOD

We used 4 groups of male white rats weighing approximately 200 gm each. The rodents of the first group were subjected to unilateral adrenalectomy under ether anesthesia. In the second group, a hemi-

thyroidectomy was carried out, and the third was subjected to hemithyroidectomy plus unilateral adrenalectomy; the animals of the fourth group were retained as controls. Four days after the operation, 5 microcuries of I^{131} were injected into each rat by the intraperitoneal route. Twenty-four hours after injection the animals were sacrificed, and then the thyroid lobes were weighed, and thereafter hydrolyzed, in 40% NaOH, under the action of heat, for 2 to 3 hours, and then the radioactivity of the samples was determined with the help of a Geiger-Müller counter.

RESULTS

It will be noted that fixation, in a thyroid lobe, is 7.53% for the unilaterally adrenalectomized animals, 23.48% for those hemithyroidectomized, 34.91% for those unilaterally hemithyroidectomized and hemi-adrenalectomized, and that the radioactivity of the thyroid lobe, in the non-operated controls, was, on an average, 16.6%. From the statistical viewpoint, the differences obtained are valid. The adrenals have suffered a compensatory hypertrophy, from 17.5 to 20.7 mg in the hemithyroidectomized animals and in those unilaterally adrenalectomized. In the unilaterally adrenalectomized rats, it was from 19.8 to 23.3 mg.

DISCUSSION

Our data show that hemithyroidectomy increases I^{131} fixation in the remaining lobe, with respect to the non-operated controls. These results were obtained by stimulating the thyrotropic hormone.

Unilateral adrenalectomy brings about a decrease in the fixation of I^{131} by the thyroid. This effect is due to the stimulation of corticotropic hormones which brings about an increase in the secretion of the metabolic hormones of the adrenals of the glucido-proteid type. It was shown that these hormones reduce the fixation of I^{131} in the thyroid.⁴ However, there are data according to which stimulating the corticotropic hormone, even in the absence of the adrenals, reduces the fixation of I^{131} by the thyroid⁵ by reducing TSH secretion. The exogenous corticotropic hormone also reduces the fixation of I^{131} , even in the adrenalectomized animal.⁶

The simultaneous stimulation of the corticotropic and thyrotropic hormones by partial extirpation of

Original language: French.

the adrenals and the thyroid brings about an increase in the thyrotropic fixation of I^{131} . At the same time, one notes an increase in the average weight of the adrenals, from 17.5 to 20.7 mg, which indicates that there has been a stimulation of the corticotropic hormone at the same time.

Our results show that, aside from stimulation by cold, there is another experimental condition in which the thyrotropic and corticotropic hormones are simultaneously secreted in excess.

The balance of secretion of the two tropic hormones thus does not apply in the cases where specific stimulation of TSH and stimulation of the corticotropic hormone take place at the same time. The hypothesis of a common precursor for both hormones,⁵ which the hypophysis possesses in limited quantities, is not in agreement with these cases.

As for the adrenals, we have had to study the enzymatic systems, and their possible competition or synergism under various circumstances. The correlations between the two tropic hormones of the hypophysis studied by the method of the combined compensatory hypertrophies, must be examined at various intervals, beginning at the operation. Similar

investigations must be carried out as regards the relationships between the other tropic hormones of the hypophysis.

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Incidence of Malignant Diseases and Need for Radioactive Isotopes in Pakistan

By **Abdur Rehman, Pakistan**

Radiation therapy, especially the use of isotopes, will be a step forward towards the advancement of medical science in Pakistan. There is, therefore, a need for isotopes for research and treatment of malignant and other diseases.

It may be stated that the incidence of carcinoma in this country is not as high as in some of the western countries. Most people in this country do not reach cancer-bearing age i.e., above forty years in the same proportion as the people in the west, owing to low expectancy of life, and therefore tissue senescence is proportionately excluded of its predisposition to cancer. Furthermore, industries of various forms are reported to have added to the cancer incidence in the west. Dye industry is predisposed to vesical cancer, shale oil industry to various forms of skin cancer. Coal industry is impregnated with potentialities for various types of carcinoma and mines with their radioactive dusts give rise to bronchial malignancy. Absence of these industries in the past in this country or their presence in a very small scale, have reduced the possibilities of industrial cancer in Pakistan. Nevertheless, numerous cases of cancer are known, and are possibly on the increase.

Table I.

1. Laryngeal	17
2. Malignant lower jaw	10
3. Carcinoma of breast	12
4. Carcinoma of penis	11
5. Carcinoma of stomach	8
6. Malignant parotid	6
7. Carcinoma of tongue	6
8. Carcinoma of rectum and anal canal	6
9. Pharyngeal carcinoma	4
10. Carcinoma of colon	4
11. Malignant testes	4
12. Fibro sarcoma of muscle and fascia	4
13. Malignant liver	4
14. Carcinoma of malignant upper jaw	4
15. Carcinoma of lip	3
16. Carcinoma of cheek	3
17. Epidermoid carcinoma of skin	3
18. Cancer thyroid	3
19. Carcinoma of lung	2
20. Malignant brain tumour	2
21. Rodent ulcer	2
22. Hyper-nephroma	2

Between January 1952 to September 1953, 4069 surgical cases were admitted in the Surgical Wards of the Dacca Medical College Hospitals, East Pakistan. Of these, 120 cases were malignant diseases. Thus 2.9 per cent of all surgical cases admitted in the hospitals were cancer, excluding the malignant diseases of the female genital tract.

The above mentioned 120 cases are classified in Table I.

Various etiologic and predisposing factors probably operate in the production of cancer in Pakistan. It is evident that carcinoma of the larynx is the commonest type of cancer in East Pakistan, probably due to chronic irritation of mucous membranes in the ary-epiglottic region by hot spiced food of this province.

Carcinoma of the lip and the mouth: also fairly common particularly in those who take "Khaini" or who indulge in chewing betel nut.

Carcinoma of the stomach: the next most common cancer. In the smaller number of cases it arose as a sequela to gastric ulcer incidence which is also fairly high in Pakistan due to the use of hot spices in food.

Carcinoma of colon: in this country chronic colitis is a common disease usually due to chronic amoebiasis but carcinoma of the colon is not proportionately higher.

Epidermoid carcinoma: epidermoid cancer commonly described in aniline dye workers or chimney sweepers is seen in East Pakistan amongst people who walk barefooted and may develop small ulcers on feet. These ulcers, due to lack of sufficient attention, turn chronic and may lead to malignancy.

For the effective control of cancer in Pakistan it is necessary that diagnostic clinics and detection centres should be started which should be equipped with the following modern instruments to detect early cancer: (a) apparatus for detecting the difference in electropotential of the malignant cells as compared with the same of the normal tissue cells of the host; (b) radioactivated chemicals for the location of cancer cells with the help of G.M. and scintillation counters; (c) electron microscopes for the ultra magnification of the malignant cells; and (d), radio-therapy units where all facilities for treatment by deep X-ray, radium and isotopes should be available.

Against the background of these requirements for the control of the malignant diseases, the present position of Pakistan is far from satisfactory. In some of the hospitals facilities are available for the administration of deep X-ray to these cancer cases. Radium has also been used in some hospitals mainly for cancers of lip, cheek, tongue, larynx, breast, uterus, vagina and vulva. It is regretted that, for various reasons, cases were not properly followed up, so that definite opinion cannot be given regarding the recurrence of the disease after the above treatment. Radioisotopes, not being available in this country, have not been used so far. In order to take advantage of the new techniques of treatment to patient, the following isotopes, if available, may be of great help to us: I^{131} (the quantity required is 20,000 millicuries per annum), cobalt-60, radioactive colloidal gold and radio chromic phosphates.

Besides the above active isotopes, super voltage X-ray therapy betatrons are also not available for use in Pakistan. A scheme for having personnel trained in the above therapy techniques and apparatus installed in Pakistan will contribute towards the future improvement of the treatment of cancer patients.

Besides the problem of cancer, leukaemia and polycythaemia vera had a statistical incidence of 30 cases from January, 1954, to June, 1955, in the Dacca Medical College Hospitals. In view of these statistics, the amount of P^{32} which could be used for the treatment of these diseases at present in Pakistan is about 2000 millicuries annually.

In Pakistan there are endemic areas for goitre and a very high percentage of these goitres undergo thyrotoxicosis secondarily apart from the number of primary thyrotoxicosis. In treating these cases on modern lines I^{131} should be made available to the

extent of 1500 millicuries per annum. In spite of differences of opinion about isotope treatment of Graves' disease, I believe Pakistan has a wide field for its clinical trial to assess its value in therapy.

The application of isotopes for clinical diagnosis is no doubt in its experimental stage, and it is believed that it can be profitably used to corroborate the diagnosis and to indicate the line of treatment for the following diseases:

1. To ascertain early metastasis of carcinoma of thyroid and testes.

2. To ascertain the malignant changes that may occur to goitres, specially nodular goitre.

3. To ascertain the circulation time in vascular diseases—Raynaud's diseases, thrombo-angitis obliterance.

All these diseases are fairly common in Pakistan. So, P^{32} and I^{131} are again helpful for the above purposes.

Metabolic studies: to understand the dynamic constituents of the body with the help of tracer substances extensive work has been done in many centres of research in western countries. In Pakistan too, some of the biochemical and physiological laboratories are in a position to start work on similar lines as soon as tracer substances are available here. For this purpose tracer substances like isotopes of potassium, heavy hydrogen, radioactive sodium calcium and iron will be necessary.

SUMMARY

Requirement of isotopes come under the following heads: (1) cancerous: diagnostic, P^{32} , I^{131} ; therapeutic, Co^{60} , radioactive gold, radioactive chromic phosphate; (2) non-cancerous: diagnostic, I^{131} ; therapeutic, P^{32} , I^{131} ; research, radioactive Na, K, heavy hydrogen, radioactive calcium and iron.

Record of Proceedings of Session 9C

FRIDAY MORNING, 12 AUGUST 1955

Chairman: Mr. V. R. Khanolkhar (India)

Vice-Chairman: Mr. F. Hercik (Czechoslovakia)

Scientific Secretaries: Messrs. I. D. Rojanski, C. Polvani
and L. F. Lamerton

PROGRAMME

P/768	The use of radioactive isotopes in diagnostic procedures	W. V. Mayneord
P/684	Experience in clinical and diagnostic application of some radioactive isotopes in the USSR	M. N. Fateyeva
	DISCUSSION	
P/181	Scanning of positron-emitting isotopes in diagnosis of intracranial and other lesions	G. L. Brownell and W. H. Sweet
P/754	The dynamic aspects of erythropoiesis in normal and pathologic states as indicated with the radioactive isotope tracer technique	J. F. Ross <i>et al.</i>
	DISCUSSION	
P/769	The speed of iodine metabolism	E. E. Pochin
P/9	Further clinical studies of thyroid and salivary gland function with radioiodine	C. H. Jaimet and H. G. Thode
P/365	The part played by the genetic factors in the development of congenital myxedema	M. Bernheim <i>et al.</i>
P/1099	The effect of estradiol on iodine fixation in the thyroid (studied by means of radioactive iodine I^{131})	M. St. Milcou <i>et al.</i>
	DISCUSSION	
P/199	Tissue specific antibodies as carriers of radioactive materials for the treatment of cancer	W. F. Bale and I. L. Spar

The CHAIRMAN: This morning's session as you know, is to consist of a continuation of papers on radioactive isotopes and nuclear radiations in medicine, with emphasis on diagnosis and studies of disease. Our program is very crowded, and I shall curtail my remarks and merely mention that we have a large number of papers covering a wide area of knowledge on various facets of the subject.

As Mr. Mayneord points out in his opening paper, the use of radioactive isotopes is already playing a part in the reshaping of the diagnostic art. Naturally it is important to know the ways in which the isotopes have been useful in this direction, as well as to realize the inherent difficulties and shortcomings of the techniques. The papers during these sessions give an excellent review of some of these problems, and I am sure that you, like myself, look forward to listening to them.

Mr. W. W. MAYNEORD (UK) presented paper P/768.

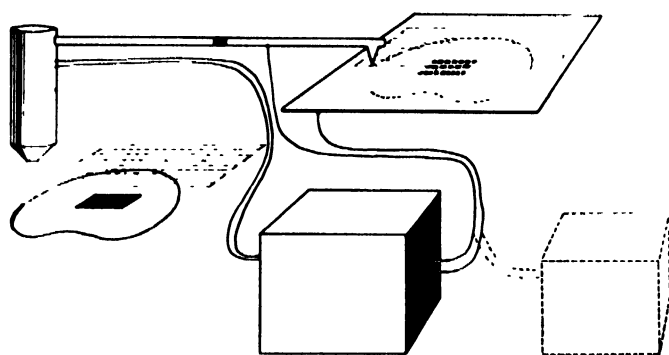
Mrs. M. N. FATEYEVA (USSR) presented paper P/684.

DISCUSSION OF P/768 AND P/684

Mr. ZIEDESSES DES PLANTES (Netherlands): I should like to ask Mr. Mayneord's opinion about the procedure by which a drawing of the outlines of the examined part of the body is obtained. In our Institute the procedure has been used which is schematically shown in Slide 1.

After or before the scanning, which is schematically drawn, a source of alternating current is connected to the registering apparatus (as is indicated with dotted lines) and a pointer is attached at the collimator of the counter tube, and then after we have obtained the scanned picture we can move the counter tube over the outlines of the parts of the body and we get a picture of these outlines superimposed on the scanned picture. We showed the procedure at the Sixth International Congress of Radiology in 1950, but published only in the proceedings and not in the journal.

Slide 2 shows a patient with thyroid cancer on whom the procedure has been applied.



Slide 1

In Slide 3 you see the result. You can see the outlines of the tumour, the head, neck, shoulders and clavicularae.

Mr. M. ROCHE (Venezuela): I was interested to hear that endemic goiter had been investigated by means of radioiodine in the USSR, although no details were given. I wonder if Mrs. Fateyeva could give us some of those details.

I would like to take this opportunity to show three slides illustrating a few of the results obtained by us in the Venezuelan Andes.

The interrupted, bell shaped curves on Slide 4 are intended to illustrate the distribution of 48-hour iodine uptake by the thyroid in Boston, USA, as given by Skanse. On the left side of the figure, you see this distribution in 70 euthyroid subjects in Caracas, the capital, as determined by Dr. Francisco de Venanzi and by me; the average is not shown here, but it is about 34% of the dose administered. On the right side of the figure, in Bailadores, a small town in the Andes, we have an average uptake of 74% and, as you can see, the distribution curve is shifted markedly to the right. A large number of the subjects exhibit uptakes of more than 80%, and some above 90%. There is no significant difference in this respect between subjects who had goiter and those who had not. In the group without goiter we made sure that the thyroid was not even palpable on careful examination, and yet many of these people had very large uptakes. I must emphasize that clinical and laboratory studies carried out simultaneously indicated that most of these subjects were euthyroid or slightly hypothyroid. The average BMR was around -15%.

In Slide 5, we see the distribution of 48-hour urinary excretion of radioiodine. As expected, this is generally very low, and again there is no significant difference between goitrous and non-goitrous subjects. Thus practically everyone in this region has an increased avidity of the thyroid for iodine; some have had to develop thyroid hypertrophy in order to carry out this efficient iodine extraction, and others are able to carry out this process without apparent anatomical hypertrophy.

We were also interested in finding out what the status of the iodine in the thyroid was, that is whether it was bound to protein or not. For that

purpose, we gave potassium perchlorate to a group of subjects on the fourth hour after I^{131} administration. In Slide 6 we may see at A the continually climbing uptake over a 7-hour period in a control group without perchlorate; at B, the flattening out of the curves after 100 mg of perchlorate in another group, and at C and D the decrease in thyroid radioactivity in the majority of those given 300 and 500 mg of perchlorate respectively. These curves are interpreted as meaning that, in this particular endemic goiter region, part of the iodine in the thyroid is in the unbound form, although what this peculiarity means is at present obscure. For purposes of comparison, six normal subjects from the expedition were given 300 mg of perchlorate. The resulting curves are shown as dotted lines.

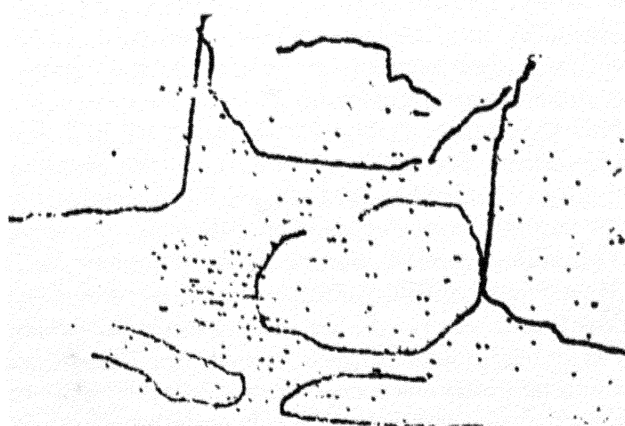
The CHAIRMAN: There are four questions put to Mr. Mayneord and one to Mrs. Fateyeva.

Mr. MAYNEORD (UK): The first question is from Mr. Sweet (USA)—“What ratios of activity between brain tumour and normal brain have you noted, with what compounds of what isotopes?”

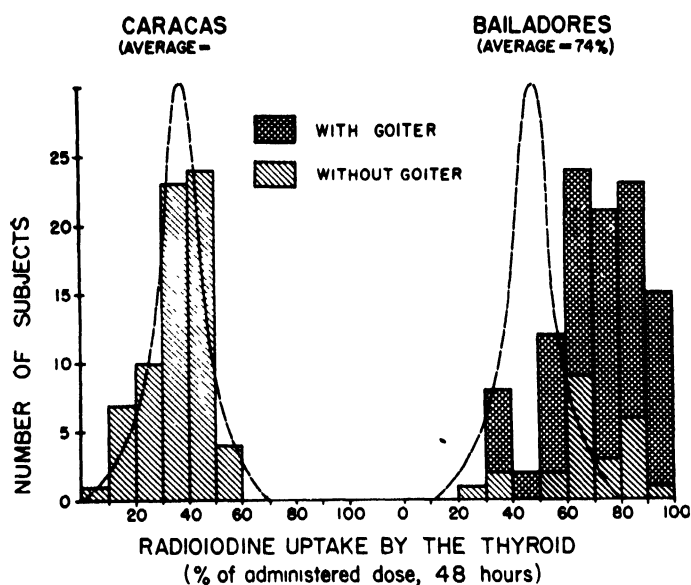
There are three variables and so there must be quite a large number of solutions. First of all, what



Slide 2



Slide 3



Slide 4

ratios of activity? The ratio of activity between brain tumour and normal brain is a very variable number. As far as I can judge, it depends so much on the specimens which you have, and upon the type of tumour. We have not yet collected all our results but my impression is that the ratios might be of the order of 5 to 30, say, with phosphorus. It is not my subject, but so far as I can judge I do not think we could make any firm correlation with the pathology. The ratio is commonly of the order of 10, and this, unfortunately, in view of the very low concentration in normal brain, is not usually sufficient for our purposes, as I am sure you agree.

As to the isotopes and compounds and dose picture, I am sorry I have not the details. Perhaps if there is time Mr. Trott, who is here, could say something about the ratios of activity between brain tumour and normal brain and the compounds of the various isotopes that were employed.

The next question is from Mr. Bacq, "Don't you think that it is also useful to know in which form I^{131} is circulating in the blood, organic, inorganic, thyroxine or 3-iodothyroxine, the last substance being probably the active form of the hormone?"

My answer is emphatically "yes." I wish I knew how to find out all this.

The next question is from Mr. Lawrence: "How sensitive is your scanner for the detection of gamma rays in microcurie amounts?"

This depends of course on the aperture and geometry. There are some very curious things about this, but my general result is that if the concentration, let us say, for colloidal gold or iodine or something of reasonable gamma energy, is of the order of 1 microcurie per millilitre then you will see the shape outlined quite clearly. If the value goes to $0.2 \mu\text{C/ml}$, you will begin very largely to lose the shapes but you will be pretty sure that there is something there. If you drop to one-tenth or one-twentieth of a microcurie you will find yourself with an analysis to find

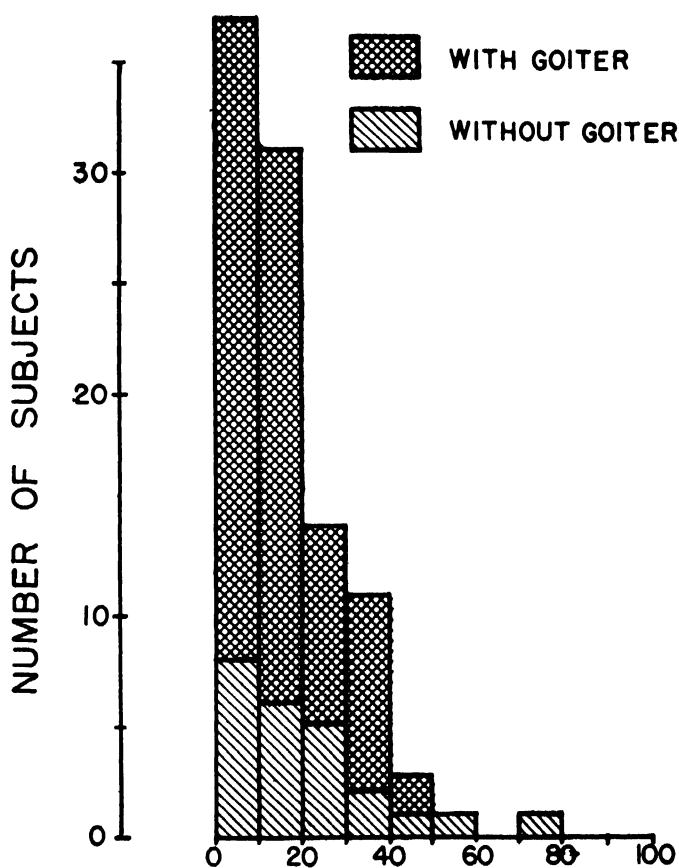
out whether it is a random distribution. This raises very interesting philosophical questions as to what is the real basis of our judgment in a number of matters as well as medical diagnosis specifically. I think it is very interesting to look at this problem from the point of view of the quantum limitation of radiation which nature has set for us and which we shall never surpass.

May I add one other thing? The absolute sensitivity of the counters is now quite high. For iodine under normal conditions we estimate about 37 per cent. I think that is our best measurement of the absolute efficiency, that is, the ratio of the number of counts to the number of quanta falling on the crystal. If we use low energy radiations this number can go up to 85%, for example with thulium or xenon radiations. We are therefore approaching the theoretical limits, and this rather worries me; we cannot go much further.

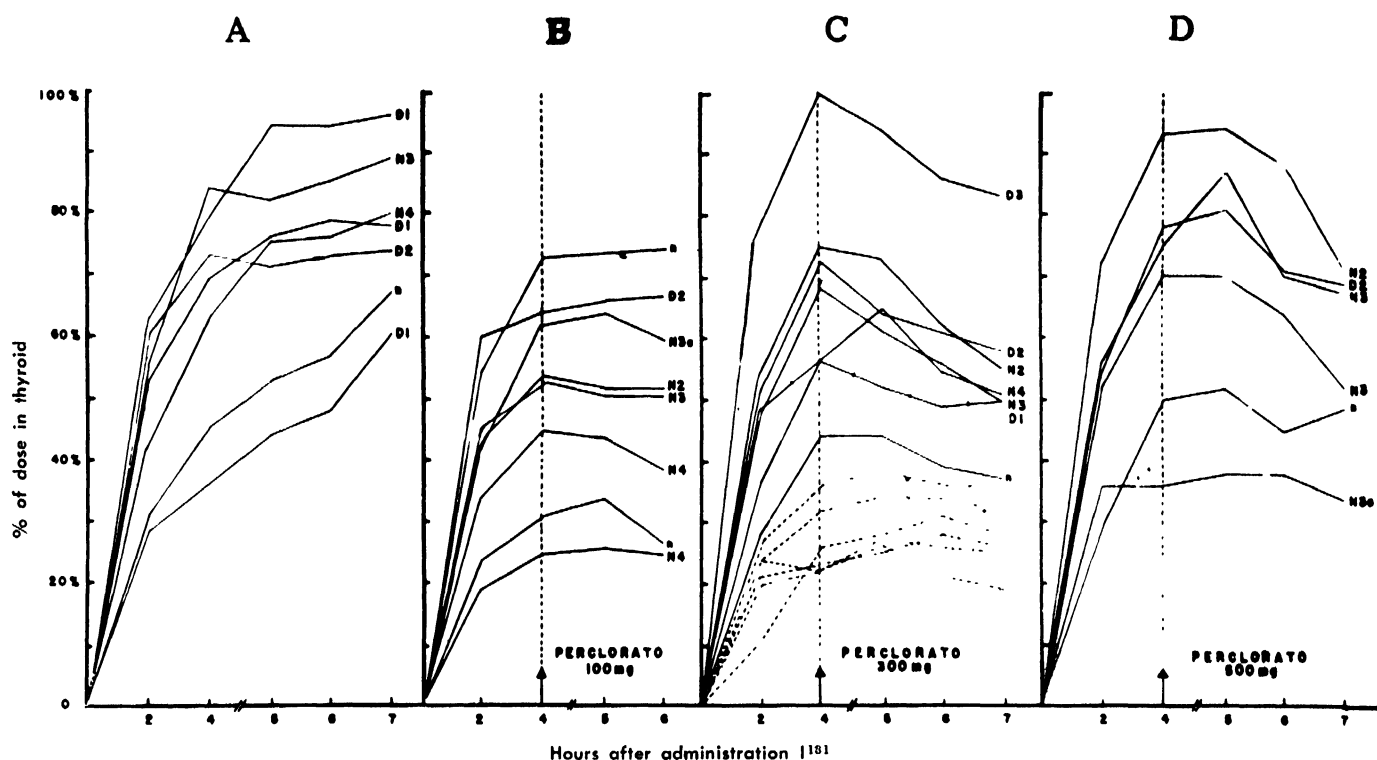
The next question is from Mr. Muller: "Could not the diagnostic radiographic sources offer particularly good possibilities for high resolution tomography, for instance of skull parts?"

Yes, that is true. A number of years ago during the war I tried a very simple tomographic technique which did work admirably on these things, but I only used it on small mechanical objects.

May I say that this scanning method is a very powerful one which we now intend to take first of all into the field of X-ray spectroscopy. It can be used for ultra-violet or infra-red microscopy, or



Slide 5



Slide 6

indeed for making images, if you wish, of ultrasound—it is a very general method.

Mrs. FATEYEVA (USSR): I can give information about two areas of the Soviet Union in which the disease is endemic and in which, in addition to clinical and statistical research, thyroid function has been studied with the help of radioactive iodine. These areas are a district in the Urals and the Chernovitsky region of the Ukrainian SSR.

The clinical picture of these two endemic forms differed. In the Urals, where the disease is marked by mild hyperthyrosis, the clinical picture showed a high uptake of radioactive iodine by the thyroid gland in the patients studied. This high uptake was noted even when the gland itself was not enlarged. But when this fact was related to the clinical picture, a precise diagnosis could be established in every individual case.

In the other area—the Chernovitsky region—hyperthyrosis was a fairly prominent feature of the clinical picture and we found a low uptake of radioactive iodine even where the gland was comparatively large. This was in line with the clinical picture of the disease.

As regards the excretion of radioactive iodine from the kidneys, the relationship is known to be reversed, a fact which our studies in the two areas confirmed. In the district in the Urals a fall in the amount of radioactive iodine excreted from the kidneys was frequently noted, whereas in the Chernovitsky region, where hyperthyrosis is a feature of the disease, we found that the amount of radioactive iodine excreted from the kidneys rose as the uptake of the substance by the thyroid gland increased.

In areas where goiter is endemic, this method gives us valuable guidance in regard to cretinism and early infantile idiocy, since in both these diseases there is no significant change in the uptake of radioactive iodine.

Mr. W. H. SWEET (USA) presented paper P/181 as follows:

Radioactive isotopes have proven useful in the diagnosis of focal disorders of the brain because normal brain presents a barrier to their entrance from the blood which is lacking in areas of focal disease. Sites of high concentration of those isotopes giving rise to gamma rays which penetrate the skull may, we think, be detected. Fundamentally, the problem of external localization of isotopes is threefold: (1) that of obtaining a suitable resolution of measurement of activity while maintaining its intensity; (2) that of securing a high activity ratio between the diseased area and normal tissue; and (3) that of presenting the information effectively.

The scattering of gamma rays as they traverse matter leads, of course, to a blurred, enlarged image at the external detector of a gamma-ray focus within the body. In an effort to exclude the scattered rays from the gamma count, Mr. Brownell conceived the idea of using the paired gamma radiation which arises when a positron undergoes annihilation. Positrons given off in radioactive decay collide within a small fraction of a second with one of the large number of electrons in the matter, as I am sure is familiar to most of you. Their mass is replaced by the energy of two 0.51 Mev quanta, the annihilation radiation. These leave the scene of the collision at 180 degrees, or "back-to-back." A pair of scintillation

counters may now be placed one on each side of the head in the same straight line directed at the mid-sagittal plane and connected to a coincidence circuit. Only when each member of the annihilation gamma-ray pair strikes its detector will a count be recorded; the scattered rays are hence rejected from consideration. Increased resolution is obtained with but slight loss of intensity.

To produce a pictorial record of the data we move the detectors automatically so as to scan the head, much as Mr Mayneord has shown in his automatic scanning equipment; stamp marks appear automatically on paper in our apparatus, one for every 10 coincidence counts, at a point corresponding to their intra-cranial origin.

Slide 7 shows the second model of the positron scanner built by the Physics Research Laboratory of the Massachusetts General Hospital. The patient lies comfortably supine with her head between the two detectors. The writing arm moves in a 1:1 relationship with the detectors so that a life-size picture of the radiation from the head emerges. This is completed by drawing the patient's profile on the paper. To aid that process, the grids on each side are lined so that the individual making the drawing need only follow the lines on the grid and transfer them to this paper.

We next illustrate the advantage of coincidence counting of the positron-evoked radiation over single channel counting of all the gamma rays.

Slide 8 (Fig. 1A of P/181) shows the picture upon coincidence scanning of the positron emitter, arsenic-74, in a phantom consisting of 6 tubes each 2 mm in diameter arranged as a star, and placed to correspond to the mid-sagittal plane of the head. Note the relatively low background and slight enlargement of the 2 mm tubes. Each symbol is 1 cm long.

The single-channel count of the same phantom with I^{131} in the 6 tubes shows in contrast a much higher background with gross enlargement of the image of the tubes Slide 9, (Fig. 1B of P/181).

The positron emitters As^{72} and As^{74} , the latter with a convenient 19-day half-life, have been our most used isotopes to date. Injected intravenously as a mixture of the arsenate and arsenite they concentrate to a useful degree in most brain tumours and brain abscesses.

The coincidence positron scan of the head, positrocephalogram or PCG as we have called it, gives in the normal person the type of picture shown in Slide 10. Over most of the cranial vault the distribution of activity is about the same, with increased density at the base due to uptake in muscle.

Slide 11 is the PCG of a patient with a large benign tumor, a meningioma, overlaying the right motor and sensory cerebral cortex. The precise size and location of the lesion in a side view stand out clearly at a glance.

From the PCG alone one cannot determine the size or depth of the lesion. This information we

glean from an additional type of scan in which we determine the degree of asymmetry of the total (not merely the coincidence) gamma counts. The nearer the lesion is to one of the detectors the greater the total gamma radiation it will encounter, because of the inverse square effect of increasing distance. The apparatus is so arranged that whenever one detector receives enough radiation to yield a specific charge in excess of the other, the stamp mark for that detector is made on the scanning paper and the charge arising from both detectors returns to zero. Arbitrarily we have chosen to indicate right-sided asymmetry with a curved mark, and left-sided asymmetry with a straight mark.

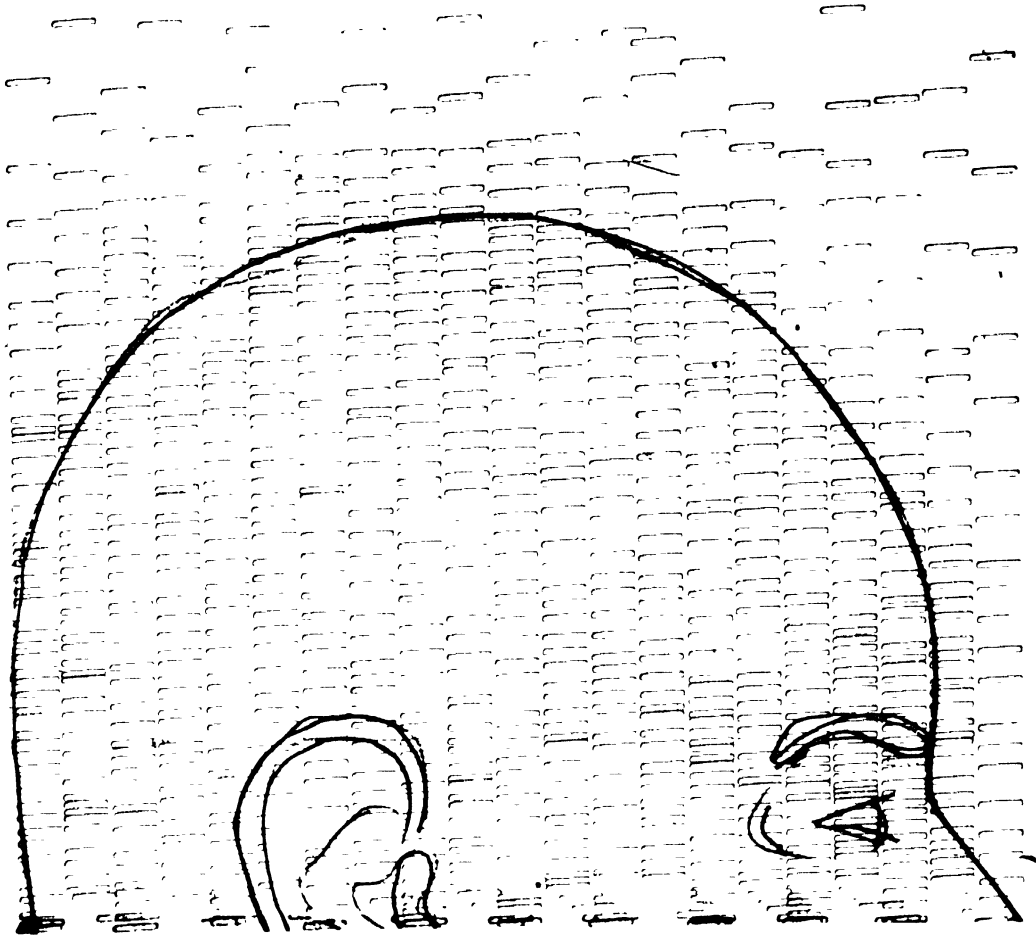
In the normal person with symmetrical disposition of the radioactive substance this scan is virtually a blank.

Slide 12, from the patient with the meningioma giving the PCG of the previous slide, is the simultaneously evolving scan of the asymmetry, the asymmetrogram, or AGG as we have denoted it. The density of the curved marks indicating superficial placement of the right-sided lesion is so characteristic of a meningioma that we are able to surmise this diagnosis in most of the cerebral convexity tumours of this type. This is a point of considerable value to me as a surgeon, because we usually have to be prepared to give such a patient a large amount of blood by transfusion in the course of the operative removal.

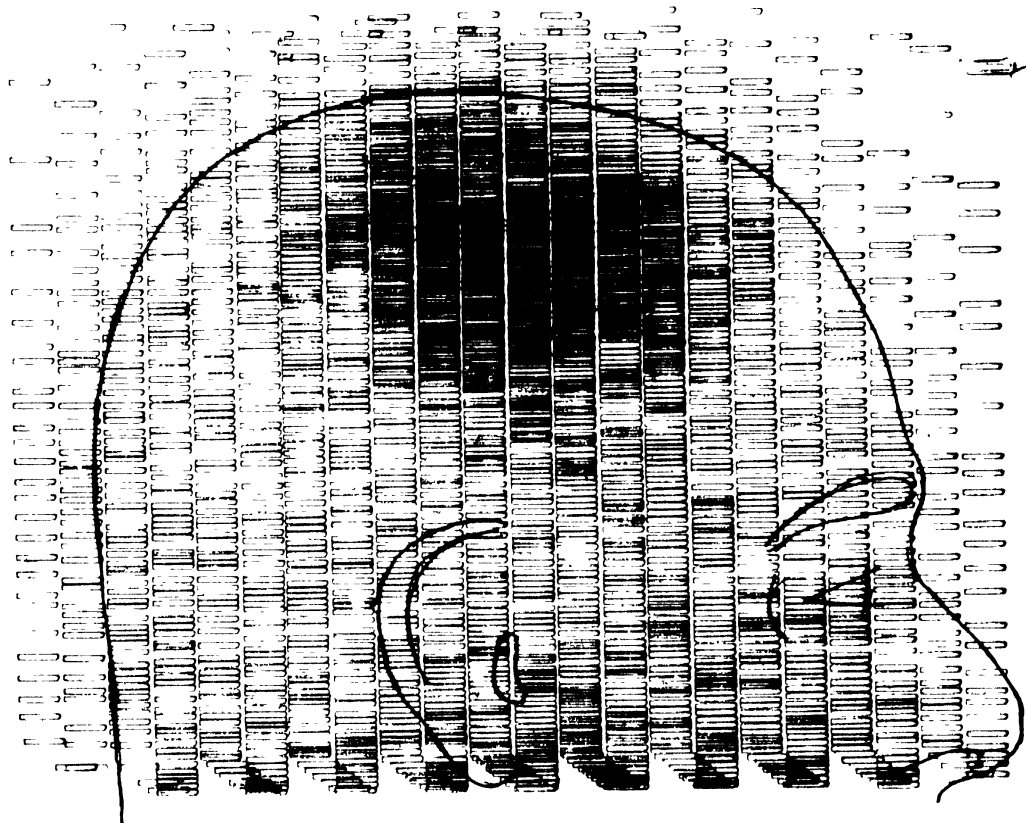
The posterior inferior part of the head deep to the heavy cervical muscles has been a difficult area in which to spot tumors by earlier isotopic methods.



Slide 7



Slide 10



Slide 11

It remains so for us, but we are having increasing success in this region.

Slide 13 is the PCG, the positrocephalogram, of a patient whose clinical symptoms and course were not suggestive of tumour. The striking increased isotopic uptake behind the ear was matched by the equally striking AGG of Slide 14. The density of the right-sided (curved) marks of asymmetry pointed to a position just deep to the bone, the site from which we removed the tumor, another meningioma. This is the first patient in whom we ventured to operate without either of the confirmatory surgical diagnostic measures of arterial or air injection. These are slightly hazardous, and we have been able to dispense with them in an increasing number of cases by virtue of the clarity of the scans.

Multiple tumours may also be depicted. Slide 15 is the AGG, asymmetrogammagram, showing that this patient had two right cerebral tumours. His history of earlier removal of a primary carcinoma of the lung made it likely that these lesions were two carcinomatous metastases and hence not fruitfully operable.

We are especially pleased that the tactic has proven effective in finding small tumours lying in or near the cerebral cortex. The presence of such lesions may be suspected by the occurrence of convulsions or other significant neurological troubles, yet arteriography and pneumography—the surgical diagnostic methods—may fail to reveal the tumour. In such circumstances these favorable neoplasms may grow to irremovable size before their presence is established. We have, to date, diagnosed on our PCG and AGG and then successfully removed three such small tumours even though their preceding air and arterial X-rays were normal.

Slide 16 (Table I of P/181) summarizes our experience with tumours. The scans were interpreted without reference to clinical data and the isotopic concentration classified in 4 categories: (1) definitely abnormal, (2) probably abnormal, (3) probably normal, and (4) normal. Our best results were obtained with the benign meningiomas, important tumours to diagnose early before they grow so large that their removal will damage the brain. In the most numerous group of malignant gliomas, the glioblastomas, we have our next best score, having diagnosed the site of abnormality in 90 per cent of them. These we are anxious also to spot as early as we can in order to give our post-operatively administered boron—slow neutron capture therapy—its best chance for success. Our worst record is in the gliomas of the brain stem and other small midline tumours which are, however, usually well shown by pneumography. Hence the two methods complement each other.

In Slide 17 (Table II of P/181) we see that abscesses also take up enough arsenic-74 so that the method spotted 7 of the 9 such lesions we have studied. Another large group of patients with focal

cerebral disease consists of those with a vascular accident other than haemorrhage. One sees from the slide that only two of these had definitely abnormal and seven had probable abnormal scans. Hence this common non-surgical disorder is not likely to be confused with the surgically amenable tumours and abscesses.

It will be noted that the patients with convulsive seizures from disorders other than those discussed earlier in the slides have occasionally shown abnormal scans. This is most likely to happen when seizures occurred shortly before the scanning. Abnormality in such circumstances must be interpreted cautiously and it may be necessary to repeat the scan after a fortnight or so.

Mr. Brownell is also in the early stages of efforts to localize lesions in the liver and pancreas with his instrument, using other positron emitters.

In summary, then, we have described a method for diagnosing surgically remediable local intra-cranial disease, in particular tumour and abscess. The painless, harmless character of the method permits its use when the index of suspicion of tumour is low.

Mr. J. F. Ross (USA) presented paper P/754, as follows:

The radioisotope tracer method has been particularly fruitful in studies of erythropoiesis and iron metabolism, and today I wish to summarize studies directed toward determining the cause of the anaemia which develops in patients with cancer and other types of neoplastic diseases.

Customarily this type of anaemia has been attributed to a "crowding out" or a "suppression" of the bone marrow. When radioisotope tracer methods were applied to determine what actually occurs in such patients, it became evident that the classical explanation of this anaemia was far from the truth.

We have studied 25 patients with leukemia or malignant lymphoma, and 38 patients with cancer, selecting cases in which blood loss, overt hemolytic anaemia and nutritional factors could be excluded as the cause of the anaemia. In other words, the cases studied had the "anaemia of cancer."

Our studies were undertaken to determine the "Dynamics of the Erythrocyte Cycle."

1. We determined the duration of survival and rate of destruction of erythrocytes using radiochromium labeled red blood cells.

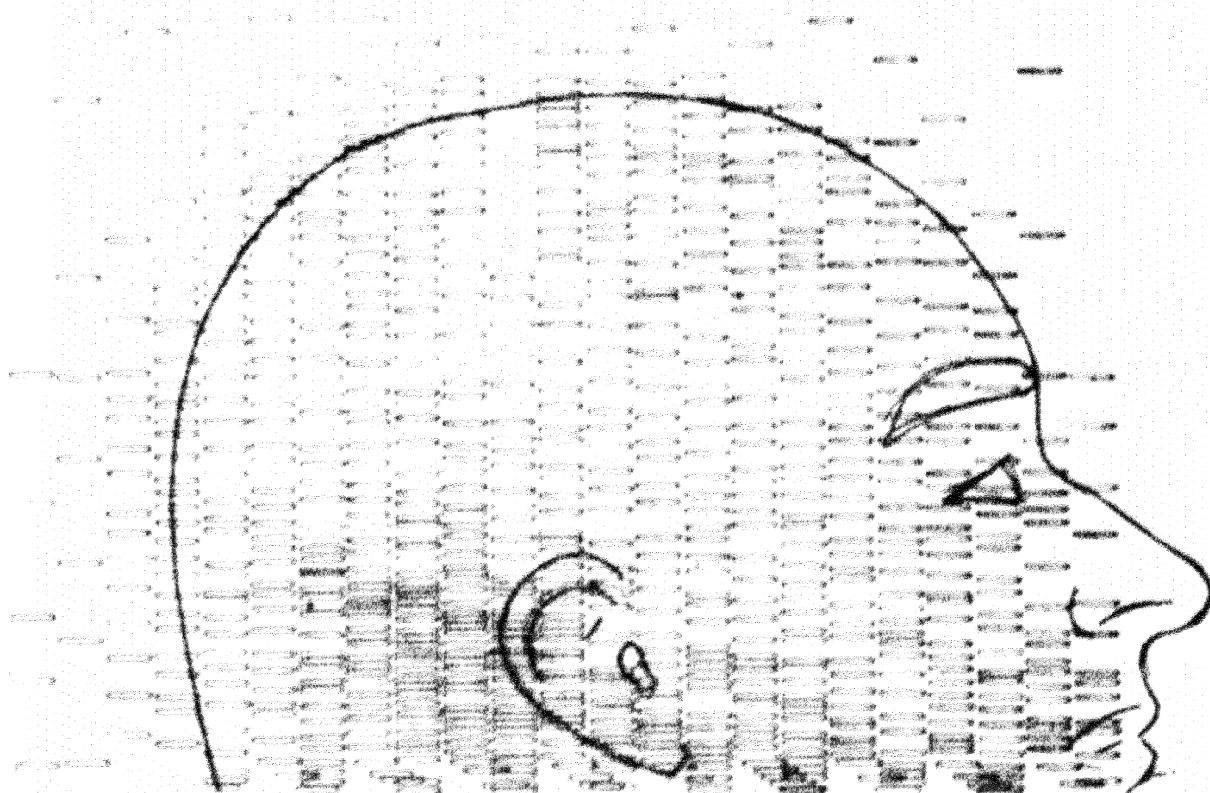
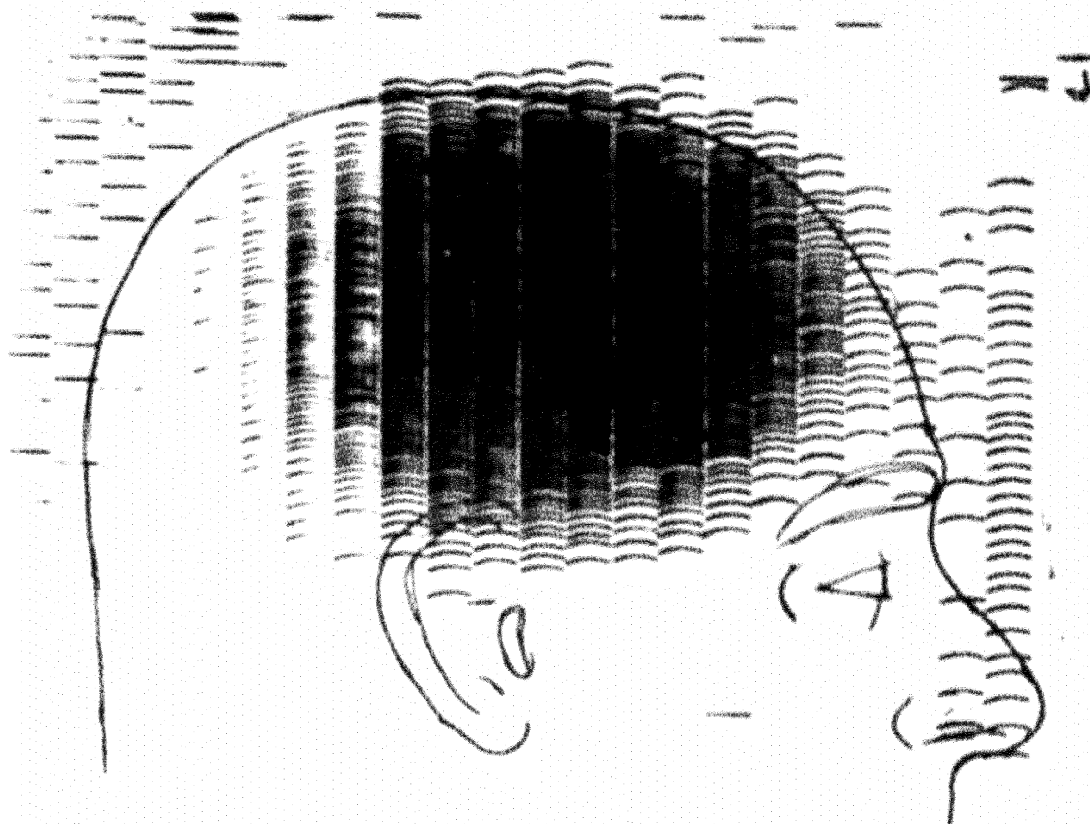
2. The rate of erythropoiesis was determined with radioactive iron.

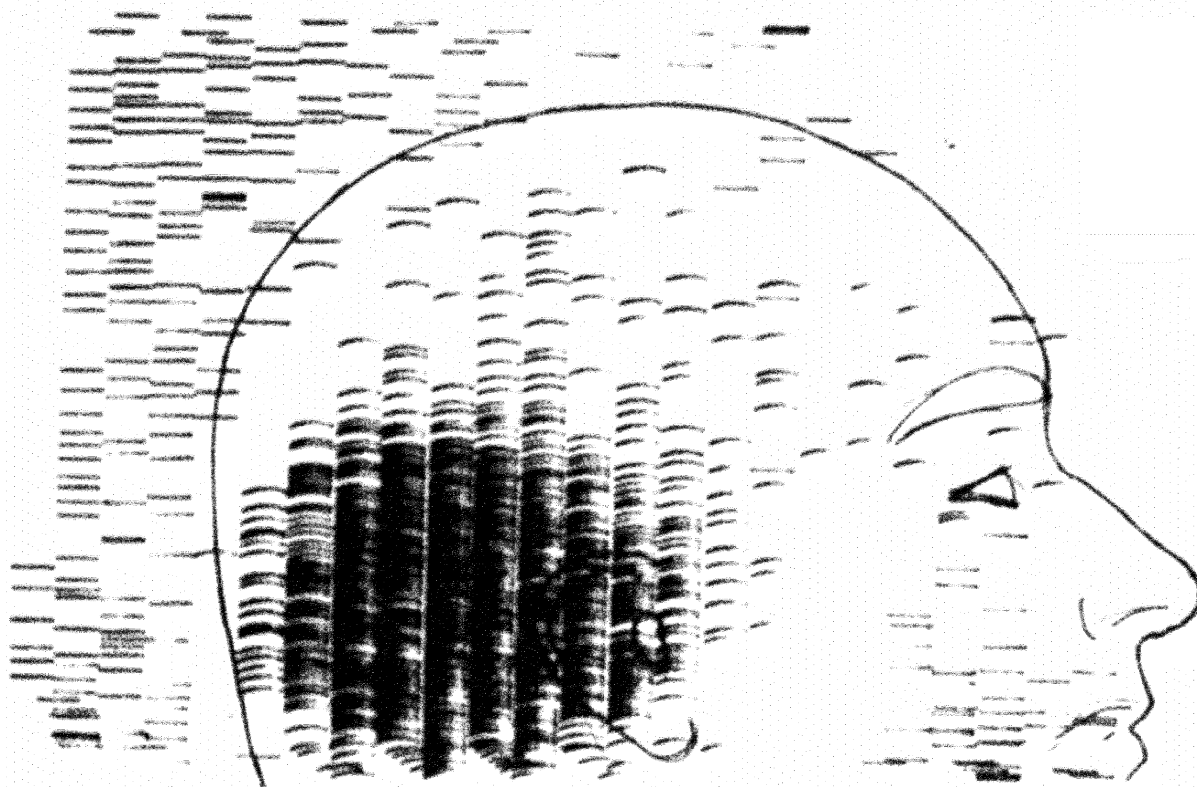
3. The sites of erythropoiesis were localized with radioiron and external monitoring techniques.

4. The sites of erythrocyte destruction were determined with radiochromium labeled erythrocytes and external monitoring.

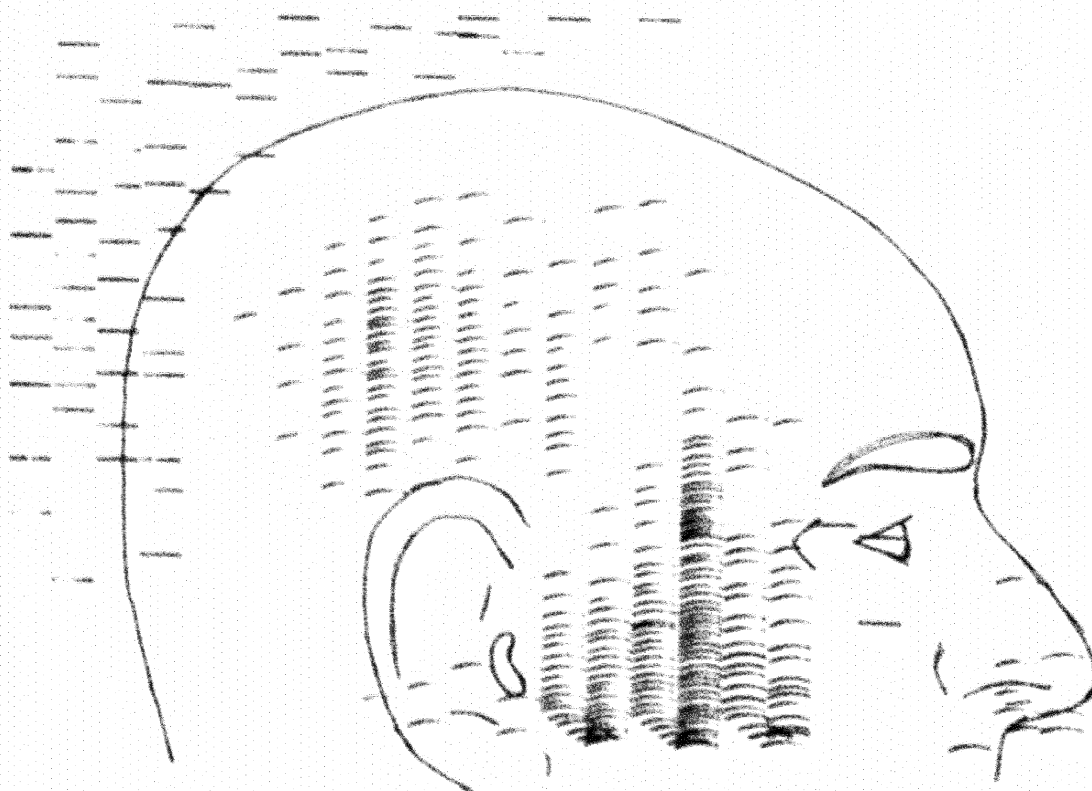
I shall detail each of these techniques and the observations made.

The following procedures are employed to evaluate the duration of erythrocyte survival and the rate of destruction by the radiochromium tracer method.





Slide 14



Slide 13

1. Erythrocytes are "labeled" by sterile incubation *in vitro* with radioactive sodium chromate.
2. They are then transfused into the subject.
3. Serial samples of blood are withdrawn over a period of days and quantitated for the amount of radiochromium-labeled cells remaining.

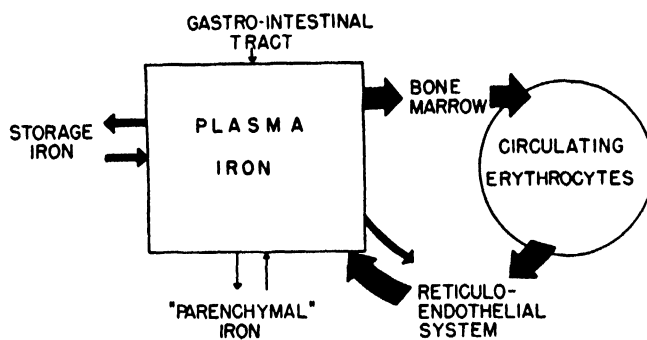
From these procedures it is possible to determine the duration of survival of the erythrocytes and the rate of their destruction.

Slide 18 illustrates such observations on a series of patients with cancer. The abscissa represents the percentage of transfused cells present in the circulation, and the ordinate the number of days after the transfusion of the labeled cells. The normal range of survival as determined in our laboratory is from 112 to 120 days as indicated by this technique. The survival of erythrocytes in patients with cancer was greatly shortened. In 18 of 22 patients studied in this fashion the duration of erythrocyte survival varied from 25 to 85 days, compared with a normal of 120 days. However, a few patients, clinically indistinguishable from the others, showed a normal duration of erythrocyte survival. Why these patients should differ in respect to red-cell survival is a most stimulating problem and remains to be determined.

In general, however, the survival of erythrocytes in patients with neoplastic disease is markedly shortened and the rate of erythrocyte destruction increased several fold. This is a major factor in the pathogenesis of the anaemia of cancer.

The second vital aspect of the erythrocyte cycle, namely the rate of erythropoiesis, has been evaluated with the radioactive iron method first published by Huff and Lawrence. The principles involved in these studies may be illustrated by consideration of the next slide.

Slide 19 represents schematically the metabolic pathways of iron in the human body. The plasma iron is seen to occupy a central position through which passes the iron for various physiological functions. By far the largest fraction goes to form erythrocytes. The tracer method consists of introducing a



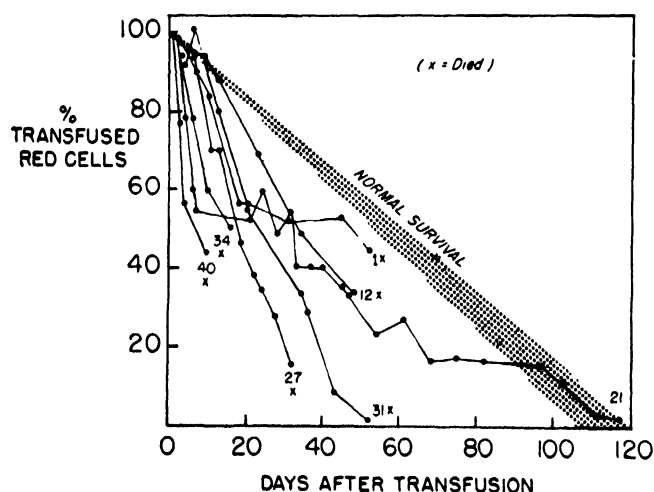
Slide 19. Metabolic pathways of iron

few micrograms of radioiron into this pool and then determining the rate and amount of the plasma iron which leaves the plasma iron pool per unit time, and the fraction and amount of the iron which is incorporated into the erythrocytes. Having determined this, the rate of erythropoiesis is readily calculated.

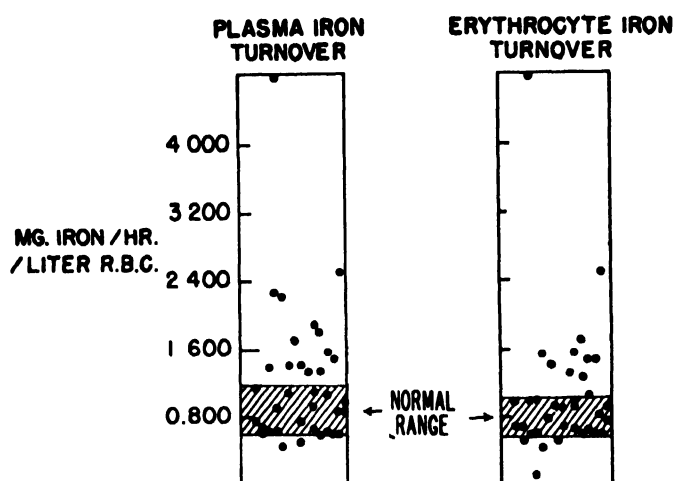
Slide 20 (Fig. 1 of P/754) illustrates the application of this method to patients with cancer. 1 to 10 micrograms of radioactive iron bound to plasma globulin is injected at zero time and samples serially drawn over a period of minutes. The plasma is assayed for radioiron content. As is evident, the rate of disappearance of the radioactive iron is greatly accelerated in the majority of the subjects with cancer, and as will be shown subsequently, this is reflected by an increased amount of iron passing through the plasma iron pool.

Slide 21 (Fig. 3 of P/754) illustrates observations on the incorporation of radioactive iron into circulating red cells. The percentage of the tracer dose present in the circulating erythrocyte mass is indicated on the abscissa and the time in days following the injection on the ordinate. The normal range is indicated by the cross hatched line and it is evident that in 25 of these 32 patients the utilization of iron for red cell formation was normal; in 4 cases it was accelerated and increased to an extent; and in only 3 patients was it slightly decreased. Thus it is evident that the utilization of iron for erythropoiesis is quantitatively and qualitatively normal in most patients with the anaemia of cancer. These observations are summarized in the next slide.

Slide 22 shows the total amount of iron passing through the plasma pool, and the amount of iron incorporated into erythrocytes per unit time is indicated in comparison with normal. It is obvious that instead of being *decreased*, the use of iron for formation of erythrocytes is either *normal* or significantly *increased*, frequently by a factor of two or three. Although erythropoiesis is normal or increased in rate it is not adequate to compensate completely for the increased rate of erythrocyte destruction in the majority of patients. Why erythropoiesis does not accelerate adequately to compensate completely for the increased rate of erythrocyte destruction is not known. This is an important and fascinating subject for further investigation.



Slide 18. Increased destruction of normal transfused red cells in cancer patients

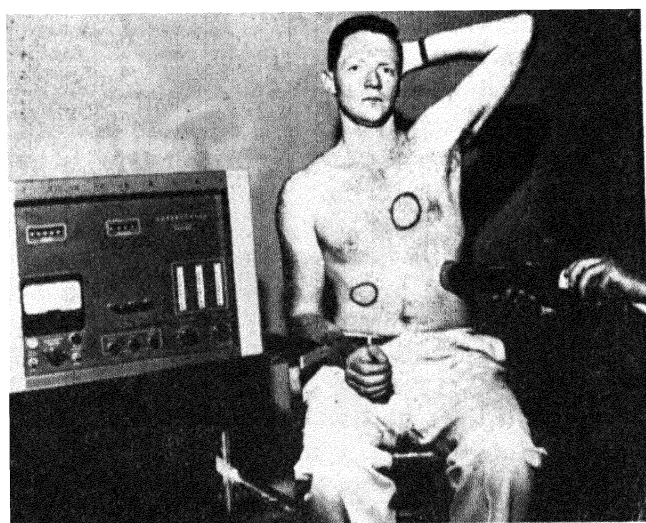


Slide 22. Plasma and erythrocyte iron turnover in cancer patients

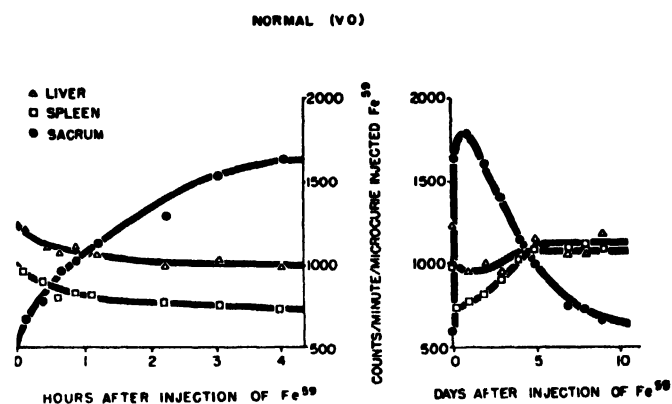
Next I will discuss studies of the metabolic pathways of iron in the body and the determination of the actual anatomic sites of erythrocyte formation and destruction. These studies are easily accomplished with external monitoring techniques and are proving to be of considerable practical as well as theoretical value, since they provide information regarding the functional state of the bone marrow and the spleen.

Slide 23 shows the procedure followed. A tracer dose of radioiron or radiochromium labeled erythrocytes is given by intravenous injection and at appropriate intervals measurements of radioactivity are made with a scintillation counter over various organs—spleen, liver, heart or sacrum—the sacral measurements being indicative of bone marrow activity.

It must be emphasized that, because of radiation scattering and the geometry differences of different organs, such measurements cannot be interpreted as indicating quantitatively a specific fraction of the administered dose, but serial measurements over the same anatomical site provide valuable information concerning the movement of iron or erythrocytes into or out of the organ.



Slide 23



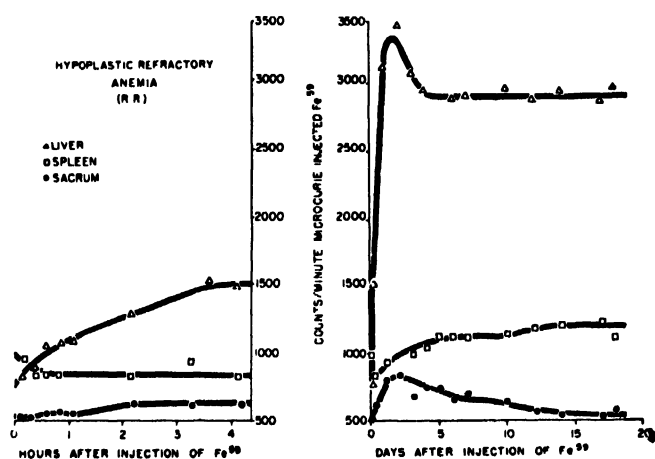
Slide 24. Body distribution of injected Fe^{59}

Slide 24 illustrates the pattern of distribution of iron in the liver, spleen and sacral bone marrow of a normal individual. The radioactivities are expressed in counts per minute per microcurie of administered iron. The left portion of the graph illustrates the changes during the first 4 to 5 hours, and the right side during the 10 days following the injection. In the normal individual there is an initial rapid assimilation of iron in the bone marrow of the sacrum, and then as this iron is released in newly formed red cells there is a rapid decrease. In contrast the liver and spleen show little assimilation of iron, and the late increase in activity reflects the presence of radioactive iron-containing red blood cells within the liver or spleen.

Slide 25 shows the markedly different pattern in a patient with hypoplastic bone marrow. In this case the marrow assimilated very little of the radioactive iron, and in contrast most of the radioiron proceeded to the liver where it remained in high concentration.

Slide 26 is typical of the observations in the majority of patients with cancer. The pattern of distribution is normal.

Finally I should like to detail the studies of the anatomic sites of erythrocyte destruction that can be determined in similar fashion by following with external monitoring techniques the deposition of radiochromium-labeled erythrocytes in various body organs. The next two slides detail such observations



Slide 25. Body distribution of injected Fe^{59}

made by Dr. James Jandl and Dr. William Castle of the Harvard Medical School, who have kindly allowed me to present their data.

Slide 27 illustrates observations in a normal subject following re-infusion of the individual's own radiochromium-labeled erythrocytes. The gradual disappearance of the labeled cells from the bloodstream is indicated in the upper graph, and in the lower graph, to which I wish to call your attention, is shown the very slight accumulation of radiochromium in the spleen and liver.

In very marked contrast, Slide 28 indicates observations in a patient with congenital hemolytic anaemia, in which there is rapid disappearance of the abnormal erythrocytes from the circulation, and marked accumulation of radiochromium in the spleen, indicating that the spleen is the site of rapid erythrocyte destruction.

Application of this method has been of considerable diagnostic value in determining the etiologic role of an enlarged spleen in patients with anaemia of obscure etiology. It has indicated the necessity of splenectomy in certain cases by clearly demonstrating the hemolytic role of the spleen.

In the several cases of anaemia of cancer studied with this method, no abnormality in splenic function was indicated.

In conclusion, I shall summarize the dynamics of the erythrocyte cycle in neoplasia and indicate the pathogenic mechanisms operating in these diseases.

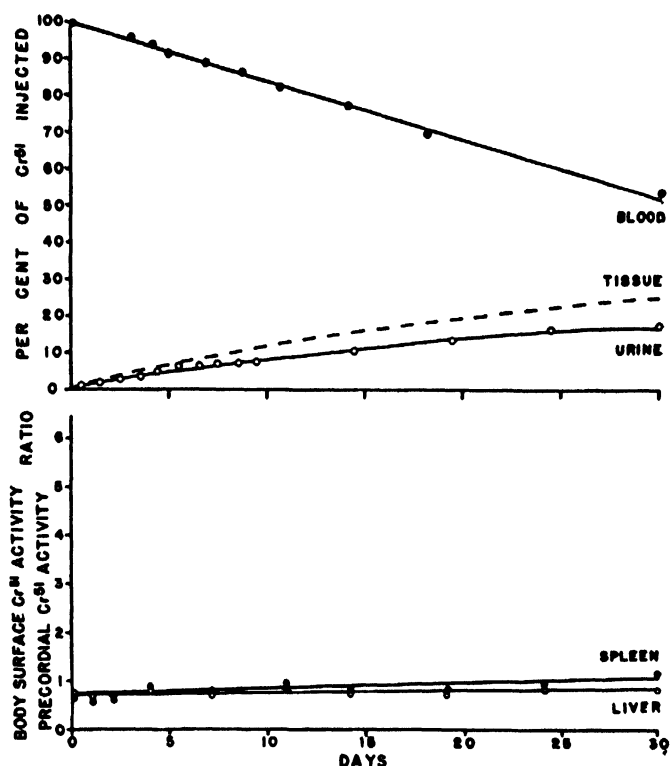
First, the life span of the erythrocyte is markedly shortened. This appears to be the most important factor in producing the anaemia of cancer.

Second, the rate of destruction of erythrocytes is correspondingly increased.

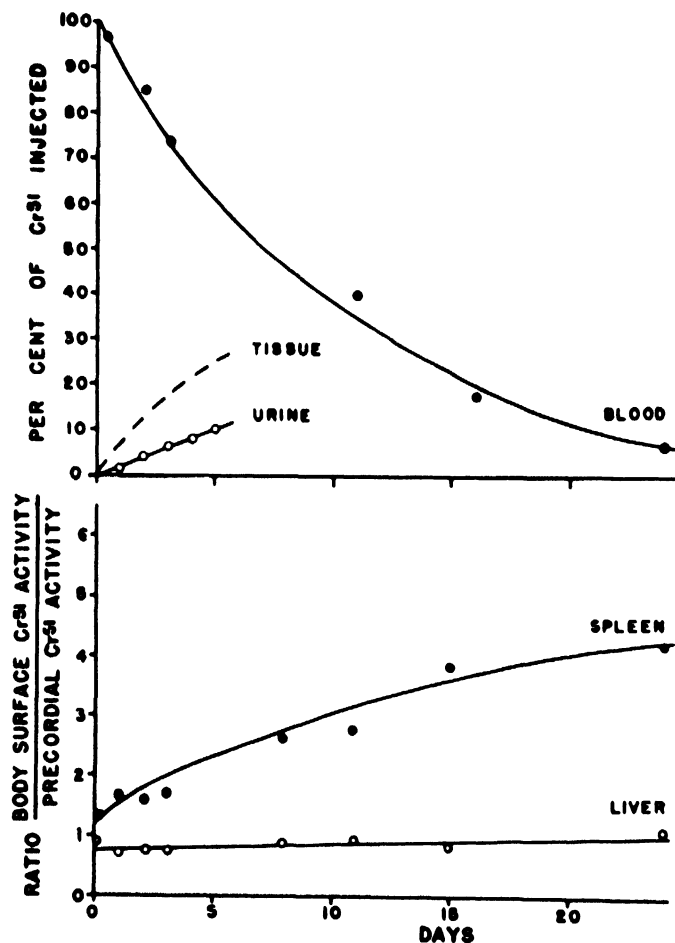
Third, the rate of erythropoiesis is increased or normal, *not* decreased as formerly believed. However, the increase is not adequate to compensate completely for the increased rates of blood destruction, and consequently anaemia develops.

Finally, the anatomic pathways of iron metabolism and the sites of erythrocyte destruction are normal.

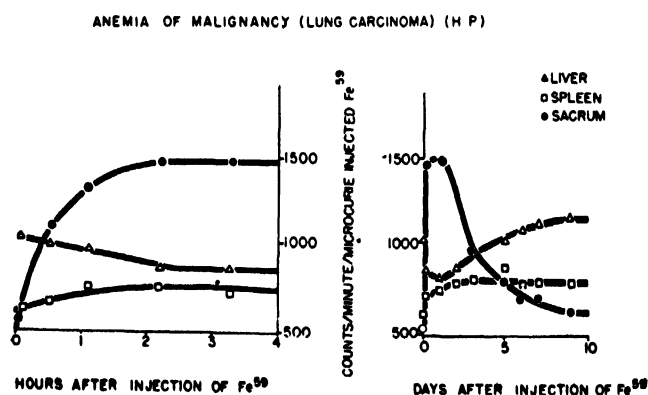
So much have radioisotope tracer methods contributed to our knowledge of the ways in which cancer affects erythropoiesis and erythrocyte survival in the host organism. In providing these an-



Slide 27. Cr⁵¹-labelled autogenous red cells. Subject: normal



Slide 28. Cr⁵¹-labelled autogenous red cells. Subject: congenital hemolytic anemia



Slide 26. Body distribution of injected Fe⁵⁹

swers, however, many new problems and questions have been raised, which, it is hoped, also will be answered in the future by the continued application of the radioisotope tracer method.

DISCUSSION OF P/181 AND P/754

Mr. H. A. KÜNKEL (Germany): In addition to the very interesting paper by Mr. Sweet, it may be of interest that by means of gamma-coincidence measurement it is possible, as we could show, to localize not only positron emitters but even quite a lot of other isotopes emitting two or more gamma quanta per decay. If the resolution time and the coincidence is short enough, it is possible to localize some further very useful biological isotopes, especially with regard to the angular distribution of the two gamma quanta.

Mr. W. F. BALE (USA): I wish to ask Mr. Sweet if he could give us a little more data regarding the relative efficiency of single channel as compared with coincidence counting as a general technique.

Mr. SWEET (USA): Could I ask my colleague, Mr. Brownell, to answer that question?

Mr. G. L. BROWNELL (USA): Comparison of different methods of detection, even from a physical point of view, is a comparatively difficult task. However, as Mr. Mayneord has pointed out, the necessary amount of activity required to obtain the scan or picture depends on the resolution that one desires in the picture or the sharpness of the picture. As a matter of fact, we have estimated that, for a given detecting system, the optimal amount of activity varies as the sixth power of the resolution. Therefore, to compare two techniques, it is necessary to compare them at the same resolution or sharpness of picture, and our estimates on this basis, on geometric grounds, are that the coincidence method can provide a picture with the same resolution as the single channel with about one-tenth of the activity.

Mr. E. E. POCHIN (UK) presented paper P/769.

Mr. C. H. JAIMET (Canada) presented paper P/9, as follows:

May I say first that I am a physician, not a chemist, as is indicated in the programme. My co-author, Mr. Thode, is a chemist.

There are two features for you to consider in this paper. The first is the function of the salivary glands in relation to the thyroid; the second is the value of the saliva test as an index of thyroid function.

In regard to the first, a thyroid enzyme tyrosine-iodinase was discovered in our laboratories; it was shown to be responsible for the iodination of tyrosine; it is present in great concentration in the parotid and submaxillary glands (but in only a few other tissues in the body); and it has been shown to act in the reverse process, the degradation of di-iodotyrosine.

These observations led us to believe that the salivary glands may play a specific part, in the human,

in the degradation of thyroxine and the recycling of iodine to the thyroid gland as iodide ion. Our clinical tests on saliva were then devised and the data obtained from these saliva counts and the saliva-to-protein bound iodine ratio seemed to confirm the views stated.

Our clinical investigations with iodide ion and labeled 1-thyroxine tracers show that the salivary glands concentrate iodide into the saliva, and regardless of whether or not they degrade thyroxine, the majority of the iodide concentrated must arise from the inorganic iodide in the blood. However—and this is important, I think—this does not mean that the saliva activity test is merely reflecting the iodide ion level in the blood, since the blood level 24 hours later varies widely from patient to patient and does not correlate at all well with thyroid function. Furthermore, the 24 hour saliva-to-blood iodide ion ratio (S/I⁻) varies by a factor of 60 with much overlapping of the groups, and the average for hypothyroids is about double that for normals. It is seen, therefore, that such aspects of salivary gland function as the ability of the glands to concentrate iodide ion into the saliva and, to some extent, the rate of saliva flow, are reflected in the accurate S-PBI. test; and these functions of the salivary gland seem closely related to the iodide ion, thyroxine, and TSH. levels in the blood.

Although perhaps less direct, there is much clinical evidence suggesting a thyroid-salivary relationship. For example, there are reports of submaxillary weight increase with thyroid weight in goiter cases: hypertrophy of the salivary glands during treatment of myxedema with tri-iodothyronine, submaxillary gland swelling during treatment of a patient with thiouracil and subsequent atrophy of one parotid gland. Others have reported atrophy of submaxillary glands in rats after thiourea administration or surgical thyroidectomy.

This, then, is a brief summary of our thinking on the first feature—the thyroid salivary interrelationship. I refer you to our references, for the biochemical details.

The second feature is our clinical use of the saliva tests—indicated as total saliva counts or, better still, the ratio saliva to blood protein bound iodine—i.e., S-PBI. ratio.

In the past 5 years we have investigated and treated a total of just under 1500 patients, using I¹³¹. At first we did only 24-hour pick-up with Geiger and scintillation counters, regional scanning when indicated for nodules and the "Conversion Ratio Test" described by Clark of Chicago. The conversion ratio test was by far the best, and formerly, when a larger tracer dose was used, it was quite good in picking up hypothyroids, although much more accurate in the normal and hyper range. As the tracer dose is reduced it becomes less accurate in the low normal and hypothyroid range. Here, however, is where our saliva tests have really

helped us. These remain accurate with relatively small tracer doses and are practical and economic to do as a routine in any laboratory measuring plasma activity. By our method of obtaining saliva—i.e., simply expectorating $\frac{3}{4}$ inch in a wax cup—very little more time is required of the patient and no extra visits are necessary.

We feel that the ultimate decision in thyroid disorders is clinical, but we do need laboratory aid in ferreting out many low grade nodular toxics and, more particularly, hypothyroidism without myxedema, both being disorders with more bizarre manifestations than we ever suspected.

I think you will be pleased with the results of saliva tests, particularly in hypothyroidism, just as we have been; and other workers such as Clark and Ryan have confirmed our findings.

Therefore, every new radioiodine test, especially if it measures a different aspect of thyroid function, will be welcomed as an addition to our battery of tests, because any combination of these, to date, has frequently failed to indicate the thyroid state. The "6-hour pick-up," the "tri-iodothyronine" and the "TSH." tests are three such excellent additions.

To summarize, we have carefully evaluated the data on radioiodine saliva tests for thyroid function on over 400 of our patients. There is, of course, some overlapping in the three groups—hypos, normals, and hypers. Note, however, the wide separation of these groups in the averaged figures. Total saliva counts in hypos averaged 19,333; in normals 2393; and in hypers 380. The S-PBI. ratio averaged 795 in hypos, 188 in normals and 10 in hypers. We tentatively suggest a level of below 40 for hypers and above 400 for hypos.

The advantage of the "saliva" test appears as follows:

1. It is equally accurate with pick-up and conversion-ratio tests in hyperthyroids.
2. It gives accurate indication of hypothyroidism when other tests are disappointing (the TSH. test excepted).
3. The S-PBI. ratio seems to follow the clinical state more closely in patients after treatment with I^{131} , thiouracil, etc. when other tests have indicated otherwise.

Finally, the excellent correlation between saliva tests and thyroid function provides strong clinical evidence for a connection between salivary and thyroid function and supports the evidence of Fawcett and Kirkwood of our laboratory that the salivary glands play an important role in the iodine cycle in the body, perhaps in the degradation of organically-bound iodine.

Mr. M. BERGER (France) presented paper P/365.

Mr. S. MILCOU (Romania) presented paper P/1099.

The CHAIRMAN: As we have made up time, I think, before we start the discussion, I will request

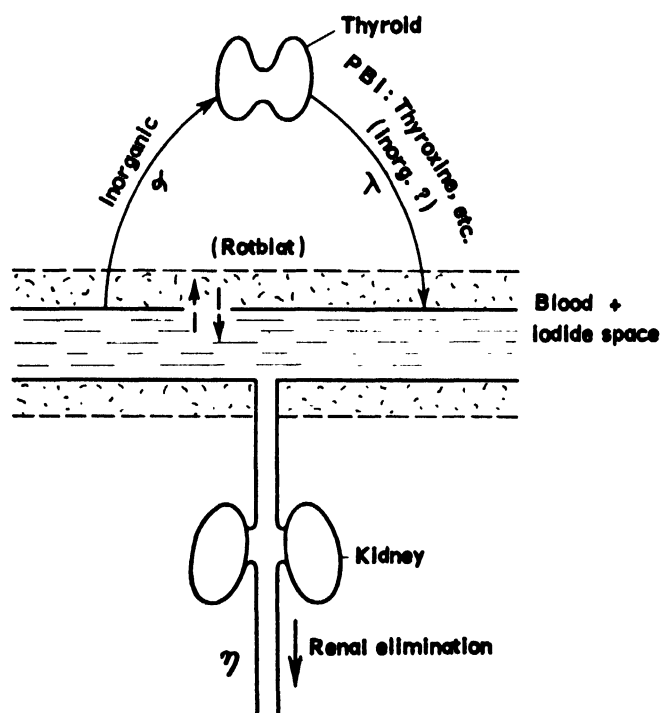
Mr. Joyet to read a small two-minute paper on "The Dynamics of Radioiodine in Normal and Pathological Functions."

Mr. G. JOYET (Switzerland): When we began using iodine-131 in the Zurich hospital, we first followed the generally accepted procedure of administering iodine by mouth and then measuring thyroid activity at a standard interval of 3, 6, 24 or 48 hours after intake. The results soon struck us as very misleading. Only in a few cases was there any correlation between biological intensity on the one hand and the measurements of thyroid uptake, on the other.

We thought it necessary to examine the principal processes through which the injected iodine passes in the body, along the lines of the experiment made by Rotblat *et al.* in London.

We studied three basic processes (Slide 29). The first, the uptake of inorganic iodine from the blood entering the thyroid is denoted by the factor alpha. The renal excretion of inorganic iodine from the blood is denoted by a second factor, eta. Lastly, the third process, the organic elimination of iodine, which, after passing through the thyroid gland, re-enters the blood stream, is denoted by the factor lambda. The thyroid activity measured with iodine-131 is the result, not of one of these processes, but of all three.

I should point out that a similar theory was evolved about three years ago by Rotblat and Marcus in London, but the lambda factor was not taken into account. However, the passage of the isotope from the blood into inter-cellular space and *vice versa* was taken into account. This we did not consider necessary, as diffusion into the inter-cellular space is effected in only a few minutes.



We can then (and this seems essential in understanding the effect of these processes on uptake) write the differential equations for these different processes, and interpret them. The calculation, which is not difficult for the physician, gives the following result: the factor alpha, which represents the intensity of passage of the inorganic iodine from the blood to the thyroid is exactly equal to the initial tangent of the curve of increased thyroid activity, i.e., to the tangent taken during the first twenty or thirty minutes after injection of iodine-131.

In the course of the calculation, we can find the eta and lambda factors as a function of the curve of thyroid activity. This appears in the calculation as the difference of two exponentials.

In studying a rather large number of patients (more than 70) over a period of eight days, we established the alpha, eta and lambda factors in a considerable number of cases by asking ourselves which of the three was the determining factor, for the biological activity of the function (Slide 30). The uptake curve appears as the difference between two exponentials in accordance with the theory. This relationship was confirmed with less than 5% error in 63 of the 70 cases.

If we examine the maximum rise (Slide 31), its formula shows us that it is a function of alpha divided by alpha plus eta, i.e., the increase in thyroid activity on the one hand and renal elimination on the other.

The increase in biological activity, as classified by the clinic, itself is shown here on the abscissa. To the right are the cases of hyperactivity (three de-

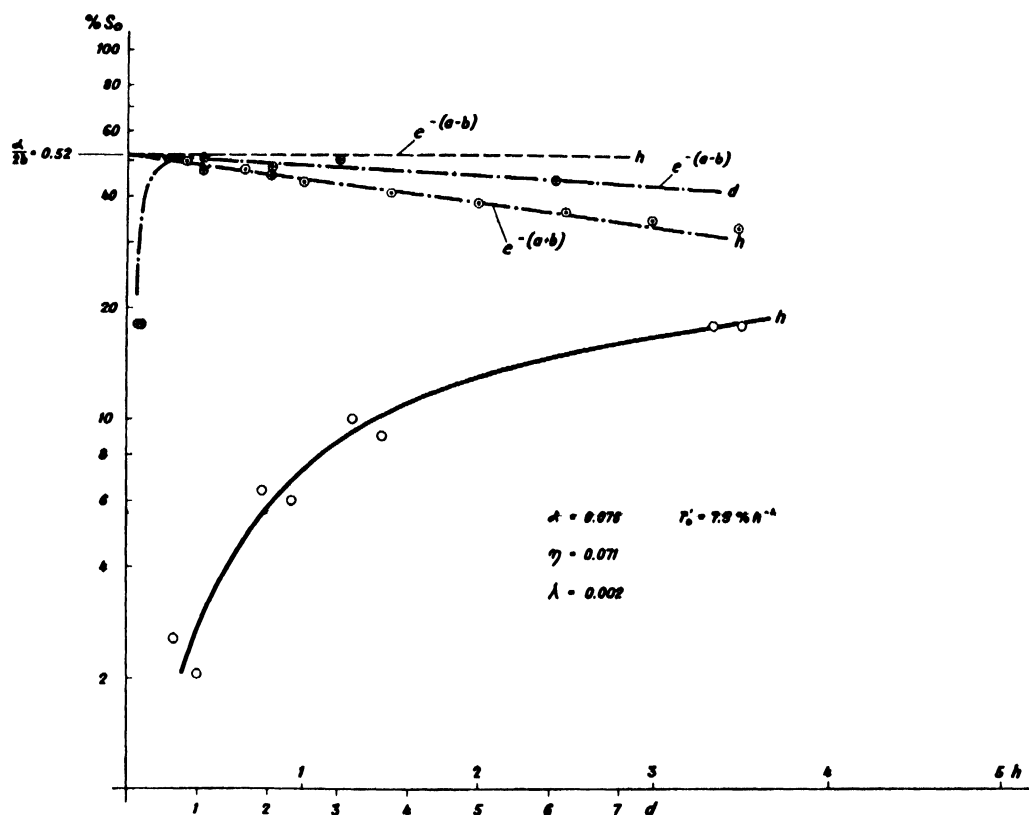
grees of hyperactivity), to the left, the cases of hypoactivity (three degrees of hypoactivity) and in the middle, the normal activities. The maximum rise is given on the ordinate.

It will be seen that there is a general tendency for the maximum rise to increase in correspondence with biological intensity of activity. But it is only the cases of hypoactivity—myxedema—that can be distinguished. Normal cases can hardly be distinguished from those of hyperactivity.

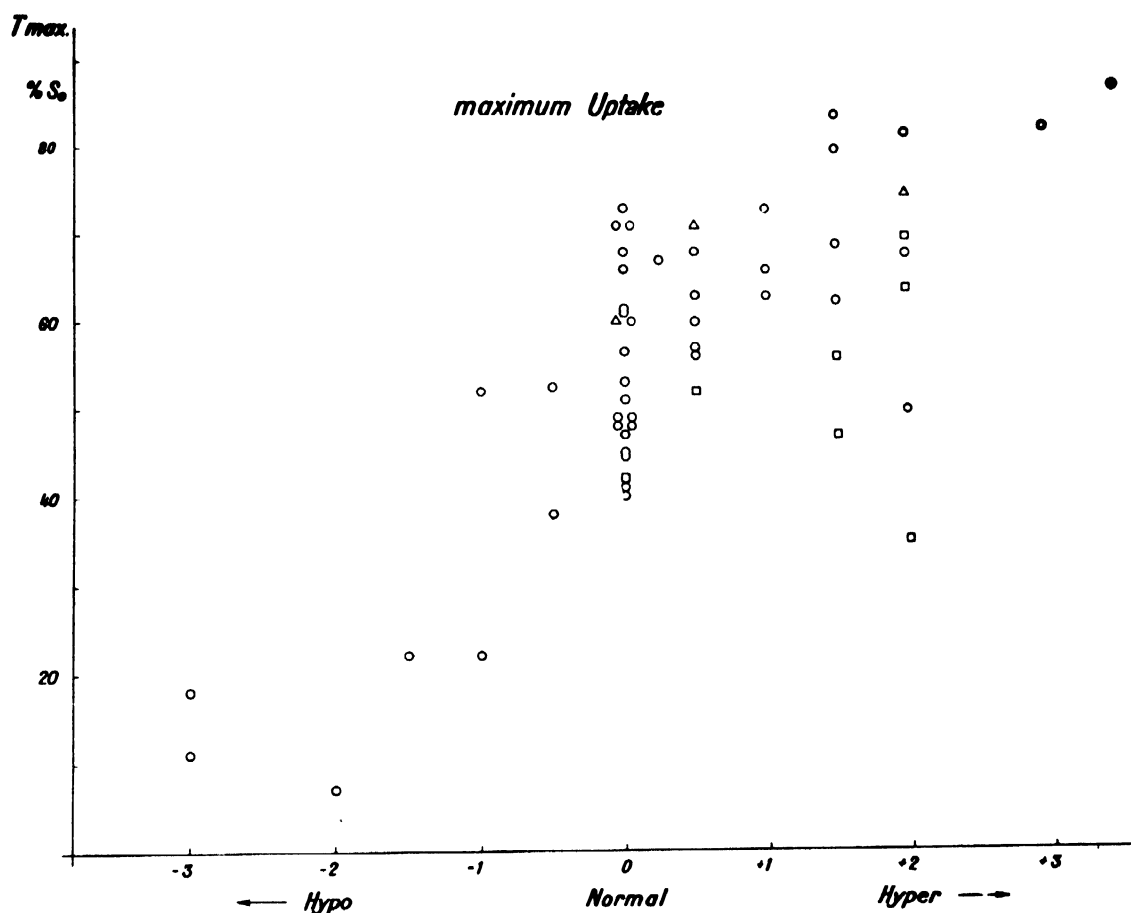
Where urinary excretion is concerned (Slide 32), it will be seen that there is no constant relationship between intensity of activity and the initial rate of excretion. This rate has therefore no significance for the biological intensity of activity, a fact which other writers have already shown, in particular, Berson *et al.* in New York.

If we take the value of the initial tangent, which is equal to alpha, as a function of the intensity of biological activity we find that this time we have a parameter, which has a wide and significant range of values (Slide 33). We can distinguish a normal field lying between 7 and 18 per cent per hour. Cases of hypoactivity can be distinguished. They are under 5 per cent. Cases of hyperactivity or Basedow's disease can be distinguished and are always above 25 per cent. Thus, we obtain a very satisfactory differentiation between the normal and abnormal fields.

If we take this initial tangent as a diagnosis test and treat a hypofunction (lower curve before treatment) with pituitary extract, we find that the tangent rises to a normal value (Slide 34).



Slide 30



Slide 31

On the other hand (Slide 35), in cases of Basedow's disease, the initial tangent is first very high, but after treatment activity is reduced to within the normal field. Thus, the initial tangent of the curve of thyroid activity enables us to distinguish normal from abnormal cases and also enables us to check the effect of the treatment given.

The latest results of this work and the pertinent literature are given in paper P/917, which appears in this volume.

DISCUSSION OF P/769, P/9, P/365, P/1099

Mr. POCHIN (UK): Mr. Clark has addressed three questions to me. The first question is: "In patients with a functioning carcinoma which is amenable to surgery, do you recommend post-surgical prophylactic radioiodine therapy?"

In our opinion, if the surgeon considers that the tumour tissue remains behind after surgical operation, then we would use radioiodine for that tumour tissue if the tumour had a differentiated histological structure; that is to say that if we were to have used radiiodine for that tumour in the full treatment, we would use it for the small amount of tumour probably remaining after operation.

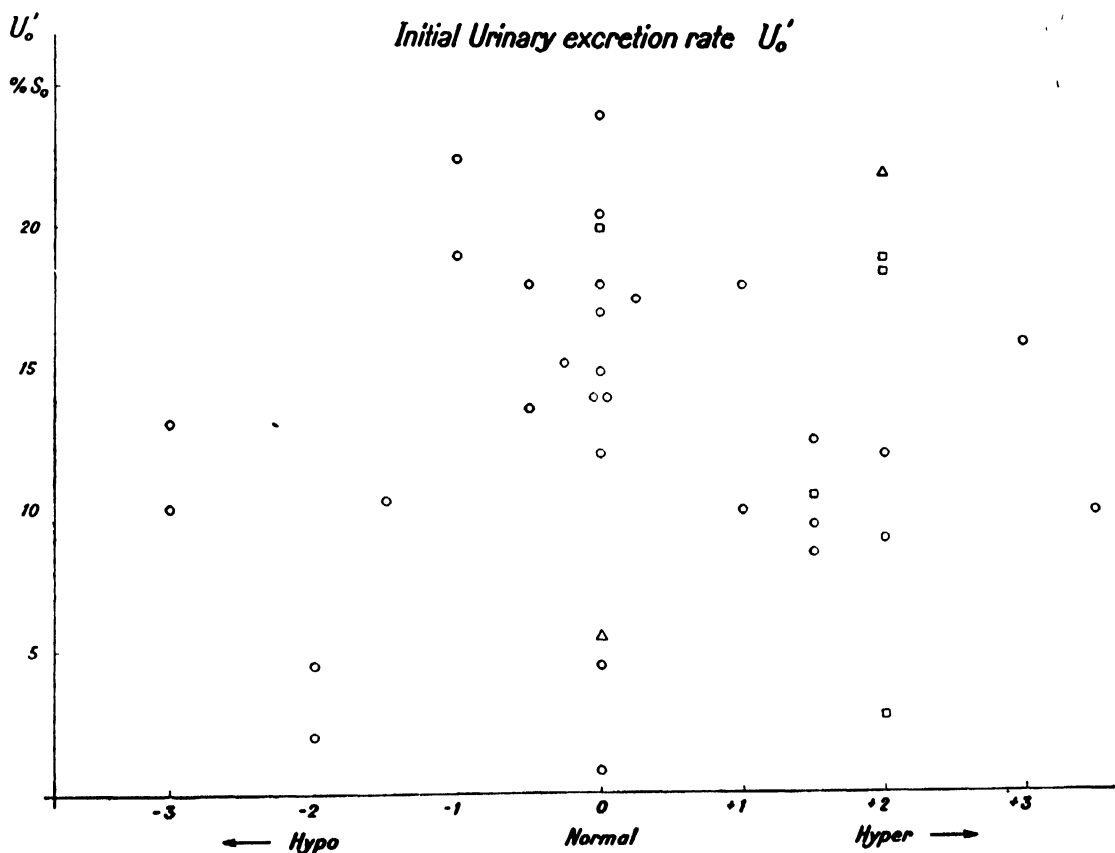
The second question is: "From your studies on the rate of turnover of iodine in tumour tissue, what would be your recommendation for the method of radioiodine therapy in cases with functioning carcinoma of the thyroid?"

I think it is important to know whether the discharge is rapid because the tumour is being stimulated or because it is simply a tumour. If it is rapid in discharge, because that is a property of tumour tissue, then I do not think that one can secure a high irradiation. If it is rapid because it is being stimulated, then one should remove the cause of stimulation to obtain higher irradiation. I do not think that any radioiodine of a different half-period would be better, and I think the only possibility is to give radioiodine and obtain as much irradiation as possible, even though much is lost because of the rapid discharge. I do not know any trick, in fact.

Thirdly: "Have you performed any chromatography on the plasma of patients with carcinoma of the thyroid to determine if an abnormal iodinated organic compound may be present?"

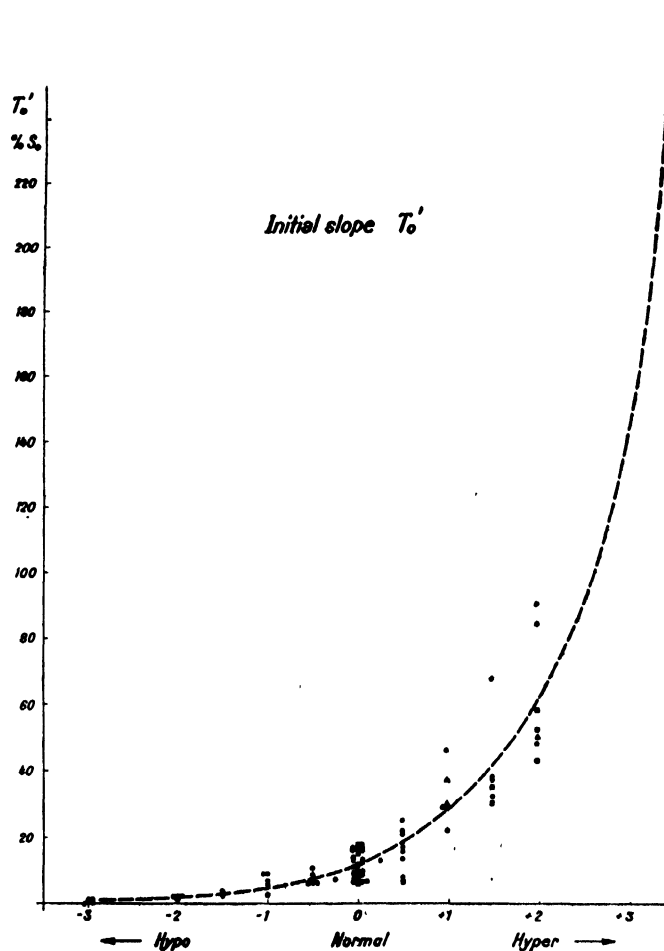
We have, and we are doing so, but have no results which go beyond those already published from other departments. We have found a high tri-iodothyronine content in one case, but we have obtained no systematic new results.

Mr. H. VETTER (Austria): I should like to comment on Mr. Jaimet's paper. In Vienna we have during the past year paid some attention to the saliva-plasma ratio and have performed some experiments, involving the use of iodine-131 as well as iodine-132. Last summer at the Oxford Conference Mr. Pochin has already suggested that the S/P ratio might be mainly determined by the behavior of

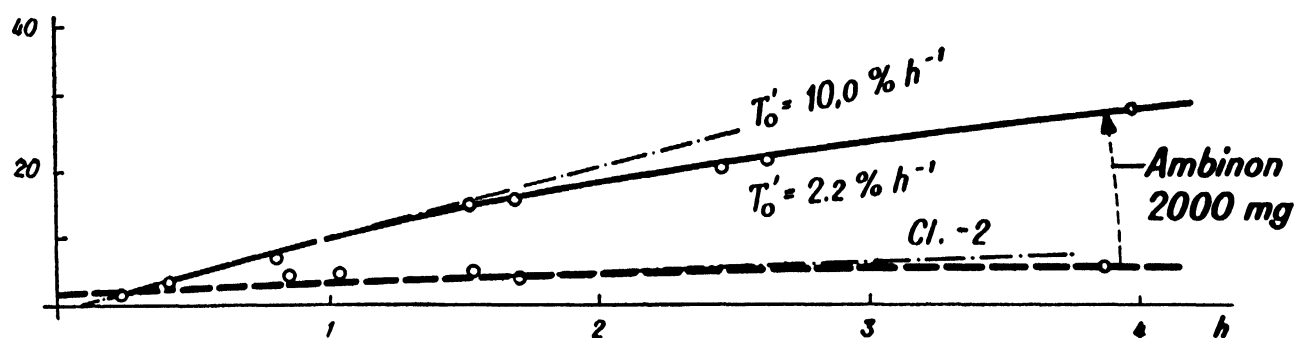


Slide 32

the plasma radioiodide concentration. In order to test this hypothesis we have measured the radioiodide clearance of plasma by the parotid gland in several subjects with various degrees of thyroid dysfunction, and have found the salivary concentration mechanism for iodide completely independent of thyroid function, and to be constant over a wide range of thyroidal clearances, the average value being around 50 ml/min. Thus the salivary/plasma radioiodide ratio reflects thyroid function only in so far as the plasma radioiodide concentration does. If either whole plasma or protein-bound iodine-131 is used as the denominator of the S/P ratio this will lead to a better discrimination because of the specificity of the behaviour of the protein-bound iodine-131. It should be noted, however, that the denominator will then be influenced by some other factors than the hormonal secretion rate, for example the renal clearance of iodide, its fecal excretion, the daily iodine intake, the volumes of the iodide and hormonal iodine spaces, and by the iodine content of the thyroid. Therefore, abnormal values for the S/P ratio will be obtained in renal failure, in chronic iodine deficiency, and in thyroidectomized or radioiodine treated patients. In addition, the S/P ratio is influenced by two further extrathyroidal factors: the degree of dilution of serous by mucous saliva which contains no iodine, and by the rate of salivary flow. I am afraid that these results have indicated to us that the S/P ratio is no better test than other tests currently in use—in fact, the saliva-plasma ratio may lead occasionally



Slide 33



Slide 34

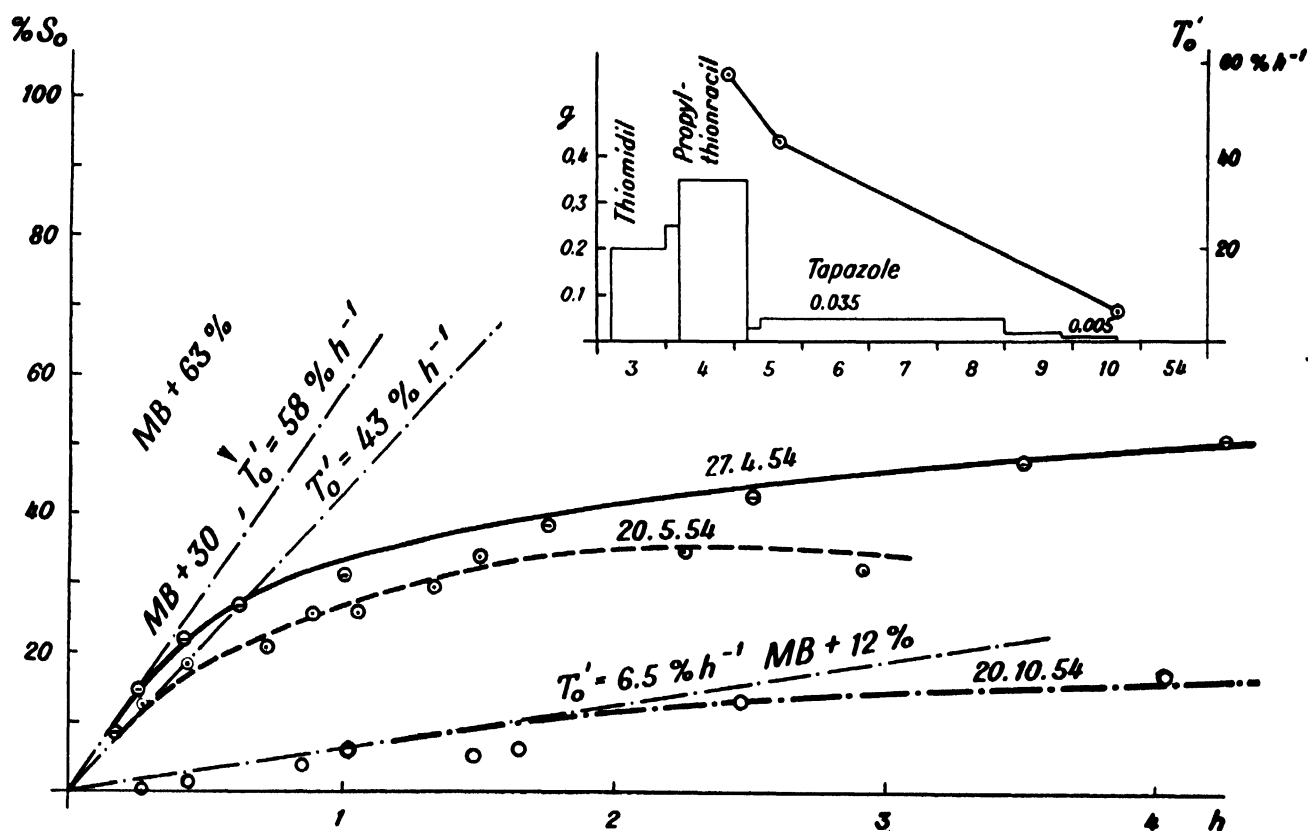
to considerable errors in the diagnosis of thyroid function. Thank you.

Mr. BERGER (France): Mr. Pora of Romania wishes to know if the authors of the paper I read have investigated the living conditions of the ancestors of myxedematous patients.

The answer is that the parents of myxedematous children lived under perfectly normal conditions from the nutritional point of view. Approximately half of them had lived in areas quite free from goitrous edema, whereas the other half had spent their early years in areas where goiter was slightly endemic. But after a few years they had settled mainly in the Lyons area, where there is no endemic goiter. Apart from this, all the relatives—I am referring to the relatives in the direct line, since it is difficult to obtain information on grandparents or more distant relatives—lived under apparently normal conditions.

The second question is by Mr. Muller of Switzerland, who asks whether it would not be possible for hyperthyroidism in a pregnant woman to produce congenital myxedema in the infant by obstructing the development of the infantile thyroid.

My answer is that this theory has already been put forward by a number of authorities. It seems quite a feasible explanation so far as the mother is concerned. Obviously no such explanation can be advanced in the case of the father. I think that the presence of any deviations from the normal in the the father compels us to assume the transmission of genetic factors by him. In regard to the mother, it is debatable whether genetic or diverse factors are involved. We believe, however, that the mother also transmits a genetic factor. We may therefore conclude that both factors are operative in the case of the mother. It would be interesting to study this problem in greater detail.



Slide 35

The third question, from Mr. Mawson of Canada, relates to the meaning of the biochemical hyperactivity mentioned in our paper.

My reply is that there is a rapid iodine metabolism marked by a high fixation of radioactive iodine twenty-four hours after injection, then later, a fixation of over 50 per cent. We also soon find a high percentage of radioactive iodic organic compounds in the serum as well as a large amount of stable proteic iodine. These different results demonstrate a rapid metabolism very similar to that found in hyperthyroidism. No clinical symptoms are found, however, as we pointed out just now in connection with endemic goiter. In regions of endemic goiter we find subjects with a very rapid iodine metabolism, but who show no clinical symptoms and in particular, no thyroid hypertrophy. I simply used the term "biochemical hyperactivity" to describe this set of facts: it is, however, open to criticism.

Mr. J. H. MULLER (Switzerland): I should like to make a small addition to Mr. Milcou's excellent statement. In a study made in collaboration with Mr. H. Aepli and published in "Experimentia" in 1948, we obtained the same results in principle with the injection of estrogen and androgen. We later made a first experiment by taking thyroid tissue from a young sheep immediately after slaughter and transferring it, under conditions ensuring survival, into a liquid containing both radioactive iodine and varying proportions of androgen and estrogen hormones. If no effect is detected, the cause of the change must be sought in the pituitary or in the pituitary region.

Mr. MILCOU (Romania): The information given by Mr. Muller bears out the explanation we have given of this mechanism.

Mr. W. F. BALE (USA) presented paper P/199.

Session 10C

RADIOACTIVE ISOTOPES AND NUCLEAR RADIATIONS IN MEDICINE (*concluded*) — DIAGNOSIS AND STUDIES OF DISEASE

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The Use of Isotopes in Biochemical and Medical Research

By A. Baird Hastings,* USA

Prior to the availability of isotopes for the marking of specific molecules, and the resulting insight it has given into the biological adventures which these molecules experience, information about metabolism was limited to what went into and what came out of organisms, organs and tissues. Now, we are not only able to follow labeled compounds in their progress through the body but we are able to follow the individually labeled atoms from one compound to another.

From Hevesy's use in 1923 of an isotope of lead, radium D, in the study of the movement of lead in plants¹ has stemmed the numerous enlightening studies of the metabolism of inorganic ions in plants and animals. From the equally important pioneer work in 1935 of Schoenheimer and Rittenberg, who employed deuterium to label a fatty acid and follow its metabolism² has come the modern concept of the dynamic state of the body constituents.

Such new knowledge about the behavior of atoms and molecules in the body has led to much better understanding of normal and abnormal physiological processes. Today, clinicians, physiologists, biochemists and biophysicists—working together—are able to attack problems that were hitherto little understood.

Since it would be impossible in a short paper to review adequately or comprehensively the numerous uses to which isotopes have been put to provide new knowledge about the metabolism of inorganic and organic compounds in the body, a limited number of examples will be chosen by way of illustration.

In order that the relations among the various types of new information may be viewed in perspective, Fig. 1 has been prepared to illustrate, in approximately quantitative terms, the principal fluid and solid phases of the body. Relative concentrations of constituents in the fluids are plotted vertically and relative masses of the fluids horizontally. The area occupied by any single ionic symbol represents its relative amount in the body. On the right of the figure, the areas marked protein, carbohydrate and fat serve to designate the pool of intracellular organic compounds that participate in intermediary metabolism and provide the energy for heat, work and synthesis. The fact that various endocrine glands provide hormones which participate in the regulation of the mass and composition of the extracellular and intracellular fluids as well as in intermediary meta-

bolic reactions, is also indicated at the sides of the figure.

This means of representing the mass and composition of the extracellular fluids, the mass and composition of the intracellular fluids, and of indicating the intermediary metabolism of the proteins, lipides and carbohydrates emphasizes the fact that the intracellular reactions are conducted in a particular intracellular ionic environment, just as the cells themselves are served by a particular extracellular ionic environment. Examples of ways in which the employment of isotopes has served to shed light on the mass and composition of the several phases and on intermediary metabolism follow.

TOTAL BODY WATER

Deuterium and tritium can both be employed to label water. A known amount of such labeled water may be injected and time allowed for the distribution of the labeled water throughout the water of the body. By withdrawing a sample of blood and analyzing it for its concentration of labeled water, the amount of water in the body may be estimated.

This method has provided physiologists and clinicians with an accurate means of following changes in the water content of the body under conditions of edema and of dehydration, and during recovery from wasting disease and trauma.^{3,4}

EXTRACELLULAR FLUIDS

The same principle may be employed to determine the mass of the extracellular fluids. For this purpose, isotopes of sodium (Na^{24}), of chlorine (Cl^{36}) and (Cl^{38}), and of bromine (Br^{82}) have been employed. (Non-labeled materials such as insulin, sulfate and sucrose which do not penetrate into cells have also been used.) Although different materials yield somewhat different absolute values, clinical investigators are thereby enabled to follow changes in the extracellular fluid mass with disease. Furthermore, if the total body water and the extracellular fluid mass have been determined, the amount of intracellular water may be calculated. Thus it becomes possible to determine whether or not a loss or gain of weight by the body is the result of a reduction or expansion of the extracellular fluid mass—or whether the intracellular fluid mass is also involved in the change in weight.

The composition of plasma and of other accessible extracellular fluids may, of course, be determined by

* Harvard University.

THE FLUIDS AND SOLIDS OF THE BODY A REGULATED, COORDINATED SYSTEM

INTRACELLULAR FLUIDS

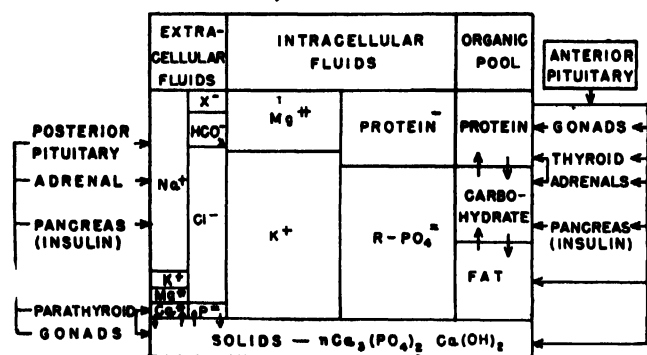


Figure 1. The fluids and solids of the body; a regulated, coordinated system

direct chemical analysis. All that have been analyzed have been found to have essentially the same concentrations of inorganic constituents. However, the rates at which the different ions are exchanged across the several membranes that separate the plasma from the other portions of the extracellular fluid pool differ widely. Such information would have been unobtainable without the availability of isotopes of sodium, potassium, calcium, phosphorus and chlorine.

PLASMA AND RED BLOOD CELLS

Plasma proteins labeled with isotopic iodine (I^{131}) or with isotopic chromium (Cr^{51}) in the chromic state have been employed to determine the volume of the circulating plasma. For the determination of the circulating mass of red blood cells, the isotopes of iron (Fe^{59}), of phosphorus (P^{32}) as phosphate, and of chromium (Cr^{51}) as chromate ion have been successfully employed.

Such measurements have been of great value in the study of traumatic and hemorrhagic shocks, anemias and of the survival times of injected erythrocytes.

Prior to the availability of isotopes of sodium and potassium, it was generally believed that erythrocytes, though freely permeable to anions such as chloride, were impermeable to cations. Using K^{42} , however, Hahn, *et al.*,⁵ in 1939, and Cohn in 1941,⁶ demonstrated a slow exchange of potassium between erythrocytes and plasma. Accurate measurements made in several laboratories since that time have established that a steady exchange of intracellular potassium occurs at the rate of about 1.5 per cent of the cell's potassium per hour.⁷ This exchange is dependent on metabolic activity of the cell. Sodium has also been found to exchange at approximately the same rate as potassium. Various theories have been proposed to account for the ion flux, but no completely satisfactory explanation has yet been given which relates the metabolic activity of the cells and the exchange of ions.

The basic information gained from these observations has been of value in blood preservation studies.

The mass of intracellular fluids in the body as a whole cannot be determined directly, but only estimated by difference, using the methods referred to above. However, such estimations have been found useful by Moore and his associates in following the course of tissue wastage and repair.⁴ It is furthermore possible to employ isotopic potassium (K^{42}) to determine the mean intracellular potassium concentration in the tissues of the body.

Studies on isolated muscle have shown that intracellular potassium is exchanged at the rate of about 15 per cent per minute (60 times faster than in erythrocytes).⁸ The rate of exchange of intracellular potassium in liver is many times more rapid than in muscle. A dependence of metabolic reactions in the liver on the intracellular concentration of potassium has been observed—a loss of intracellular potassium leading to modification in several aspects of carbohydrate metabolism.⁹

Isotopic phosphorus (P^{32}) has been widely used, not only to determine the rates at which phosphates are exchanged between extracellular and intracellular fluids, but more importantly the turnover rates of phosphorus among the metabolically important intracellular organic phosphates.¹⁰

The ability of investigators to now explore the mass, the composition and the relative rates of important metabolic reactions unfolds a whole new world for the study of disease processes—knowledge which has led and will continue to lead to improved therapeutic procedures.

BONES AND TEETH

The solid phase depicted in Fig. 1 designates the organic matrix of the skeletal structures and their minerals—i.e., primarily hydroxy-apatite, $3\text{Ca}_3(\text{PO}_4)_2 \cdot \text{Ca}(\text{OH})_2$, etc. It is currently thought that the bone salt is composed of minute crystals of hydroxy-apatite, $3\text{Ca}_3(\text{PO}_4)_2 \cdot \text{Ca}(\text{OH})_2$, which present a very large surface area to the surrounding body fluids and from which adsorption of ions or ion exchange occurs. Such ions include those of carbonate, citrate, sodium, etc. McLean has described the bone mineral in the following terms: "Each structural complex may be regarded as including interior unit cells wholly within the crystals; surface unit cells, sharing some of their sides with interior cells; ions bound by surface forces; and a surface hydration shell, containing ions in equilibrium, both with the surrounding medium and with the cell surfaces." The mineral phase of bone is thus composed of crystals of hydroxy-apatite which internally are stable and surface salts which are readily mobilized.¹¹

The evidence upon which this concept of the bone salt is based has been derived in large part from experiments with isotopic calcium, phosphorus and CO_2 .

More explicitly, by use of radioisotopes in the investigation of the skeletal system, new knowledge has been gained concerning the structure and dyna-

mics of the mineral phase and the matrix of bone. Employing exchange techniques it was found that there exist at least two phases in the mineral constituent of bone: (1) the surface of the apatite crystals, including the hydration shell, which is in very rapid equilibrium with the circulating fluid, and (2) the interior of the crystal, which equilibrates much slower. The uptake of radioactive isotopes can be explained by three different processes:¹² (1) heteroionic exchange on the surface, that is, exchange of ions such as sodium for calcium; (2) isoionic exchange, (Ca^{45} for Ca^{46}) and (3) recrystallization, a process which involves the partial or total remodeling of the apatite crystal and allows exchange of ions within the crystal lattice.

For the maintenance of homeostasis the surface of the microcrystals assumes the greatest importance. From X-ray, electron microscope and exchange studies, this surface was calculated to be about 80–100 acres in a 70 kg man. Maximally, about 25 per cent of the calcium and phosphorus are readily exchangeable. It is this fraction of the bone mineral which can readily replenish Ca or PO_4 depletions of the blood, without necessarily requiring the interaction of the parathyroid hormone. Studies of calcification in bone using radioactive phosphorus¹³ showed that this element is deposited selectively at sites of active bone metabolism, such as in the epiphysis and callus sites. In rachitic rats, the deposition of P^{32} in callus sites is decreased; vitamin D increases the P^{32} uptake in rachitic animals, while it has no effect on normal animals. The exchange of Ca^{45} in rachitic rats is reduced,¹⁴ and the administration of vitamin D increases it.

In a study of the formation of the bone matrix, S^{35} as Na_2SO_4 has been used.¹⁵ Rapid deposition as chondroitin sulfate throughout the epiphyseal cartilage has been observed. The rate of turnover, however, is not the same in all parts of the epiphysis. It is most rapid at the junction to the diaphysis. From this it has been concluded that new calcification is associated with an accelerated synthesis and removal of chondroitin sulfate in the adjacent cartilage—vitally important in the understanding of calcification.

Studies of problems of clinical importance, such as the removal of foreign substances from the bones and teeth, the treatment of rickets and of osteoporosis and the healing of fractures are greatly facilitated by the use of isotopes.

INTERMEDIARY METABOLISM

The availability of isotopes of carbon (C^{11} , C^{13} , C^{14}), hydrogen (H^2 , H^3), oxygen (O^{18}), nitrogen (N^{15}), sulfur (S^{35}) and phosphorus (P^{32}), has made possible the unraveling of many of the details of intermediary metabolism not previously known, and in the correction of wrong concepts of metabolic activity.

Beginning with Schoenheimer and Rittenberg's classic work on the turnover of fats and proteins¹⁶

biochemists have employed numerous isotopically labeled materials to explore metabolic reactions *in vivo* and *in vitro*, in many tissues and many species. The work has achieved such proportions that it is quite impossible to do more than present a few examples.

If one general conclusion is to be drawn, it is the remarkable way that reactions involving the metabolism of carbohydrates, fats and proteins are intertwined through short chain carbon units. The rate at which one possible reaction of a compound, such as pyruvate, may occur at the expense of another, determines the relative amounts of the components of the cell's metabolic mixture. And these rates in turn are influenced by the ionic composition of the environment and in specific instances by the hormones present.¹⁷

CARBON DIOXIDE INCORPORATION

In 1936, Wood and Werkman discovered that heterotrophic organisms can utilize CO_2 and showed that this was a C-3 to C-1 union with the formation of a four-carbon acid. In 1940, while studying the metabolism of isotopic lactate, the author and his associates accidentally found that the liver glycogen of rats injected with $\text{NaHC}^{11}\text{O}_3$ and unlabeled lactate contained radioactive carbon.¹⁸ After much scepticism and considerable difficulty, it was established that this radioactive carbon, which had originally been C^{11}O_2 in the body, had in fact been incorporated into the glycogen molecule. Indeed, the amount was considerable, indicating that about 12 per cent of the glycogen carbon had been derived from CO_2 carbon. (Later experiments with rabbit liver *in vitro* have confirmed these early *in vivo* observations.)¹⁹

The fact that CO_2 could be fixed by and played a role in the intermediary metabolism of mammals originally came as quite a surprise, but studies of the enzymic reactions involved, from the laboratories of Wood²⁰ and Ochoa²¹ have clearly identified the points of CO_2 incorporation, which provide it with a necessary metabolic role in normal liver metabolism.

PROTEINS

Early experiments of Schoenheimer¹⁶ with N^{15} -labeled amino acids showed the "dynamic state" of proteins and set the stage for further study of synthesis and intermediary metabolism of proteins.

One line of investigation has been the study of turnover times of proteins. The first experiments with radioactive tracers were those of Tarver and associates with S^{35} -methionine.^{22,23} Use of radioisotopes have the advantage that short time periods (minutes to hours) could be used and the differential rates of turnover in various organs and protein fractions could be observed. These authors confirmed the more rapid turnover rate of visceral proteins as opposed to muscle proteins, and conclusively demonstrated that the liver was necessary for the formation of plasma albumin, but not for formation of plasma globulins.

The technique of turnover measurements has been used by many investigators to study gross differences in metabolism under different growth conditions, in particular in tumors, e.g., Zamecnik.²⁴ These studies, in general, have shown some increase in synthetic activity of cancer tissue and a marked decrease in rates of protein degradation. Further uses of this technique have led to measurement of the life of human red blood cells in normal subjects and in subjects with polycythemia vera, sickle cell anemia, and pernicious anemia.²⁵

A second line of investigation has been the study of the mechanism of protein synthesis. Bloch and co-workers²⁶ have isolated the separate enzymatic steps in glutathione synthesis. This study required the use of C¹⁴-glycine as substrate, since synthesis could not be demonstrated by purely chemical means, because, in the *in vitro* system employed, there was a net destruction of glutathione. This led to the isolation of glutathione synthetase and to information on the mechanism of this reaction and its requirement for ATP.

Anfinsen and co-workers^{27,28} with the use of C¹⁴-labeled carbon dioxide and amino acids, have demonstrated net synthesis of such proteins as plasma albumin, ovalbumin, insulin and ribonuclease in *in vitro* systems. In applying this technique to the study of the mechanism of protein synthesis, they have shown differences in radioactivity of a given amino acid isolated from different positions in a protein, indicating that simultaneous incorporation of amino acids into all parts of the protein does not occur.

The introduction of isotopes has qualitatively changed the study of the intermediary metabolism of the amino acids and has shown the complex interconversions occurring not only between the amino acids themselves, but between amino acids and other tissue constituents. Outstanding in this field has been the elucidation of the metabolic reactions of the aliphatic amino acids by Minor Coon and his co-workers.²⁹

A metabolic synthesis of great importance is that of the heme of hemoglobin which has been investigated by Shemin and co-workers.³⁰ They have shown that succinyl CoA and glycine condense, followed by decarboxylation to give δ -amino levulinic acid, two molecules of which condense to form the basic pyrrole unit of heme.

An area of study which has developed almost completely from the application of isotopic tracers is the metabolism of "one-carbon" compounds, which involves the amino acids—methionine, serine, glycine and histidine—the nucleic acid and phospholipide bases, and the vitamins B₁₂ and folic acid. Important in these developments, have been the study of transmethylation and transulfuration studied with isotopes by du Vigneaud,³¹ Tarver,³² and the finding by Buchanan³³ that labeled formate was incorporated *in vivo* into the uric acid of pigeon excreta. A large number of investigators, du Vigneaud, Buchanan,

Stekol, G. Greenberg, Welch, Sakami, Sprinson, Winnick, etc., and their many co-workers have contributed importantly to this field. They have clarified the interconversions of the compounds cited above, have demonstrated the involvement of formate and formaldehyde as normal metabolic intermediates in amounts, undetectable by other than isotopic means,³⁴ have elucidated the role of the folic acid vitamins as carriers of formate and formaldehyde, and have thrown some light on the points of involvement of vitamin B₁₂.

One may confidently expect new light on clinical problems involving abnormal states of protein metabolism to result from such basic studies as those cited above.

NUCLEIC ACIDS

Experiments using the isotopic tracer technique have been particularly fruitful in the field of nucleic acids. In the past decade such experiments have shown the general outline, and many of the detailed steps, in the biosynthetic pathways of purines and pyrimidines, and have clarified the roles of folic and vitamin B₁₂ in these pathways. These have greatly aided our understanding of the biochemical lesion in pernicious anemia. Experiments with a variety of animal and plant species have shown that there is essentially no interconversion of the two classes of nucleic acids—that there is no conversion of pentose nucleic acid, (RNA), to desoxypentose nucleic acid, (DNA), or *vice versa*. They have shown further that, in contrast to almost all other constituents of the body, desoxypentose nucleic acid is not in "dynamic equilibrium" with the other substances present in tissues. Instead, it appears to be formed or "turned over" only at the time of cell division. In between cell divisions, the molecule is extremely stable. This finding is in agreement with our concept that DNA is an important part of the gene, and that a gene molecule should be a stable substance in order to transmit the hereditary characters of plants and animals with fidelity.

Nucleic acids are important parts of viruses, and the reproduction of viruses has been studied by a number of ingenious experiments using substrates labeled with two or three different isotopes. The reproduction of bacterial viruses (bacteriophages) has been studied by a group at the University of Chicago. They have shown that the phosphorus for the synthesis of new viruses comes in part from the host.³⁵ The relative amount from each depends on the size of the virus: small strains of bacteriophage get about 70 per cent of their phosphorus from the host bacteria, large strains only about 25 per cent. Further experiments showed that 'phage particles derive almost half of their purines from the labeled purines of the host. Using triply labeled substrates (P³², N¹⁵, and C¹⁴, they showed that the virus DNA is derived in part from the medium (by new synthesis) and in part from the bacterial DNA, but not from bacterial RNA. The evidence was interpreted

as showing that the infecting particle is disrupted within the host cell and new particles are formed from some sort of subunit. It has been estimated that there are about five such subunits in small phages and 25 in the larger ones. Genetic experiments provide data in agreement with these estimates.

It had been postulated that in early embryonic development the pentose nucleic acid of the egg is converted to desoxypentose nucleic acid for cell division. Experiments with P^{32} in developing sea urchin embryos showed that this was not the case: the biosynthetic pathways of pentose nucleic acid and desoxypentose nucleic acid are quite separate.³⁶ This has been confirmed by other experiments in which C^{14} -labeled purines were used.

A large number of experiments with a variety of animals has shown that there is a close correlation between the rate of synthesis of desoxypentose nucleic acid, as measured by the incorporation of P^{32} , labeled adenine, or labeled pyrimidines into DNA, and the rate of mitosis observed cytologically.³⁷ The DNA of the cells of the adult nervous system, for example, in which cell division is quite rare, has an extremely low rate of renewal. In contrast, about one-third of the DNA of the thymus gland, where mitoses are numerous, is renewed each day.

The work of Buchanan and his colleagues³⁸ in which the heavy isotope, carbon-13, was used, revealed the source of the atoms of the purine ring. By studying the synthesis of uric acid in the pigeon, they showed that carbons 2 and 8 are derived from formate, carbon-6 is derived from CO_2 by a process of CO_2 fixation, and carbon-4 and -5 and nitrogen-7 are derived from glycine, which enters the purine ring as a unit. The other nitrogens, 1, 3, and 9, are derived from aspartic acid and the amide N of glutamine. This was confirmed and extended by D. W. Wilson and his group, who used C^{14} and showed that the purines of the nucleic acids of the mammal are formed in exactly the same way.³⁹ The work of a number of investigators has revealed some of the intermediates in the formulation of purines from glycine, via glycine amide ribotide and amino-imidazole carboxamide ribotide.⁴⁰ A folic acid derivative has been implicated as the coenzyme for the transfer of formyl or "active one-carbon compound" into the purine ring.⁴¹

More recently, experiments with labeled materials have shown some of the intermediates in the biosynthesis of pyrimidines from oxaloacetic acid. These include ureidosuccinic acid and orotic acid. Carbon 2 of the pyrimidine ring is formed by CO_2 fixation and the 5-methyl carbon of thymine is derived from formate. The transfer of this formyl group also requires the folic acid derivative as coenzyme. The clarification of the role of folic acid in these biosynthetic pathways led to the synthesis and use of the folic acid antagonists, such as aminopterin and amethopterin, in the treatment of leukemia. 6-Mercaptopurine, an analogue of adenine, has been valu-

able in treating leukemia and certain other forms of cancer.⁴²

Thus, we see that isotopes have been invaluable in the study of the origin and the role of nucleic acids—subjects of basic importance to an understanding of genetic influences and of mutation.

LIPIDES

Isotopes have played a dominant role in unraveling the details of lipid metabolism since the classic experiments of Hevesy, Chaikoff, Artom and their associates, on the incorporation of P^{32} into phosphatides (Reviewed by Chaikoff⁴³), and those of Schoenheimer and his school using deuterium to demonstrate the dynamic state of body lipides and the interconversion of fatty acids of different chain lengths.

The former studies have led to a great number of researches on phosphatides (Reviewed by Artom⁴⁴), while the latter led into many experiments on the metabolism of isotopically labeled fatty acids. Among the most notable of the latter have been those of Gurin,⁴⁵ Weinhouse,⁴⁶ Popják,⁴⁷ and their colleagues. In addition, interest has also turned to the assembly of lipid molecules from constituent moieties. In this connection, glycerol has received attention because it forms the "backbone" of many lipid types, and because of its close relationship to carbohydrate metabolism.^{48,49} Recently, beautiful studies on phosphatide assembly have been carried out in the laboratories of Kornberg⁵⁰ and Kennedy⁵¹ using P^{32} and C^{14} .

In the steroid field, recent knowledge of the origin of cholesterol carbon stems from the brilliant experiments of Bloch⁵² and Popják⁵³ and their collaborators on cholesterol and squalene biosynthesis. Bergstrom has contributed valuable information on the metabolism of bile acids.⁵⁴ The problem of intestinal absorption of the lipides has been re-examined, using labeled lipides, by Chaikoff,⁵⁵ Reiser⁵⁶ and Bergström.⁵⁷

The use of isotopes in studies of disease involving dislocation of lipid metabolism is probably only now just about to flower—particularly in relation to abnormalities of steroid hormone metabolism, arteriosclerosis and obesity.

CARBOHYDRATE METABOLISM AND HORMONE ACTION

The use of isotopes of carbon has been of particular value in achieving understanding of the intermediary metabolism of carbohydrates, both in excised tissues and in whole organisms. To some extent, it has also been possible to gain more insight into the manner in which certain hormones exert their influence on metabolic reactions. Examples from both of these intimately related areas of investigation will be cited.

C^{14} has been used in the recent and rapid advances made in determining the biogenesis⁵² and intercon-

version of the steroid hormones.⁵⁸ The chemical incorporation of C^{14} into biologically active steroid hormones has been successfully achieved,⁵⁹ and the metabolism of these hormones in normal and diseased states is being actively pursued. Insulin has been labeled⁶⁰ with I^{131} , S^{35} and its metabolic fate determined, insofar as this type of labeling will permit. Concentration of I^{131} by the thyroid has been known for many years and has found so many well known clinical applications that they will not be reviewed here.⁶¹

Apart from the above examples, isotopes have become an almost essential tool in investigation of the role of hormones as regulators of biochemical processes. More subtle changes in intermediary metabolism are readily demonstrable by the use of labeled carbohydrates, amino acids and lipides. The regulation of the metabolism of these substances by hormones has rapidly developed in the last five years, as for example, the role of hormones in the regulation of carbohydrate metabolism. C^{14} -labeled glucose has been studied in experimental animals, in isolated tissue preparations and recently in man. In the intact animal isotopic glucose has made possible the estimation of the body glucose pool, rate of utilization and rate of production, and to a limited extent, the over-all regulation of these processes by hormones. Thus, Feller *et al.*⁶² have estimated that the glucose pool in the diabetic rat is twice that of the normal, but is turned over at a normal rate. No major defect in glucose oxidation was observed. Stetten *et al.*⁶³ estimated the rate of endogenous production of glucose and the influence of insulin and adrenalcortical hormones on this process. Basic work of this type, originally confined to experimental animals has now been applied to studies in man.⁶⁴ Several laboratories have studied the action of hormones on a biochemical level using isolated tissue preparations.⁶⁵

DIABETES

As a concrete example of the type of information one may obtain by the use of isotopes in the study of diabetes, I shall cite a few experiments from our own laboratory.

The metabolic lesion, or lesions, in diabetes has long been under debate—the question in its simplest terms has been whether an inadequate supply of insulin leads to an under-utilization of glucose or an overproduction of glucose by the whole organism. Either or both of the processes might be expected to result in an increased concentration of glucose in the blood.

Diminished glucose utilization in diabetes may be accounted for by a decrease in the rate at which glucose is phosphorylated to glucose-6-phosphate. Whether this is the result of diminished glucokinase activity as suggested by Cori⁶⁶ or a decreased intracellular concentration of glucose as suggested by Levine⁶⁷ and Park⁶⁸ and their associates is not clear at the present time.

Using glucose uniformly labeled with C^{14} , it has been found that diaphragms from diabetic rats use less glucose than those from normal rats.^{69,70,71} Furthermore, it was found that the addition of insulin, *in vitro*, to both normal and diabetic diaphragms increased the uptake of glucose. The glycogen formed from the labeled glucose by the diabetic diaphragms was reduced below normal, and the addition of insulin increased the glycogen produced by both the normal and diabetic muscle. It thus appeared clear that a lack of insulin led to under-utilization of glucose. However, since muscle does not contain glucose-6-phosphatase, it is not a producer of glucose and hence such experiments gave no information about glucose production in diabetes.

On the other hand, liver does contain glucose-6-phosphatase and is the principal producer of glucose from glucose-6-phosphate. Experiments on rat liver were therefore carried out using C^{14} -labeled glucose and unlabeled pyruvate as substrates in one flask and unlabeled glucose and C^{14} -labeled pyruvate in a paired flask which made it possible to determine both the amount of glucose taken up and the amount of new glucose produced by the liver. The results of such experiments are presented in Fig. 2.

The glucose output by the liver expressed as micromoles of glucose produced per gram of liver per 90 minutes incubation is plotted from zero, upward; the glucose uptake expressed in the same units is plotted from zero, downward. It is evident that in normal liver the glucose output but slightly exceeds the glucose uptake; whereas in diabetic liver, the glucose output is greatly increased above normal values and the glucose uptake is decreased to much below normal values. It would thus appear that glucose utilization in the liver in diabetes is impaired as it is in muscle but that, in addition, glucose production by the liver is markedly increased.¹⁷

The question then became, would insulin added to diabetic liver *in vitro* correct these biochemical abnormalities? In contrast to the prompt effect of insulin added *in vitro* on the glucose uptake of diabetic muscle, no such effect was demonstrable on liver. However, when insulin was administered to diabetic animals, 48 hours before they were sacrificed, the glucose output was returned to normal values, and the glucose uptake was even greater than normal.

This raised the question of what was the time sequence of metabolic changes produced in the diabetic animals following the injection of insulin.⁷² A group of diabetic rats was injected with insulin and sacrificed at various time intervals from 15 minutes to 48 hours after the first injection. The data obtained from the study of excised diaphragm muscle and excised liver in these experiments are shown in Figs. 3 and 4. Dashed lines, marked *N*, designate normal values.

The top curve (*A*) of Fig. 3, shows that before the injection of insulin, the ability of the muscle to

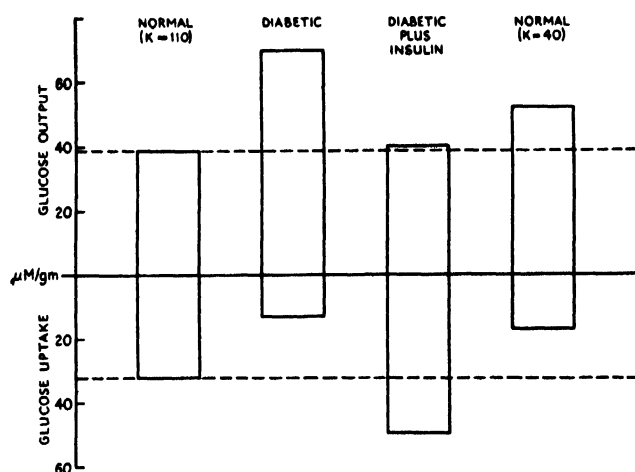


Figure 2. Glucose output is plotted upwards and glucose uptake is plotted downwards from the mid-line, for normal, diabetic and insulin-treated diabetic rat livers. Normal values are indicated by the dashed lines. All incubations were carried in an isotonic medium containing potassium in a concentration of 110 millimoles per liter—except that shown on the far right, where the potassium concentration of the incubation medium was 40 millimoles per liter

form glycogen from glucose was only about half-normal. Within 10 minutes after the injection of insulin, the conversion of labeled glucose to glycogen had risen to more than three times its normal value. Subsequently, this rate gradually fell toward normal. This experiment confirms the diminished ability of diabetic muscle to take up glucose and convert it to glycogen, and demonstrates the prompt *in vivo* action of insulin on muscle tissue.

The middle curve (B) shows the greatly diminished ability of the diabetic liver to convert glucose to glucose-6-phosphate. It will be noted that this is a much lower value than that given for net glucose uptake in Fig. 2. This is due to the fact that by combining experiments on labeled glucose with experiments on labeled fructose (which, once phosphorylated, can not again appear as fructose) we were able to calculate the actual rates of glucose phosphorylation.⁷³ These data revealed that the diabetic livers phosphorylate glucose at only about one-sixth the rate of normal livers.

In spite of the continued administration of insulin, glucose phosphorylation by the liver was not appreciably affected until more than six hours had elapsed after the first injection.

The bottom curve (C) shows similarly that the rate of conversion of pyruvate to fatty acids was much impaired in the diabetic liver—a biochemical lesion that was not restored to normal values until about 24 hours had elapsed after the first injection of insulin. This is consistent with the studies of others who have found that fatty acid synthesis is increased by insulin.^{74,75} These experiments demonstrate that, in contrast to muscle, the effect of insulin on liver is greatly delayed. Additional data obtained in this series of experiments is presented in Fig. 4.

The top curve (A) shows that the blood sugar concentration of the animals used in these experiments returned to normal about six hours after in-

sulin administration was begun. That this response was delayed, in spite of a prompt effect of insulin on the glucose uptake of muscle, indicates the existence of an additional biochemical abnormality.

The middle curve (B) shows that the liver of diabetic animals converted labeled pyruvate to glucose at a rate that was almost twice the normal value; furthermore, that this rate remained elevated until about 24 hours after the administration of insulin was begun. This shows that gluconeogenesis was elevated in the diabetic and did not respond rapidly to insulin administration.

Since one reason for the increased ability of the diabetic liver to make glucose might be an increased glucose-6-phosphatase activity, the diabetic livers were analyzed for this enzyme by Ashmore *et al.*⁷⁶ The results are shown in the bottom curve (C) of Fig. 4. It was found that the diabetic livers had glucose-6-phosphatase activities that were twice normal and that the continued administration of insulin did not restore these enzyme activities to normal until 12 hours had elapsed. The correlation between the rate of return of gluconeogenesis and of glucose-6-phosphatase activity from abnormal to normal values leads to the conclusion that the overproduction of glucose in the diabetic animal may indeed be due to an abnormal amount of this enzyme.

From our observations, therefore, it may be concluded that the diabetic state is one in which there is both diminished glucose utilization *and* increased glucose production—the former lesion being the result of diminished ability of the peripheral tissues to

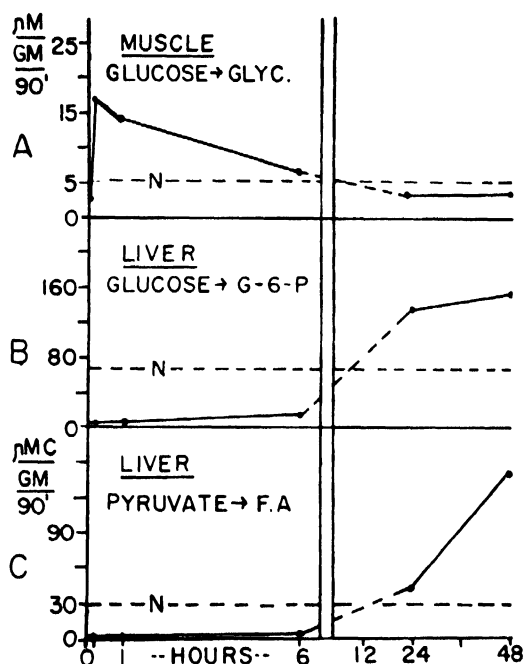


Figure 3. Biochemical sequence of events after insulin administration to diabetic rats. Curve A represents micromoles of glucose- C^{14} converted to glycogen per 90 minutes incubation, as a function of time following insulin administration to diabetic rats. Curve B represents micromoles of glucose converted to glucose-6-phosphate per gram of liver and Curve C represents micromoles of pyruvate converted to fatty acids per gram of liver from the same animals. The broken line marked N designates normal values for normal animals

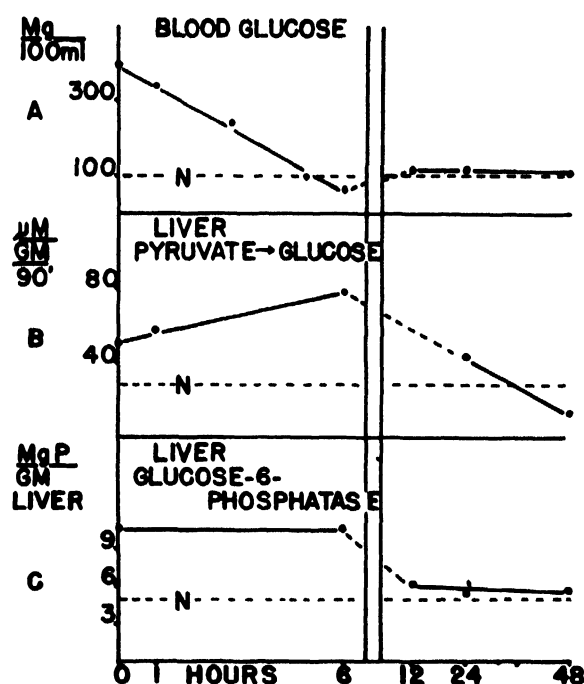


Figure 4. Curve A represents the blood glucose concentration in mg per 100 cm³ of diabetic animals following the administration of insulin. Curve B shows the changes produced in the micromoles of pyruvate-C¹⁴ converted to glucose by the livers of the same animals. Curve C designates the glucose-6-phosphatase activity measured as milligrams of phosphate released from glucose-6-phosphate per gram of liver per 30 minutes

phosphorylate glucose, the latter lesion being the result of an increased ability of the liver to split glucose-6-phosphate. The effect of insulin on muscle is a prompt, direct one—but on the liver, its effect is delayed and indirect.

It is of interest that recently Max Miller and his associates at Western Reserve University have reported data derived from the administration of C¹⁴-labeled glucose to normal and diabetic human subjects. From an analysis of their data, they have concluded that, as in our diabetic animals, severe diabetes in the human is also characterized by increased glucose production as well as decreased glucose utilization.⁶⁴ Here is another example of a medical mystery that could not have been elucidated unless labeling of molecules had become possible.

IONIC ENVIRONMENT AND METABOLIC ACTIVITY

This brief survey was begun with reference to the mass and composition of the extracellular fluid environment of the cells and to the mass and composition of the intracellular fluid environment of the organelles and enzyme systems. The manner in which the ionic composition of the intracellular environment influences the metabolic activity of cells is a subject of active investigation in many laboratories. Here is an area of research in which the use of isotopes to measure rates of ionic exchange and rates of specific reactions will prove invaluable. For example, in our own laboratory, it has been found that a diminished intracellular potassium ion concentration in normal liver leads to diminished glucose uptake, diminished

glycogen formation and increased glucose output.¹⁷ These metabolic changes, an instance of which is given in Fig. 2, are qualitatively similar to those observed in diabetic livers.

Experiments such as those cited illustrate the principle that, in dealing with the integrated metabolic reactions of the body, one is concerned with a coordinated system comprised of enzymes—coenzymes—hormones—and a homeostatic ionic environment, the maintenance of which is essential for normal metabolic activity. A study of such systems is only now made possible by isotopes.

CONCLUSION

Many other examples might have been cited on how the constituents of the body, formerly believed to be stable and static, actually are in a state of dynamic balance—now increasing, now abating—at rates characteristic of the particular tissue and process involved.

This change in concept has already led to important revisions in our search for therapy in metabolic diseases. Instead of radical replacement, we now seek those which alter rates of existing enzymatically catalyzed reactions by altering substrate concentrations and enzymatic activities.

Although much has been learned through the use of isotopes, this author believes that much greater insight into biological processes lie before us. Thus far we have made a good beginning toward describing the systems with which we are dealing, but we are far from understanding how they work together so harmoniously.

Investigators have shown remarkable ingenuity and skill in taking the systems apart—in isolating and characterizing the individual enzymatic systems concerned with the metabolic work of the cell. We seem to be at the threshold of the solution of the problem of how the energy of oxidation is utilized for the energy of synthesis. But even when the characterization of the individual enzymes is complete, and the link between metabolism and synthesis is solved, there will still remain the problem of how the numerous reactions are so coordinated in the cells, the tissues and, ultimately, the whole organism, to the end that the metabolism of the organism, as a whole, may proceed in the orderly fashion which we recognize as normal.

Isotopes have given us insight into old biological phenomena, they have opened up new vistas of which we previously had had no knowledge. It remains largely for the future to reveal the manner by which we may use this new knowledge for the conquest of disease.

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The Use of Isotopes in Analysis of Metabolic Disorders

By DeWitt Stetten, Jr.* USA

Attempts on the part of the biochemist to contribute to the understanding of the disease process are complicated by the fact that the experimenter generally imposes abnormal conditions upon the biological preparation in the course of securing his information. Except for purely analytical information derived from the study of excreta, it is always necessary to subject the animal to some insult, some disturbance, in the course of an *in vivo* experiment. This insult may range from the removal from or addition to the diet of some constituent to excision of an organ, slicing or homogenization of that organ, isolation of an enzyme from such a homogenate and ultimate study of the kinetics of the reaction which it catalyzes. At the end of such procedures, one is forced to evaluate the contribution to some process which results from disease as opposed to the contribution arising from the trauma of the experimental procedure.

Perhaps the least traumatic useful experimental procedure devised thus far is the administration of some normal body constituent in which one or more atoms are replaced by isotopic atoms. Under suitable conditions, the alterations in body processes resulting from such an experimental procedure are negligible, permitting the direct assessment of certain quantities which are otherwise difficult to obtain.

An example of such an approach to the analysis of a complex pathological process is to be found in studies by my colleagues and myself of the metabolic defect in gout¹. This disease, peculiar to the human, comprises an acute intermittent arthritis of characteristic distribution and drug responsiveness, and a defect in the body's handling of uric acid. It has been the latter aspect of the disease upon which we have concentrated.

To measure the quantity of uric acid in the human body, and its rate of regeneration or turnover, experiments were designed in which tracer amounts of uric acid-1, 3-N¹⁵ were injected intravenously and the uric acid of the urine was followed with regard to N¹⁵ content.² It was found, in accord with expectations, that the decline in N¹⁵ content of urinary uric acid followed a hemilogarithmic course, permitting the direct calculation of (a) the quantity of uric acid which mixed promptly with injected uric acid, the miscible pool, and (b) the rate at which

new uric acid was entering this pool. In normal subjects the miscible pool of uric acid was found to be about 1 gram in size and the turnover rate constant ranged from 50 to 75 per cent per day. In every case, the rate of entry of newly formed uric acid exceeded the urinary excretion, often by about 20 per cent, leading to the conclusion that uric acid was disposed of by routes other than urinary excretion. The finding of N¹⁵ in urea and ammonia of the urine, a finding which was uninfluenced by sulfonamide bacteriostasis of the intestinal tract,³ confirmed the idea that uricolysis occurred in normal man, although the tissue site of this destruction of uric acid is still open to some question.

The striking change noted in the gouty population was an enormous increase in the size of the miscible pool of uric acid when measured by this isotope dilution method. So large were some of the measured pools as to suggest that the surface layers of the solid urate masses, tophi, found in the cartilages in gouty individuals, were contributing to the miscible pool. This idea was confirmed experimentally by the analysis of a tophus excised from a patient who had received isotopic uric acid.⁴ Uricosuric drugs, such as salicylates, were found to mobilize this miscible yet solid phase urate and it has since been noted clinically that tophi may on occasion completely disappear under prolonged medication with such drugs.⁵

The source of the increase in quantity of miscible uric acid in gout has also been studied. To this end, glycine, a known precursor of uric acid in animals, has been labeled with N¹⁵ and fed to gouty and to normal subjects.⁶ In certain gouty patients, great increases, up to 3-4 times normal, were detected in the rate at which glycine nitrogen contributed to uric acid. It has therefore been concluded that in the gouty individual, uricogenesis, the generation of uric acid, is greatly enhanced, and that dietary amino acid may serve as a source of this extra uric acid. The conversion of glycine to uric acid in man has been studied under a variety of conditions and it has been found that 4-amino-5-imidazolecarboxamide, itself a uric acid precursor, markedly interferes with the entry of glycine into uric acid.⁷

A quite unrelated metabolic defect in man which has been studied by similar techniques is creatinuria. Creatine, normally present in urine only in trace amounts, is abundantly excreted in a variety of clin-

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ical conditions. Among these are progressive muscular dystrophy and thyrotoxicosis. Creatine has long been known to reside largely in skeletal muscle, where it undergoes reversible phosphorylation-dephosphorylation in the course of varying muscle activity. Each day some 2 per cent of total muscle creatine is cyclized to creatinine which appears to be a useless product to the organism and is rapidly excreted in the urine. Creatine is synthesized, not in muscle, but in kidney and especially in liver, by a well-defined series of reactions in which glycine, arginine and methionine participate. Essentially all of the creatine discharged by the human liver is consumed by the musculature, so that but little escapes normally into the urine.

The question which was set to be resolved was that of the source of the massive creatinuria in various disease states. Did this creatine represent material which leaked out of a diseased muscle or was it freshly synthesized creatine which, because of reduced muscle mass, failed to gain entry into muscle?

To explore this question, glycine- N^{15} was fed to patients with severe creatinuria due to progressive muscular dystrophy.^{8,9} Since glycine nitrogen is the immediate source of one of the three nitrogen atoms of creatine, a label is introduced into creatine by this device. If the creatinuria were a consequence of leakage of creatine from muscle, it would be anticipated that, since creatinine is known to arise solely from creatine of muscle, urinary creatine and urinary creatinine should be similarly labeled. This proved not to be the case. Shortly after ingestion of glycine- N^{15} the creatine of urine contained a far higher concentration of N^{15} than did the creatinine. With the passage of time, the isotope concentration in urinary creatine fell rapidly whereas that of creatinine fell very slowly indeed. From these results, seen now in three patients with progressive muscular dystrophy, it is concluded that the creatinuria is clearly not a consequence of leakage of creatine out of muscle. Rather it appears that the creatine of the urine is freshly synthesized material of hepatic origin which has never mixed with the reservoir of creatine in muscle. The creatinuria is therefore a consequence of diminished functional muscle mass. The liver, in these patients, perseveres in its function of creatine synthesis, unaware that there is insufficient functioning muscle to absorb creatine at normal rate. The unabsorbed surplus of hepatic creatine is what appears in the urine.

Recently we have had the opportunity to perform a similar experiment on a thyrotoxic patient with creatinuria.¹⁰ On the basis of the presumed mode of action of thyroid hormone and speculations as to the relative diffusibilities of creatine and phosphocreatine, there was reason to anticipate that in this situation creatine might indeed leak out of muscle. However the experimental results indicate that here, too, the creatine of the urine is freshly synthesized, unmixed with muscle creatine. It may be inferred

that hepatic overproduction of creatine is responsible for the creatinuria in this disease.

The application of isotopes to the study of the metabolic defects in diabetes has been most rewarding. Of the host of experimental results, a very few will be selected as examples. Most of the studies of this disease have been in animals rather than in humans, in view of the ease with which diabetes is produced in experimental animals.

The very earliest isotope studies in diabetic animals¹¹ revealed that a major defect was a failure of lipogenesis, fatty acid synthesis, in this disease. Originally this was demonstrated in the intact animal with the aid of the sole isotope available for the purpose at that time, deuterium. Since then, the observation has been amply confirmed using C^{14} and specifically it has been shown that liver slices from diabetic animals have far less capacity to synthesize long chain fatty acids from acetate than do slices from normal livers.¹² The ramifications of these observations are too numerous to list here but it should be mentioned that no similar restriction of acetoacetate or cholesterol synthesis occurs in this disease.

A second defect in the diabetic rat, also studied both in the intact animal and in the isolated tissue, is the impairment of conversion of glucose carbon to CO_2 .¹³ The results of studies of this type have clearly characterized diabetes as a disease of glucose underutilization and have distinguished the results of hypoinsulinism from those of hyperadrenocorticism. In the latter condition, a marked enhancement of gluconeogenesis, not manifest in alloxan diabetes, was readily demonstrable.¹⁴ These latter conclusions were based on experiments in which glucose production by tissues of the animal was measured by the dilution of the isotope in intravenously injected glucose- C^{14} .

From the foregoing examples it will be seen that the application of the isotopic tracer technique has proven of considerable value in the elucidation of pathological processes. The present indications are that it will grow in usefulness and will become one of the keystones of research in this area. The experiments conducted thus far are probably only crude beginnings of what is before us. Many isotope techniques, such as radioautography and activation analysis, are only beginning to be exploited. With increasingly sensitive measuring equipment, with increasing variety of isotopic compounds becoming available, it is impossible to foresee the limits of this powerful research tool.

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The Use of Radiosodium and Radiopotassium Tracer Studies in Man

By B. A. Burrows*† and J. F. Ross,‡ USA

INTRODUCTION

Changes in body sodium and body potassium have long been clinically important as separate metabolic abnormalities. Recently, interest in the ratio of body sodium to body potassium has been stimulated by the discovery of a sodium-retaining and potassium-losing adrenal hormone, aldosterone. This hormone is excreted in increased amounts both by the uncommon patient with an adrenal tumor and by patients with a variety of chronic diseases, such as congestive heart failure, hepatic cirrhosis, and the nephrotic syndrome. The investigation of changes in the body content of electrolytes by conventional balance studies is possible only for a few days or weeks because of the difficulties and cumulative inaccuracies of the methods. However, it is possible by repeated radiosodium and radiopotassium tracer studies to derive the same information in regard to overall balance changes with greater accuracy and over a longer period of time. Tracer studies also furnish a base-line value for initial body content of sodium and potassium which is otherwise unobtainable.

It is also possible to estimate the distribution of sodium in the body between extracellular and non-extracellular phases by serum sampling at appropriate intervals following intravenous administration of the tracer dose of radiosodium. Simultaneously, isotope dilution measurements of the body potassium, which is chiefly intracellular, can be made to determine changes in the ratio of body sodium to body potassium as well as changes in body potassium alone.

RADIOSODIUM AND RADIOSULFATE DISTRIBUTION

It is inaccurate to consider the body fluids to be divided into distinct compartments except in a dynamic or functional sense. However, it is informative to obtain an approximate measurement of the amount of body sodium which is extracellular, if changes in body sodium are to be interpreted. Extracellular

sodium content may change with fluctuations in extracellular fluid volume and obscure opposite changes in intracellular sodium content, as the greater part of "exchangeable" sodium (the sodium with which a tracer dose of radiosodium is mixed) is extracellular. An estimation of extracellular sodium appears possible if a comparison is made between the rates of distribution of radiosodium and radio-sulfate in the first two hours following simultaneous intravenous administration of tracer doses of these radioisotopes (Fig. 1).

Radiosulfate has been chosen as the reference substance for radiosodium distribution because it has been shown to mix rapidly with a volume of distribution comparable to that of other molecules presumably confined to extracellular fluid, and to be distributed thereafter at a much slower rate.¹ The apparent volume of distribution at eighteen minutes, correcting the dose for urine excretion, corresponds closely to the volume obtained by extrapolation of the slower distribution slope back to zero time. This has been taken as an index of extracellular fluid volume.

A comparison between the early mixing of radiosodium and radiosulfate with body fluids shows similar curves, with the result that the twenty minute apparent volume of distribution of radiosodium is equivalent to the radiosulfate space as an index of extracellular fluid volume. After this early mixing interval the radiosodium curve becomes almost flat, so that at twenty-four hours in normal subjects the apparent volume of distribution of radiosodium is approximately 1.3 times the twenty-minute value (Fig. 2).

The radiosodium and radiosulfate spaces have been compared in a group of patients undergoing elective surgery to determine if the reaction to surgical trauma would influence the rate of distribution of these radioisotopes. Patients were selected to exclude gross abnormalities in fluid and electrolyte metabolism before and during surgery. In such patients no changes in the rates of distribution could be detected following the operative procedure. Patients with gross accumulation of edema fluid, such as occurs in the nephrotic syndrome, showed no greater difference between radiosodium and radio-sulfate rates of distribution, although it is apparent that in such patients equilibration occurs more slowly than in normal subjects.

Including work by J. H. Sisson,§ M. M. Ashley,¶ M. A. Jewell,¶ E. S. Dell,† J. F. Kelly,† D. J. Davis,† G. J. Hine,† L. A. Sarkes,† T. Peterst and A. A. G. Lewis**

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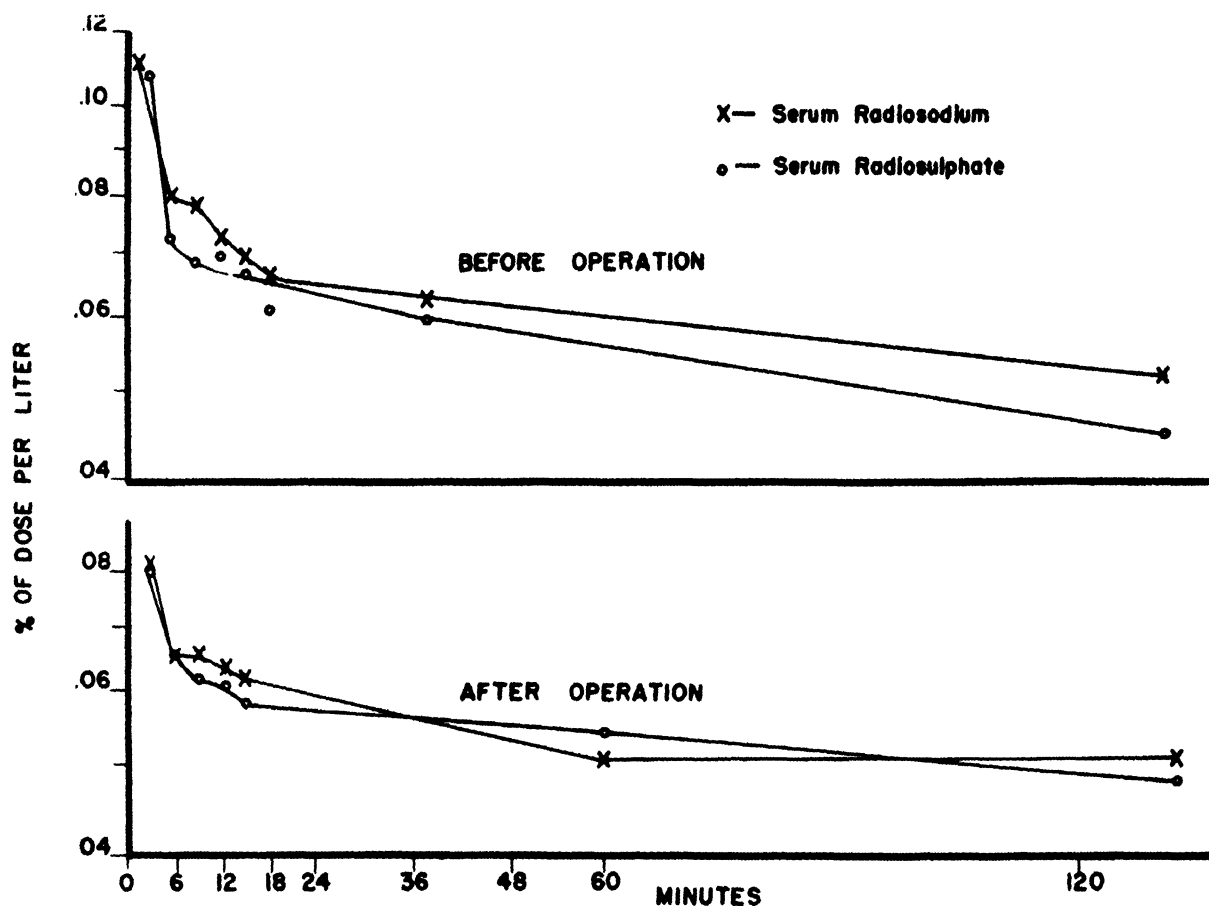


Figure 1. Radiosodium and radiosulfate distribution. The serum radioactivities (per cent of dose per liter) for radiosodium (Na^{24}) and for radiosulfate (S^{35}) following simultaneous intravenous administration of tracer doses are plotted against time in minutes. The curves are similar in this early mixing period both before and after surgical operations. The "apparent volume of distribution" (in liters) at any given time is obtained by dividing the dose minus urine excretion (100 minus per cent of dose in urine) by the serum radioactivity (per cent of dose per liter)

It would appear reasonable in patients without abnormal fluid accumulation to partition "exchangeable" sodium between extracellular and non-extracellular portions, using the twenty-minute sodium space as an index of extracellular fluid volume. In patients with edema or ascites the twenty-minute sodium space is probably considerably less than the actual extracellular fluid volume, but a comparison between simultaneous radiosodium and radiosulfate spaces should indicate an appropriate time at which time to make these calculations.

RADIOPOTASSIUM DISTRIBUTION

The determination of body potassium by the isotope dilution technique is not influenced by the amount of potassium in extracellular fluid, as most body potassium is intracellular. Therefore, in contrast to changes in body sodium, changes in body potassium are not due to expansion or contraction of extracellular fluid volume. The determination of "exchangeable" potassium at twenty-four hours following a tracer dose can be used as an index of changes in body potassium provided equilibration of the tracer radioisotope with body potassium is nearly complete (Fig. 3).

Rates of radiopotassium distribution obtained in patients with a great variety of metabolic abnormal-

ities, with and without potassium depletion, are apparently similar. Therefore, the same portion of body potassium is measured in diseased patients as in normal subjects by this technique.

Changes in body potassium determined by repeated tracer studies have corresponded to the changes shown by conventional metabolic balance studies, even when the gain in body potassium has amounted to one-half the initial "exchangeable" potassium value. This suggests that the same portion of body potassium is involved in both repletion of potassium-depleted subjects and in exchange with the radioactive tracer, indicating that the tracer measures the metabolically active potassium of the body (Fig. 4).

This metabolically active body potassium, related to body weight, is reduced in a variety of metabolic and other chronic diseases. The cell mass may also decrease in relation to body weight with a gain in body fat or fluid, so that such low potassium values do not necessarily reflect a reduction in intracellular potassium concentration in any or all of the body cells. Conversely, a loss of fat or fluid may reduce weight and mask changes in intracellular potassium content. However, in terms of a reduction in either cellular function or mass in relation to body weight,

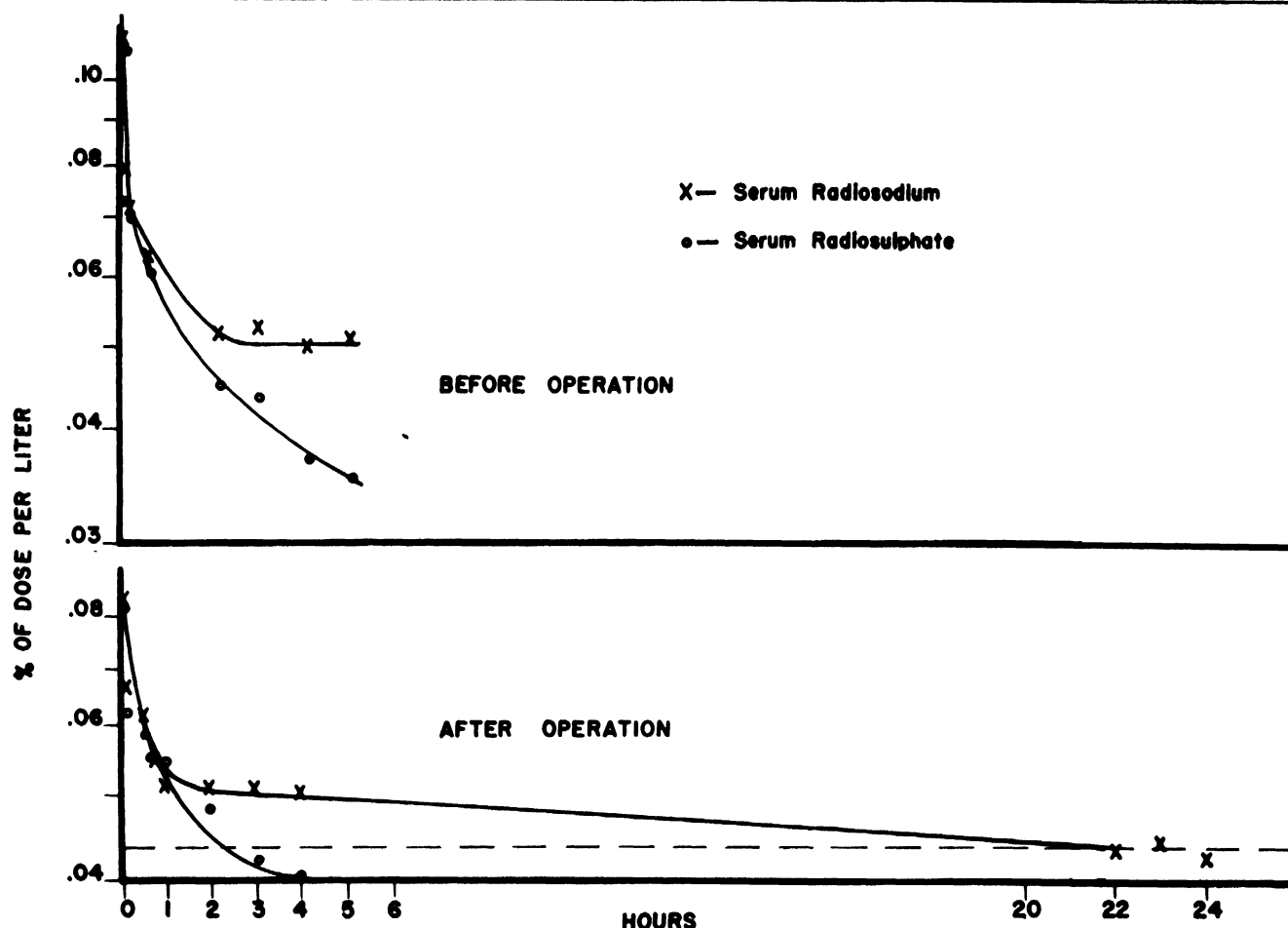


Figure 2. Radiosodium and radiosulphate distribution. The serum radioactivities (per cent of dose per liter) for radiosodium (Na^{24}) and for radiosulphate (S^{35}) following simultaneous intravenous administration of tracer doses are plotted against time in hours. The serum radiosodium curve approaches a plateau at twenty-four hours. The "exchangeable" sodium (in milliequivalents) is obtained by dividing the dose minus urine excretion (100 minus per cent of dose in urine) by the sodium specific activity (per cent of dose per milliequivalent)

these measurements may correlate with the debilitating effects of chronic illness (Fig. 5).

A decrease in the "exchangeable" potassium related to body weight may be associated with a low, normal, or high serum potassium concentration. The serum potassium level may change without significant alteration in "exchangeable" potassium. Patients with hepatic cirrhosis who had low serum sodium concentrations associated with abnormal fluid retention were restored during recovery to normal electrolyte values with little change in body potassium. This indicates that the abnormal serum sodium concentration in these patients was not due to an osmotic effect of depleted intracellular potassium. Also, patients with Addison's disease and others with Cushing's syndrome responded to therapy with restoration of normal serum potassium values without much change in body potassium. Potassium-depleted patients, however, showed a marked increase in body potassium with restoration of serum potassium values to normal.

These results would indicate that the serum potassium concentration is not a reliable index of body potassium content or changes in body potassium in many disease conditions. However, in patients with

true potassium depletion, a restoration of serum potassium values to normal would indicate a gain in body potassium, if not complete restoration of body potassium stores.

SIMULTANEOUS MEASUREMENT OF "EXCHANGEABLE" SODIUM AND POTASSIUM

As has been indicated, the separate determination of body sodium and potassium by the isotope dilution technique is of value in studying clinical disorders of electrolyte metabolism. However, it may be important to make these measurements simultaneously in certain acute situations and in patients in whom abnormalities in adrenal hormone secretion are suspected.

Mixtures of Na^{24} and K^{42} have been counted in biological samples by several different techniques.^{8,4,5} Either separation or differential counting of these radioisotopes is necessary because their half-lives are short and quite similar (12.4 and 15 hours). Most published methods are either insensitive, laborious, or require large serum samples when permissible human tracer doses of these isotopes are given.

With suitable scintillation counting equipment, it is possible to obtain accurate differential counting

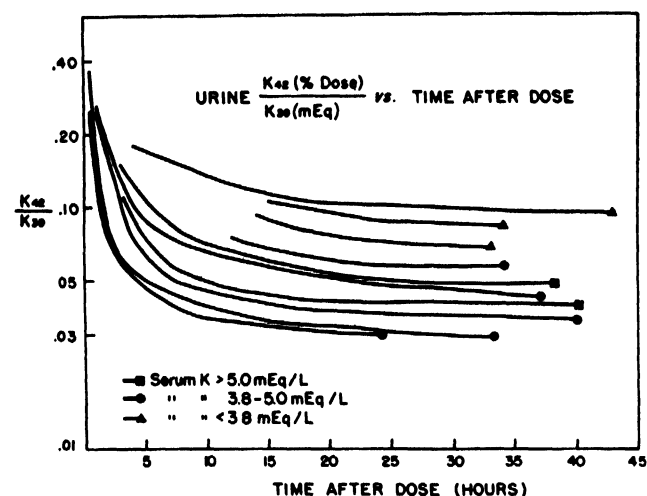


Figure 3. Radioactive K distribution. When a tracer dose of K^{42} is administered, either orally or intravenously, there is rapid mixing of the K^{42} with the naturally occurring K^{39} in the extracellular fluid compartment and exchange of the K^{42} with the K^{39} inside cells immediately commences. This exchange produces a fall in the urine ratio of K^{42} to K^{39} specific activity plotted against time. The urine specific activity, when it reaches a constant level, approximates the specific activity of the potassium in the body. The final specific activity, expressed as per cent of the dose of K^{42} per milliequivalent of K^{39} , is inversely proportional to the amount of "exchangeable" K^{39} in the body. This ratio varied from 0.03, representing a body potassium of 3300 milliequivalents, to 0.1, representing a body potassium of 1000 milliequivalents. There was, therefore, in this group of patients a three-fold variation in the amount of body potassium available for exchange with the tracer dose of K^{42} . Irrespective of abnormal serum potassium concentrations, differences in body potassium content, or the presence of metabolic disease, equilibration of the administered K^{42} with the body K^{39} is virtually complete at twenty-four hours. This point is taken to calculate "exchangeable" potassium by dividing the dose minus urine excretion (100 minus per cent of dose in urine) by the potassium specific activity (per cent of dose per milliequivalent)

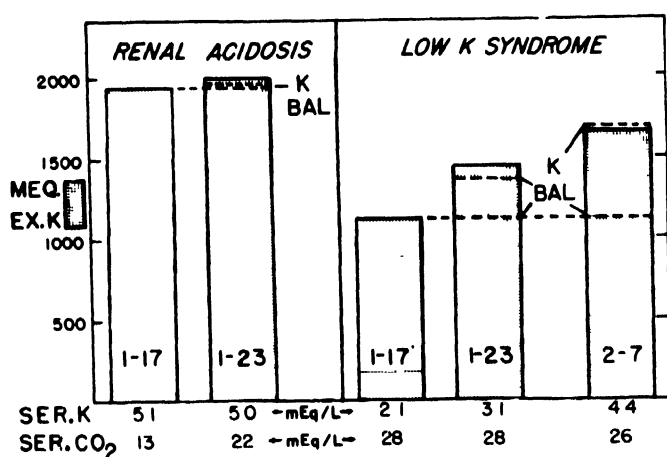


Figure 4. Radioactive K distribution, compared to K balance. The "exchangeable" potassium, in milliequivalents, is shown by shaded columns. Increases in exchangeable K^{42} are indicated by the area of the columns above the lower dotted line, which represents the initial exchangeable potassium value. The dates on which the "exchangeable" potassium was measured are indicated on the columns. The cumulative potassium balances between the dates of the "exchangeable" potassium measurements are indicated on the same scale between the lower and upper dotted lines. The first patient, with renal acidosis, was run on the artificial kidney with a marked reversal in the clinical and laboratory evidence of acidosis. The "exchangeable" potassium increased slightly after the extracorporeal dialysis, and this small change in body potassium was confirmed by a small positive potassium balance. (This patient was under the care of and balance studies were performed by Dr. John Merrill and Dr. Marcel LeGrain at the Peter Bent Brigham Hospital, Boston). The second patient had marked potassium depletion due to chronic diarrhea. With treatment the "exchangeable" potassium increased by almost one half the initial value, with an associated increase in the serum concentration from 2.1 to 4.4 milliequivalents per liter. The change in the body potassium content, as measured by this technique, agreed with the cumulative positive potassium balance²

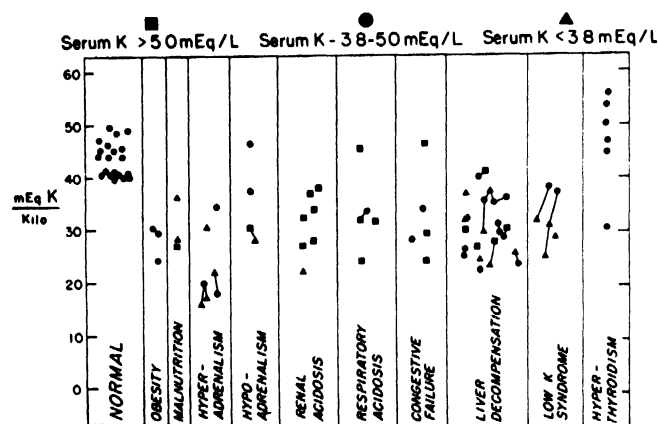


Figure 5. Radioactive K distribution, related to body weight. The exchangeable potassium is expressed as milliequivalents of potassium per kg of body weight. Repeated measurements of "exchangeable" potassium where there were changes in serum potassium concentration are connected by lines. Patients with hyperadrenalism include two patients with Cushing's syndrome before and after subtotal adrenalectomy, and two patients on prolonged cortisone treatment who showed either hypokalemic alkalosis or some features of Cushing's syndrome. Patients with hypoadrenalism include two patients with Addison's disease, one before and after treatment, and one patient with panhypopituitarism. The "exchangeable" potassium in patients with liver decompensation remained relatively constant on repeated measurement, but due to loss of weight in the form of edema fluid, this value related to body weight, sometimes rose. Patients with low potassium syndrome showed increases in "exchangeable" potassium with little change in body weight. In hyperthyroidism there were slight increases in this index with weight loss

Table I. Counting Efficiencies for a 3-ml Sample in Well-type Scintillation Phosphors (%/Disintegration)

Isotope	NaI(Tl)		Plastic	
	β -rays	γ -rays	β -rays	γ -rays
Na^{24}	0	40%	7%	13%
K^{42}	0	4%	30%	1%

of Na^{24} and K^{42} in a 3-ml sample following tracer doses of 200 μ c in human subjects.⁶ This method depends on the direct relationship of the energy of the incident gamma-rays to the resultant height of the pulses obtained from the NaI crystal. The more energetic Na^{24} gamma rays can be selectively counted using pulse height discrimination (Fig. 6).

K^{42} has the more energetic beta rays. These can be preferentially counted in a plastic phosphor which is relatively insensitive to gamma rays.

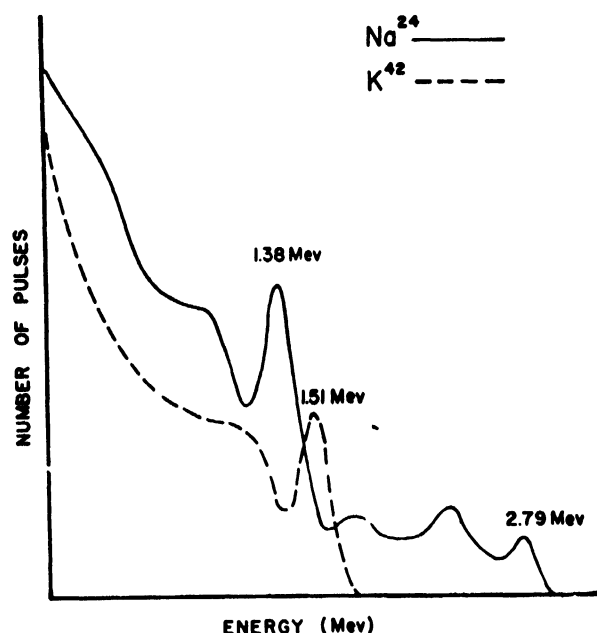


Figure 6. Gamma ray energy spectra of Na^{24} , K^{42} . The radioactivity measured in a well-type NaI(Tl) crystal (number of pulses) of separate samples of Na^{24} and K^{42} , plotted against gamma-ray energies (Mev). The relative contribution of each radioisotope to a mixed sample depends on pulse height selection by scintillation counter

The separate contributions of Na^{24} and K^{42} to the activity of a mixed sample can be determined by counting the sample consecutively with the two well-type scintillation phosphors and solving suitable equations. The activity of each radioisotope can be

then related to the appropriate standard representing the dose, correcting for decay.

This technique permits frequent sampling of serum following the simultaneous administration of tracer doses of radiosodium and radiopotassium. The distribution curve for each radioisotope can then be employed for the estimation of "exchangeable" sodium and potassium and "extracellular" sodium in human subjects.

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The Application of Radioisotopes to the Study of Cancer Induction

By Charles Heidelberger,* USA

CARCINOGENIC HYDROCARBONS

The pioneer experiments by Yamagiwa and Ichikawa, who, in 1915, first succeeded in producing skin cancer by the application of coal tar to the ears of rabbits, culminated in 1932 with the isolation of 3,4-benzopyrene from coal tar, and the demonstration by Cook and Kennaway that it and dibenzanthracene, pure chemical compounds, could induce tumors in experimental animals. This field of chemical carcinogenesis was actively pursued in the non-isotopic era by the above mentioned English workers and by Fieser and Shear at Harvard, who synthesized hundreds of related compounds and assayed them for carcinogenic potency. Thus, although the carcinogenic hydrocarbons became invaluable tools for the production at will of tumors in experimental animals, their potentialities as tools for studying the biochemical mechanisms of carcinogenesis remained unfulfilled. Some progress has been made in understanding the relationship between the chemical constitution and biological activity of these compounds as exemplified by the "K region" theory of the Pullmans, but a large gap in our knowledge between the electron density in an organic molecule and the complex biological processes leading to cancer remained unbridged.

Some excellent beginnings in understanding the metabolism of these compounds were made by Berenblum and others, which led to the identification of several metabolites of the carcinogenic hydrocarbons, particularly of benzopyrene. This work was carried out using fluorescence and ultraviolet spectroscopy, but this method often suffered from a lack of accurate quantitation, and in most cases the recoveries of administered doses were extremely small. When the long-lived radioisotope of carbon, C^{14} , became available it was feasible to launch a long-term detailed study of the mode of action of these compounds, and considerable strides in our understanding of this process have already been made. Some of the applications of isotopic tracers to the biochemistry of cancer have been reviewed.¹

The first step in these tracer studies involved the synthesis of the labeled compounds, which was soon accomplished in the cases of dibenzanthracene,² methylcholanthrene,³ and benzopyrene.⁴ A study was

then initiated in which the labeled dibenzanthracene was administered to mice by a variety of routes, and the radioactivity excreted and in the various tissues was determined at several times. In accordance with expectations, quantitative recoveries were obtained, and it was shown that the material was excreted primarily through the bile and into the feces. Furthermore, the radioactivity present in the hydrocarbon, although ample for tracer experiments, was not sufficient to produce any complicating biological effects on the process of tumor induction.⁵ Since no information about the chemical state of the radioactivity was available, chemical fractionations were carried out on tissues and excreta, which showed that extensive metabolic degradation, mostly to carboxylic acids, had taken place.⁶ These acids no longer retain the characteristic dibenzanthracene ring-system, which accounts for the low recoveries obtained in the earlier metabolic experiments by the use of optical methods that require the presence of the unchanged hydrocarbon or closely related derivatives.

In order to assess the relationship between metabolism and carcinogenic activity, the rates of elimination from the site of subcutaneous injection of labeled dibenzanthracene, methylcholanthrene, and benzopyrene were studied under closely comparable conditions. It was found that dibenzanthracene was retained at the site of injection for the longest period of time, benzopyrene for the least, and methylcholanthrene for an intermediate time.⁷ This is in accord with and explains earlier observations that dibenzanthracene is the most potent carcinogen of the three at very low doses. At higher doses benzopyrene is the most powerful, indicating that above a minimal level benzopyrene is sufficiently potent to initiate tumors in spite of its rapid elimination. Thus, it is evident that for the correct evaluation of carcinogenic potency, information on the rate of metabolism must be available.

In the identification and quantitative determination of metabolites of dibenzanthracene, the radioactive carrier technique has been uniquely applicable. If a non-radioactive compound, suspected of being a metabolite, is added to a solution containing labeled metabolites and is isolated and purified rigorously, any radioactivity present in the compound must

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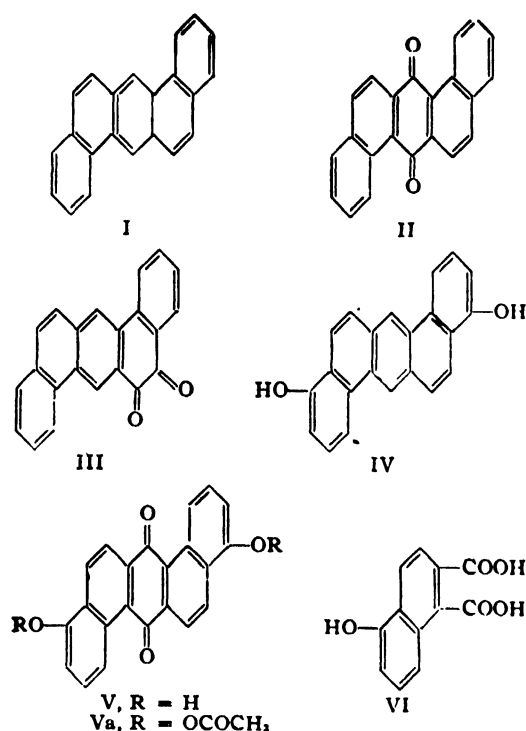


Figure 1. Metabolites of dibenzanthracene

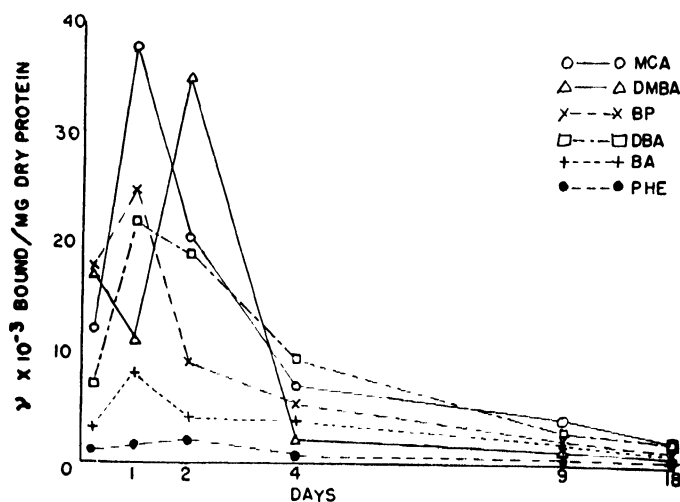


Figure 2. Hydrocarbons bound to soluble proteins

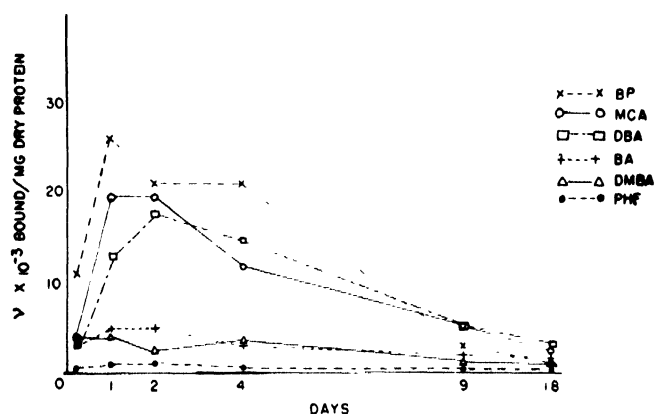


Figure 3. Hydrocarbons bound to particulate proteins

have been due to minute amounts of that metabolite and represent a quantitative analysis of its presence. By means of this technique, microgram quantities of the compounds indicated in Fig. 1 have been shown to be metabolites of dibenzanthracene in tissues and excreta.^{8,9} Previously, compounds hydroxylated in positions similar to that in compound IV had been shown to be metabolites, but the identification of the 9,10-quinone (II), the 3,4-quinone (III), the dihydroxyquinone (V), and the dicarboxylic acid (VI) disproved a formerly held generalization that metabolic oxidation occurs only in regions of the molecule that are not the most chemically reactive, and thus represent new pathways of aromatic hydrocarbon metabolism. However, it appears likely that these compounds represent detoxication products of the hydrocarbon and are not directly involved in the carcinogenic process.

In order to gain more direct information about the carcinogenic process it appeared necessary to study the interaction of the carcinogenic hydrocarbons with cancer-susceptible tissues. A valuable lead in this direction was furnished by the work of the Millers, who demonstrated the presence of bound dye in the livers of rats fed the hepatic carcinogen, *p*-dimethylaminoazobenzene. This binding occurred only in liver, and a series of excellent correlations was found between this biochemical reaction and the process of tumor induction.¹⁰ That protein-binding to susceptible tissues also occurs with the carcinogenic hydrocarbon benzpyrene was demonstrated by fluorescence methods by E. C. Miller.¹¹ This lead has been followed making use of the tracer technique with labeled hydrocarbons. A new method was developed for the clean separation of the epidermis and dermis of mouse skin from the fat and connective tissue, which permitted homogenization and separation of the dermal and epidermal proteins into particulate and soluble proteins.¹² The binding of labeled dibenzanthracene to these protein fractions was studied at various time intervals after a single application to the skin, and the peak specific activities were reached at one day for the soluble and at two days for the particulate proteins. This binding was not due to adsorption or coprecipitation and did not involve the nucleic acids.¹²

Because it was not possible to separate the skin homogenates into nuclear, mitochondrial, microsomal, and supernatant fractions, attention was turned to the submaxillary gland where such a separation could be accomplished by differential centrifugation. This is a tissue in which tumors are rapidly induced following direct injection of carcinogenic hydrocarbon solutions. The time course of the protein-bound radioactivity in these fractions was measured following a single injection of isotopic dibenzanthracene. There was essentially no bound radioactivity in the nuclei, but a large peak was reached at three days in the soluble fraction. The mitochondria and microsomes had intermediate and

similar specific activities. This evidence suggests, although it does not prove, that the soluble fraction may be the one most concerned in the initiating steps of chemical carcinogenesis.¹²

A good correlation was also found between the amount of protein-binding following direct injection of labeled dibenzanthracene into a number of mouse tissues and their susceptibility to tumor induction.

In order to determine more critically whether this phenomenon is directly related to the carcinogenic process, a number of chemically similar hydrocarbons were synthesized with radiocarbon and their binding to the skin proteins following a single topical application was measured quantitatively under standardized conditions. The results obtained are shown in Figs. 2 and 3. Phenanthrene (Phe) is not carcinogenic, 1,2-benzanthracene (BA) is very weakly carcinogenic, 1,2,5,6-dibenzanthracene (DBA) is of moderate carcinogenic power, and benzpyrene (BP), methylcholanthrene (MCA), and 9,10-dimethylbenzanthracene (DMBA) are all very potent carcinogens. It will be noted from Fig. 2 that there is a perfect correlation between the quantity of radioactivity-bound and the carcinogenic potencies of the applied hydrocarbon in the soluble protein fraction. In Fig. 3 a similar correlation is found with the exception that dimethylbenzanthracene (DMBA) is not appreciably bound to the particulate fraction. This is a further suggestion that the soluble fraction may be of greater importance than the particulate in tumor induction. In addition, labeled 1,2,3,4-dibenzanthracene, a compound reported to be non-carcinogenic, was synthesized and studied in a similar fashion. This compound is also extensively bound to the proteins, and the reason for this apparent discrepancy has not yet been explained.

Because of the likelihood that the binding phenomenon represents a very important step in the chemical induction of tumors, it was felt that its definition in chemical terms would represent an important contribution to our knowledge of the mechanism of the carcinogenic process. Accordingly, studies of the structure of the bound derivative of dibenzanthracene are being carried out. The situation can be schematically represented as in Fig. 4, where Aa represents individual amino acids in the protein, and M is the bound metabolite of dibenzanthracene. When the protein is treated with pepsin, which hydrolyzes it to the small polypeptide level, the binding is unaffected. This completely rules out the possibility of ionic bonds or Van der Waal's forces being involved, and leaves only the alternative of a covalent chemical bond. When the pepsin treated peptide fraction is hydrolyzed with alkali to the free amino acid level, it has been shown that some metabolite remains bound to amino acids, and that some is liberated, presumably in altered form (M'). The M' fraction contains primarily free carboxylic acids. In searching for a compound which might satisfy this requirement, 2-phenylphenanthrene-3,2'-dicar-

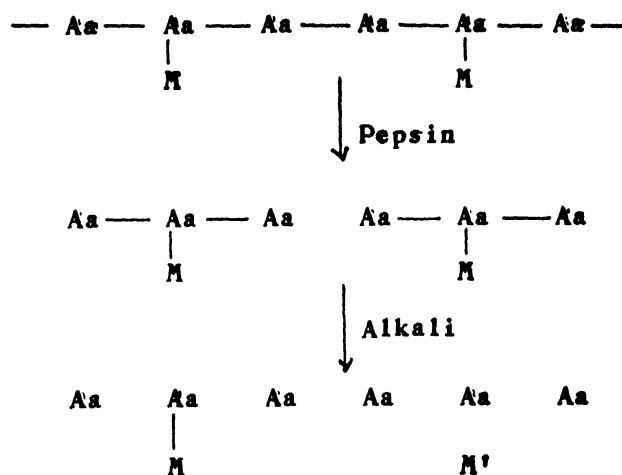


Figure 4. Schematic representation of protein-bound metabolite

boxylic acid (PDA), indicated in Fig. 5, was tried as a carrier and was shown to account for the major portion of the carboxylic acid fraction. It was present in alkaline hydrolysates in amounts equivalent to 5 per cent of the original bound material. However, when the hydrolysis was carried out in the presence of permanganate, PDA accounted for 25 per cent of the total bound radioactivity.¹³

It then became of importance to know whether this acid was bound to the protein as such or whether it was produced as an artifact from some precursor still containing the ring during the degradation procedure. In order to test this, the proteins were treated with hydrazine, which if the acid were bound to amino acids through amide linkages, would give rise to one or both of the hydrazides whose structures are shown in Fig. 6. These two compounds were then synthesized as carriers and both carried equal amounts of radioactivity, which in all amounted to 25 per cent of the original isotope in the proteins. Thus, it is evident that PDA is bound as such to two peptide chains and to a single peptide chain (presumably through terminal or lysine amino groups) as indicated in Fig. 6. Further work aimed at the elucidation of the remaining radioactivity is being continued.

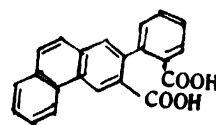


Figure 5. 3-phenylphenanthrene-2,3'-dicarboxylic acid (PDA)

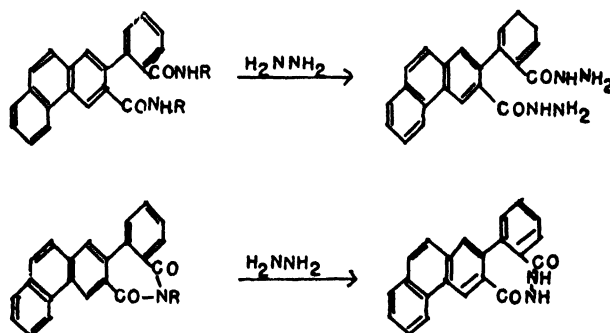


Figure 6. Reactions of hydrazine with protein-bound PDA

AZO DYES

The group of azo dyes that are derivatives of *p*-dimethylaminoazobenzene produce liver tumors in rats after prolonged feeding. The work of the Millers has shown that with these highly colored substances, a great deal of metabolic information could be obtained without isotopes. However, when it became necessary to study the oxidative removal of the N-methyl groups, a series of these compounds of varying carcinogenic activity were synthesized with radio-carbon and their metabolism studied *in vivo*.^{14,15} It was shown that the methyl groups are converted by rats into respiratory carbon dioxide, and the presence of radioactivity in choline and creatinine demonstrated that these carbons gave rise to the synthesis of labile methyl groups, which then participated in the metabolism of the animal. Serine was demonstrated as an intermediate, showing that the demethylation went through the oxidation level of formaldehyde. However, in a series of substituted dyes of different carcinogenic activities, no significant differences were found in methyl-group metabolism.

The metabolism of an aminoazo dye labeled in each of the two benzene rings has been studied by the Millers, but the results have not as yet been reported.

ACETYLAMINOFLUORENE

This compound, a potent carcinogen that produces tumors at many sites when fed to rats, has been labeled⁴ with C¹⁴ and its metabolism has been studied. In comparing the distribution of radioactivity following feeding of 2-acetylaminofluorene-9-C¹⁴ and the same compound labeled in the methyl carbon of the acetyl group, Morris *et al.*¹⁶ found considerable differences. There was a greater conversion to respiratory carbon dioxide and less urinary excretion of the methyl-labeled carcinogen, which suggests that deacetylation occurred to a considerable extent. Most of the isotope was excreted in the urine, and radioactivity was present in many tissues. The Millers have shown that acetylaminofluorene is bound to the proteins of the liver, a tissue susceptible to carcinogenesis by this compound¹⁷ and this has been confirmed by the Weisburgers,¹⁸ who also demonstrated that the compound was excreted in the urine in the form of several hydroxylated derivatives. The effect of a riboflavin-deficient diet considerably altered the tissue distribution of radioactivity, but did not affect the amount bound to liver proteins.¹⁹ It is of interest in this connection that riboflavin deficiency does not affect the incidence of tumors induced by acetylaminofluorene.

CONCLUSION

The fact that three series of compounds, as chemically unrelated as the carcinogenic hydrocarbons, the aminoazo dyes and acetylaminofluorene and which produce different types of tumors at different sites, all combine chemically with the proteins of sus-

ceptible tissues suggests very strongly that this may represent a common mechanism of tumor induction. This phenomenon lends support to the "deletion" theory of carcinogenesis.¹⁷ According to this theory the carcinogen combines with a protein which is an enzyme or enzyme system essential for the control of growth. The carcinogen complex results in the inactivation of its metabolic function and during subsequent cell divisions some cells might survive with the loss of this enzyme system. Such cells would lack a growth control mechanism and would thus be cancer cells. If this theory is correct, it could explain the induction of tumors by ultraviolet light, radiation, viruses and other causative agents which could by different means lead to the inactivation of the same enzyme systems. It is therefore imperative that continued studies on the mechanism of chemical carcinogenesis aimed at the eventual elucidation of the chemistry of the carcinogens involved and the nature of the proteins with which they interact be vigorously pursued. Once this is understood it should be possible to determine the enzymatic and metabolic function of these proteins. If this can be accomplished, the problem of carcinogenesis may well have been solved. The solution of this baffling problem would have important implications in endogenous cancer in humans and should lead to new advances in the chemotherapy of cancer and perhaps even make prophylaxis available.

This review demonstrates the tremendously important contributions that isotopes have made towards the fundamental studies of the mechanism of the induction of tumors and these contributions also extend to cancer research, biochemistry, biology, and medicine. The significant advances that have been made in the relatively short time that isotopes have been readily available point the way towards even more progress in the future, and firmly establish isotopes as indispensable tools for the understanding of nature, the alleviation of disease and suffering, and which if they continue to be used peacefully and with humility, will bring to humanity a bright new era of health, prosperity and understanding.

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Dental Uses of Radioisotopes

By W. W. Wainwright,* USA

It was predicted by Leicester¹ that radioisotopes would provide the most promising tools for investigation of the hard structures of the teeth. Dental enamel being acellular and dense, is the least subject to change of any tissue of the body. Under these circumstances Leicester points out that any changes which do occur will be gradual and very slight. This prediction has been amply confirmed. It has been 16 years since publication of the first paper in this country, by Manly and Bale,² in which radiophosphorus was used as a tool in the study of tooth metabolism. After an initial period of exploration of the technics and possibilities of this new tool, radioisotopes lately have been overshadowed by the results of studies obtained with their use, and by the more complete data obtained by a correlation of all methods of investigation. The major contributions with radioisotopes have been made (a) in the elaboration of technics for the study of calcification and calcified tissues, especially the formation of teeth, (b) the discovery of new functions of tooth structure, (c) the examination of pathological changes in teeth and alterations of function, and finally (d) an evaluation of therapeutic technics and the establishment of new principles of dental therapy.

The gradual disappearance of emphasis on the tool, the radioisotope, and the concentration of interest on the investigations on dental problems has made it increasingly difficult to segregate publications in which radioisotopes have been used in dental investigation. Possibly one measure of the use of radioisotopes would be to add up the shipments of isotopes distributed for dental use. This would not be quite adequate because of the number of associate investigators involved in fundamental studies where dental tissues have been made available in the course of other work. The investigator attempting to survey the literature in preparation for a project therefore finds there are two types of dental investigators using radioisotopes. First are those whose primary object is the investigation of some dental problem. Second are the ones where other objectives come first, but, secondarily dental studies are included in the investigation (as, for example, in studies of the general skeleton, or broadly, on complete studies of the metabolism of specific substances). In indexing and abstracting journals, these several types of investigations are listed quite differently. The first is given

primarily in dental journals and when these have been published in the English language they are indexed in the Index to the Dental Literature and do not usually appear in medical and biological indexing journals. The second group of investigations usually is indexed in medical, biological, chemical or basic science indexing journals but seldom appears in dental indices.

A few major reviews of dental uses of radioactive materials have appeared: Armstrong,³ McCauley,⁴ Leicester,^{1,5} Wainwright,⁶ Bartlestone,^{7,8} Lussier⁹ and Council on Dental Research.¹⁰ Additional reviews for special purposes have been published by Armstrong,¹¹ Bartlestone,^{12,13} Copp,¹⁴⁻¹⁷ Archinow,¹⁸ Schwartz,¹⁹ Wainwright,²⁰⁻²⁴ Belgorod,²⁵ Symposium,²⁶ Alyea.²⁷

This review is an attempt to cover publications up to May 1955 on the uses of radioisotopes by dental investigators in the United States. For more complete coverage of this subject than can be given in this review, the reader is referred to the reviews mentioned.

STUDIES OF METABOLISM, ADSORPTION AND EXCHANGE OF RADIOISOTOPES IN TEETH AND BONE

The earliest studies using radioactive isotopes in any type of biological investigation were on the metabolism of teeth and bone. The pioneer work of Hevesy and his co-workers^{28,29} demonstrated a marked ability of the teeth and jaw bones to take up quantities of minerals. The first studies were, in general, on the subject of the metabolism and utilization of radiophosphorus³⁰⁻³⁷ and sodium³⁸ and later of calcium^{39,40} and strontium.⁴¹ Soon it was shown that other processes besides calcification were involved in the deposit of these minerals, and terms such as turnover,⁴² adsorption,⁴³⁻⁴⁸ and exchange⁴⁹⁻⁵¹ were soon introduced. Adsorption was used to indicate the deposit of minerals on the surface of a structure such as tooth enamel at a time when calcification was not active, for example, after formation and eruption of the tooth. Later it was found that not only was there an uptake on the surface of structures but there was also a release of material from the solid phase to a surrounding liquid phase. In this case, the term turnover was used at first, and subsequently the term exchange.

Manly and Bale² found that the uptake in teeth was considerably less than that of bone and that

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the incisor teeth of rats acquired radiophosphorus both by exchange and by calcification. They were able to demonstrate that phosphorus exchange occurred primarily in the inorganic portion of calcified structures. Hevesy and Armstrong⁵² found only a minute amount of parenterally administered radiophosphorus, 0.005% per gram, in the enamel of cats, whereas, in the dentin there was about 10 times this amount. Volker and Sognnaes⁵³ and Sognnaes and Volker⁵⁴ found that a large portion of the radiophosphorus was deposited in the outer layers of enamel and was derived from the saliva. In unerupted teeth, on the other hand, they found the same quantity in the enamel as in the dentin. Wasserman, Blayney, Groetzinger and De Witt⁵⁵ and Blayney, Wasserman, Groetzinger and De Witt⁵⁶ found that pulpless teeth acquired only negligible quantities of radiophosphorus in the enamel through the saliva and that about 10% of the radiophosphorus present in the tooth crown was acquired by means of the periodontal vessels through the cementum and thus from the root to the crown. Barnum and Armstrong⁵⁷ found in cats that the uptake of phosphorus by enamel was about $\frac{1}{20}$ of that of the dentin. After five days, one gram of ashed enamel contained only 0.00546% of the original subcutaneously administered dose of radiophosphorus. The dentin contained 0.00878% whereas it was 2 to 7 times this in bones and still higher in marrow. They showed that phosphate could be transferred from the saliva to the enamel and then to the dentin and also in the reverse, from the blood to the dentin and then to the enamel. Koss and Ginn⁵⁸ traced radiosodium after parenteral administration into the teeth of animals; the largest fraction not being present in the mineral phase. Campbell and Greenberg⁵⁹ found that radiocalcium under *in vivo* conditions was deposited in about equal amounts in bone and whole teeth. Armstrong⁶⁰ showed that powdered enamel and dentin acquired radiophosphate from solutions under *in vitro* conditions, thus accounting for the uptake of radiophosphorus and other minerals by formed calcified structures on the basis of ion exchange. Hodge, Van Huysen, Bonner and Van Voorhis⁶¹ found this to follow the Freundlich adsorption isotherm. Armstrong,⁸ in commenting on these mechanisms, states that although they have been demonstrated, the rates of exchange are so slow that it would be many years before it would be possible to renew the phosphate, for example of one gram of enamel.

During a complete investigation of the metabolism of radioactive gallium, English and Dudley⁶² studied its distribution in the jaws and teeth of rats and rabbits. Ga⁷² was found, radioautographically, to locate primarily on the surfaces of bones, the walls of the pulp chamber and root surfaces of teeth. There was a lesser uptake in compact bone and still less in trabecular structure and in the dentin of the root. None was observed in the dentin of the crown or in the enamel.

FLUORINE

Following the observation that fluoride containing water supplies provided a partial resistance to dental caries, Volker, Hodge, Wilson, and Van Voorhis⁶³ studied the ability of enamel, dentin, bone and hydroxyapatite to adsorb fluoride. As a result of these studies, they proposed the possible benefits of topical application of fluoride solutions to the surfaces of teeth. As a result of the marked interest in this clinical procedure, a number of studies followed, testing the effects of fluoride deposition in other parts of the body. Volker, Sognnaes and Bibby⁶⁴ showed that parenterally administered fluorine was largely excreted in the first 30 minutes. By this time there had been some uptake in the teeth, mostly in the root dentin and in growing areas of the rat incisor. Other studies were made on the ability of tooth surfaces to take up fluoride.⁶⁵ Hein, Bonner, Brudevold and Hodge⁶⁶ found that teeth scrubbed with a brush and water picked up relatively little fluoride, whereas teeth repeatedly scrubbed with pumice took up greater amounts of fluoride on the surface. Brushing with water removed some of the fluoride but almost all of it could be removed in 5 seconds by brushing with pumice. Brushing with calcium phosphate tooth paste removed about half of the fluoride in 30 seconds. They observed that the uptake in superficial opacities or white spots on the enamel was much higher than in sound enamel. In a further study McCann and Bullock⁶⁷ found that there are at least 5 reactions by which fluorine is taken up on the surface of powdered enamel and dentin. The first is by exchange with carbonate which is assumed to be bicarbonate. This amounted to 0.1% if a 2 ppm F solution was used on dentin. If the solution was a 2% one this amounted to 1.3% uptake, whereas the 2% solution against enamel only gave an uptake of 0.2%. The second reaction is by adsorption with identical amounts acquired. The third reaction is by the precipitation of magnesium fluoride, the magnesium being supplied to the distilled water from the teeth. The quantity in this case is about half that of the previous reactions. The fourth is by the precipitation of calcium fluoride in roughly the same quantities for dentin as before, however, the amount on enamel was 1.0%. The last reaction is that of the formation of fluorapatite. The amount is determined by difference from the above figures and in dentin for the 2 ppm solution is 0.4% uptake; in the 2% solution it is 1.2%; and in enamel, for the 2% solution it is 0.2% uptake. McCann and Bullock point out these phenomena in order that we may be able to interpret published data on the uptake of fluorine by powdered enamel and dentin. They note that the *in vivo* situation in the presence of saliva is bound to be different (for example, the magnesium effect is not to be expected) and also that adsorbed fluorine and precipitated calcium fluoride are gradually converted to fluorapatite during the course of time.

A very interesting radioautographic study of the uptake of radioactive fluorine on the surface of teeth was made by Myers, Hamilton, and Becks in 1952.⁶⁸ They found that 2% of the fluoride ions applied with a cotton pledget were transferred to the tooth. The major portion of this could be removed by rinsing. The localization of the fluoride fixed on the surface of the tooth was primarily in defects and discolored areas of enamel. From these studies it is apparent that the reaction of topical fluoride on the surface of the tooth is a complex one, and apparently most of the uptake is in damaged areas of the tooth.

Several studies have been made on the metabolism of injected fluoride. For example, Wills⁶⁹ showed that intravenously administered fluorine was found within 1 minute in the stimulated submaxillary salivary secretion of the cat. Within 21 minutes, 0.082% of the original dose had appeared in the saliva. By comparison, within 32 minutes of the administration of a 3 times greater dose of chlorine 0.365% had appeared in the saliva. When the fluorine was injected and the submaxillary gland stimulated after a waiting period of 50 minutes, in the following 21 minutes of stimulation, 0.020% of the original dose of fluorine was secreted in the saliva. The ratio of salivary fluoride to plasma fluoride concentration was 0.098 whereas the ratio of salivary chloride to plasma chloride concentration was 0.389.

The interest in possible toxic effects of fluoride has resulted in four publications in 1954 on the metabolism of fluorine. Wallace-Durbin⁷⁰ found that there was no significant deposition of F^{18} in soft tissues with the possible exception of the kidney. There was some fecal excretion of F^{18} . After 9 hours, approximately 10% of the oral dose was found in the cecum, large intestines, and feces. The distribution was the same whether the F^{18} was given orally or by vein. In the soft tissues no age difference was to be observed, in contrast to the higher uptake in the teeth and bones in younger animals. Likins, Zipkin, Steere and McClure⁷¹ found that in the rat, 20 doses of 10 mg F each to weanling female rats resulted in an increase in fluoride content by 30 times in the bone ash and 15 times in the incisor and molar dentin. These authors⁷² also tested the effect of previous administration of fluoride on the deposition of radioactive calcium. They could find no effect on the deposit of this substance in bone. Harris⁷³ tested the effect of acute and chronic administration of sodium fluoride on thyroid iodine metabolism in rats by measuring the thyroid uptake of I^{131} . Fluorine had had no discernible effect on thyroid iodine metabolism.

The Manly and Hodge flotation method of separating powdered enamel and powdered dentin in a bromoform mixture was tested by Binford and Sognnaes.⁷⁴ Radioactive powdered dentin containing P^{32} was mixed with non-radioactive enamel. Separation was quite successful, there being no radioactivity in the enamel nor in the bromoform solution.

This confirms the previous tests of this method which were made on the basis of microscopic examination of the particles separated. By means of the use of radiophosphorus-labeled dentin, Binford and Sognnaes were able to make a rapid, direct test of this separation technic.

RADIOAUTOGRAPHIC METHODS AND SPECIAL TECHNIQS IN DENTAL INVESTIGATION

The ease with which ground sections can be prepared made it readily possible to apply the technics of contact or survey radioautography in dental investigations. Sognnaes and Shaw⁷⁵ and Sognnaes, Shaw, Solomon and Harvold⁷⁶ developed technics for the simultaneous radioautographic study of both hard and adjacent soft structures. Wainwright⁷⁷ applied the technics of nuclear track radioautography to undecalcified bone sections and outlined simple radioautographic technics. Bartlestone⁷⁸ outlined the fundamentals of the radioautographic process. Martin and Slater⁷⁹ presented a technic of permanently mounting the radioautographic emulsion on the surface of ground tooth sections. Myers, Jennings and Becks⁸⁰ preferred a radioautographic technic in which a superimposed weak roentgen exposure was made, to assist in orientation. Bartlestone and Wolff⁸¹ adapted a stripping emulsion technic to radioautography of tooth sections and achieved a high degree of precision for grain density radioautographs. Kinnersly⁸² reported the application of nuclear track radioautography to beta emitters from tooth specimens. For some years radioautographic exposure time has been estimated on the basis of specimen activity. Existing formulas were converted to a network chart for exposure calculation by Wainwright, Anderson, Hammer and Lehman.⁸³ They included exposure calculation for short-lived isotopes where the exposure time was in the order of less than one half of a half-life. In this period the exposure time does not vary in a linear fashion with the activity of the specimen. The chart is also useful for estimating the minimum activity required in a specimen for radioautographic exposure. In another chart they point out the desirability, when a selection is possible, of using an isotope of weaker emission in order to improve radioautographic sharpness.

Suggestions and plans for the arrangement of radioisotope laboratories have been presented by Sognnaes and Shaw⁸⁴ and Quimby and Braestrup.⁸⁵

A method of much interest to the clinician has been presented by Neumann and Di Salvo⁸⁶ using a Geiger-Mueller tube counter small enough to be introduced into the mouth. It has a window smaller than the crown of an individual tooth. They were able to measure the beta radiation from P^{32} from an individual molar tooth in a live monkey. Using this technic after the injection of 500 μ c they were able to obtain "high" counts from a single molar tooth. After unilateral trigeminal nerve resection including the motor fiber, they found a difference in activity

from the unoperated side, greater than 2 or 3 times the background; whereas in control animals molars on opposite sides of the jaw gave equal activity. Neumann and Di Salvo were able to accomplish these results because of the small size of the counter tube and also because they were able to shield the tube from radiation from other parts of the subject. This technic is of intriguing interest to the clinician who wishes to study pulpal circulation and the results of healing after surgery.

RADIOIODINE

The increasing use of radioiodine in the treatment thyroid disease has made possible a great many human *in vivo* investigations. Bartlestone, Mandel, Oshry and Seidlin⁸⁷ announced the localization of radioiodine in the teeth of human beings undergoing thyroid therapy. In a long series of subsequent articles Bartlestone,⁸⁸⁻⁹¹ and others cited in other sections of this paper, demonstrated the ability of the living human tooth to transmit iodide ion through enamel, dentin and cementum. Especially interesting is the finding that the dead tracts of dentin (transparent dentin) are more freely permeable to iodine than is normal dentin. This is in contrast to the classical concept of translucent or metamorphosed tracts of the dentin, which resisted the penetration of dyes and hence resulted in the name, dead tract. Bartlestone showed that enamel defects and carious areas of enamel and dentin are highly penetrated by radioiodine. He also showed cementum penetration in human teeth whereas in other investigations dyes did not have the power to penetrate human cementum. In experiments tracing the behavior of radioiodine in the penetration of enamel, Bartlestone showed that cat enamel is so permeable to this substance that the isolated tooth is easily penetrated from the surface of the enamel and that within 2 hours iodine uptake can be measured with an external Geiger counter over the thyroid gland. These remarkable findings have revolutionized our ideas of the metabolic and physical behavior of teeth and their relation to their nutrient environment.

Wainwright, Butt, Hauptfuehrer and Dute⁹² confirmed the findings of Bartlestone and found the deposition of radioiodine to occur in the teeth in periods as short as five hours and to persist for periods as long as 19 days after the administration of the iodine.

Santy¹⁰⁶ reporting in 1952 states that the only side effect from the administration of radioiodine for thyroid therapy is occasional transient symptoms of radiation sickness. He mentions that if nausea, vomiting or malaise, swelling of the thyroid, or fever occur, they are not significant and disappear within a day or two.

Schneyer⁹³ in 1953, and with Tanchester⁹⁴ in 1954 pointed out that there were damaging effects from the administration of radioiodine to the sali-

vary glands in humans which resulted in a decrease in salivary secretion and salivary amylase activity. In one case these changes were subsequently followed by a rampant type of atypical caries characterized by large white opacities along the gum line of the teeth. In one tooth the destruction was so great as to permit the crown to snap off. A possible factor in the localization of iodine in teeth has been mentioned by Ash⁹⁵ who produced increased deposition of radioiodine in the molars of rats which had been traumatized by blows from a gold foil plugger. Ten days after the trauma, I¹³¹ was administered intraperitoneally and the activity of the traumatized and control teeth measured at 6, 12, 24, 36 and 48 hours. The uptake in the traumatized molar peaked before that in the thyroid gland, parotid gland, liver, kidney and other tissues, and reached a figure 3 to 5 times that of the control teeth.

The possibility of experimental error introduced through storage of teeth containing radioiodine was pointed out by Butt and Hauptfuehrer.⁹⁶ They found that if teeth were stored at room temperature there was a relocation of the iodine and a slow diffusion through the teeth. This could be controlled by simple storage in the freezing compartment of the refrigerator.

CALCIFICATION

As soon as it had been shown that radioactive isotopes could be used for the study of calcified tissues, that radioautographic exposures could be made from bone and tooth sections, and an understanding was achieved by the processes of metabolism, adsorption and exchange, investigations were started on the process of calcification. As yet, thin window counting tubes had not been introduced which would make possible ready measurement of the weak beta emission of Ca⁴⁵, so Pecher⁹⁷ investigated the possibility of using Sr⁹⁰ as a substitute since its chemical behavior was similar. Pecher showed that in many respects strontium could substitute for calcium in the process of calcification; however, the similarities were not complete. More detailed studies have been made by Jarabak and co-workers.⁹⁸⁻¹⁰⁰ This group of workers was able to distinguish between ion exchange on the surface of bones and teeth and the actual calcification process. From these observations they were able to show that the uptake of radio-calcium is essentially similar to that of alizarin, the time honored dye which has been used to demonstrate sites of calcification. The precision of location of areas of calcification, however, is much greater with calcium-45 than with the dye. Radioautographic studies enabled Jarabak and co-workers to examine in detail all areas of the skull and teeth in growing rats and to show the dynamics of remodeling of the skull. Myers¹⁰¹ (1955) studied the deposition of radio-calcium in the lower incisor and neighboring structures of the rat. He noted that calcium-45, during the formation of the enamel of the continuously growing incisor, deposited in both the roentgeno-

lucent and roentgenopaque enamel. In other words the enamel which is already calcified but is immediately adjacent to the forming areas of enamel continues to acquire calcium salts.

Jarabak¹⁰² used these methods to study the healing of fractures with the aid of calcium-45. Rats whose right radius had been fractured were sacrificed at various subsequent periods of 5 to 72 days. One day before sacrifice they were injected intraperitoneally with 20 μ c of Ca⁴⁵. Five days after fracture Ca⁴⁵ was deposited in the primary callus with some diffuse deposition in the surrounding bone. Shortly, thereafter, there was a generalized deposition in the region of the callus. At this second stage of repair, the callus itself showed the highest deposition. Thirty-six days after fracture the diffuse deposition had disappeared and calcium-45 was deposited in the outer portion of the callus; there was some organization of the fracture and the beginning of formation of a medullary canal. After 72 days, the different areas of bone formation surrounding the callus began to coalesce especially in the periphery of the shaft.

TOOTH FORMATION

The fascinating possibilities of investigation of calcification and tooth formation by means of radioautographic studies with Ca⁴⁵ have enabled investigators to study the actual substance calcified; rather than to use some indirect method of study, such as the appearance of a dye along with the calcification process (albeit a dye with toxic properties in some concentrations) or the somewhat indirect methods of histology in which the appearance of cells and staining properties are used to detect the beginnings of calcification. Bevelander¹⁰³ introduced a method whereby he could observe calcification of the matrix of the shell of molluscs through the microscope under living conditions. He was able to introduce radioactive calcium and radioactive phosphorus into these *in vivo* arrangements and subsequently to prepare radioautographs and to identify the appearance of globules of calcification. The method, of course, suggests the obvious possibility of studying modifying factors in the calcification process. In a series of papers, Greulich,¹⁰⁴⁻¹⁰⁶ working with Leblond and Belanger has studied the detailed functions of radiocalcium and radiophosphorus in the formation of enamel and dentin. They have been able to identify several phases of calcification and matrix formation by means of labeling with C¹⁴. Sodium bicarbonate-C¹⁴ was injected into newborn rats and radioautographic studies of decalcified sections made 4 hours to 15 days later. These showed that the C¹⁴ labeled matrix is a permanent acquirement of these structures quite in contrast to the lack of fixation of C¹⁴ labeled substances formed in other organs. Usually C¹⁴ in inorganic structures in other parts of the body is quite labile and moves rapidly from its original location; however, in the organic matrix of teeth it is fixed quite permanently and is

not removed by decalcification. They describe the process of dentin matrix formation and calcification as follows: The C¹⁴ is laid down presumably in a collagen which first appears as predentin; next, large amounts of carbohydrate material are added to the framework of the matrix, changing it from predentin to dentin; lastly, calcium phosphate crystals appear in the matrix of the dentin. Nicholas and Denzler¹⁰⁷ are exploring the possibility of applying radioisotopes to studies of phosphatase activity.

THE NATURAL RADIOACTIVITY OF HUMAN TEETH

Since there are many naturally occurring radioisotopes and since some of these are radioactive heavy metals such as radium, it is interesting that De Ment¹⁰⁸ has been able to produce evidence of their deposition in teeth. He suggests that the major activity is due to P⁴⁰ with an upper limit of concentration of 10⁻⁴% with traces of uranium, thorium, radium and C¹⁴. Examination of prints of the original radioautographs have satisfied this author that the exposures, which are necessarily long, are actually from the specimens and are not due to artifacts and represent radioactivity naturally occurring in the teeth. The identification of the deposited radioisotopes remains to be clarified.

STUDIES OF TOOTH PERMEABILITY

The differences in uptake of phosphate and sodium ions in teeth suggested more detailed study of tooth permeability, especially with respect to the different microscopic structures of the enamel. Reviews on this subject have been published by Hodge,¹⁰⁹ *Nutrition Reviews*,¹¹⁰ and Wainwright.^{111,112} The early findings were greatly extended by Sognnaes and Shaw,¹¹³ and Sognnaes, Shaw, Bogoroch and Sweeney.¹¹⁴ By isolating the teeth of monkeys and placing cups over the crowns of the teeth they were able to control the introduction of radioisotopes and to direct them toward the tooth either internally from the blood supply or externally from the surface of the tooth. After intravenous injection of P³² they found the highest activity in the dentin to be next to the pulp chamber. The activity gradually decreased to the dental enamel junction. Phosphate did not pass the dental enamel junction and did not enter the enamel, when applied only from the pulpal side. When applied externally, the highest concentration was found at the enamel surface, gradually decreasing to the dental enamel junction, which was not passed in the 5 hours of the experiment. The enamel of teeth which had only recently erupted and had not yet reached the line of occlusion took up 10 times the amount of phosphorus found in the enamel of mature teeth. The dentin of newly erupted teeth took up twice as much P³² as that of mature teeth. The amounts in the enamel and dentin of the recently erupted teeth were about the same in contrast to the much reduced uptake in the enamel of mature teeth, which took up only $\frac{1}{5}$ as much as the dentin of the same tooth. It was not possible to change the

distribution of P^{32} in dentin or enamel by diluting the external solution with water or by the addition of 20% sucrose. When the pulps were removed from the teeth the uptake of P^{32} was greatly reduced especially around the walls of the pulp chamber; however, some did get into the dentin presumably through the root surface. (See also Volker, Gilda and Ginn;¹¹⁵ Gilda, McCauley and Johansson;¹¹⁶ and McCauley and Gilda.¹¹⁷) When the teeth were tested with I^{131} and H^3 the enamel and dentin proved to be much more permeable than they were to P^{32} . Within 5 hours both I^{131} and H^3 passed from the pulp through the dentin and enamel to the surface.

The availability of radioisotope-labeled compounds has made it possible to test the behavior in teeth of a number of specific substances. For example, Wainwright and Lemoine¹¹⁸ demonstrated in freshly extracted human teeth that C^{14} -urea was able to penetrate diffusely through intact enamel and dentin and to reach the pulp chamber within 18 hours. The urea followed the general direction of histologic elements of the enamel and dentin. The softer radiation from C^{14} permitted a more precise interpretation of the radioautographs than is possible with P^{32} and showed that the penetration occurred diffusely through calcified structure. Diffuse penetration was also observed by Wainwright¹¹⁹ when nicotinamide, thiourea and acetamide were applied to the enamel. The time, however, varied greatly; in the case of nicotinamide diffuse penetration was observed through 0.4 mm of enamel within 79 minutes. Urea penetrated 0.5 mm within 20 minutes; while thiourea and acetamide penetrated the entire thickness of the enamel, 1.0 mm and 0.3 mm, respectively within 34 minutes. After slightly longer periods all four compounds penetrated the dento enamel junction at intervals and entered the dentin. In a few of the teeth the pulp was reached. Identification was made of the urea molecule as the penetrating agent by means of radiochromatographic examination of dentin extracts of two teeth which had been treated with C^{14} -urea on the enamel surface.

In a series of studies in which the same four compounds were placed in the pulp chambers of freshly extracted teeth, Wainwright and Belgorod¹²⁰ found that each compound penetrated through approximately 3.5 mm of dentin to reach the dento enamel junction within 20 minutes. At 37° acetamide penetrated the dentin 4.1 mm, and faintly through the enamel 2.1 mm, within 22 minutes. Acetamide was the only compound in this series of experiments which penetrated the dento enamel junction when placed in the pulp chamber. Wainwright¹²¹ reported on the movement of methionine from the pulp chamber through the dentin in a banded pattern with a high uptake at the wall of the pulp chamber then a band of reduced uptake followed by a band of greater uptake.

The observation by Bartlestone of broad bands of penetration by radioiodine through the exposed root

surfaces of human teeth to the pulp chamber was confirmed by Wainwright^{122,123} for short periods of time and a review of possible factors presented.

LEVELS OF ENAMEL PERMEABILITY

In contrast to the previously reviewed experiments, when plutonium citrate solution was applied to the surface of freshly extracted teeth, Wainwright¹²⁴ found that although there was surface uptake, gross defects were not penetrated. The defects were easily stained by silver nitrate. This, together with the finding of rapid diffuse penetration of enamel by small organic molecules, suggested at least 3 levels of enamel permeability. The first one is exemplified by plutonium citrate which localizes on the surface only and does not penetrate intact enamel or grossly damaged structure. The second level is the classical concept of enamel permeability as judged by the behavior of dyes and silver nitrate, in which damaged structure can be permeated but not intact structure. The third level of permeability is that exemplified by small organic molecules which rapidly penetrate intact enamel. For these diffusely penetrating substances the dento enamel junction is not a barrier to further penetration into the dentin.

Muehleman¹²⁵ with the assistance of Wainwright and Hauptfuehrer further investigated this problem with the purpose of trying to artificially modify enamel permeability. He attempted this by pretreating the enamel with solutions of compounds of different valence. In this study with S^{35} -thiourea as the tracer Muehleman was able to show detailed changes in enamel permeability whereby penetration was enhanced in the middle layers of the enamel and decreased just below the surface and at inner enamel layers. In view of these findings it is evident that there are an infinite number of levels of enamel permeability depending upon the immediate circumstances of the environment with respect to the saliva, foods, organisms, plaques, etc., etc.

PROPERTIES OF ALTERED TOOTH STRUCTURE

Areas of sclerosis of the dentin have been examined by Leung and Gilda¹²⁶ and Boyd.¹²⁷ In some cases the sclerosed tracts were resistant to the penetration of I^{131} and P^{32} as well as to basic fuchsin; however, other tracts admitted the radio tracers but not the dye. In attempting to work with a more uniform structure Boyd and Blum¹²⁸ studied the uptake of I^{131} in ivory. They found that the uptake was not uniform and varied from specimen to specimen, conditioned by factors other than, or in addition to the degree of hydration.

Even the stress of chewing has been shown to affect the ability of teeth to take up P^{32} . Neumann and Di Salvo¹²⁹ and Neumann¹³⁰ found a greater uptake of P^{32} in nonfunctioning teeth than in teeth in occlusion. These were not teeth which were newly erupted but teeth whose opponents had been extracted and the experimental teeth allowed to remain with no occlusion for the same period of time as

the control teeth. They proposed that function may manifest itself as a factor affecting the ionic balance and turnover rate, perhaps by means of molecular dislocations from the cyclic strain of vigorous chewing. Myers¹³¹ studied the effect of a phosphorus deficiency upon the uptake of P^{32} while on a recovery diet. He found that after being on the recovery diet for two weeks the formerly deficient animals showed a slightly higher uptake in rat molars and incisors than did either the *ad libitum* or paired feeding controls. In the bones the difference was much more marked as the formerly deficient animals had a 10 times higher uptake in the femur.

DENTAL CARIES

In addition to the observations on carious changes in teeth incident to other experiments, there have been a few studies with isotopes in relation to this disease. Reviews and comments on this subject are to be found in the papers of Bodecker,¹³² Bagnall¹³³ and Sognnaes.¹³⁴ Hoppert and Shirley¹³⁵ were unable to find any essential difference in the uptake of P^{32} in the normal teeth of caries resistant and caries susceptible strains of albino rats.

Leicester, Thomassen and Denzler¹³⁶ studied the uptake of cadmium-115 in the molar teeth of rats. In radioautographic observations they found the surface uptake to be higher when the cadmium was administered in drinking water rather than parenterally. In further radioautographic studies, Thomassen¹³⁷ found that there was very little uptake of radioactive cadmium in rat teeth during the period of formation; however, when radiocadmium was administered over a long period of time in drinking water, a layer of cadmium was adsorbed on the surface of the rat molars. This layer, Thomassen states, may have a caries inhibiting or enhancing effect, depending on the amount of cadmium deposited.

THE SEAL BETWEEN FILLINGS AND TOOTH

Radioactive calcium chloride solutions have been used by a number of investigators to test the marginal seal of fillings. Armstrong and Simon¹³⁸ reported on the efficiency of the marginal seal of all popular types of filling materials. They found that all of the filling materials leak at the margin by this test. If anything, amalgam fillings leaked the least while gold foil and silicate cement fillings not only leaked completely around the margin but also through the substance of the filling. When autoseal acrylic restorations were tested by Sausen and Simon,¹³⁹ the brush technic of placement gave a more impermeable seal than pressure pack did. Storage in a humid atmosphere preserved the seal better than when the filling was immersed in water. They found no penetration of the acrylic material itself by the calcium solution. Wainwright¹⁴⁰ observed the same characteristics of silicate cement fillings. He also found that some were penetrated throughout their structure by calcium chloride solu-

tion whereas others were penetrated around the margin and base of the filling and also the underlying discolored dentin. In further tests of the sealing properties of silicate cement Harris¹⁴¹ reported that with careless technic (without control of slab temperature and moisture to the filling) there was gross penetration of radioactive calcium chloride solution around the filling and into the dentin. However, if the rubber dam was applied, slab temperature controlled, a powder-liquid ratio of 4 to 3 was used, and the filling covered with cocoa butter for 25 minutes before it was trimmed, only slight penetration occurred around the filling and not to the base of the cavity. Brown¹⁴² in similar studies of autopolymerizing acrylic found less penetration around the filling when the brush technic was used. When he tested zinc phosphate cements he found remarkably less penetration when a thick mix was used than when a thin mix was used. Crawford¹⁴³ found that whereas in previous experiments when fillings had been placed in freshly extracted teeth and immediately tested by immersion in calcium chloride solution, if the amalgam fillings and gold inlays were allowed to remain in the teeth for periods of several weeks to seven years before extraction, there was no difference in the penetration around amalgam fillings and gold inlays. In commenting on these studies Crawford and Larson¹⁴⁴ come to the conclusion that present types of filling materials have too many undesirable characteristics and that a new and different material is needed.

EFFECTS OF MEDICAMENTS AND CAVITY LINERS ON DENTIN PERMEABILITY

Amler^{145,146} and Amler and Bevelander¹⁴⁷ have tested the effect of several drugs commonly used after cavity preparation for the purpose of cleaning or disinfecting the cavity, or of sealing the floor of the cavity. Contrary to clinical opinion the precipitation of silver from silver nitrate by eugenol followed by phenol and the application of cavity varnish served only to increase the permeability of dentin to P^{32} in solution as phosphate. As a matter of fact, the permeability was greater as time increased from 7 to 70 days. Even such an innocuous material as 70% ethyl alcohol used to swab the cavity produced a slight increase in permeability of the floor of the cavity. Two per cent sodium fluoride produced an increase in permeability of dentin by 7 days; however, after 14 days there was apparently a decrease in permeability. Bartleson⁷ in a human *in vivo* experiment treated the axial wall of a cavity with silver nitrate, filled the cavity with cement, and after 12 hours gave radioiodine by mouth. Radioautographic examination showed an increased uptake of I^{131} in the area of silver nitrate application. In extracted teeth Martin¹⁴⁸ and Martin and Slater⁷⁹ also found that silver nitrate application served to increase the permeability of dentin. Sodium fluoride application did not stop P^{32} penetration nor did zinc

ferrocyanide. Zinc phosphate cement did block the P^{32} . The precipitation of calcium fluoride by first applying sodium fluoride to the cavity wall and then calcium chloride produced a sealing effect and prevented the penetration of radioactive phosphate solution through the dentin of the floor of the cavity. They proposed that this procedure be tested clinically since it more nearly approaches the wishes of the clinician for the protection of freshly cut dentin and the underlying pulp tissue.

A classical example of laboratory testing of a clinical procedure for the purpose of selecting among numerous procedures the ones most desirable for clinical trial is presented in a paper by Zander, Glenn and Nelson¹⁴⁹ in which they examined three general types of cavity liners for the protection of freshly cut dentin and the underlying pulp from the acid of silicate cement fillings. Three groups of substances were applied to the floor of the cavity. The first included various types of films or varnishes as liners; all of these failed to stop the penetration of the radioactive phosphoric acid of the cement. The second group was composed of compounds which would actively neutralize the acid of the cement and prevent its affecting the freshly cut dentin underneath. Of these, only calcium hydroxide and calcium hydroxide plus zinc oxide prevented the penetration of the radioactive phosphoric acid of the cement. The third group was composed of various combinations of the first two groups. Of these only the films containing calcium hydroxide were not penetrated. Twelve combinations of the successful films containing active compounds were tested for damage to the pulp. The histologic studies showed that the least damaging response was obtained from a film containing 5 parts calcium hydroxide, 5 parts zinc oxide, 0.1 parts pigments, 2 parts polystyrene and 87.9 parts chloroform. This liner produced only occasional histologic changes in the pulp of a very minor nature. These changes, they felt, were due to the chloroform content of the liner.

EFFICIENCY OF ELECTROPHORETIC THERAPY

Sausen¹⁵⁰ tested the efficiency of the electrophoretic procedure for the transport of substances through the dentin of the tooth. The experiments were performed on living dogs. Cavities were prepared with about $1\frac{1}{2}$ mm of dentin between the cavity and the pulp. It was found that $0.1N$ Ca^{45} ions could be moved from the floor of the cavity to the wall of the pulp within 60 seconds if a current of 0.5 milliamperes was applied. When 0.4 milliamperes was applied, much less calcium was transported through the dentin and less than this figure gave only negligible quantities. In tests of the amount of current which the pulp could tolerate, it was shown that current greater than 1 milliampere produced intracellular vacuolization of odontoblasts.

Sausen also tested the effect of concentration gradient on the movement of substances under elec-

trophoretic current. He showed that a $1.0N$ solution of calcium chloride moved not only more rapidly but gave $2\frac{1}{2}$ times the ultimate concentration at the pulpal wall. Because of the poor conductivity of enamel, calcium ions could be moved through it at currents which would not damage the pulp. Sausen tested the retention time of the calcium ions along the pulpal wall and found that they remained in the original maximum concentration as long as 4 days. After 10 days, however, some calcium had been lost on the pulpal side of the band of penetrated dentin. The distribution then remained approximately the same for two weeks more. Tests of the diffusion of calcium ions without electrophoretic current showed that the $0.1N$ solution required 7 to 8 minutes to reach the pulpal wall through $1\frac{1}{2}$ mm of dentin. This time was reduced to 5 minutes when the $1.0N$ solution was used. When electrophoretic current was applied the time for ion diffusion was reduced to 30 seconds.

This greater efficiency is achieved in spite of the reduced conductivity of the more concentrated solution. The difference cannot be accounted for on the basis of diffusion alone and is a property of the electrophoretic process. Under Sausen's standard conditions of electrophoresis, ($0.1N$ calcium solution and 0.5 milliamperes direct current), 80% of the maximum ultimate concentration of calcium ions at the pulpal wall was achieved in 3 minutes and the maximum in 40 minutes.

It is understandable how so many conflicting reports on the use of electrophoresis in the treatment of teeth could arise. For example, in Sausen's findings regarding the amount of current to be applied, there was a tremendous difference between 0.5 ma and 0.4 ma and below the latter figure no effect of electrophoretic current could be found. Since current, concentration gradient and time were significant factors, it is conceivable that many clinicians have not used satisfactory conditions to achieve the desired transport of ions. Sausen has opened a large area for investigation.

TISSUE EFFECTS OF RADIATION FROM RADIOISOTOPES

Dental studies on the effects of radiation from radioisotopes administered from external sources and also from injected radioisotopes have been reported by Burstone,¹⁵¹⁻¹⁵⁵ Bruce¹⁵⁶ and Street and Street.¹⁵⁷ In a summary of these studies, Burstone¹⁵⁸ reports that the radiation from isotopes causes: (1) cell death; (2) alterations of organ function; and (3) in some instances neoplastic growth. In the mouth, for example, there have been reported alterations in the growth of teeth and in the secretory cells of the salivary glands. Burstone lists the processes by which these changes occur as: (1) direct breakdown of molecular structure; (2) the effect of breakdown of the intercellular substances on the cells themselves; and (3) the direct effect on the molecules of the genetic system.

The oral effects of atom bomb radiation have been described by Bernier,¹⁵⁹ De Coursey,¹⁶⁰ and Er-lacher.¹⁶¹ They note that the severest symptoms in the mouth occur primarily in the third to sixth week after exposure.

RADIOISOTOPES FOR ROENTGENOGRAPHY

Spangenberg¹⁶² and Spangenberg and Pool¹⁶³ have produced roentgenograms without the use of X-ray machines. By selecting a suitable radioisotope with an emission similar to that needed for dental X-rays they have obtained sources powerful enough to actually expose X-ray film for experimental dental use. Their source was cesium-131. Their experimental source was weak, requiring exposure times in the order of hours. From their calculations, however, they find that there is no reason why sources of sufficient strength cannot be prepared at the present time. The great practical value of such X-ray sources is immediately apparent when one considers the problems of transportation in the field, for example, where power supplies for X-ray machines are not available.

DENTAL SOCIETY ACTION

The First District Dental Society of New York through its Atomic Energy Program Committee, (chairman, Dr. Theodore Kaletsky), aided dental investigators desiring to use radioisotopes by sponsoring symposia and meetings, and distributing fundamental information on the use of radioisotopes in dentistry. The activities of the committee are reported by Kaletsky¹⁶⁴ and in editorials.¹⁶⁷⁻¹⁶⁹ After the initial work of this committee, much of these activities have been carried on by the Council on Dental Research of the American Dental Association.^{170,171}

CONCLUSION

The availability of radioactive compounds has opened for dentistry a flood of new and provocative information. Already this has stimulated dentists to undertake new fundamental investigations of dental caries and other dental diseases and to develop improved methods of treatment. It should be pointed out that the new investigator should not hesitate to acquire training in the use of radioactive materials. He should note that the simplest technics have been the most productive; in fact, the photographic effect which first enabled Becquerel to discover radioactivity has become an important research technic.

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Use of Tritium-Containing Water as an Indicator of Water Metabolism in Normal and Postoperative Subjects

By P. Fallot* and A. Aeberhardt,† France

Fluid balance disturbances are frequently encountered in medical and surgical practice. A variety of methods is used to detect them, such as daily measurement of the patient's weight, the drawing-up of a fluid balance sheet affording a comparison between the total intake and output of water, the study of the various fluid compartments of the body in terms of the diffusion "spaces" of certain chemical substances, radioactive or inactive.

Various compounds have been used in order to determine the total water content of the body: antipyrine, urea, etc. However, they could not be used for a study of the kinetics of water exchange, since such studies have meaning only if the water itself is used as an indicator.

The use of heavy water, deuterium oxide, recommended by llevesy in 1934 and used by one of us,² is satisfactory, but requires the use of delicate methods (densitometry, mass spectrometry), for the dosages needed.

The use of tritium-containing water, proposed by Pace and his group in 1947,³ offers some advantages which are well worthwhile: work with tracer doses, relative ease of measurement, low cost, nearly complete absence of any risk to the patient and personnel, very soft beta emission of relatively short biological half-life in the organism (6 to 10 days).

Some criticisms of the use of tritium as a water indicator, while they are valid on the theoretical plane, are less important in clinical studies in that (a) the isotopic selection effect remains lower than the physiological variations observed in some individuals, (b) the interchange phenomena between tritium and the labile H atoms of the constituents of the body are of the order of 1 or 2%, and (c) the introduction of tritium into organic compounds by the metabolic route is a very slow process and it remains negligible for the limited duration of the experiment.

Among the important problems which still have not been answered, those which bear on the transfer of fluids from one compartment to the other and that of absorption of water by the intestine are of peculiar interest. In this particular field, namely the kinetics of fluid exchange, there is a relative dearth

of material. On the other hand, many studies have brought interesting experimental findings to light as regards the total water volume and the biological half-life of tritium; however, these data still do not allow accurate correlation between them and the normal (age, sex, outer temperature, exercise, etc.) or pathological states (heart, endocrine or kidney conditions, shock and stress, in particular).

We propose in this first paper, to study the metabolism of water from these various standpoints, using tritium as an indicator.

METHOD USED FOR THE MEASUREMENTS

We developed a method for the measurement of the tritium contained in the water of the body fluids, which is simple, quick and suitable for clinical use by unskilled personnel.

Our method consists of decomposing the water extracted from the biological fluids by a toluene carrier, and then to measure the radioactivity of the evolved hydrogen in an ionization chamber.

Water Conversion

We finally chose purified small chips of calcium, which decomposes water into calcium hydroxide and hydrogen. The conversion involves 2.5 ml of water. We were led to carry it out *in vacuo* in a closed vessel, leaving the reaction products in contact just long enough for the reaction to be complete (about 24 hours).

Measurement of Hydrogen Radioactivity

Hydrogen is transferred, under manometric control, to a 1300 ml ionization chamber. The radioactivity is determined once atmospheric pressure has been established in the chamber by reading the potential difference between the terminals of a 10^{12} ohm leak resistor following dc amplification.

The ionization current is determined 15 to 20 minutes after filling, once the parasitic charges have been eliminated.

The measurements are referred to an aliquot of the tritium-bearing water administered, or to standard sources supplied by Châtillon and Harwell.

The complete technical details are described in a paper shortly to be published.

Justification of the Method

The distribution of the hydrogen atoms of the water between the two reaction products raises no

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problems under our experimental conditions. The volume of gas obtained is insufficient to fill the chamber at atmospheric pressure. Now, 2.5 ml of water are obtained from 3 ml of whole blood. There is no objection to collecting this quantity of blood, in man, several times in succession at short intervals.

The "isotopic selection effect," which may modify the distribution of tritium between the hydrogen and the calcium hydroxide formed, does not come into account; we always operate under the same conditions and do not endeavor to make absolute measurements. Furthermore, since contact is maintained between the products of the reaction for 24 hours, exchange phenomena are likely to lead to a homogeneous distribution of tritium between the hydrogen atoms of the gas and those of the calcium hydroxide.

The possible "memory effect" is limited by renewing the calcium after each conversion, and cleaning the flasks after each operation. No residual radioactivity can be detected under our experimental conditions.

Measurement of the volume of the gases obtained and control of the pressures with our gauges during over 250 operations showed that the yield of the conversion was constant and in the vicinity of 98%. Thus, reproducibility was very satisfactory.

Results Obtained

Plotting the deflection read in millivolts as a function of specific radioactivity of tritium in microcuries per ml of water for specific radioactivities between 0.02 microcurie per ml and 1 microcurie per ml we obtained a straight line. The coefficients of the equation have the following value:

$$a = 475 \pm 2.8 \text{ (standard deviation)}$$

$$b = 18.5 \pm 0.6 \text{ (standard deviation)}$$

The lower limit of measurement is 0.02 microcuries per ml, which corresponds to a total measured amount of tritium of 0.05 microcuries.

The upper limit has been set at 1 microcurie per ml of water, but some measurements which were carried out for specific radioactivities of up to 5 microcuries per ml showed that the equation of the straight line remains applicable.

PRACTICAL APPLICATIONS

We have applied the method to a study of the absorption of water by the intestine following the ingestion of tritium-bearing water, both in normal subjects, and in patients suffering from some medical and surgical conditions.

Complementary information of the total water volume and on the biological half-life of tritium in man can be derived from an examination of the results obtained.

Standard Operating Procedure

A standard operating procedure has been developed. The hospitalized subject, at rest and fasting

since the night before, ingests 100 ml of water containing 4 mc of tritium at 10 a.m. This amount remains under the tolerance dosage accepted for this radioelement (10 mc). The subject stays in bed for the whole duration of the test, namely 2 hours.

Venous blood collections (5 to 10 cm³) on powdered heparin are carried out following ingestion, according to the following schedule: 5 min, 15 min, 30 min, 45 min, 60 min, 90 min, and 120 min. The patient returns to his customary diet. 24 hours following ingestion, a new blood collection is made. On the following day, a urine sample is taken following evacuation of the urinary bladder. On the 8th and 15th days, blood samples are collected, in order to check how the equilibrium of the tritium concentration in the two fluids under study (blood and urine) is being maintained.

Blood collections are made by the venous route, despite the criticisms of Code and his group:⁴ the repetition of arterial puncture at the suggested intervals offers some difficulties; and, what is more, our experimental results have not shown the variation indicated by these authors, which motivated their criticism of the venous route.

This experimental technique has been implemented, either in the form of a single injection, or in that of 3 injections 24 hours apart. Even in the case of repeated tests, the total dose received by the subject does not exceed 0.3 rcp per week. Where repeated tests were carried out, the collection of blood was made before the 2nd and 3rd injections, in order to determine the residual radioactivity. This may be considered constant for the duration of the test (2 hours).

Presentation of Experimental Results

The results of the various dosages carried out with the blood or the urine, are expressed in μc per ml of water. By plotting these values as a function of time, one gets a curve which gives the variations of the tritium concentration in those fluids. We used standard coordinates for the results pertaining to the first two hours, and hemilogarithmic coordinates for those given by the prolonged experiments.

The curves thus obtained provide information on the absorption of water by the digestive route under physiological conditions, as well as data on the diffusion of water in the fluids of the body. Aside from this, additional information is given on the time needed in order to achieve equilibrium of the tritium in the body (making it possible to compute the total volume of the water in the body) and on the biological half-life of tritium in man.

Normal Subjects—Single Ingestion

We have carried out this test in 10 normal adults, of different sexes and various age groups. The curves obtained may be classed in two types (Figs. 1 and 2). Type 1 curves show an ascending branch which reaches a plateau toward the 90th or the 120th minute, thereafter to be maintained until the

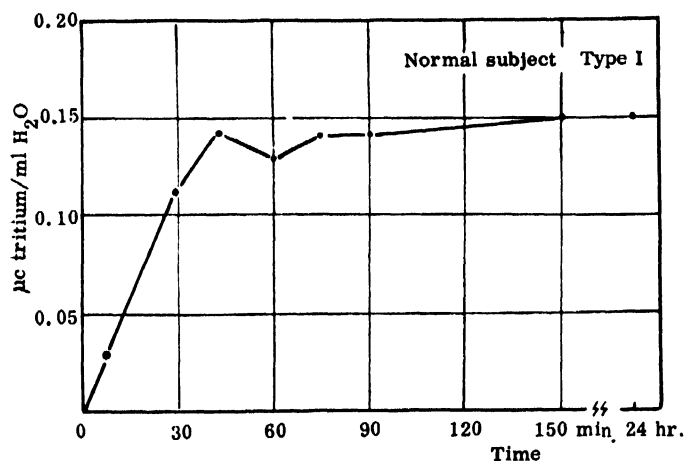


Figure 1. Normal subject—type I

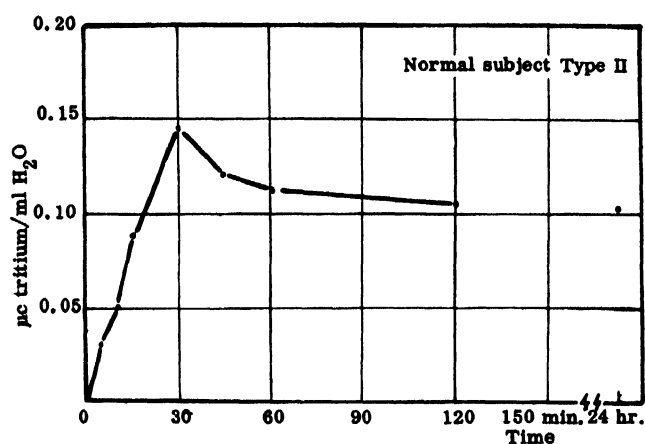


Figure 2. Normal subject—type II

24th hour. Type 2 curves show an initial branch which climbs rapidly; the maximum is reached between the 30th and 60th minute, in general about the 45th minute, and this is followed by a descending branch of the exponential type. Its analysis leads to the resolution of several components; starting at the 120th minute, the slow component become predominant, and the curve is practically identical with a small negative slope straight line until the 24th hour.

The curves obtained from the 10 subjects examined break down approximately as follows: four of Type 1, six of Type 2. A study of the curves pertaining to the normal subjects, published by Howard⁵ shows that this writer obtained curves of both types, those of Type 2 seem to have been more numerous than those of Type 1 (4 out of 6).

Discussion

Examination of the curves suggests the following comments: the initial ascending branch, common to both types of curves, represents the absorption of water by the digestive tract, a phenomenon which takes place at a faster rate than distribution throughout the body. The differences observed correspond to unequal rates of absorption or diffusion. Since the times required to achieve equilibrium are comparable in both types of curves, it would seem that the rate of absorption is the preponderant factor during the first period. We propose to clarify this point by studying the absorption of salt and glucose solutions of the hypo-, iso- and hypertonic types, since the rate of absorption by the digestive tube is related to the tonicity of the ingested solution (Visscher *et al.*⁶).

The tritium space is derived from the data obtained once equilibrium is reached, at the 120th minute. This space is closely similar to the total water volume (difference 1 to 8%) of the system, as Moore⁷ showed it for the rabbit, by comparison with the desiccation method.

The curves drawn in hemilogarithmic coordinates for the values obtained from the blood or urine taken from the second day on, show an exponential decay with a single component and make it possible

to compute the biological half-life of the tritium present in the system.

Normal Subjects — Repeated Ingestions

In order to obtain reference curves in normal subjects, we carried out the repeated ingestion test with two subjects (Fig. 3). The residual radioactivity, prior to each new ingestion, was subtracted from the experimental values found, in order to afford a comparison of the curves. Thus, we have curves which show, for each ingestion the kinetics of absorption and diffusion. The curves obtained look alike and have similar characteristics, in particular, all three belong to the same type (Type 2 in the case of Fig. 3), and the maximum is reached between the 30th and 45th minute in the three cases.

This study shows that, under conditions made as closely similar as possible, the curves obtained in a given subject are the same, which expresses a certain constancy in the water exchanges process.

Additional Data

We have collected, in Table I, the data derived from studies carried out on normal subjects, for total water volume, total water percentage with respect to body weight, and biological half-life of tritium following the ingestion of tritium-containing water.

Our results may be compared with those published by various authors who used tritium or deuterium oxide.^{8,9,10}

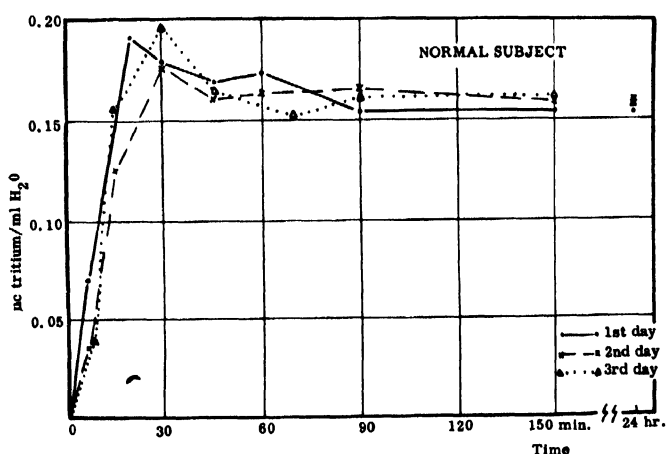


Figure 3. Normal subject

Table I. Total Water and Biological Half-Life in the Normal Subject

Subject	Sex	Age	Weight (kg)	Equilibrium reached (min)	Total water (ml)	Total water as % of body weight	Biological half-life of tritium (days)
T.R.	M	35	63,300	120	34,600	55	5
R.A.	F	23	49,900	90	27,000	54	9
M.H.	F	48	54,000	150	32,000	59	6
L.J.	F	38	56,300	150	34,600	61	—
D.L.	M	67	58,200	90	37,600	64	9
A.M.	M	24	60,000	150	37,800	63	10
D.H.	M	57	60,000	150	30,800	51	6
N.J.	M	26	73,000	105	41,100	56	11
V.G.	M	37	76,000	120	49,400	65	—
N.A.	M	38	83,000	120	51,900	62.5	—

Applications to Medical Pathology

In some conditions affecting the endocrines, liver or heart, the changes observed concern the slope of the ascending part of the curve, its type, the time required to reach the maximum if any (Type 2 curve), the time required for equilibrium to appear, and the value of the latter. The biological half-life also shows definite variations, such as an increase or a reduction.

Let us mention as an example, the results obtained in cooperation with Dr. Canivet in a case of alcoholism: the slope of the ascending branch of the curve is normal, and the maximum is reached at the 60th minute. This curve, similar to that of Type I, still differs from it, to the extent that it shows, from the 60th minute on, a faster decrease than normal. There is no equilibrium, which makes it all but impossible to determine the volume of total water, and, in addition, the curve of tritium concentration in the urine, established over a period of a fortnight, shows changes in the slope which spread over several days, and which we are unable to relate to the patient's fluid ingestion.

Applications to Surgical Pathology

The repeated ingestion test was carried out with patients operated on by Dr. Soupault (Hospital St.

Antoine, Paris), under the conditions indicated above: a curve was plotted before the operation, and two more 24 and 48 hours respectively after the procedure. The curves obtained for the second and third days show important modifications with respect to the control curve.

Figure 4 shows the curves obtained before and after a cholecystectomy, in a woman 45 years of age. In particular, one notes a change in the type of curve and definite modifications in the conditions of equilibrium. Now, the metabolism of water, in general, is much affected during surgical operations. The changes observed in the curves thus obtained must make it possible to judge the fluid balance status of the operated patient and the disturbances in the absorption of water by the intestine. The over-all results will be presented in a subsequent study.⁵

CONCLUSION

This paper describes a rapid, accurate and sensitive method for the measurement of tritium in biological fluids. In view of the ease with which it can be used, its application in routine hospital practice may well be envisaged.

The method has been applied to a study of the intestinal absorption of water in the normal subject, as well as in some medical and surgical conditions. Interesting data concerning the kinetics of the ingested water are obtained by examination of the experimental curve. These, in addition, supply data on the total volume of water in the human body, and on the biological half-life of tritium in the system. Preliminary results obtained in surgical pathology are described.

These studies may lead to a clarification of the manner in which wound and shock patients should be rehydrated. Specifically, they should provide a determination of the moment at which the normal digestive route is to be substituted for the intravenous route in rehydration. The importance of this last point is not to be minimized, particularly under circumstances where wound and shock patients might appear in large numbers, so that the reserve of injectable solutions would be inadequate.

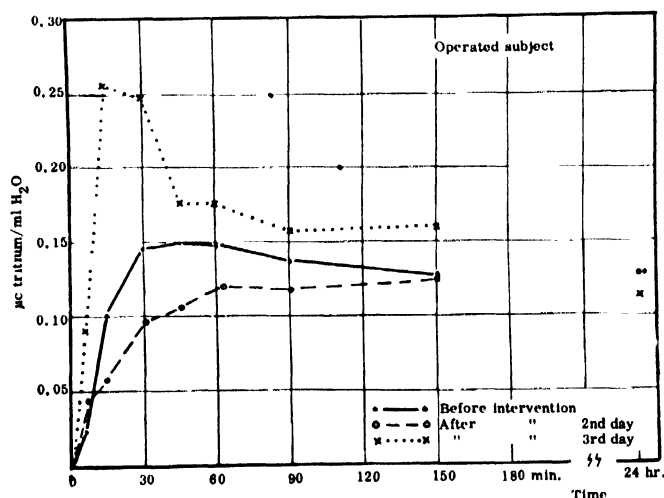


Figure 4. Operated subject

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Studies of the Use of Rb⁸⁶ as a Tracer of Potassium in Man

By George E. Burch, C. Thorpe Ray and S. A. Threefoot,* USA

With recent interest in electrolyte metabolism, particularly as related to membrane potentials, carbohydrate and protein metabolism, as well as over-all disturbances in various disease states in man that influence the expressions of disease and management, it is important to know more about kaliokinetics of intact man. K⁴², because of its short half-life, is not suitable for the study of kaliokinetic phenomena over prolonged periods. It has been shown, however, that Rb enters into the metabolic pool of potassium in a number of biologic systems¹⁻¹¹ to an extent that radiorubidium, Rb⁸⁶ ($T_{1/2}$ 19.5 days), may be suitable as a tracer of potassium to define certain aspects of kaliokinetic phenomena that require relatively long periods of observation. For example, it has not been possible to determine satisfactorily by direct experiment the time required for equilibrium of distribution of an injected sample of K³⁹ or K⁴² to become established, or to determine certain parameters of biologic decay rates of K³⁹ either in normal or disease states. Surely, the kaliokinetic phenomena that can be labeled and traced for a period of a few hours following administration of a tracer dose of K⁴² can be investigated with this short half-life material. If it could be shown that Rb⁸⁶ traces K³⁹ sufficiently accurately to reveal aspects of kaliokinetics requiring prolonged periods of observation, this tracer would have applications in biology and medicine. Furthermore, there are certain kaliokinetic phenomena that can be investigated only by the tracer method.

Studies were undertaken to evaluate Rb⁸⁶ as a tracer of potassium in control human subjects and subjects with chronic congestive heart failure. These investigations were so designed that it was possible to obtain information not only about rubidiokinetic phenomena but, as shown below, about interesting aspects of potassium metabolism as well in control subjects and in subjects with a diffuse chronic state of edema.

METHODS AND MATERIAL

Six adult subjects, 3 controls and 3 with chronic congestive heart failure, were studied (Table I) continuously under controlled metabolic conditions for two months.^{12,13,14} The rapid rate of disappearance of Rb⁸⁶ alone or with K⁴² (as Rb⁸⁶Cl and K⁴²Cl in water) was determined by rapid collection (5 and 10 second intervals at first and 10 minute intervals later) of blood from a median basilic vein following a rapid (2 to 8 second) injection of the tracers into the median basilic vein of the opposite arm. One subject received the Rb⁸⁶ and K⁴² simultaneously orally. The plasma was quickly separated (1 to 2 minutes) from the red blood cells (RBC) by centrifugation of one aliquot and whole blood studied in another aliquot. Urine was collected continuously from an in-dwelling bladder catheter. After the initial 120 minutes of study of rapid plasma and erythrocytic exchange, the subjects were followed in bed with close surveillance, separate collections being made of urine, stool, sputum, vomitus and other biologic fluids as passed or obtained. After the samples were measured separately, they were pooled for 24 hour periods and measured again.

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Table I. Clinical Data on the Six Subjects Studied. Subjects 701 and 702 Received Simultaneously 50,000,000 cpm of Both K⁴² and Rb⁸⁶ Intravenously

Subject no.	Age (yr)	Sex	Body weight (kg)	Diagnosis	Dose	
					cpm	mc
601	34	F	72.8	Tuberculous adenitis under treatment	200,000,000	1.0
602	57	M	71.1	Anxiety state	200,000,000	1.0
603	39	M	55.8	Rheumatic heart disease, moderate congestive heart failure, neurofibromatosis	200,000,000	1.0
604	45	M	55.8	Cor pulmonale, severe congestive heart failure	200,000,000	1.0
701	51	F	51.2	Rheumatic mitral insufficiency, no congestive heart failure	50,000,000*	0.25
702	34	F	80.0	Hypertension, moderate congestive heart failure	50,000,000*	0.25
					50,000,000*	0.25

* Refers to 50,000,000 cpm K⁴² and 50,000,000 cpm Rb⁸⁶.

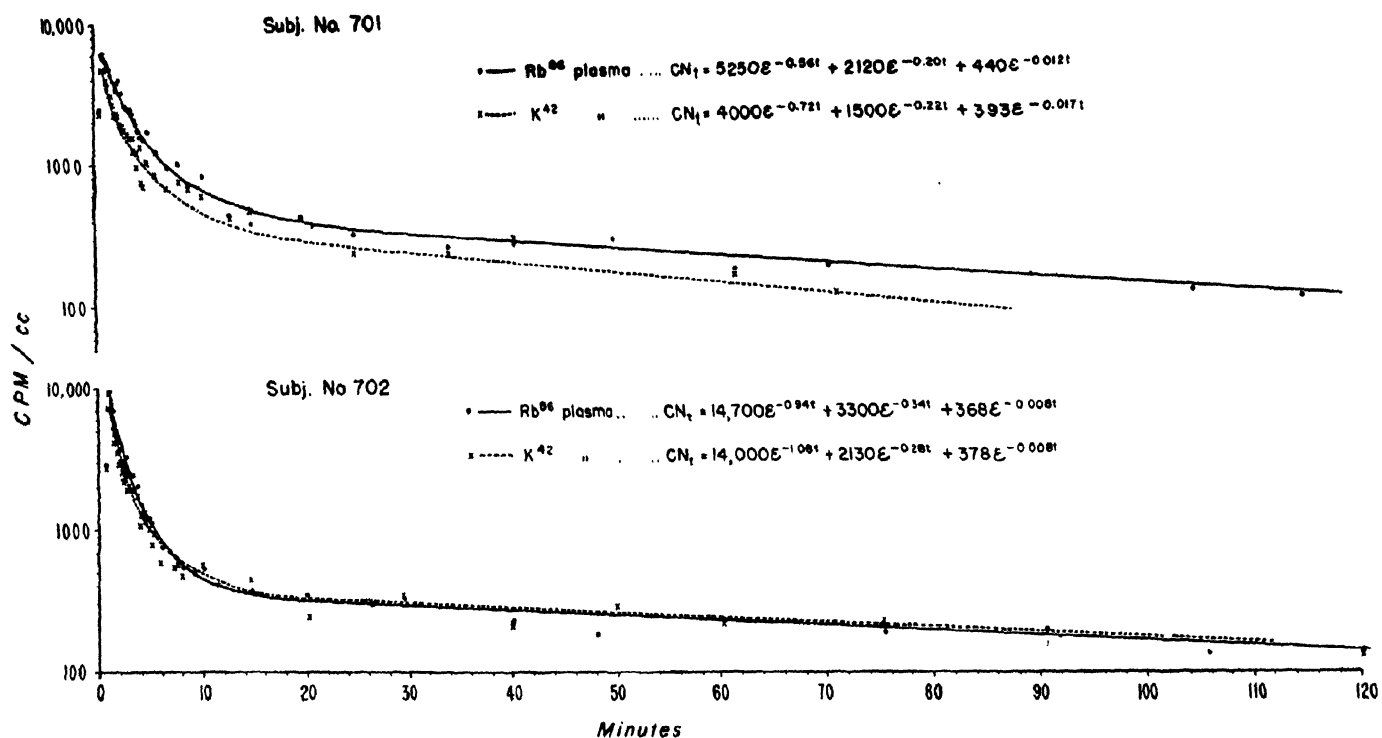


Figure 1. Concentration-time course curves in plasma of K⁴² and Rb⁸⁶ administered simultaneously intravenously to two subjects. The formulae describing the strikingly similar curves are shown

Table II. The Distribution and Rate of Turnover of Rb⁸⁶ and K⁴² Between the Plasma and Other Compartments

	Subject 602		603		604		701		702	
	Tracer	Rb ⁸⁶	Tracer	Rb ⁸⁶	Tracer	Rb ⁸⁶	Tracer	Rb ⁸⁶	Tracer	K ⁴²
q = proportion of total tracer not in plasma		0.9705								
$1 - q$ = proportion of total tracer in the plasma		0.0295		0.9555		0.9727		0.9437		0.9365
R_s = proportion of plasma tracer leaving plasma per minute		0.592		0.0445		0.0273		0.0563		0.0635
R_e = proportion of nonplasma tracer returning to plasma per minute				0.619		0.558		0.4524		0.545
$R_s \times (1 - q) = R_e(q)$ = proportion of total body tracer moving in or out of the plasma per minute		0.0180		0.0288		0.0157		0.0270		0.0370
$R_e(q) + R_s(1 - q)$ = proportion of total body tracer moving in and out of the plasma per minute		0.0175		0.0275		0.0153		0.0255		0.0347
		0.0350		0.0550		0.0306		0.0510		0.0694
										0.0326
										0.0436

Equations of decay curves:

Subject

602 Rb⁸⁶ = $30,050e^{-0.701t} + 5800e^{-0.140t} + 1090e^{-0.00245t}$

603 Rb⁸⁶ = $28,500e^{-0.782t} + 6900e^{-0.177t} + 1650e^{-0.00641t}$

604 Rb⁸⁶ = $51,000e^{-0.843t} + 8850e^{-0.170t} + 1680e^{-0.00480t}$

701 Rb⁸⁶ = $5250e^{-0.86t} + 2120e^{-0.20t} + 440e^{-0.012t}$; K⁴² = $4000e^{-0.72t} + 1500e^{-0.22t} + 393e^{-0.017t}$

702 Rb⁸⁶ = $14,700e^{-0.94t} + 3300e^{-0.34t} + 368e^{-0.00765t}$; K⁴² = $14,000e^{-1.08t} + 2130e^{-0.277t} + 378e^{-0.00765t}$

Table III. Urinary Excretion of Rb⁸⁶ and K⁴²—First 120 Minutes

Subject	Dosage		Appearance time		Recovery				Total urinary volume (ml)	Average rate of urinary flow (ml/min)
	Rb ⁸⁶ (cpm)	K ⁴² (cpm)	Rb ⁸⁶ (min:sec)	K ⁴² (min:sec)	Total cpm		% inj. dose			
					Rb ⁸⁶	K ⁴²	Rb ⁸⁶	K ⁴²		
702	5 × 10 ⁷	5 × 10 ⁷	0:50 to 3:55	0:50 to 3:55	245,194	428,926	0.44	0.85	113.4	0.92
703	5 × 10 ⁷	5 × 10 ⁷	< 13:30	< 13:30	1,303,747	1,912,324	2.61	3.82	336.0	2.71

Rb⁸⁶ and K⁴² were counted for all samples in the conventional manner, previously described,¹⁵ with an end-mica-window Geiger-Müller counter. Whole blood and the erythrocytes were laked by freezing and thawing for counting. Na²³ and K³⁹ were measured in all samples except the feces with a Beckman flame photometer,¹⁶ and Cl³⁵ was determined by the method of Schales and Schales.¹⁷ Dietary intake, fluid intake and output, and daily weighings were recorded.

The methods and theoretic considerations for the determination of space and mass were previously described.¹⁸

The calculations of clearance¹⁴ were obtained from the equation:

$$\frac{UV}{B} = \text{ml/min cleared} \quad (1)$$

where U is concentration per ml in the urine, V the urinary flow in ml/min and B the concentration per ml in plasma.

RESULTS

The Early Concentration-Time Course of Rb⁸⁶ and K⁴² in the Blood Plasma

The concentration-time course of Rb⁸⁶ and K⁴² in plasma for the first 120 minutes after simultaneous sudden (4–8 seconds) intravenous administration of the two tracers is summarized by Fig. 1. The equations defining the regression curves are shown on the illustration. Similar curves for Rb⁸⁶ alone were obtained for the other subjects. There were no definite significant differences between the curves for the control subjects and those with chronic congestive heart failure.

These curves also resembled those obtained previously in similar types of subjects under the same circumstances for Na²⁴, Na²², Cl³⁶ and Hg²⁰³, differing only quantitatively.^{19,20,21,22}

From the regression curves transfer rates into and out of the plasma were calculated,^{23,24} the results being summarized by Table II.

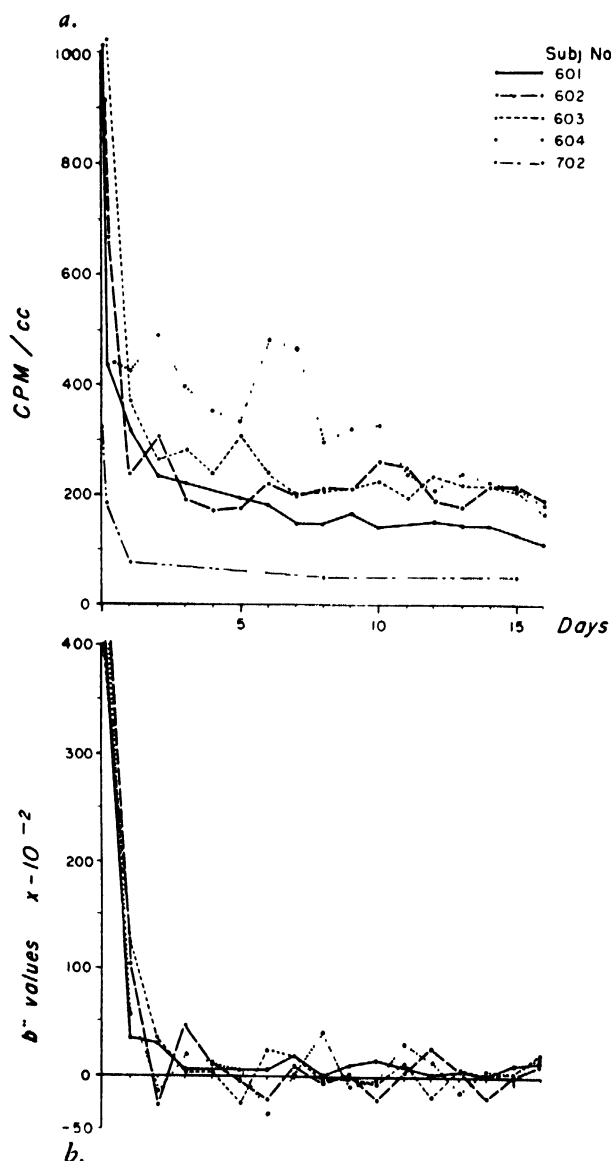


Figure 2. (a) Concentration-time course of Rb⁸⁶ in the plasma of five subjects for the first sixteen days of observation. (b) Rate of change in concentration expressed as the slopes (exponent "b") of the regression curves

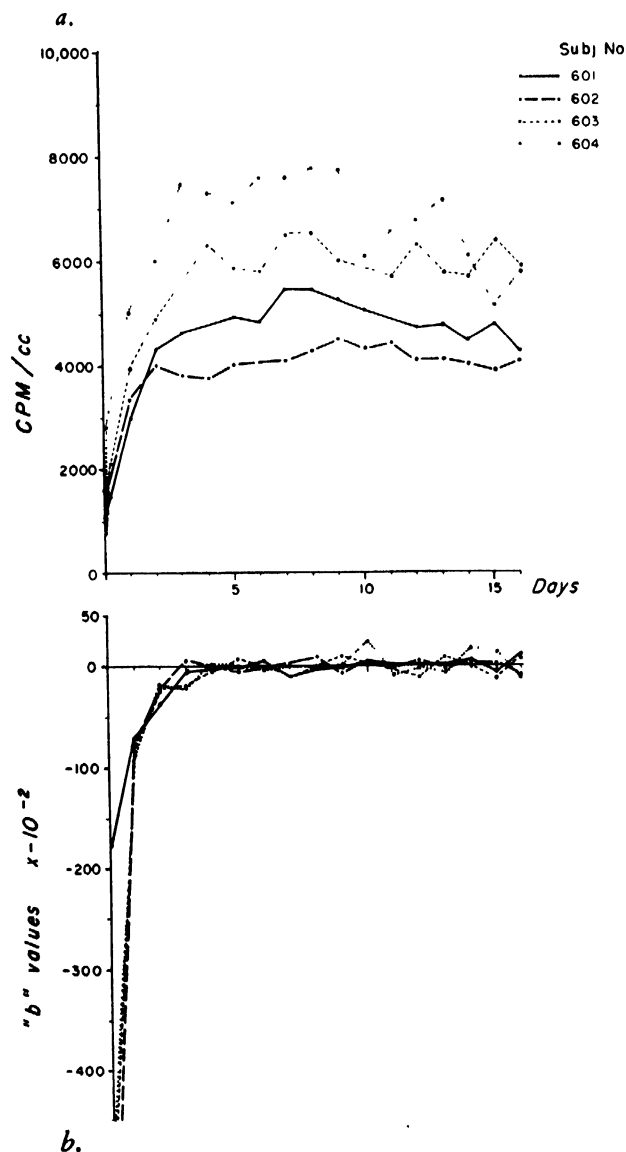


Figure 3. (a) Concentration-time course of Rb⁸⁶ in erythrocytes for the first sixteen days of study. (b) Rate of change in the concentration of Rb⁸⁶ in the erythrocytes expressed as the time course of the slope (exponent "b") of the regression curves

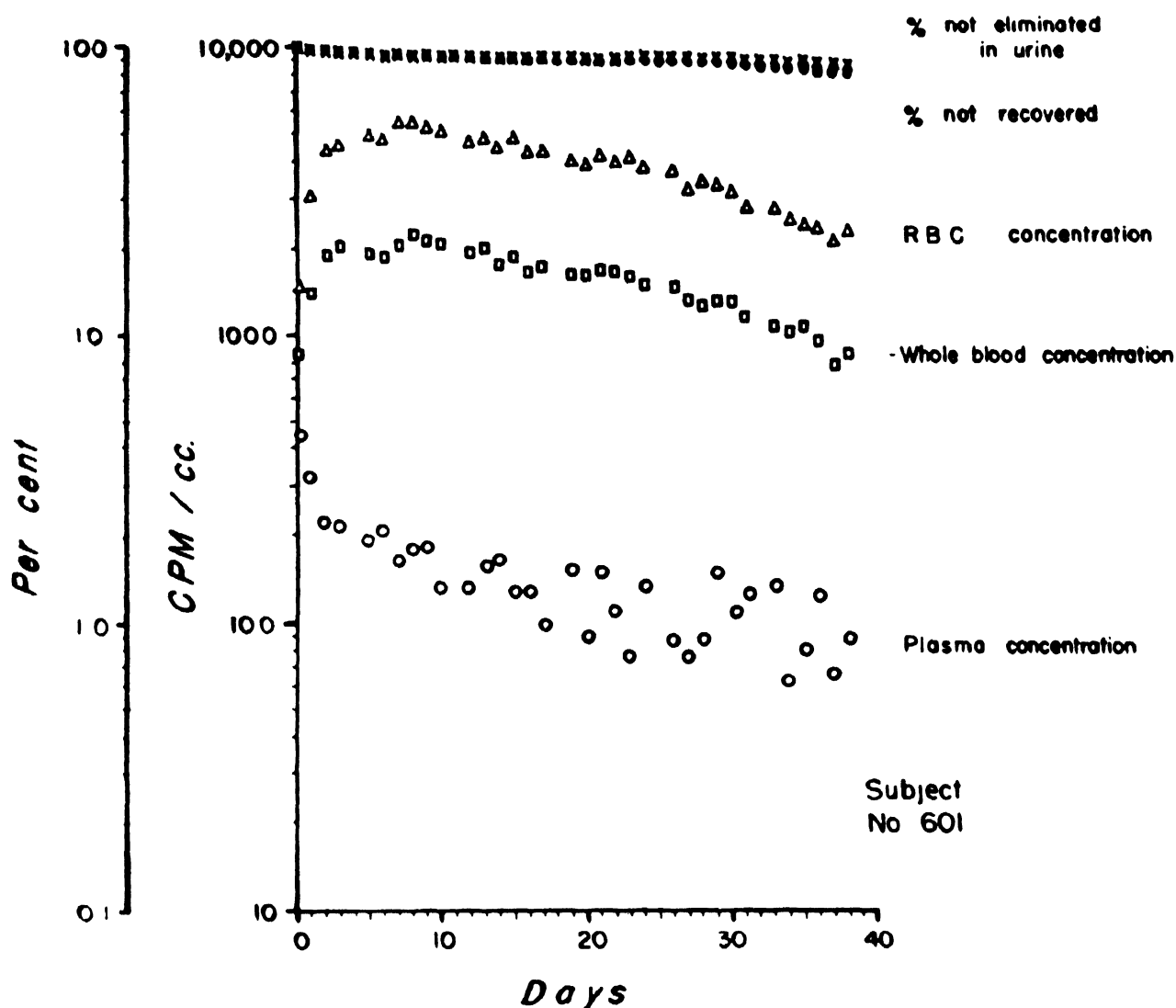


Figure 4. Biologic decay curves Rb⁸⁶, control. Concentration-time course of Rb⁸⁶, in erythrocytes, whole blood and plasma of a control subject. Urinary excretion and total recovery are expressed as the percentage of the administered dose remaining in the body and not collected in the biologic fluids studied

Comment

The data show K⁴² and Rb⁸⁶ to behave impressively similarly during the first 120 minutes of study. These rates of disappearance from the plasma were slower than C¹³⁶,²¹ more rapid than Na²⁴ and Na²²,^{19,20} and at about the same rate as Hg²⁰³.²² Because of the small numbers of subjects studied and individual variations, especially during congestive heart failure, the significance of these differences remains unknown, the rate of injection and mechanical mixing being among the important variables.

The striking similarity between concentration-time course curves of K⁴² and Rb⁸⁶ supports the possibility of metabolic similarities between K⁴² and Rb⁸⁶ but does not establish this relationship, since the kinetics involved so early after injection are more physical in character than chemical. However, the Rb⁸⁶ ion, with an atomic mass almost twice that of K⁴², is approximately the same in the hydrated state as the K⁴² ion.²⁵

Equilibrium of Distribution

The relatively long half-life of Rb⁸⁶ makes it possible to observe directly the approximate time at which equilibrium of distribution of a tracer dose occurs, data which may also reflect to some extent the situation for K⁴². Since it was not possible to sample each Rb⁸⁶ compartment separately, equilibrium of distribution could be studied indirectly only. The time at which the concentration-time course curves reach a constant slope or the "b" values, the exponent defining the rate of regression of the concentration-time course curve, became constant (Fig. 2) for the plasma and the erythrocytes (Fig. 3) reflects to some degree the time at which equilibrium of distribution is achieved. It is evident that this was not possibly reached until 5 days and probably was never reached, since the concentration of Rb⁸⁶ declined more rapidly in the plasma than did that for excretion in the urine or for total excretion (Figs. 4, 5).

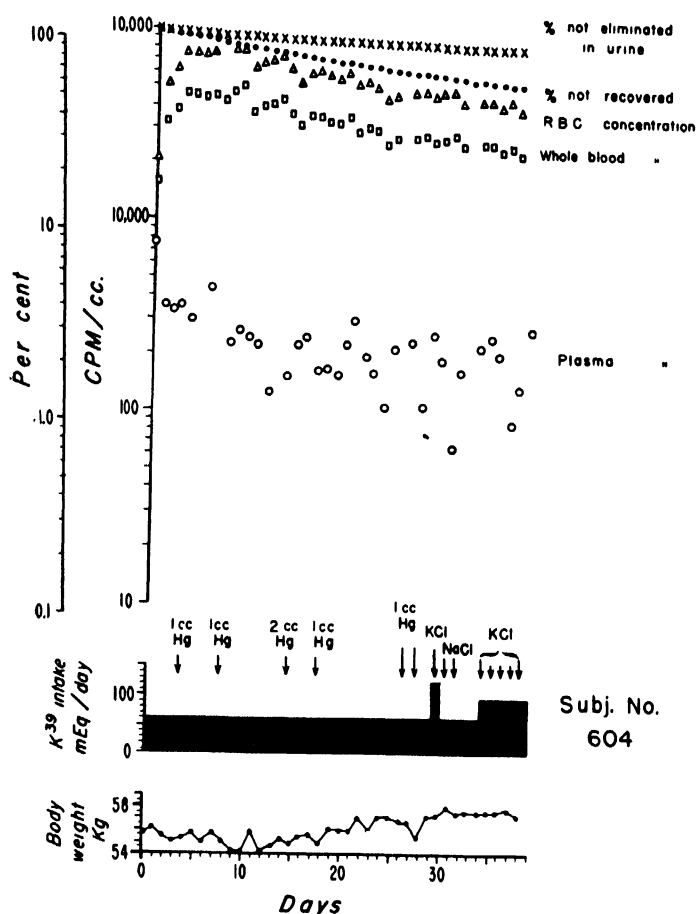


Figure 5. Biologic decay curves Rb^{86} , severe CHF. Concentration-time course of Rb^{86} in erythrocytes, whole blood and plasma and the time course of urinary and total excretion. Dietary intake of potassium and drugs administered are shown

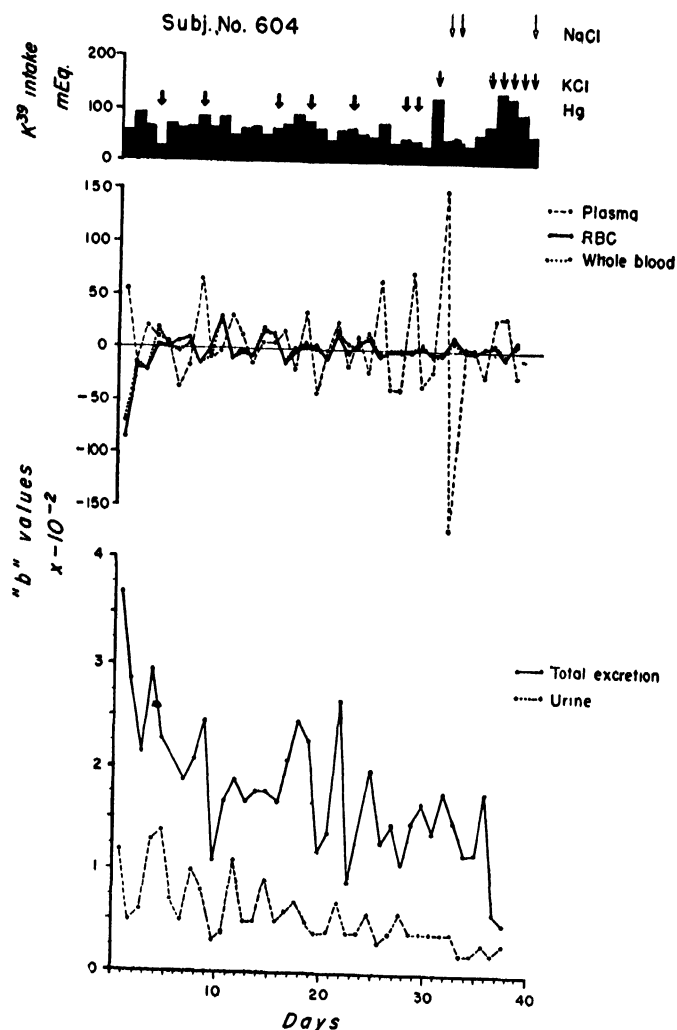


Figure 7. Time course of biologic decay curves, severe CHF. Data for a subject with severe congestive heart failure present as in Fig. 6 showing the relationship to dietary intake of potassium, drugs and total excretion

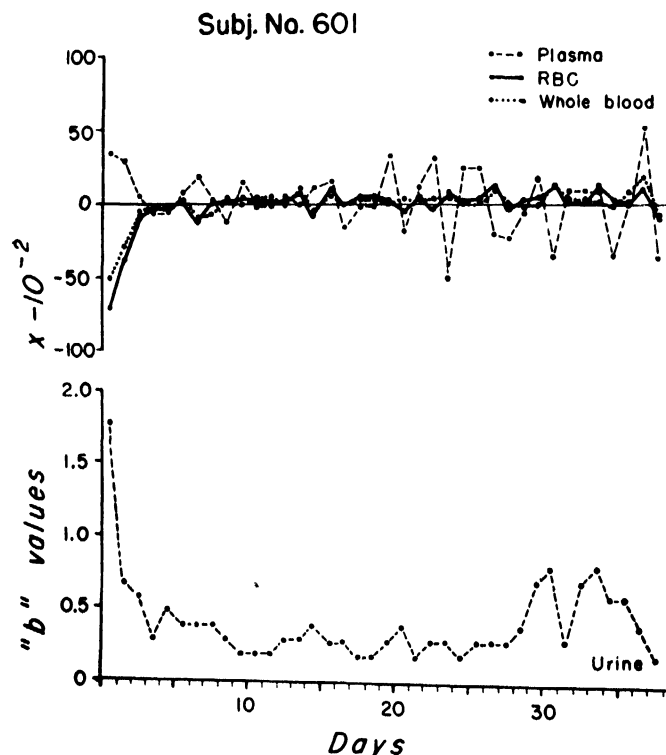


Figure 6. Time course of biologic decay curves, control. Daily variations in the slopes (exponent "b") of the concentration-time course curves of Rb^{86} in the erythrocytes, whole blood and plasma and of the time course of urinary excretion of Rb^{86} in a control subject

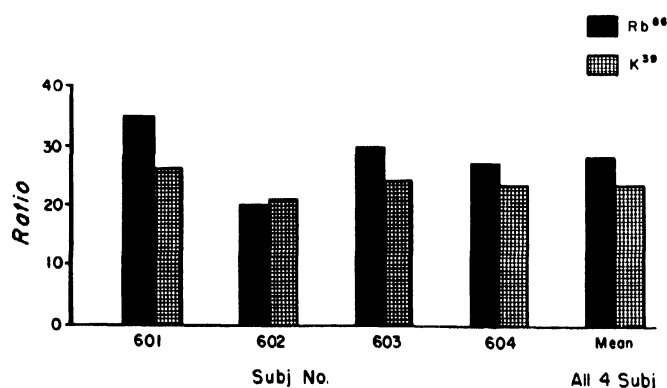


Figure 8. The average ratio of concentration of Rb^{86} and K^{39} in the erythrocytes to that in the plasma for the period of observation

Comment

It was not possible to estimate accurately the time at which equilibrium of distribution occurred, although the concentration-time course curves for plasma and erythrocytes and the time course curves for the "b" values revealed that this could not have occurred in less than 5 days. The more prolonged time course of concentration showed that it prob-

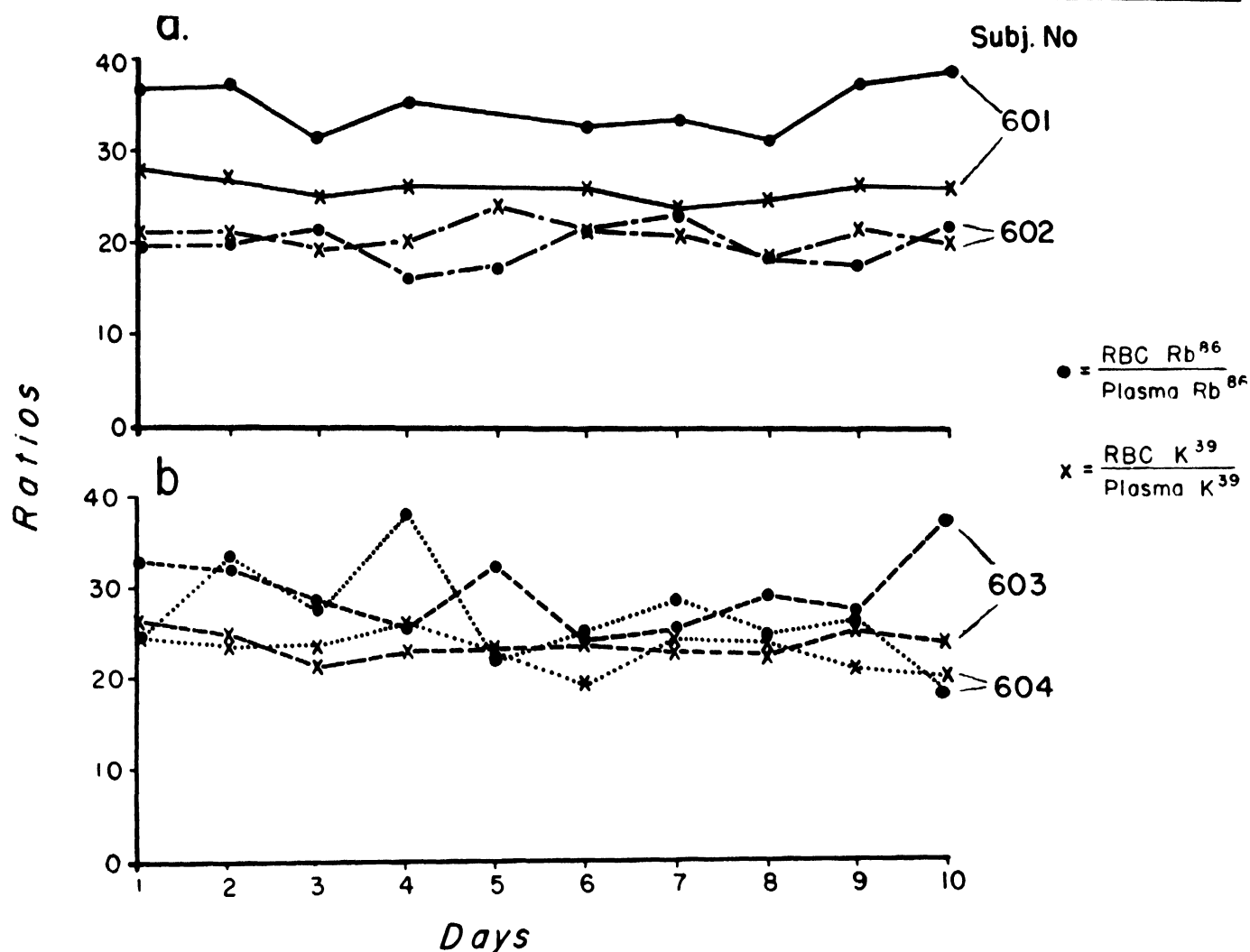


Figure 9. Daily variations in the ratio of the concentration of Rb⁸⁶ and potassium in the erythrocytes to that in the plasma

ably was never fully established. The similarities in kinetics for K⁴² and Rb⁸⁶ suggest that these findings probably hold for K³⁰ and K⁴² as well.

Because of the relatively small plasma compartment (extracellular) and relatively large RBC compartment and the rapid rate of turnover in the plasma and relatively slow turnover for the erythrocytes, the times at which equilibrium of distribution is established cannot be determined accurately by such indirect methods. It was not established in the 4 to 6 weeks of continuous study. The progressive rise in RBC concentration of Rb⁸⁶, with a progressive decline in plasma concentration, is definite evidence that for the first 5 days these two compartments had not yet established equilibrium of distribution. This lack of equilibrium must be even more true for the brain and bone, which turn over their potassium even more slowly than RBC. Thus, sampling of the RBC compartment reflects more accurately the kaliokinetic and rubidiokinetic processes for the body as a whole than does the plasma but not absolutely.

Furthermore, the compartment of interest should be observed directly to produce reliable data concerning its kinesis.

Biologic Decay Rates

The concentration-time course of Rb⁸⁶ in the plasma, RBC, and whole blood, as well as the time course of percentage of the injected dose remaining in the body recorded continuously for 40 or more days, permitted a determination of the values of $C_{1/2}$ (time required for the plasma, RBC, or whole blood to reach $1/2$ concentration with the t_0 being measured from the 5th day after injection of the tracer), $U_{1/2}$ (time required for $1/2$ of the injected dose to be excreted in the urine), and $E_{1/2}$ (time required for $1/2$ of the injected dose to be excreted by all routes studied).

Typical time course curves for a control subject and a subject with chronic congestive heart failure are shown in Figs. 5 and 6. The biologic decay rates of Rb⁸⁶ obtained from the mean slopes of the decay curves and from mathematical analysis of the "b" values are summarized in Table III.

The percentage remaining in the body was determined from the equation:

$$\% N_t = - \frac{\sum_{s=1}^t K_s}{N_0} \times 100 \quad (2)$$

where N_t is the percentage remaining on the t^{th} day and N_0 is the amount injected or present at t_0 .

The $C_{1/2}$, $U_{1/2}$, and $E_{1/2}$ values revealed a slower rate of turnover of Rb^{86} in the subjects with congestive heart failure than in the control subjects. The regressive curves (Figs. 4 and 5) and the time courses of b values (Figs. 6, 7) showed a much greater variation in the concentrations and the rates of excretion for the subject with congestive heart failure than in the control subjects. The various plots (Figs. 4-7) revealed the influence and relationship of dietary intake of K^{39} , body weight, and mercurial diuretic upon the parameters of biologic decay rates.

Comment

The time courses of the b values, C_b , U_b , E_b , indicate further the usefulness of Rb^{86} as a tracer not only of rubidium but also of K^{39} . The short half-life of K^{42} makes it unsuitable for such observation. Furthermore, the curves of C_b , U_b , E_b , reflected the wide variations in potassium and rubidium masses and spaces. Furthermore, the large variations revealed the importance of making many successive measurements of the various biologic fluids to obtain a fairly accurate impression of rubidiokinetic and kaliokinetic phenomena. A single measurement at any given time provided an unsatisfactory impression of the metabolic state of potassium or rubidium.

These curves also revealed the instability of the metabolism of potassium and rubidium in congestive heart failure in comparison with relatively small oscillations as in the control subject. The wide fluctuations or "hunting" about a mean in congestive heart failure was also observed previously for Na^{22} , Na^{23} and Cl^{36} . The wide variations in the *milieu interieur* may have been responsible for, or at least associated with, the variations in the clinical state of the subjects with congestive heart failure.

Figures 8 and 9 summarize the ratios of erythrocyte to plasma Rb^{86} and to K^{39} . These illustrations indicate a disproportionate distribution of Rb^{86} and K^{39} but at the same time a sufficiently close similarity to support further the possible usefulness of Rb^{86} as a tracer of K^{39} .

The Excretion of Rb^{86} , K^{42} , and Potassium, Sodium and Chloride

The urinary excretory relationship of Rb^{86} to that of potassium, sodium, and chloride over prolonged periods was observed in four subjects, and the relationship of urinary excretion of Rb^{86} to that of simultaneously administered K^{42} was observed for shorter periods in two subjects. The simultaneously intravenously administered Rb^{86} and K^{42} appeared in the urine in from 50 seconds to 3 minutes and 55 seconds whereas after oral administration the tracers appeared in the urine within 13 minutes (Table III). Other aspects of the rates of urinary excretion of Rb^{86} and K^{42} are shown in Fig. 10. Excretion expressed as counts per minute, as a %

of that present in the body at any given time, as concentration and as clearance, showed K^{42} to be excreted more rapidly than Rb^{86} . There was a constant qualitative similarity between Rb^{86} and K^{42} , although the ratio of Rb^{86} to K^{42} for the excretory parameters was about 0.52 and 0.68 for the two subjects.

The relationship of clearance and excretion of Rb^{86} to that of K^{42} is shown in Fig. 11. The preferential excretion of potassium over rubidium is apparent.

The specific activity of K^{42} in the plasma and urine, as well as the "specific activity" of ($\text{Rb}^{86}/\text{K}^{39}$ ratio), are shown in Fig. 12. Since the urinary specific activity of K exceeded that of Rb^{86} , it is apparent that radiorubidium was not a precise tracer of potassium excretion. "Skimming" or excretion before entry into cellular compartments was reflected in the higher specific activity in the urine than in the plasma and by the rapid decline of specific activity in both urine and plasma, which was most pronounced in the subject who received the tracers intravenously.

The total recovery of Rb^{86} in 4 subjects under balance conditions varied from 19% to 50% of the injected dose. Of that recovered, 20% to 25% was in the feces of the subjects without diarrhea, and in one subject with diarrheal state it was about 50%. The remainder, 39% to 78% of injected dose, was excreted in urine. The total duration of the observations was limited by the low count levels in plasma and urine, although a major part of the injected isotope remained in the body in the cellular compartments. The lag in the loss of Rb^{86} from the cellular compartments resulted in less effect of intake on excretion than with isotopes in the extracellular compartment such as Na^{22} and Cl^{36} . In the balance studies 0.5% to 2.0% of the potassium calculated to be in the body²³ was excreted per day, which was more rapid than the excretory rate of Rb^{86} in these subjects. Even if Rb^{86} traced potassium precisely, differences in excretion of potassium and Rb^{86} would be expected, since they are principally intracellular in location. Since lags in exchange occur, excretion of potassium is disproportionately made up of recently ingested potassium, which has not had time to come into an equilibrium of distribution with the potassium of the body. Over short periods of time (1 to 2 hours) a constant relationship of Rb^{86} to K^{42} and K^{39} might be expected, but when observations are conducted over periods of time sufficiently long to manifest lags in exchange, variations in dietary intake and changes in kaliokinesis, a constant relationship of potassium and Rb^{86} would not be expected. The addition of KCl to the dietary intake of potassium had an inconstant effect on the excretion of Rb^{86} . The administration of an organic mercurial diuretic increased the excretion of Na and Cl in all instances, but in three of the four subjects, this effect was not observed for Rb^{86} and potassium.

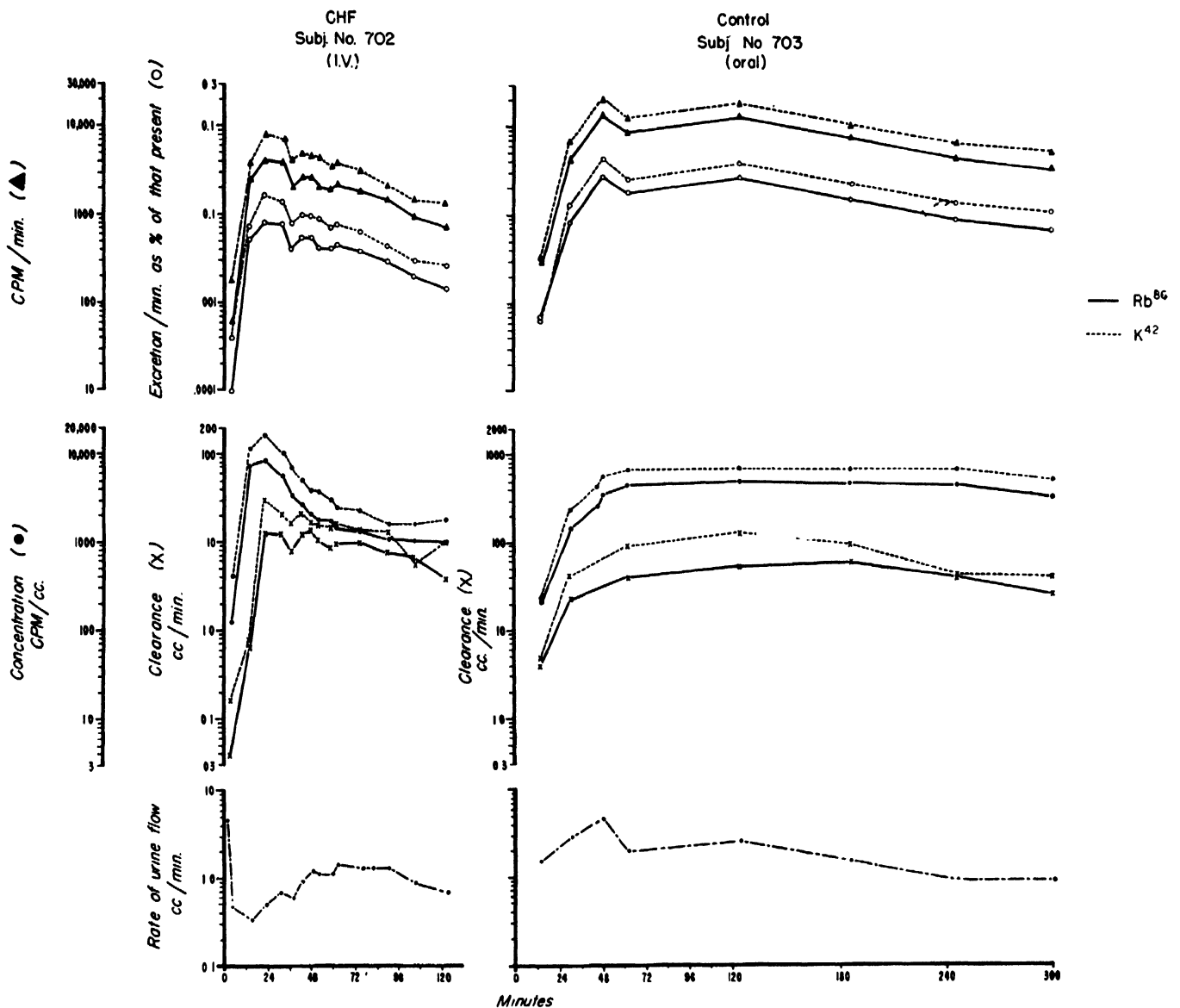


Figure 10. Time course of rates of urinary excretion and clearance of Rb⁸⁶ and K⁴² administered simultaneously intravenously (Subject No. 702) and orally (Subject No. 703)

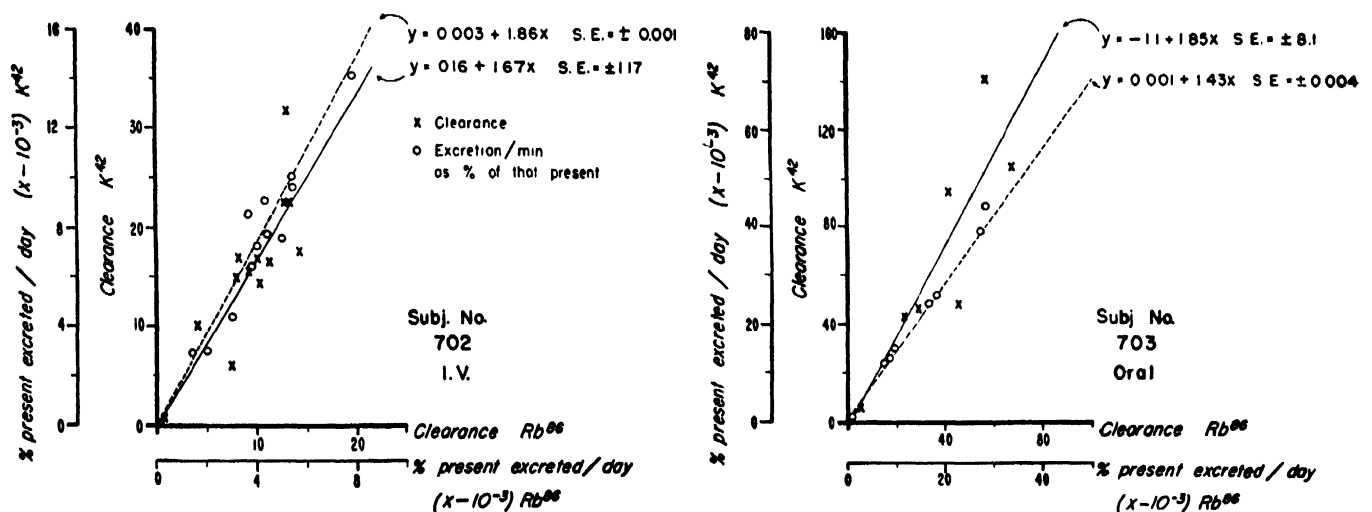


Figure 11. The relationship of urinary clearance and rate of excretion of K⁴² to that of Rb⁸⁶ in two subjects immediately after administration

The relationship of daily urinary clearances of Rb^{86} and potassium is shown in Fig. 13. In Subject No. 601 the open circles represent the values while the subjects received large supplementary doses of KCl. The variations are shown in greater detail in Fig. 14 along with "specific activity" of Rb^{86} in erythrocytes, plasma and urine. There was no constant quantitative relationship in urinary clearance, although a qualitative relationship is apparent. There were wide fluctuations in urinary specific activity of Rb^{86} . Variations in excess of the biologic decay rates indicate lags in exchange, skimming or changes in kaliokinetic and rubidiokinetic phenomena.

GENERAL DISCUSSION

Apparently Rb^{86} traces closely the exchange of K^{39} between the plasma and other compartments of the body for the first few hours after the intravenous administration of a tracer dose. This was also found to be true for erythrocytes *in vitro*.¹ The extent to which this can be applied to specific compartments about the body of intact man has not been determined. This should be evaluated carefully for each particular experimental approach. This is evident from the studies on excretion, which reveal a less rapid (52 to 68% of K^{42}) excretion of Rb^{86} and K^{39} and K^{42} . Nevertheless, in spite of these differences, considerable information concerning K metabolism not available through the use of K^{42} can be obtained from the use of Rb^{86} . For precise or crucial measurements and studies, K^{40} and K^{42} must be employed if it is at all possible.

These experiments have revealed direct information concerning the rubidium ion, and with certain reservations indirect information concerning the potassium ion. Surely, the wide fluctuations in plasma and urine concentrations of Rb^{86} , urinary clearance, exchanging space and mass of Rb^{86} and K^{39} reflect

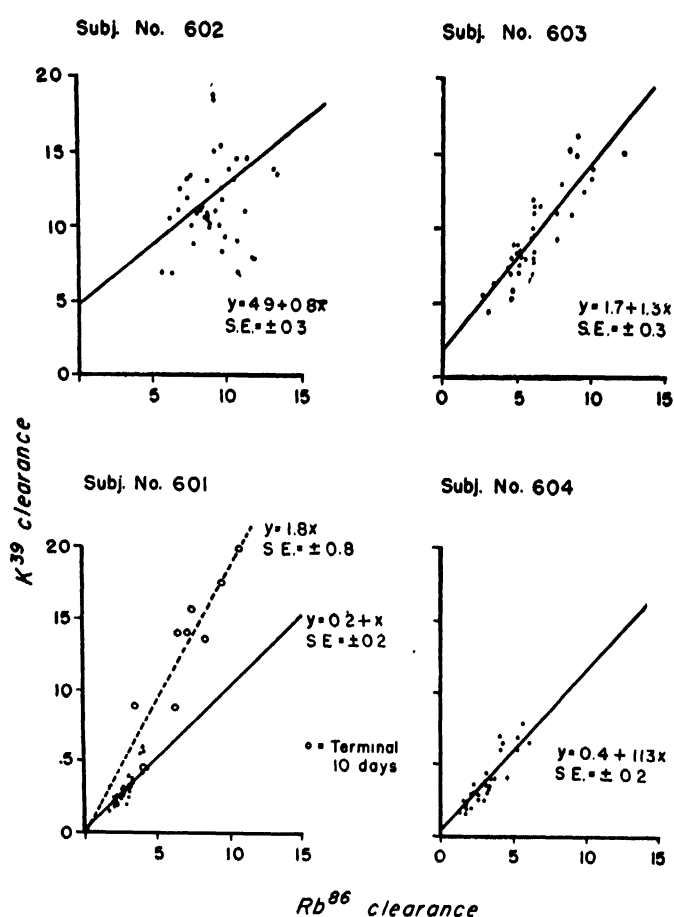


Figure 13. Relation of daily clearances of K^{39} to those of Rb^{86} (cc/min). Open circles for Subject No. 601 represent values obtained on KCl intake

accurately the true physiologic circumstances. That these fluctuations are greater in the subjects with chronic congestive failure is most probably correct even though errors in magnitudes exist. The biologic decay rates for Rb^{86} , and indirectly an index of that for K^{39} , the influence of mercurial diuretics and the dietary intake of K^{39} , the relationship of Rb^{86} concentration in the blood to change in body weight are among the many bits of data that Rb^{86} provided as a tracer of K^{39} .

From the data presented, the time courses of exchanging space and mass of K^{39} were estimated.¹³ These curves can be visualized by a glance at the illustrations. These calculations, as well as others, show clearly the possible hazard in the use of a single measurement rather than many successive ones. For reliable information, continuous curves are necessary. Furthermore, accurate measurements of space and mass for the body as a whole are virtually impossible because equilibrium of distribution is never established, and certainly not nearly established for the first 5 days even in man without congestive heart failure. With lags in exchange, slow turnover rates in some compartments and variations in dietary intake and output make the calculations of exchangeable space and mass almost impossible. These studies have shown the importance of considering the plasma a segment of the extracellular or minor compartment of Rb^{86} , K^{42} and K^{39} and

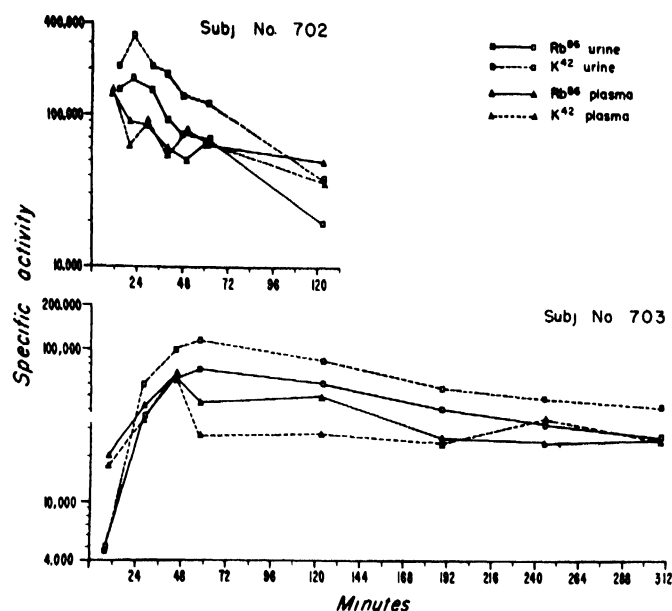


Figure 12. Early time course of specific activity in urine and plasma of K^{42} and Rb^{86} following simultaneous Intravenous (Subject 702) and oral (Subject 703) administration of the two tracers

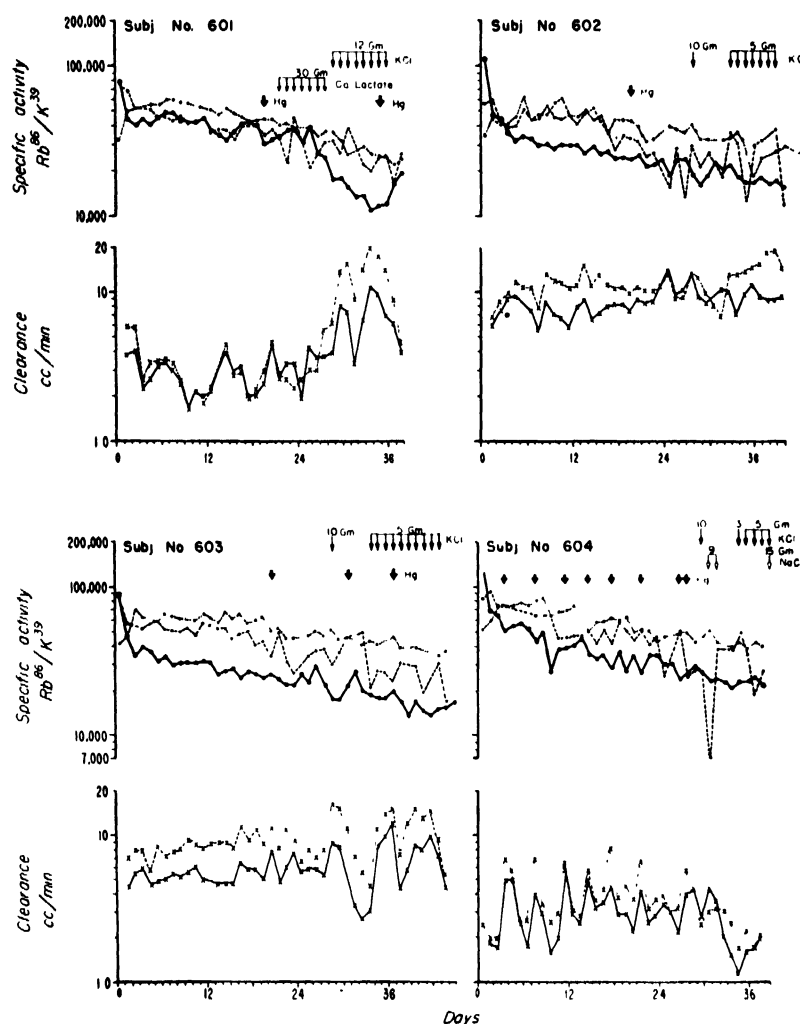


Figure 14. The relationships of specific activities to clearances of Rb⁸⁶ and K³⁹. The time at which drugs were administered is indicated

the intracellular or major compartment, which contains the major mass and space of rubidium and potassium. These studies with Rb⁸⁶ and K⁴² and those with Na²² and Cl³⁶ reveal the importance of at least sampling the intracellular compartment when tracing Rb and K and the extracellular compartment when tracing Na and Cl and attempting to obtain an impression of the kinetics for the body as a whole. To sample the minor compartment only can lead to significant quantitative and qualitative errors.

SUMMARY

Studies of six subjects, 3 control and 3 with congestive heart failure, with Rb⁸⁶ and K⁴² as well as K³⁹, Na²³, Cl³⁵ revealed that Rb⁸⁶ traces K⁴² and K³⁹ in the blood fairly accurately during first 120 minutes after rapid intravenous administration. It was excreted 52% to 68% as rapidly as K⁴² and K³⁹. Significant information concerning kaliokinetics not obtainable with short life K⁴² was obtained by the use of Rb⁸⁶. Its usefulness and limitations in long term studies of kaliokinetics was evident from the data obtained.

Such phenomena as biologic decay rates, exchanging space and mass, equilibrium of distribution, urinary clearance, fecal excretion and other rubidio-

kinetic phenomena were observed directly with the Rb⁸⁶.

ACKNOWLEDGEMENT

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The Use of C¹⁴-Labeled Compounds in Tuberculosis Research

By D. Koch-Weser, B. J. Tricou, W. R. Barclay and R. H. Ebert,* USA

The purpose of this paper is to demonstrate the value and the limitations of various experimental procedures employing C¹⁴-labeled compounds in tuberculosis research. In our laboratories several techniques have proven to be useful: the uptake and turnover of C¹⁴-acetate by tubercle bacilli *in vitro* under various experimental conditions, the labeling of tubercle bacilli with C¹⁴-acetate to investigate their distribution in the living animal, the exposure of tubercle bacilli *in vitro* to C¹⁴-labeled antituberculous drugs, such as isonicotinic acid hydrazide (Isoniazid—INH), paramino-salicylic acid (PAS) and isoniazid of glucuronolactone (INH-G), and the tracer studies with C¹⁴-labeled INH in tuberculous guinea pigs and in surgical specimens obtained from tuberculous patients.

C¹⁴-ACETATE UTILIZATION BY TUBERCLE BACILLI *IN VITRO*

Acetate is one of the fundamental building stones for organic matter and, therefore, it could be expected that its utilization by tubercle bacilli represents basic metabolic activity. Part of the carbon from acetate is taken up and retained by the bacilli to build up the bacterial substance and part is turned over into CO₂ and released.¹ This uptake and turnover of C from acetate by tubercle bacilli under various conditions was quantitated with the use of C¹⁴-sodium acetate with the C¹⁴ in the methyl group. Tubercle bacilli (strain R₁R_v) were grown in Dubos' liquid Tween-albumin medium.² Their growth rate was measured by nephelometric readings.³ One aspect of their metabolic activity was determined by successive tetrazolium reduction tests.⁴ Triphenyl-tetrazolium chloride (TTC) is a colorless water-soluble substance, which is reduced quantitatively by dehydrogenase systems of the bacilli to red insoluble formazan. The formazan is extracted from the medium by ethyl acetate and measured colorimetrically. The results of these tests were expressed in μmol TTC reduced per nephelometric unit (N.U.). One N.U. in our laboratory corresponds to 1.05 μg of dry tubercle bacilli or 200,000 viable organisms.⁵

The C¹⁴-acetate uptake and turnover of these bacilli were measured in Conway dishes (Fig. 1). Four ml of tubercle bacilli suspension in the strongly buffered medium were injected into the outer well and C¹⁴-acetate was added. The center well contained 1 ml

of N NaOH, which absorbed the liberated C¹⁴O₂. The ground glass cover of the unit was sealed to the dish with a paraffin-petrolatum mixture. At any time samples from both wells could be removed through the rubber diaphragms without danger of secondary contamination. To facilitate comparison and conclusions all data reported in this paper refer to uptake and turnover in 24 hours after the addition of the C¹⁴-acetate. For the determination, the bacilli from the outer well were separated from the medium by repeated centrifugation and washing in Tween-water, in which they were finally suspended. After determining the number of bacilli in N.U., they were plated on copper planchettes and their radioactivity measured in a windowless continuous gas flow counter under helium. After correction for self-absorption the amount of C¹⁴ taken up by the bacilli was expressed in disintegrations per minute (dpm) per N.U. The C¹⁴O₂ absorbed by the NaOH was precipitated as barium carbonate, plated on copper planchettes and counted in an identical manner. Results were expressed in dpm produced per N.U. bacilli.

Early in the experiments it was observed that with increasing density of the bacterial suspension, decreasing amounts of C¹⁴ were taken up by the bacilli and turned over to C¹⁴O₂ (Fig. 2). This was believed to be due to a "crowding" effect. Probably the optimal amount of O₂ is not available to the bacilli in the denser cultures. TTC reduction (Fig. 2) did not decrease with the "crowding" due to the fact that the presence of O₂ is not necessary for TTC reduction, which can take place under anaerobic conditions.⁶ As a consequence of this finding, bacterial suspensions were diluted or concentrated to the same density (N.U./ml) in each experiment.

Such identical suspensions took up and turned over to C¹⁴O₂ proportional amounts of C¹⁴-acetate, when this was added to the outer well in increasing amounts (Fig. 3). The explanation is that the C¹⁴ given as acetate enters a pool of unlabeled C, which is in excess of the metabolic requirements of the bacilli. More or less C¹⁴-acetate in the medium means only more or less labeling of the pool and, therefore, the results are proportional to the amount of C¹⁴-acetate added.

To demonstrate that C¹⁴-acetate uptake and turnover really measure some metabolic activity of the bacilli, these were submitted to different temperatures

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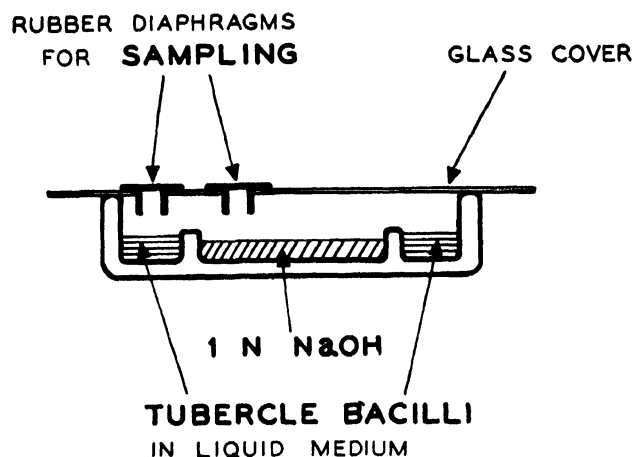
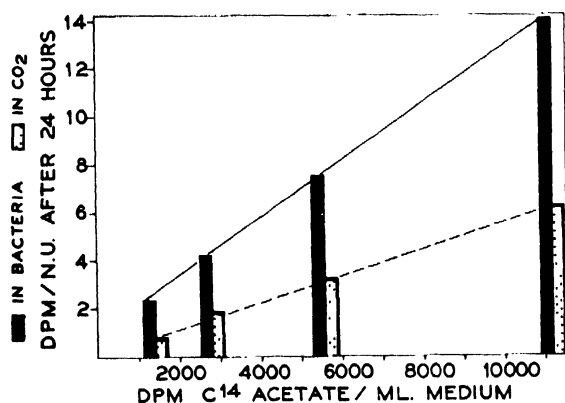
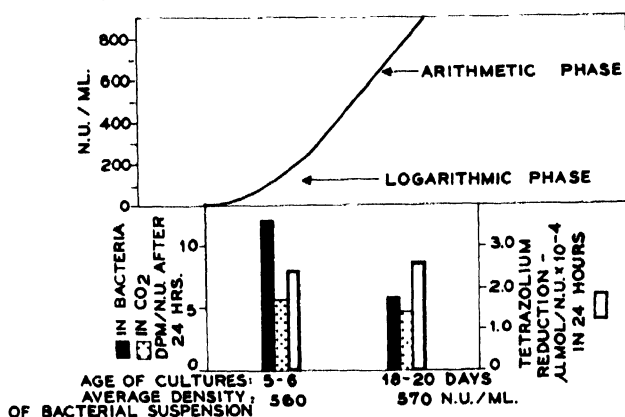


Figure 1. Cross section through a Conway dish

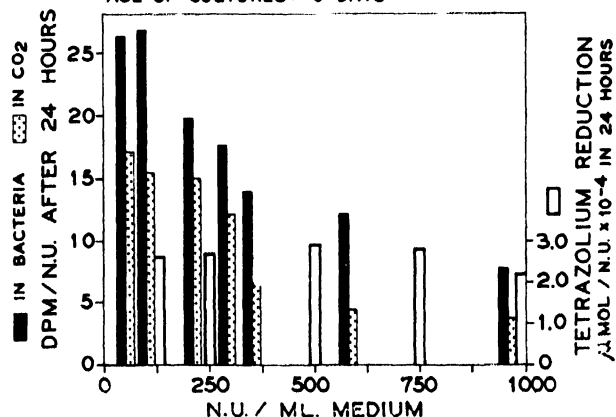
INCREASING AMOUNT OF C^{14} ACETATE IN MEDIUM
 CONSTANT DENSITY OF BACTERIAL SUSPENSION: 340 N.U./ML
 AGE OF CULTURES: 6 DAYS

Figure 3. C^{14} -acetate utilization by tubercle bacilli as a function of C^{14} -acetate concentration in the medium

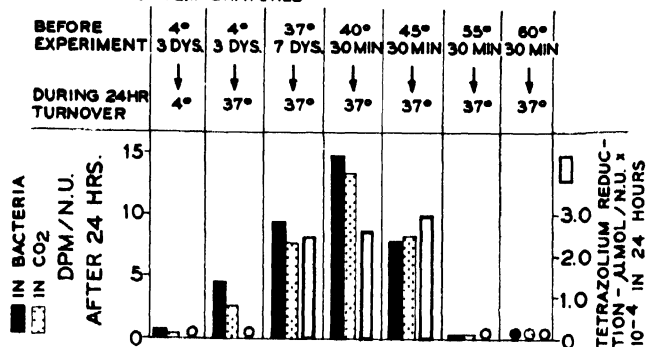
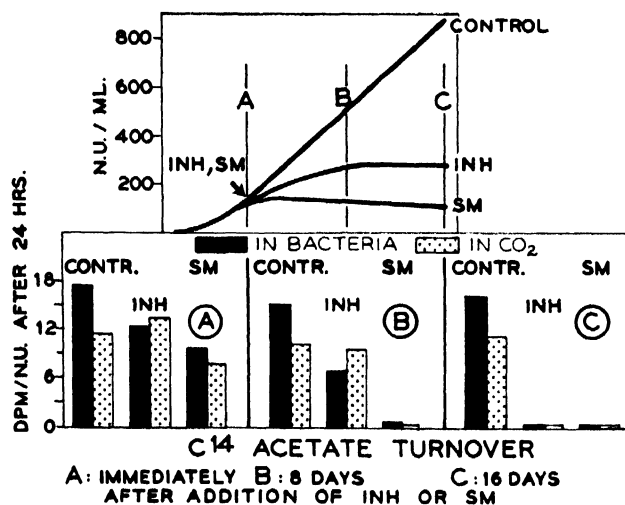
AMOUNT OF C^{14} ACETATE: 11250 DPM/ML.

Figure 5. C^{14} -acetate utilization and tetrazolium reduction by tubercle bacilli in the logarithmic and arithmetic phases of growth

CONSTANT AMOUNT OF C^{14} ACETATE: 11,250 DPM/ML.
 INCREASING DENSITY OF BACTERIAL SUSPENSION
 AGE OF CULTURES: 6 DAYS

Figure 2. C^{14} -acetate utilization and tetrazolium reduction by tubercle bacilli as a function of bacterial density

CONSTANT AMOUNT OF C^{14} ACETATE 11,250 DPM/ML.
 CONSTANT DENSITY OF BACTERIAL SUSPENSION: 460 N.U./ML.
 AGE OF CULTURE: 10 DAYS
 VARYING TEMPERATURES:

Figure 4. C^{14} -acetate utilization by tubercle bacilli after exposure to different temperaturesFigure 6. Growth inhibition of and C^{14} -acetate utilization by tubercle bacilli after exposure to streptomycin or isoniazid

before and during the turnover period (Fig. 4). After being previously exposed to 4°, bacilli took up and turned over small amounts of acetate while remaining at that temperature. If the C^{14} -acetate utilization of these previously refrigerated bacilli took place at 37°, uptake and turnover were considerable. Refrigerated bacilli did not reduce TTC. After exposure to more

than 55° no acetate utilization and TTC reduction was detected. Not represented in Fig. 4 are the findings that neither medium without bacilli nor formalin treated bacilli utilized any C^{14} -acetate. It seems that under certain circumstances tubercle bacilli, which do not grow (at 4°) and do not reduce TTC, still utilize reduced amounts of acetate.

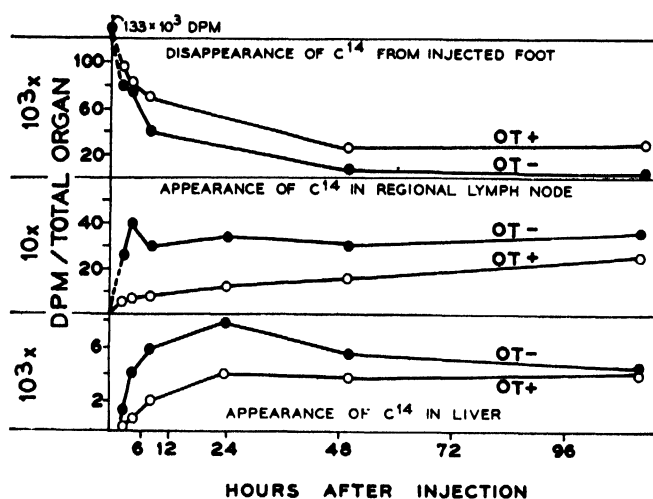


Figure 7. Distribution of C¹⁴-acetate labeled tubercle bacilli in guinea pigs

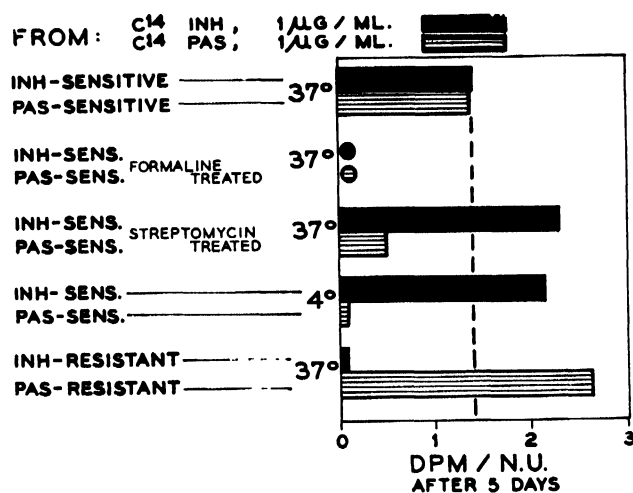


Figure 9. Uptake of C¹⁴ from C¹⁴-isoniazid and C¹⁴-para-aminosalicylic acid by tubercle bacilli under various experimental conditions

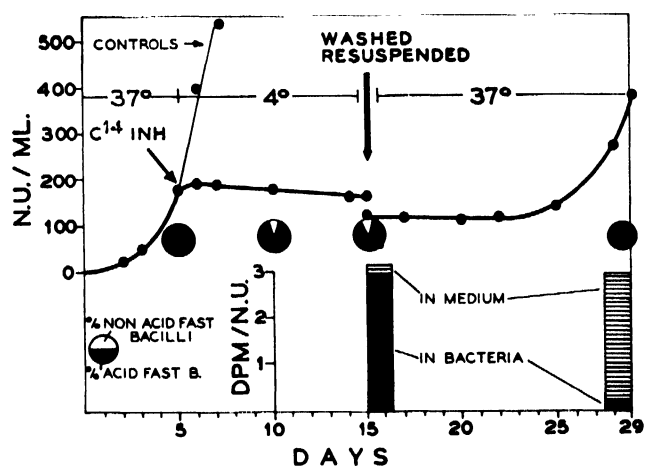
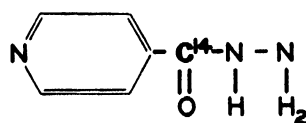


Figure 11. Reversibility of C¹⁴-isoniazid uptake by tubercle bacilli at 4°

Tubercle bacilli in liquid media multiply initially in a logarithmic rate which is followed by arithmetic multiplication. It was observed (Fig. 5) that during the logarithmic phase of growth the bacilli took up considerably more C¹⁴ and turned over slightly more C¹⁴-acetate to C¹⁴O₂ per N.U. than in the arithmetic phase. TTC reduction was the same in both phases. This lends support to the theory that the logarithmic phase of growth depends on the available O₂,⁷ which



ISONIAZID

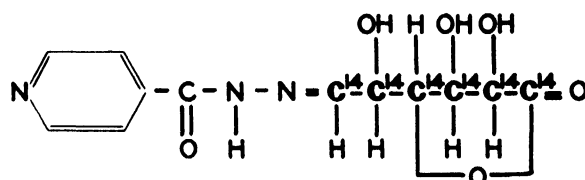


Figure 8. C¹⁴-labelling of isoniazid and of isoniazid of glucuronolactone

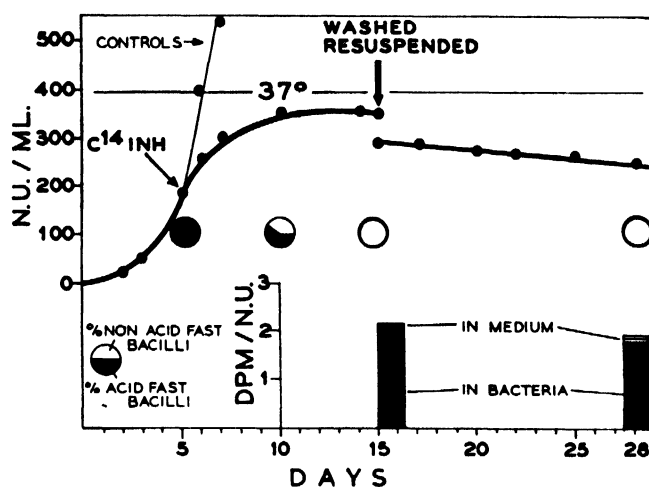


Figure 10. Irreversibility of C¹⁴-isoniazid uptake by tubercle bacilli at 37°

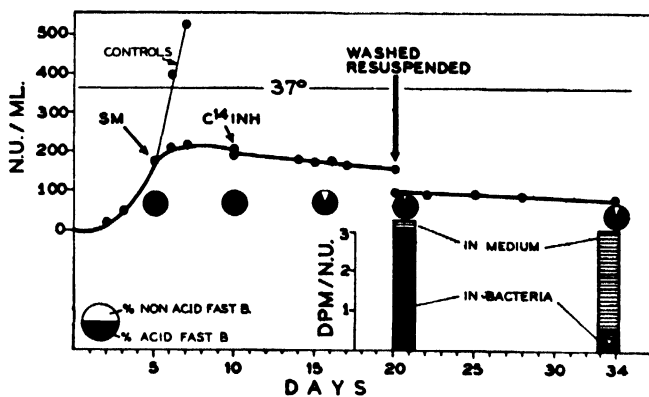


Figure 12. Reversibility of C¹⁴-isoniazid uptake by tubercle bacilli at 37° after previous exposure to streptomycin

also seems necessary for optimal acetate utilization, shown in the experiment on "crowding" (Fig. 2).

If streptomycin (SM), 5 µg/ml, was added to suspensions of growing tubercle bacilli (Fig. 8), growth and TTC reduction was very rapidly inhibited, while the addition of INH, 1 µg/ml, was followed by delayed cessation of growth and TTC reduction.⁸ Determination of the C¹⁴-acetate utilization at intervals after the addition of the antituberculous drugs to the

medium showed a good correlation with the other signs of inhibited bacterial metabolism.

These basic experiments show that both the C^{14} uptake and the turnover to $C^{14}O_2$ from C^{14} -acetate is a very sensitive indicator of metabolic activity of the tubercle bacilli. In addition we hope to use the quantitative relationship between uptake and turnover. Older bacilli with less available O_2 (Fig. 5), bacilli submitted to 45° (Fig. 4) and bacilli exposed to INH (Fig. 6) turned over relatively more C^{14} -acetate to $C^{14}O_2$ than did younger, undamaged bacilli, which took up more C^{14} . The hypothesis that greater production of $C^{14}O_2$ signified less building up of bacterial substance, is at present being tested by using other types of damage and by extracting the different components of normal and damaged tubercle bacilli after a C^{14} -acetate utilization period.

THE DISTRIBUTION OF C^{14} -ACETATE LABELED TUBERCLE BACILLI IN VIVO

The fate of tubercle bacilli injected into the experimental animal has been studied for many years.^{9,10} No simple and satisfactory experimental procedure has been found. When tubercle bacilli were grown in liquid medium containing C^{14} -acetate they became very radioactive, up to 800,000 dpm/mg dry weight, within 7 days. When these bacilli were washed and resuspended in fresh medium, a large part of the C^{14} was released into the medium and as $C^{14}O_2$ into the air. It was assumed that such bacilli injected into an animal would continue to metabolize and lose the radioactivity. If these bacilli, however, were pasteurized at 60° for 30 minutes and then washed and resuspended in fresh medium they retained 98% of their radioactivity for more than 4 days. Such bacilli were used (Fig. 7) to investigate the distribution in the uninfected guinea pig with no skin reaction to old tuberculin (OT —) and in the previously infected guinea pig with a positive skin test to old tuberculin (OT +). Suspensions of bacilli were injected subcutaneously into the foot. Animals were sacrificed at different time intervals, their organs removed, weighed, homogenized in an all glass homogenizer, diluted and aliquots were plated on copper planchettes. The number of disintegrations was determined in the continuous gas flow counter and expressed as dpm per total organ. The injected foot was completely digested by 10N NaOH, homogenized, diluted, neutralized with acetic acid using bromothymol blue as an indicator, plated and counted in an identical manner. The results obtained are reported (Fig. 7). They indicate that the previously infected, OT sensitive guinea pigs localize bacilli at the site of the injection more than the uninfected animals do. This technique may be valuable in the study of natural and induced host resistance.

THE EXPOSURE OF TUBERCLE BACILLI TO C^{14} -LABELED ANTITUBERCULOUS DRUGS IN VITRO

With the increasing role and success of chemotherapy in the treatment of tuberculosis, the study

of the mode of action of antituberculous drugs has become of great importance. C^{14} -labeling of such compounds has proven to be a valuable technique.

The *in vitro* Uptake of C^{14} -isoniazid by Tubercle Bacilli

Isonicotinic acid hydrazide (Isoniazid-INH) labeled with C^{14} in the carboxyl position (Fig. 8) was synthesized by Murray and Langham.¹¹ This compound was added, in the concentration of 1 μ g/ml, to liquid Dubos' medium, in which tubercle bacilli were growing. The growth rate was followed by nephelometric readings. The bacteriostatic concentration of the drug previously had been found to be 0.05 μ g/ml.¹² After five days the bacilli were removed from the medium by centrifugation, washed repeatedly in Tween-water and aliquots of the last suspension in Tween-water were plated on copper planchettes. They were counted in the continuous gas flow counter under helium. After correction for self-absorption the radioactivity of the bacilli was expressed in dpm per N.U. It was found that INH-sensitive bacilli took up either the labeled compound or its labeled metabolites and bound it firmly so that it could not be removed by repeated washings (Fig. 9). The relationship of the C^{14} -INH bound to the bacilli and the concentration of the drug in the medium can be expressed by Freundlich's equation for physical adsorption. In contrast to labeled isonicotinic acid hydrazide (C^{14} -INH) similar compounds without antituberculous activity, such as C^{14} -nicotinic acid, C^{14} -nicotinamide and C^{14} -isonicotinic acid were not taken up by tubercle bacilli in any significant amount.¹³ Formalin treated tubercle bacilli did not bind C^{14} -INH, while tubercle bacilli either at 4° or after previous exposure to streptomycin (SM) retained even a greater amount of the labeled drug. It seemed that tubercle bacilli, which were still viable, but metabolically less active retain more C^{14} -INH than more actively metabolizing organisms. Bacilli were made resistant to at least 2 μ g/ml of INH by successive passages through media containing increasing amounts of the drug. These INH resistant bacilli took up only an insignificant amount of C^{14} -INH given in the concentration of 1 μ g/ml (Fig. 9).

The *in vitro* Uptake of C^{14} -Para-Amino-Salicylic Acid by Tubercle Bacilli

Para-amino-salicylic acid (PAS) labeled with C^{14} in the carboxyl position was investigated using the same technique described for C^{14} -INH. C^{14} -PAS also was taken up by PAS-sensitive bacilli, but, above the level of 1 μ g/ml of medium the amount of drug bound was constant and independent of the concentration in the medium. The drug is not initially adsorbed, but metabolized and 1 μ g/ml of medium is more than the bacilli can utilize. In contrast to C^{14} -INH, C^{14} -PAS was not taken up significantly at 4° and its uptake by bacilli previously inhibited

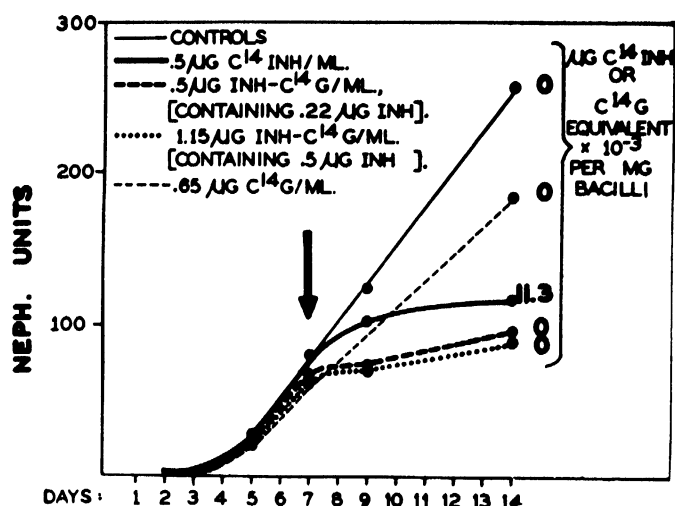


Figure 13. Growth of and uptake of radioactivity by isoniazid sensitive tubercle bacilli after the addition of C¹⁴-isoniazid, C¹⁴-glucuronolactone and isoniazid of C¹⁴-glucuronolactone

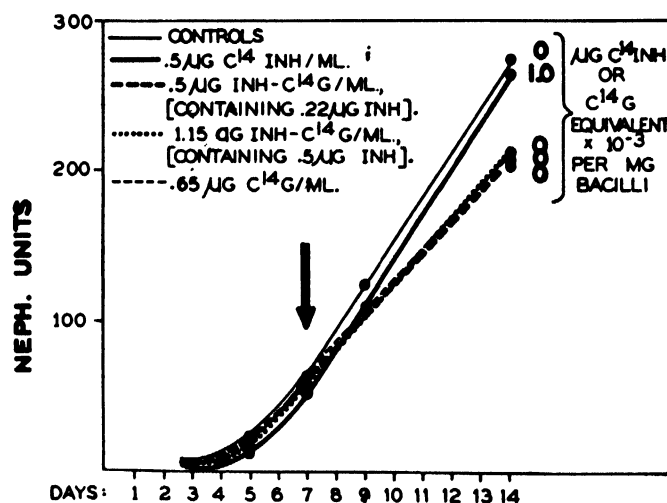


Figure 14. Growth of and uptake of radioactivity by isoniazid-resistant tubercle bacilli after the addition of C¹⁴-isoniazid, C¹⁴-glucuronolactone and isoniazid of C¹⁴-glucuronolactone

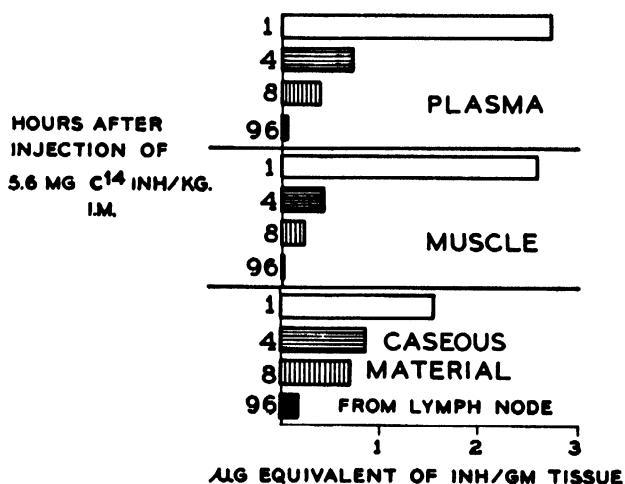


Figure 15. Distribution of C¹⁴-isoniazid in the tuberculous guinea pig

by SM was markedly decreased. PAS-resistant bacilli, however, retain quite significantly more C¹⁴-PAS, probably because they are unable to metabolize the drug and eliminate the metabolites (Fig. 9). These differences in the uptake of the two C¹⁴-labeled antituberculous drugs form the basis for continued studies on their mode of action.

Reversible Binding of C¹⁴-isoniazid by Metabolically Inhibited Tubercle Bacilli

The difference in uptake of C¹⁴-INH by actively metabolizing untreated bacilli at 37° and by bacilli inhibited either at 4° or by previous exposure to SM was investigated. C¹⁴-INH (1 μg/ml) was added to growing tubercle bacilli (Fig. 10). Growth slowed and finally ceased after approximately one more generation. Ten days after the addition of C¹⁴-INH all bacilli were non-acid-fast and did not reduce TTC.⁸ These bacilli were washed twice and then resuspended in fresh Dubos' medium. A sample of the bacterial suspension was taken for C¹⁴ determination. Bacilli and medium were separated by centrifugation, the bacilli washed, plated and counted as described above. The medium was filtered (Seitz)

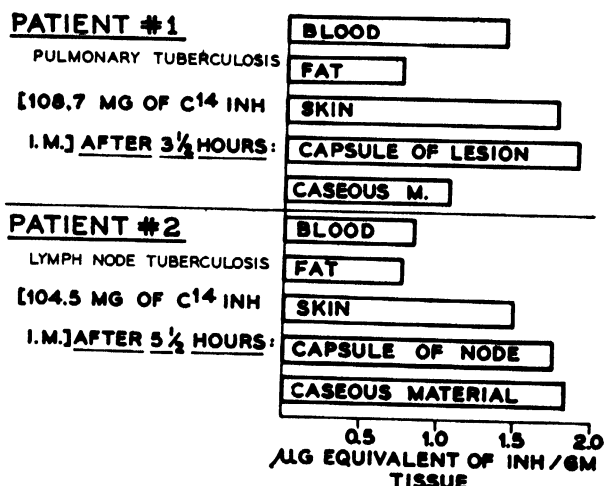


Figure 16. Distribution of C¹⁴-isoniazid in different components of surgical specimens obtained from tuberculous patients

to remove any residual bacilli and also plated and counted. The resuspended bacilli did not resume growth and remained 100% non-acid-fast for another 13 days, when the C¹⁴ determination in medium and bacilli was repeated. It was found that the bacilli retained the C¹⁴-INH, not releasing it into the medium. If in parallel experiments C¹⁴-INH was taken up in larger amounts either at 4° (Fig. 11) or after previous exposure to SM (Fig. 12), the bacilli remained almost completely acid-fast. Resuspended in fresh medium at 37° they released the radioactive compound into the medium to a very large extent. Those exposed to INH at 4° resumed growth within 10 days after resuspension. It seems, therefore, that actively metabolizing tubercle bacilli adsorb and bind INH irreversibly, while bacilli with inhibited metabolism adsorb it reversibly.

Differential C¹⁴-labeling for the Investigation of the *in vitro* Antituberculous Activity of INH-G

To overcome the development of resistance to INH *in vivo* and *in vitro* would be of great theoretical and practical importance. It was reported^{14,15} that isoniazid of glucuronolactone (INH-G) is tuber-

culostatic for INH-resistant strains. By labeling the glucuronolactone part of the molecule (Fig. 8) it was hoped to confirm these reports. C^{14} INH is taken up by INH-sensitive bacilli and not adsorbed to INH-resistant strains. Therefore, the glucuronolactone compound (INH- C^{14} G) to be effective against both, should be taken up by both, the INH-sensitive and the INH-resistant strains. Figure 13 shows that C^{14} -glucuronolactone (C^{14} G) alone had a slightly retarding effect upon the growth of INH-sensitive bacilli, which is believed to be due to changes in the medium. While C^{14} -INH inhibited these bacilli and was taken up, INH- C^{14} G inhibited growth without the C^{14} G part of the molecule being bound by the organisms. Figure 14 indicates that INH-resistant bacilli were not inhibited by INH- C^{14} G, except for the slight retardation of growth due to glucuronolactone alone. Neither C^{14} INH nor INH- C^{14} G was bound by these INH-resistant bacilli. It is concluded that INH-G does not affect the tubercle bacilli as an intact compound, but only through its INH content, which is released from the molecule before it gets in contact with the tubercle bacilli. It is ineffective against INH-resistant strains.

THE DISTRIBUTION OF C^{14} -ISONIAZID IN VIVO

Isotope tracer techniques provide an extremely sensitive and relatively simple method of determining small amounts of drugs in tissue and body fluids. Since INH is bacteriostatic in quite low concentration (0.05 μ g/ml of medium), the use of C^{14} -INH was indicated to study the distribution of the drug in animal and human tissues. With Dr. L. J. Roth's group from the Department of Pharmacology of the University of Chicago, studies in guinea pigs and on human surgical specimens were undertaken. Guinea pigs infected previously with tubercle bacilli strain H₈₇Rv, were injected intramuscularly with C^{14} -INH. They were sacrificed at different intervals and plasma, various organs and caseous material from the center of tuberculous lymph nodes were prepared and their C^{14} content determined as described above for C^{14} -acetate containing tissues. Figure 15 represents the values for plasma, muscle and caseous material from the lymph node. Though initially the concentration of C^{14} -INH is greater in plasma and muscle than in the caseous material, this retains it longer and above bacteriostatic levels for at least 96 hours.

These results were confirmed¹⁶ by analyzing surgical specimens from two patients, which preoperatively had been injected with C^{14} -INH intramuscularly. (Fig. 16). Three and one-half hours after administering the tracer dose the capsule of a resected tuberculous pulmonary lesion contained more C^{14} than the blood and the skin, which retained surprisingly high amounts. The caseous material itself contained amounts far above the bacteriostatic level. In the other patient capsule and caseous material from a tuberculous cervical lymph node showed a

significantly higher concentration than blood five and one-half hours after giving C^{14} -INH. This demonstrates that INH certainly penetrates the caseous lesions in bacteriostatic concentrations.

We present these data mainly to demonstrate the usefulness of the various procedures. Therefore, we report only the most significant results. The importance of this presentation lies in the fact that identical and similar procedures could be used to solve problems in many other infectious and parasitic diseases.

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The Absorption of Vitamin B₁₂ and the Pathogenesis of Vitamin B₁₂ Deficiency

By D. L. Mollin and E. Lester Smith,* UK

It is widely recognised that vitamin B₁₂ deficiency may occur in association with gastric atrophy; it generally follows total gastrectomy if the patient lives long enough and can develop after partial gastrectomy. Vitamin B₁₂ deficiency is also found in patients with intestinal anastomoses, strictures and blind loops who develop a megaloblastic anaemia and occurs in one third of the patients with a megaloblastic anaemia associated with steatorrhoea of unknown origin.¹ It is found in the nutritional disorders of the tropics²⁻⁵ and in subjects existing on strictly vegetarian diets.⁶ The precise incidence of the deficiency is uncertain, partly because it may be present for several years before obvious clinical manifestations appear⁷ and partly because its presence may be masked by deficiency of other haemopoietic factors.¹

The pathogenesis of the deficiency in these conditions is investigated by studying the absorption of the vitamin administered orally. Until recently such investigations were limited to untreated anaemic patients with uncomplicated vitamin B₁₂ deficiency, because the only means of demonstrating that the vitamin had been absorbed was to show that an alteration in the haematological state followed oral treatment. The introduction of vitamin B₁₂ labelled with the isotopes of cobalt^{8,9} has led to the development of methods whereby its absorption may be studied in both treated and untreated patients, as well as in normal subjects. The purpose of this paper is to describe how radioactive vitamin B₁₂ has been used to investigate the absorption of the vitamin by normal subjects and by patients with vitamin B₁₂ deficiency.

THE PREPARATION OF LABELLED VITAMIN B₁₂

The first form of labelled vitamin B₁₂ (cyanocobalamin) to be used was Co⁶⁰ cyanocobalamin.^{8,9} The long half-life (5 years) of the Co⁶⁰ limited the amount that could be given with safety and therefore also the number of tests that could be carried out on any one individual. For this reason batches of vitamin B₁₂ labelled with the shorter-lived isotopes of cobalt (Co⁵⁶, Co⁵⁷ and Co⁵⁸) were prepared.^{10,11} If carrier-free samples of these isotopes are used,

vitamin B₁₂ of high specific activity can be prepared. With such material the absorption and excretion of the vitamin can be studied more accurately and new methods of investigation can be developed.

The cobalt isotopes were incorporated into the cyanocobalamin molecule biosynthetically, because chemical exchange could not be brought about¹² and direct neutron irradiation of the crystalline vitamin gave only low specific activities.¹³ The radioactive cobalt salts were added to a practically cobalt-free medium, which was fermented by means of *Streptomyces griseus*.⁹ The scale ranged from a few hundred ml in shakeflasks to 10 litres in stirred bottles. The requirements of very high specific activity and good conversion efficiency dictated the use of limiting levels of cobalt, which led to low titres (usually less than 1 µg per ml) and increased the difficulties of purification. The radioactive vitamin was extracted by standard methods,⁹ purified by chromatography on paper and crystallised, in yields ranging from 0.1 to 3 mg. Cobalt-60 yielded products of specific activities up to 0.8 mc per mg. Cobalt-58, made by pile irradiation of nickel, was probably diluted by traces of cobalt in the target material and gave a product of no higher specific activity.¹⁰ Carrier-free Co⁵⁶ and Co⁵⁷, made by cyclotron treatment of iron (by Philips-Roxane, Amsterdam and at Birmingham University), gave vitamin B₁₂ with specific activities of 12 and 13.5 mc per mg respectively.

METHODS OF STUDYING THE ABSORPTION OF RADIOACTIVE VITAMIN B₁₂

The absorption of radioactive vitamin B₁₂ may be studied in four ways: (a) by estimating the amount absorbed from measuring the faecal excretion of radioactivity after oral doses of radioactive vitamin;¹⁴ (b) by measuring the urinary excretion of radioactivity after oral doses accompanied or followed by a large injection of non-radioactive vitamin;¹⁵ (c) by measuring radioactivity over the liver after oral doses of radioactive vitamin;¹⁶ and (d) by measuring the radioactivity of serum after oral doses of radioactive vitamin of high specific activity.¹⁷

MEASUREMENT OF THE FAECAL EXCRETION OF RADIOACTIVITY

This method, introduced by Heinle and his colleagues¹⁴ and used by several groups of workers,¹⁸⁻²¹

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is the most suitable method for quantitative study of absorption. Oral doses of 0.5 to 2.0 μg of labelled vitamin B₁₂ are given to the fasting subject and the faecal excretion of radioactivity after the dose is measured. The difference between the amount administered and the amount excreted is considered to represent the amount of labelled vitamin absorbed. The amount of radioactivity excreted may be estimated by measuring the radioactivity of aliquots of the faecal samples in a scintillation counter. It can also be estimated by measuring radioactivity in a ring counter with Geiger-Müller tubes in parallel. If a suitable instrument is designed, the total faecal excretion of radioactivity after an oral dose can be measured at one time without the need for sampling.²² In the studies reported below we initially used a ring counter with eight Geiger-Müller tubes in parallel. More recently we have been using a counter with 32 or 64 tubes in parallel. Such counters give 1600 to 3500 counts per minute above background per microcurie of radioactive cobalt. It is essential that the test material used in all quantitative studies should be free from breakdown products of the vitamin; inorganic cobalt and certain other radioactive breakdown products are not absorbed by normal subjects or by patients showing poor absorption of the vitamin.^{23,24}

The results of absorption-excretion tests can be expressed as *micrograms of vitamin absorbed* or as *percentage of the administered dose* excreted. The former method is preferred by us, for reasons given elsewhere.²¹ This technique has been used to diagnose syndromes involving malabsorption of vitamin B₁₂; to investigate the pathogenesis of its deficiency in these conditions; to study the relation between intrinsic factor and the intestinal absorption of the vitamin and to study its absorption in normal subjects. It has also been employed for the assay of intrinsic factor in various concentrates and in gastric juice.

THE DIAGNOSIS AND INVESTIGATION OF VITAMIN B₁₂ DEFICIENCY STATES

Pernicious Anaemia

To demonstrate convincingly that a patient with vitamin B₁₂ deficiency is suffering from pernicious anaemia it is necessary to show that he is unable to absorb the vitamin until a potent source of intrinsic factor is also administered.²⁵ Although this can be established by haematological methods,²⁶ such studies are tedious and can only be made on patients in moderate or severe relapse. If a patient is in the early stages of the disease, or if he has been already treated with vitamin B₁₂, it is extremely difficult, sometimes even impossible, to make a firm diagnosis by haematological methods. Patients with pernicious anaemia in relapse or in remission, however, can readily be distinguished from control subjects by faecal excretion studies with radioactive vitamin B₁₂.^{14,18-21,24}

Tables I and II summarise the results of faecal excretion tests on a group of control subjects and patients with proved pernicious anaemia. The oral dose given was 0.6 μg Co⁵⁸ cyanocobalamin and the results have been expressed as the amount of vitamin B₁₂ absorbed. The patients with pernicious anaemia absorbed much less vitamin B₁₂ than did control subjects when the dose was given alone (Table I). When the test dose was given with 30 mg of the same intrinsic factor concentrate to 23 of the patients with pernicious anaemia, the absorption of the vitamin always increased, but the effect of the intrinsic factor differed widely from patient to patient (Table II).

Such a clear-cut distinction between control subjects and patients with pernicious anaemia is not always found. Mollin and Baker²⁴ reported that control subjects not suffering from pernicious anaemia or allied conditions sometimes excreted as much radioactivity as patients with pernicious anaemia. They pointed out that the oral test doses were given to the fasting subjects and therefore under conditions in which the secretion of gastric juice and intrinsic factor might be depressed. Subnormal amounts of vitamin B₁₂ might be absorbed in these circumstances even by normal subjects. They therefore gave an injection of a parasympathetic stimulant, "Carbachol" (carbamylcholine chloride), which they hoped would stimulate the secretion of intrinsic factor, to the control subjects who had absorbed subnormal amounts of vitamin B₁₂ and to patients with pernicious anaemia. Fifteen minutes later they gave a further test dose of the radioactive vitamin B₁₂. The control subjects then absorbed normal

Table I. The Absorption of Co⁵⁸ Cyanocobalamin by Control Subjects and Patients with Pernicious Anaemia (Oral Test Dose: 0.6 μg)

Group	No. of subjects	Range (μg)	Mean and its standard error (μg)
Pernicious anaemia	33	0.00 to 0.21	0.07 $\pm$ 0.01
Control subjects	35	0.30 to 0.55	0.40 $\pm$ 0.01

Table II. The Absorption of Co⁵⁸ Cyanocobalamin by 23 Patients with Pernicious Anaemia Given an Oral Dose of 0.6 μg Alone and Then with 30 mg of One Batch of Intrinsic Factor Concentrate

	Range (μg)	Mean and its standard error (μg)
0.6 μg Co ⁵⁸ cyanocobalamin alone	0.00 to 0.20	0.07 $\pm$ 0.01
0.6 μg Co ⁵⁸ cyanocobalamin + 30 mg of I.F.	0.15 to 0.56	0.32 $\pm$ 0.02
Increase in absorption with 30 mg of I.F.	0.08 to 0.56	0.26 $\pm$ 0.03

amounts of Co⁵⁸ cyanocobalamin; absorption by the patients with pernicious anaemia was unchanged and remained grossly subnormal.

Megaloblastic Anaemias Other Than Pernicious Anaemia

The observations reported above indicate how conveniently the pathogenesis of vitamin B₁₂ deficiency may be studied with the radioactive vitamin. By use of this material it has already been established that patients who have undergone total gastrectomy are unable to absorb radioactive vitamin B₁₂ unless this is given with intrinsic factor;^{18,27} these observations confirm earlier views that the stomach is the sole site of intrinsic factor formation in man.^{28,29}

Table III summarises the results of absorption-excretion tests in patients with megaloblastic anaemias other than pernicious anaemia. All these patients were suffering from vitamin B₁₂ deficiency. The patients with partial gastrectomy, with intestine diverticulosis, strictures, anastomoses and blind loops (intestinal "loop" syndrome) or with steatorrhoea failed to absorb significant amounts of vitamin B₁₂. The patient with a nutritional megaloblastic anaemia associated with a diet grossly deficient in animal protein absorbed normal amounts of the radioactive vitamin.

The simultaneous administration of intrinsic factor failed to potentiate the absorption of vitamin B₁₂ in the patients with intestinal "loop" syndrome and in two of the patients with steatorrhoea, indicating these patients could not absorb the vitamin B₁₂-intrinsic factor complex. Absorption of the vitamin was potentiated by intrinsic factor, however, in the patients with partial gastrectomy and in three patients with steatorrhoea. The results in these patients were similar to those found in Addisonian pernicious anaemia. These patients also had achylia gastrica and

gastric atrophy. It therefore appears that the gastric lesion of Addisonian pernicious anaemia may arise as a result of gastro-intestinal disturbances.

The Absorption of Radioactive Vitamin B₁₂ After the Administration of Antibiotics

The absorption of Co⁵⁸ cyanocobalamin was studied in two patients with idiopathic steatorrhoea and in 5 patients with intestinal "loop" syndrome before and after attempted sterilisation of the gut by treatment with aureomycin and terramycin. The second dose of radioactive vitamin was given four days after beginning antibiotic therapy. Absorption of the vitamin in the patients with idiopathic steatorrhoea was not improved by this treatment, but appreciable improvement occurred in the other groups. The significance of this observation is still uncertain, but if this difference in response is constantly observed it would be a method of distinguishing between the two conditions and so would be of diagnostic and therapeutic importance.

THE MECHANISM OF VITAMIN B₁₂ ABSORPTION

The Absorption of Vitamin B₁₂ by Control Subjects

In the studies described in the previous section the absorption of vitamin B₁₂ by control subjects and by patients with vitamin B₁₂ deficiency has been compared by estimating the amounts absorbed from oral doses of 0.6 µg of radioactive vitamin B₁₂. The results indicate that this is a suitable dose for demonstrating gross abnormalities of vitamin B₁₂ absorption. To determine the maximum amount of vitamin B₁₂ that can be absorbed by normal subjects from a single dose we have used larger doses of the radioactive vitamin. The doses have ranged from 2.0 to 5.0 µg Co⁵⁸ cyanocobalamin—bigger doses than this have not been used in order to avoid as far as possible absorption of the vitamin by direct diffusion.²⁴

Normal subjects varied in their ability to absorb the vitamin but in every one the amount of B₁₂ absorbed appeared to be limited. The absorption by 10 normal subjects ranged from 0.5 to 1.3 µg/Co⁵⁸ cyanocobalamin.³⁰ Similar observations have been reported by Glass and his colleagues,³¹ who determined the amount of radioactive vitamin absorbed by measuring the hepatic uptake of radioactivity after oral doses. They gave oral doses of 0.5 µg to 50 µg of vitamin B₁₂ labelled with Co⁶⁰ to normal subjects and found that, as the dose increased, a progressively smaller proportion was absorbed. Almost all of the dose of 0.5 µg was absorbed, but only about 1.5 to 2.0 µg vitamin B₁₂ was absorbed from doses of 50 µg. On the basis of these observations they postulated the presence of a partial intestinal block to absorption of the vitamin.

Their conclusion can be criticised, because the limitation to absorption they observed could have been due to other factors. For example, the amount of vitamin B₁₂ absorbed must be in part determined by the amount of intrinsic factor secreted at the time

Table III. The Absorption of Co⁵⁸ Cyanocobalamin by Patients with Megaloblastic Anaemias Due to B₁₂ Deficiency Other Than Pernicious Anaemia (Oral Test Dose: 0.6 µg)

Condition	No. of subjects	0.6 µg Co ⁵⁸ cyanocobalamin alone	0.6 µg Co ⁵⁸ cyanocobalamin + 50 mg I.F.†
		(µg)	(µg)
Partial gastrectomy	5	0.00 to 0.2	0.30 to 0.42
Intestinal "loop" syndrome*	5	0.00 to 0.12	0.05 to 0.14
Steatorrhoea (i)	2	0.07 to 0.08	0.04 to 0.05
(ii)	3	0.00 to 0.10	0.28 to 0.36
Nutritional megaloblastic anaemia	1	0.37	0.36

* This group includes 2 patients with jejunal diverticulosis; 3 patients with stricture anastomoses and blind intestinal loops.

† Intrinsic factor concentrate.

the dose is given, for the amount of the vitamin absorbed from a single dose by control subjects can be increased by the administration of "Carbachol" or by giving the dose with a potent source of intrinsic factor.^{20,30} It is probable that a partial intestinal block to absorption of the vitamin does exist (see below), but the evidence for such a block in normal subjects is at present unsatisfactory.

Little information is at present available about differences between absorption by normal subjects and by patients with conditions other than megaloblastic anaemias. Absorption of the vitamin appeared to be depressed in patients suffering from infection,^{14,20} and it has been suggested that its absorption may be depressed in old people.³² Certain patients with atrophic gastritis absorb less vitamin B₁₂ than normal subjects but in others the absorption is within the normal range.³³ It is clear that this method is suitable for studying factors affecting the absorption of vitamin B₁₂ in control subjects, but few results have so far been published on this aspect.

The Relationship between Intrinsic Factor and the Intestinal Absorption of Vitamin B₁₂

This has been studied in patients with pernicious anaemia in remission by estimating the amount of the vitamin absorbed from successive oral doses of Co⁵⁸ cyanocobalamin given with gastric juice or intrinsic factor concentrates.²¹ Patients with pernicious anaemia were chosen so that interference from naturally secreted intrinsic factor would be minimal. If a constant amount of Co⁵⁸ cyanocobalamin is given with different amounts of the same intrinsic factor concentrate, or of pooled gastric juice, to the same patient, then the amount of the vitamin absorbed is directly proportional, over a certain range, to the amount of intrinsic factor concentrate or gastric juice administered (Fig. 1). If, on the other hand, different

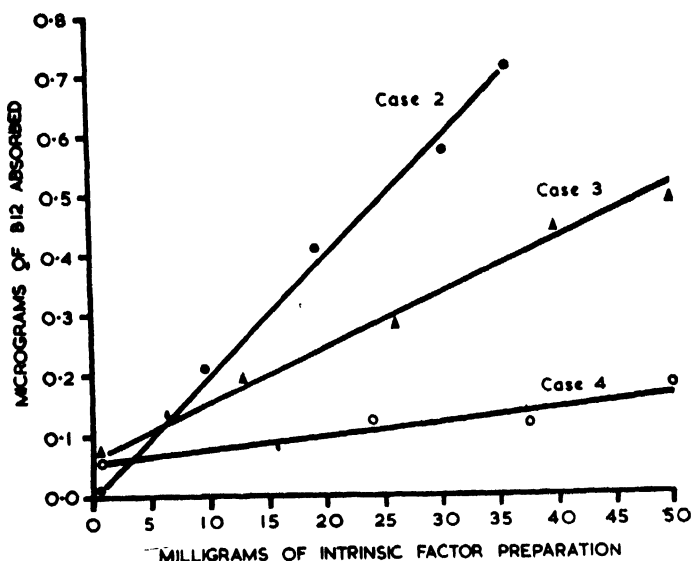


Figure 1. The amounts of vitamin B₁₂ labelled with cobalt-58 absorbed by three patients with pernicious anaemia when successive 0.8 µg doses were administered with various amounts (0–50 mg) of intrinsic factor preparation

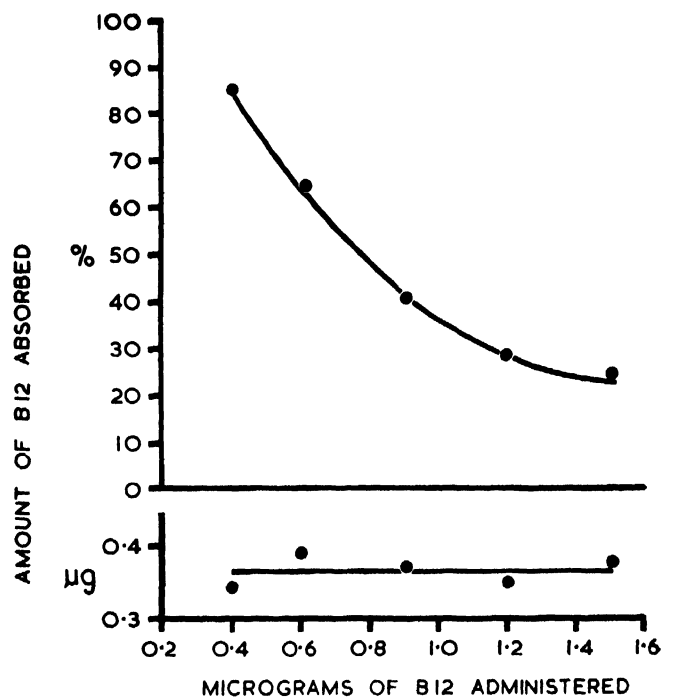


Figure 2. The amounts of vitamin B₁₂ labelled with cobalt-58 absorbed by a patient with pernicious anaemia, when successive (0.4–1.5 µg doses were administered with 20 mg of intrinsic factor preparation. The amount absorbed is expressed as a percentage of the administered dose in the upper curve; in the lower curve the amount absorbed is expressed in micrograms

amounts of Co⁵⁸ cyanocobalamin are given with the same amount of intrinsic factor concentrate to the same patient the amount of the vitamin absorbed is constant, provided each dose is large enough to saturate the amount of intrinsic factor given (Fig. 2). These observations suggest that intrinsic factor potentiates absorption by entering into some form of physico-chemical combination with the vitamin.

Beyond a certain point the amount of Co⁵⁸ cyanocobalamin absorbed from a single dose did not increase on increasing the amount of intrinsic factor given with the dose (Fig. 3). The level at which this "block" occurred differed in different patients. Glass, Boyd, Stephanson and Jones³⁴ have recently reported that the absorption of vitamin B₁₂ increases up to a certain point in pernicious anaemia patients with increase in the amount of intrinsic factor given, but that further increase in the amount of intrinsic factor concentrates then reduces the amount of radioactive vitamin absorbed. They suggested that this might be due to presence of "B₁₂-binding" substances in the intrinsic factor concentrates they used. Callender and Evans³⁵ also noticed this phenomenon when they administered exceedingly large amounts of intrinsic factor with radioactive vitamin B₁₂ and suggested that it was due to the presence of non-radioactive vitamin B₁₂ in the intrinsic factor concentrates interfering with the absorption of the radioactive vitamin B₁₂.

Repeated doses of Co⁵⁸ cyanocobalamin were given to one patient with idiopathic steatorrhoea together with various amounts of intrinsic factor concentrate.

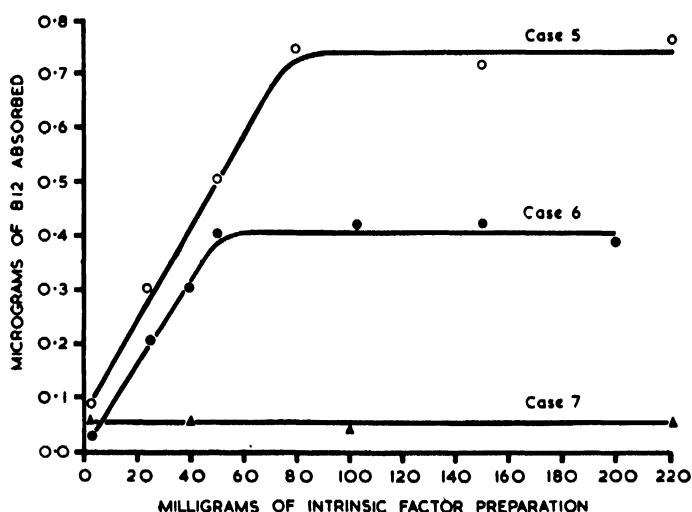


Figure 3. The amounts of vitamin B₁₂ labelled with cobalt-58 absorbed by two patients with pernicious anaemia (Cases 5 and 6) and one patient with steatorrhoea (Case 7), when successive doses were administered with varying amounts (0–220 mg) of intrinsic factor preparation

This failed to potentiate the absorption of the vitamin even in doses far greater than those effective in pernicious anaemia (Fig. 3). It has been reported, however, that much larger amounts of intrinsic factor may produce some increase in the absorption of B₁₂.²³

The Assay of Intrinsic Factor Activity

The demonstration that radioactive vitamin B₁₂ is absorbed by a test subject indicates that the subject is capable of secreting intrinsic factor. It is uncertain how far differences in the amount of the vitamin absorbed can be interpreted directly in terms of differences in amount of intrinsic factor secreted, for presumably the ability of control subjects to absorb vitamin B₁₂-intrinsic factor complex also varies. Further, studies of the absorption of radioactive vitamin B₁₂ can give no information about intrinsic factor secretion in these patients with megaloblastic anaemia who are unable to absorb the vitamin-intrinsic factor complex (Table III).

The results recorded in the preceding section indicate that the intrinsic activity of both gastric juice and intrinsic factor can be assayed quantitatively by tests on patients with pernicious anaemia in remission. This can be done by comparing the activity of the unknown material against that of a standard batch of intrinsic factor concentrate. The correct conditions for such assays are discussed elsewhere.²⁴

URINARY EXCRETION OF RADIOACTIVITY

Although the absorption of vitamin B₁₂ can be studied quantitatively by measuring the faecal excretion of radioactivity, the method is time consuming and demands considerable co-operation from the patient. A simpler method for demonstrating that radioactive vitamin B₁₂ had been absorbed was introduced by Schilling.¹⁵ He demonstrated that if oral doses of 1 to 2 µg of the radioactive vitamin are given to normal subjects or patients with pernicious anaemia,

no radioactivity appears in the urine. If, however, a large parenteral injection of non-radioactive vitamin B₁₂ is given two hours after the oral dose, considerable amounts of radioactive material are excreted in the urine of normal subjects, but little or none appears in the urine of patients with pernicious anaemia, unless a potent source of intrinsic factor is given with the oral dose.

These observations have been confirmed and extended by others.^{35,36} Different workers have used various amounts of labelled vitamin B₁₂ for the oral dose, and the amount and timing of the injection has varied. In Table IV is given the urinary excretion of radioactivity by control subjects, patients with pernicious anaemia and patients with vitamin B₁₂ deficiency associated with sprue syndrome. They were given at injection of 1000 µg non-radioactive vitamin at the same time as an oral dose of 0.6 µg of Co⁵⁸ cyanocobalamin. The radioactivity of 50 ml aliquots of the urine passed in 24 hours after injection was measured in an annular-type scintillation counter.

Significantly less vitamin was excreted by the patients than by the control subjects. When the test dose of Co⁵⁸ cyanocobalamin was given to the patients with a source of intrinsic factor, the urinary excretion of radioactive vitamin increased in the patients with pernicious anaemia but was unchanged in the patients with steatorrhoea who were unable to absorb the vitamin B₁₂-intrinsic factor complex.

Callender and Evans³⁵ compared the amounts of vitamin B₁₂ absorbed, as estimated from the faecal excretion of radioactivity, with the amount of radioactivity excreted in the urine. There was an over-all correlation between amount of radioactive vitamin absorbed and approximately one third of the absorbed dose excreted in the urine. The ratio however varied widely from patient to patient. This ratio also varies widely in the same patient, given successive oral doses of Co⁵⁸ cyanocobalamin with different amounts of intrinsic factor concentrate.³⁶ This test is therefore a simple and convenient way of demonstrating gross abnormalities of vitamin B₁₂ absorption. The admini-

Table IV. The Urinary Excretion of Radioactivity by Control Subjects and by Patients with Pernicious Anaemia and Idiopathic Steatorrhoea Given Oral Doses of 0.6 µg Co⁵⁸ Cyanocobalamin. (A parenteral injection of 1000 µg of non-radioactive vitamin B₁₂ was given at the same time as the oral dose.)

	No. of subjects	Percentage of oral dose excreted			
		Dose alone		Dose + 50 mg I.F.	
		Range	Mean and its standard error	Range	Mean and its standard error
Control subjects	10	15.9 to 25.7	20.7 ± 2.6	—	—
Pernicious anaemia	10	0.0 to 10.5	2.9 ± 1.2	8.5 to 26.0	14.4 ± 1.8
Idiopathic steatorrhoea	4	0.0 to 5.0	1.2	0.0 to 2.0	0.50

stration of large injections of non-radioactive vitamin does, however, depress the absorption of radioactive vitamin B₁₂,^{21,35} so that the test is less satisfactory than the faecal excretion method. The urinary excretion method can also be used for the qualitative evaluation of the intrinsic factor activity of test preparations. Different preparations can be given in consecutive tests on the same patient. A significant increase in the urinary excretion of radioactivity is associated with an increased absorption of the vitamin, but increased absorption of the vitamin is not invariably associated with a significant increase in the urinary excretion.³⁶

THE HEPATIC UPTAKE OF RADIOACTIVITY

Glass *et al.*¹⁶ have demonstrated that if Co⁶⁰ cyanocobalamin is given by mouth to normal subjects, radioactive material accumulates in the liver and can be measured, once the intestines are cleared of radioactivity, with a directional scintillation counter. In patients with pernicious anaemia the surface counts over the liver do not increase above background unless the dose is given with a potent source of intrinsic factor. In a patient with sprue syndrome the radioactivity over the liver did not increase when the dose was given alone or with intrinsic factor. Glass and his colleagues³⁷ have suggested that the amount of radioactive vitamin B₁₂ absorbed can be measured by this method. The amount of the vitamin absorbed is calculated by comparing the hepatic uptake after an oral dose of Co⁶⁰ cyanocobalamin with that after the parenteral administration of a known amount of the radioactive vitamin to the same patient.

Our preliminary observations with radioactive vitamin B₁₂ of high specific activity indicate that there is an over-all correlation between the changes in radioactivity over the liver and the amount of radioactive vitamin absorbed, as determined by faecal absorption studies.³⁰ It is however still uncertain how accurately the changes in the hepatic uptake reflect differences in the amount of the vitamin absorbed. Nevertheless this does appear to be a convenient and satisfactory method for investigating the absorption of vitamin B₁₂ and for qualitative evaluation of the intrinsic factor activity of gastric juice and intrinsic factor concentrates. If radioactive vitamin B₁₂ of high specific activity labelled with the shorter-lived isotopes of cobalt is used, the technique is simplified and the accuracy of the method considerably improved.

SERUM RADIOACTIVITY AFTER THE ORAL ADMINISTRATION OF RADIOACTIVE VITAMIN B₁₂

The changes in serum vitamin B₁₂ concentrations that result from the oral administration of single physiological doses of the vitamin are too small to measure by microbiological assay,²⁴ but significant changes in serum radioactivity follow the oral administration of doses of 1.0 to 2.0 µg of Co⁶⁰ cyanocobalamin of high specific activity.¹⁷

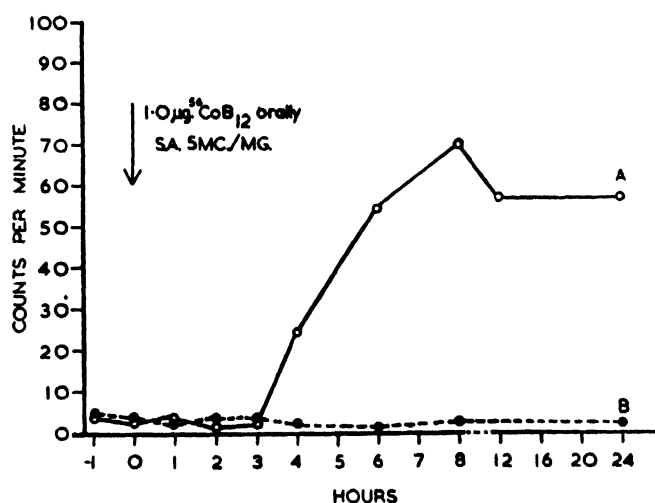


Figure 4. The changes in the serum radioactivity in a normal patient (A) and in a patient with pernicious anaemia (B) given an oral dose of 1.0 µg Co⁶⁰ cyanocobalamin (specific activity 5 mc/mg)

Figure 4 shows an example of the changes in serum radioactivity after oral administration of 1.0 µg Co⁶⁰ cyanocobalamin (specific activity 5.0 mc/mg) to normal subjects. The radioactivity of 10 ml samples of serum was measured in an annular-type scintillation counter. No increase in the serum radioactivity occurs in the first three hours after the dose, but the radioactivity rises to a peak of 8 to 12 hours and then slowly falls over a period of days. When the oral dose is given to patients with pernicious anaemia, no radioactivity appears in the serum. When the dose is given to these patients along with a potent source of intrinsic factor, the changes in the serum radioactivity are similar to those observed in control subjects. The clearance of radioactivity from the serum after such a dose appears to be similar whether the pernicious anaemia patients are in remission or relapse. Investigations with this method are still at a preliminary stage, but it is apparent that it can be used both for demonstrating defective absorption of vitamin B₁₂ and for investigating factors effecting its absorption and subsequent distribution.

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The Application of Radioactive Isotopes to the Study of the Biochemistry of Muscles

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A study has been carried out in this laboratory by means of radioactive isotopes on the biochemistry of muscles with an experimentally induced disease. In physiological and biochemical studies the muscle denervation technique (lesion or sectioning of the motor nerve) has long since been practiced as well as tenotomy and lately E-avitaminosis which causes a severe dystrophy of the skeletal muscles. In all these cases the changes in the chemical composition and activity of muscle enzymes are usually studied.

In some earlier studies carried out in this laboratory, rather interesting chemical peculiarities of the functioning denervated muscle were noted, along with the changes occurring in the muscles at various periods following denervation. It has been shown¹ by *in vivo* experiments that upon electric stimulation of the denervated muscle (the gastrocnemius of rabbits 7–12 days after severing of the sciatic nerve) fatigue sets in while lactic acid production undergoes but a comparatively slight increase, and the content of glycogen, creatine phosphate and ATP is almost completely maintained. Similar phenomena were observed in the muscles of curarized rabbits.

The evidence thus obtained seems to suggest that the activity of the denervated muscle is mainly due to the substances supplied by the blood and that fatigue sets in as soon as the muscle becomes no longer capable of utilizing these substances.

During the last few years this laboratory was engaged in studying the biochemistry of muscles in which atrophy is brought about by denervation and tenotomy, or dystrophy by E-avitaminosis. Not only did we succeed in ascertaining some changes in the chemical composition of the morbid muscles, but also in showing that administration of ATP to the rabbit slackens the course of the atrophic and dystrophic processes in the muscles. Dystrophy caused by E-avitaminosis is known to be accompanied by degenerative changes in the skeletal muscles, by a loss of the muscle tissue. ATP injections reduce the rate of the dystrophic process and prolong the life of the E-avitaminosis animal.² When administered to dogs and rabbits ATP slackens the course of the atrophic process in the denervated³ and tenotomized muscles.⁴ These findings led to a further study of

the metabolism of the skeletal muscles and myocardium, in which tracer elements have been used.

THE BIOCHEMISTRY OF MUSCLES IN E-AVITAMINOSIS DYSTROPHY

E-avitaminosis in animals is attended with symptoms somewhat similar to those of progressive muscular dystrophy in man. It has been referred to as alimentary dystrophy of the muscles. E-avitaminosis was induced in young four to six-weeks-old rabbits by keeping them on a suitable diet. 25–35 days after the beginning of the experiment the rabbits were intravenously injected with a solution of Na_2HPO_4 containing P^{32} . Two hours later the animals were sacrificed, the muscles being promptly removed and the rate of phosphorus incorporation to various phosphoric compounds estimated. The results obtained showed⁴ that the rate of phosphorus incorporation into the total and acid-soluble phosphorus fraction, the inorganic phosphate, creatine phosphate and ATP is considerably higher in dystrophic than in healthy muscles. The rate of P^{32} incorporation into creatine phosphate is approximately four times, and in ATP three times as high in the dystrophic as in the control muscles (Fig. 1).

The P^{32} content of the inorganic phosphate of the rabbits' blood plasma determined simultaneously with a study of P^{32} incorporation into the muscle phosphates, was shown to be similar in both the dystrophic and control rabbits. Since the blood supply of the dystrophic muscles is not increased it may be as-

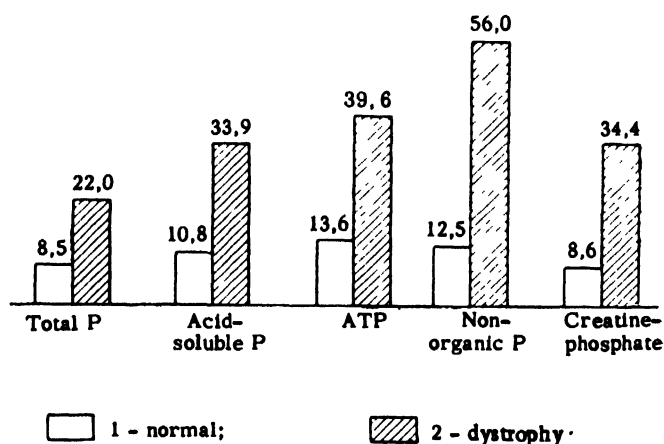


Figure 1. Specific activity of muscle phosphoric compounds in normal and dystrophic rabbits

sumed that the muscle supply of the inorganic labeled phosphorus is equal in both the dystrophic and normal rabbits. Hence, the higher rate of P^{32} incorporation into the phosphoric compounds of the dystrophic muscles should be related to the greater metabolic rate of these muscles. The respiratory rate of the muscles in rabbits rises considerably under E-avitaminosis.

The rate of P^{32} incorporation into phosphoric compounds of the muscles is an indication of their renewal rate; this has been shown to be considerably higher in dystrophic muscles than in normal ones.

Since the efficiency of the dystrophic muscles is greatly reduced while the rate of respiration is increased, it has been suggested⁵ that in alimentary dystrophy of the muscles the processes of respiration and phosphorylation are dissociated. On this assumption, the energy released by oxidation of the organic substances cannot be utilised for the synthesis of ATP and creatine phosphate, so that the conditions for the utilisation of the chemical energy of the muscles become unfavourable. This assumption was based on the reduced ATP and creatine phosphate content of the dystrophic muscles. The higher rate of P^{32} incorporation into phosphoric compounds (particularly into ATP and creatine phosphate) of the dystrophic muscles refutes the assumption that in the E-avitaminotic muscles oxidation and phosphorylation are dissociated.

It will be seen from the above evidence that the decrease in the phosphoric compounds of the dystrophic muscles is by no means an indication of the rate of breakdown and synthesis in the muscles. The use of the tracer method brought to light a considerable rise in the turnover rate of the muscle phosphoric compounds in dystrophic animals while their content in the muscles is reduced.

Alimentary muscle dystrophy in young rabbits during the first stage of the disease is characterised by an increase in weight, which during the last stage, is succeeded by a considerable loss of weight. Hence at certain stages muscle dystrophy is associated with some degree of starvation. It seemed therefore necessary to ascertain to what extent the above change in the turnover rate of phosphoric compounds of muscles of the experimental animals results from dystrophy, and not from starvation.

Experiments on starving rabbits have shown that during starvation the rate of P^{32} incorporation into the total and acid-soluble phosphorus, as well as into inorganic phosphate, ATP and creatine phosphate of the muscles not only does not increase, but even slightly decreases as compared with control rabbits.⁴ Hence, it may be concluded that the increased turnover rate of phosphoric compounds in the E-avitaminotic rabbit muscles is typical of the dystrophic process, but not of starvation.

The experimental results obtained in this laboratory on the turnover rate of phosphoric compounds in the E-avitaminosis muscles conform with the

literature data⁶ on E-avitaminotic rats treated with P^{32} ; it should, however, be noted that in these studies the P^{32} content was estimated directly in the tissues, and not in the phosphoric compounds extracted from them. Such experiments do not give any idea of the rate of turnover of individual phosphoric compounds.

The rate of turnover of phosphoric compounds in E-avitaminosis has been studied not only in the skeletal muscles, but in the myocardium as well. It should be pointed out that morphological and functional changes in the skeletal muscles and in the myocardium of E-avitaminotic animals seem to be different. While in the skeletal muscles there occur drastic degenerative changes and a considerable loss of the muscular mass in the myocardium the morphological changes are slight and its mass does not undergo any noticeable changes.

Unlike the skeletal muscles the turnover rate of phosphoric compounds in the myocardium of E-avitaminotic rabbits was found to be even slightly reduced.

The same isotope method has been applied for a study of the renewal rate of proteins of the skeletal muscles and myocardium in E-avitaminosis. Such experiments are the more important as dystrophy is accompanied by a drastic reduction of the mass of skeletal muscles while individual protein fractions show considerable changes. The relative content of the connective tissue of the muscles is increased whereas that of the proteins which form part of muscle fibrils undergoes a decrease. The question remained open as to the dynamic state of muscle proteins in dystrophy.

The renewal rate of proteins of the skeletal muscles and myocardium has been followed up with the aid of S^{35} -methionine administered to the rabbits subcutaneously whereupon (on the next day) the animals were sacrificed. The methionine content in the extracted proteins of the skeletal muscles and myocardium was determined by the radioactivity of S^{35} .

It has been found that the rate of total protein renewal in the skeletal muscles of dystrophic rabbits undergoes an increase. The renewal rate of collagen which is generally low, does not change at all. Hence, it may be suggested that the greater renewal rate of total proteins of the muscles is due to the increased renewal rate of the proteins of the muscle fibrils.

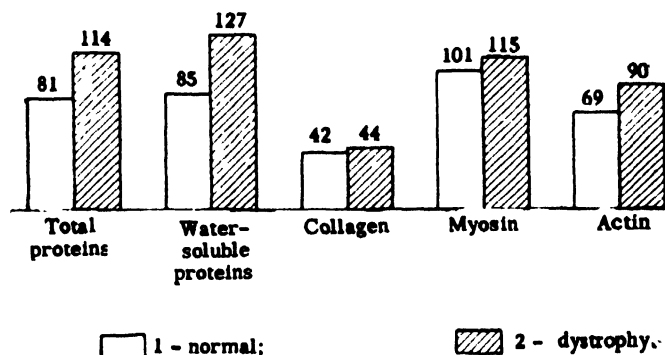


Figure 2. Specific activity of muscle proteins in normal and dystrophic rabbits

This conclusion has been experimentally substantiated by a study of the renewal rate of the water-soluble proteins and actin fractions; these values proved to be higher in the muscles of dystrophic rabbits than in those of the control ones (Fig. 2).

In the case of the myocardium proteins in E-avitaminosis, the results proved different. Actually E-avitaminosis calls forth a considerable reduction of the renewal rate of total proteins as well as of the water-soluble protein fraction of the myocardium (Fig. 3).

The question arises whether the higher renewal rate of proteins of the dystrophic muscle fibril is due to an increased permeability to methionine of the blood capillaries. To check this assumption, along with the radioactivity of protein sulphur in dystrophic and control muscles, the radioactivity of sulphur, forming part of non-protein substances (non-protein sulphur) has also been determined. This sulphur should also contain S^{35} of the methionine administered to the rabbits. The data obtained showed that the radioactivity of the non-protein sulphur in dystrophic muscles varied within the same limits as in the control muscles.

Hence, the high rate of protein renewal in dystrophic muscles cannot be accounted for by an increased uptake of the administered methionine sulphur due to a change in the permeability of the blood capillaries (Fig. 4).

The following are the conclusions to be drawn from the isotope study of the biochemistry of the E-avitaminotic muscles: (1) the rate of turnover of a number of phosphoric compounds is increased as

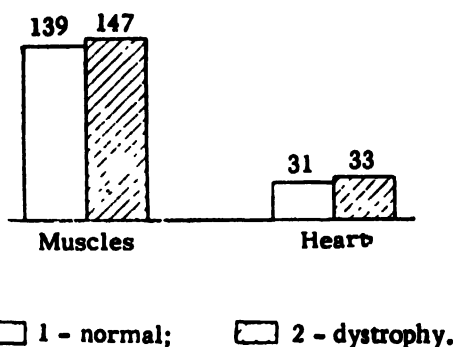


Figure 3. Specific activity of non-protein sulphur in skeletal muscles and myocardium in normal and dystrophic rabbits

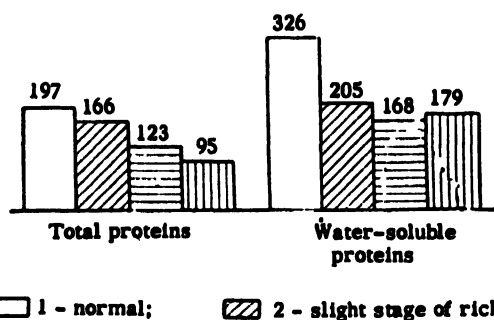


Figure 4. Specific activity of total and water-soluble proteins of skeletal muscles in normal and rickety rats

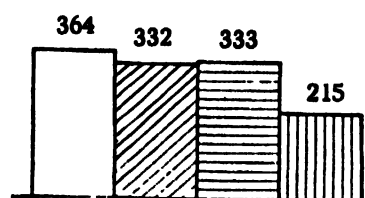


Figure 5. Specific activity of total proteins in the myocardium of normal and rickety rats. Shadings mean same as in Fig. 4

well as that of proteins of the dystrophic skeletal muscle fibrils in animals affected by E-avitaminosis; (2) the renewal rate of the myocardium proteins proved to be lowered. It may be assumed that the increased turnover rate of phosphoric compounds and proteins in those fibres of the skeletal muscles which did not yet undergo complete degeneration, is a result of some compensatory processes. In the remaining part of muscle fibres not yet involved into the dystrophic process, the physiological function of the skeletal E-avitaminotic muscles is to a certain degree preserved due to an increase in the muscle metabolism.

This suggestion complies with the evidence obtained in other experiments which will be reported below.

THE RENEWAL RATE OF PROTEINS IN D-AVITAMINOSIS

In D-avitaminosis considerable changes in the metabolic processes are to be noted. However, so far only disturbances of the mineral metabolism in D-avitaminosis have been the principal object of study. The data to be discussed below concern the renewal rate of proteins of the skeletal muscles and myocardium in young rats kept on a D-avitaminosis ration. The study was carried out in such a way as to prevent loss of weight of the experimental animals. The severity of the rickets was estimated by means of radiography.

The rate of renewal of proteins of the skeletal muscles and myocardium was studied by means of S^{35} methionine. The animals were killed a day after subcutaneous injection of the amino acid. The skeletal muscle and myocardium proteins were extracted and the radioactivity of the protein sulphur was then estimated.

The data obtained showed that beginning with the stage of slight D-avitaminosis there occurs a decrease in the renewal rate of the muscle proteins. This applies to the total protein fraction and to that of water-soluble proteins as well. The turnover rate of proteins of skeletal muscles proved to be particularly low in the acute stage of D-avitaminosis.

The data obtained are of particular interest as D-avitaminosis is accompanied by a decrease of the muscular function (Fig. 5).

Similar data have been obtained in a study of the myocardium proteins; the turnover rate of proteins is markedly lowered in the acute stage of D-avitaminosis (Fig. 6).

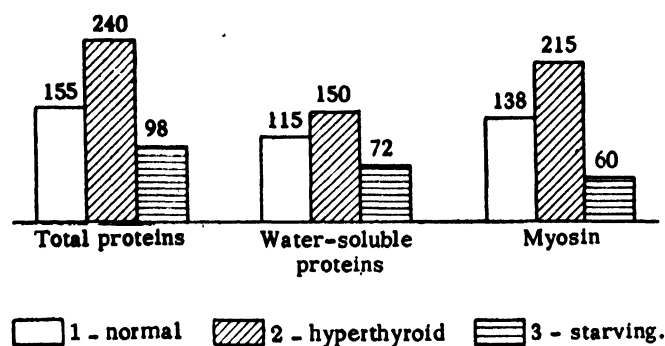


Figure 6. Specific activity of muscle proteins in normal, hyperthyroid and starving rabbits

It will thus be seen that in D-avitaminosis of rats not associated with a loss in weight the protein turnover rate is reduced in the skeletal muscles and myocardium.

THE RENEWAL RATE OF PROTEINS IN HYPERTHYROIDISM

Hyperthyroidism was induced in rabbits through administration of tablets prepared from dried thyroid ground to powder. In 30–40 days the rabbits lost 30–40 per cent of original weight. At this stage of hyperthyroidism the rabbits were injected subcutaneously with S^{35} -methionine and killed a day after the injection. Proteins were extracted from the skeletal muscles, and the radioactivity of their sulphur estimated.

In hyperthyroidism accompanied by a loss of mass of the skeletal muscles the renewal rate of total protein, of the water-soluble protein fraction and of myosin underwent an increase (Fig. 7).

Hyperthyroidism is generally known to call forth a decrease of function of the skeletal muscles as well as some change in the biochemistry of the muscles resembling those accompanying dystrophy. In hyperthyroidism, as in muscle dystrophy induced by E-avitaminosis, the renewal rate of proteins proved to be increased in the remaining part of the muscle fibres which have not up to the present time undergone any changes.

A study was carried out on the renewal rate of skeletal muscle proteins accompanying atrophy

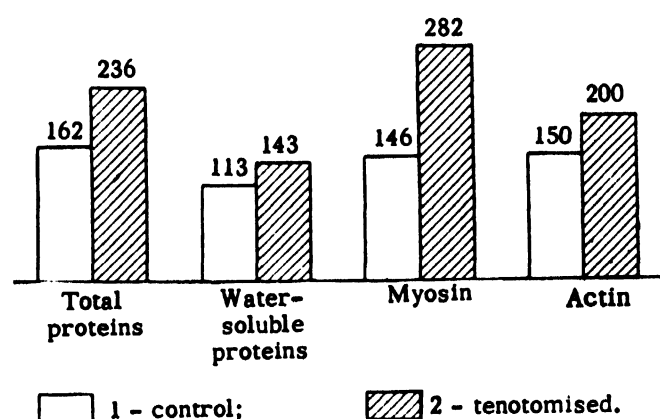


Figure 7. Specific activity of proteins in control and tenotomized muscles of rabbits

caused by tenotomy or sectioning of the motor nerve. The results are presented below.

THE RENEWAL RATE OF PROTEINS AND PHOSPHORIC COMPOUNDS IN TENOTOMIZED MUSCLES

Experiments were carried out on rabbits subjected to a unilateral tendon severance. The gastrocnemius muscles of both limbs were examined 30–40 days after the operation. During this period a considerable atrophy of the tenotomized muscles set in accompanied by a loss of weight varying from 40% to 60%. The connective tissue supply of the atrophied muscle increased while protein content of muscle fibres decreased (water-soluble protein, myosin, actin).

A day before the experiment the rabbits were injected subcutaneously with S^{35} -methionine and then sacrificed. Proteins were extracted from the atrophied gastrocnemius and the contralateral muscle of each animal and sulphur radioactivity determined.

It was found that the renewal rate of the total proteins increased in the atrophied muscles. The renewal rate of the water-soluble protein fraction in the atrophied muscles also increased.

A much greater increase was noted in the turnover rate of myosin of the atrophied muscles and in most experiments of actin as well (Fig. 8).

Hence, in muscles atrophied due to tenotomy there occurred an increase of the protein turnover rate along with a loss in weight and a diminution of the protein content of the muscle fibrils. An exception is presented by collagen; turnover rate in muscles is not high and does not change during atrophy.

As to the non-protein sulphur radioactivity in tenotomized muscles, this did not change, just as in muscle dystrophy caused by E-avitaminosis. Hence the conclusion is indicated that the turnover rate of proteins in muscles atrophied as a result of tenotomy has no bearing on the permeability of the blood capillary walls (Fig. 9).

In estimating the above findings, it should be noted that although in muscle atrophy caused by tenotomy the mass of muscle tissue was greatly reduced there occurred an increase in the turnover rate of the proteins of the muscle fibres which have not yet undergone any profound changes. Hence, here too one may assume that in the muscle fibres not yet involved in the atrophic process, the metabolism resulting in a higher renewal rate of proteins is enhanced by way of compensation.

By means of the tracer method the turnover rate of phosphorus compounds was studied in muscle atrophy caused by tenotomy.⁷ In these studies 27–35

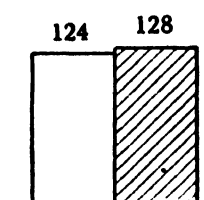


Figure 8. Specific activity of non-protein sulphur in control and tenotomized muscles of rabbits. Shadings mean same as in Fig. 7.

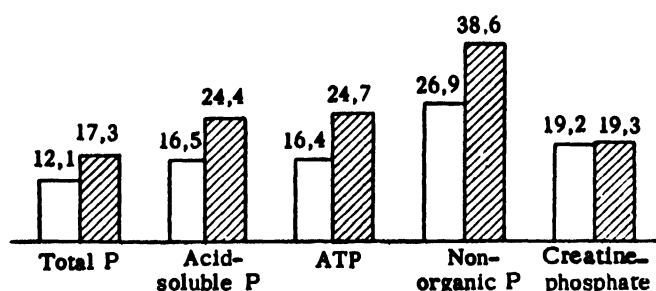


Figure 9. Specific activity of phosphoric compounds in control and tenotomized muscles of rabbits. Shadings mean same as in Fig. 7

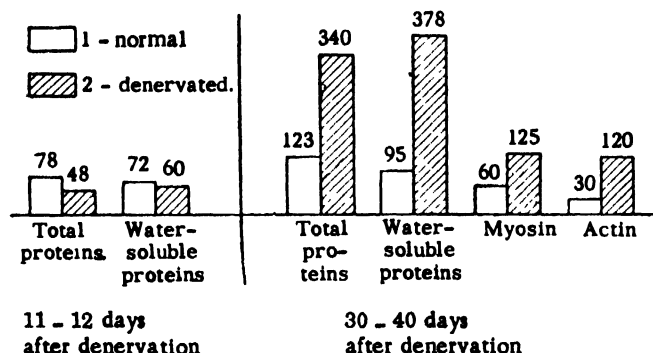


Figure 10. Specific activity of proteins in control and denervated muscles of rabbits.

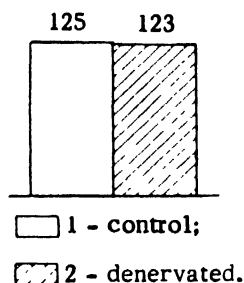


Figure 11. Specific activity of non-protein sulphur in control and denervated muscles of rabbits. Unshaded, control; shaded, denervated

days after sectioning of the tendon, when the loss of weight of the tenotomized gastrocnemius muscles reached 43–60%, the rabbits were injected subcutaneously with Na_2HPO_4 containing P^{32} and the radioactivity of phosphoric compounds extracted from the muscles was determined the turnover rate of the phosphoric compounds (total phosphorus, acid-soluble phosphorus, ATP) in tenotomized muscles proved to rise. Thus in muscle atrophy caused by tenotomy there occurs an increase in the turnover rate of phosphoric compounds of the remaining muscle fibrils along with an increase in the renewal rate of proteins (Fig. 10).

THE RENEWAL RATE OF PROTEINS IN DENERVATED MUSCLES

Experiments were carried out on rabbits with muscle atrophy caused by unilateral sectioning of the sciatic nerve. The animals were injected subcutaneously with S^{35} -methionines prior to being sacrificed at different periods after the operation. The proteins were extracted from the muscles and the radioactivity of their sulphur determined. It was found that the turnover rate of the muscle proteins somewhat

decreased 10 or 12 days after the operation. At this stage of atrophy caused by denervation the decrease in muscle weight is comparatively small. At a later period following nerve sectioning (21–40 days) when the reduced weight of the atrophied muscle reached 33–65% the turnover rate of total proteins, of water-soluble protein fraction, myosin and actin increased by 2–3.5 times (Fig. 11).

While following up the renewal rate of muscle proteins during atrophy induced by sectioning of the sciatic nerve, the animals were killed at different periods (4, 8, 12, 16, 20, 24 and 48 hours) after the administration of S^{35} -methionine. It has been found that irrespective of the time of killing the renewal rate of the muscle proteins in the last stage of atrophy has considerably increased.

Just as in the tenotomy experiments the radioactivity of muscle proteins was tested along with that of sulphur of non-protein muscle compounds. The radioactivity of non-protein sulphur in muscle atrophy resulting from nerve sectioning does not change. It will thus be seen (Fig. 12) that the drastic increase in the protein turnover rate accompanying acute muscle atrophy as caused by nerve sectioning cannot be accounted for by an increased supply of methionine. This might have been caused by a change in the permeability of the blood capillaries.

Thus the renewal rate of the muscle tissue proteins, with the exception of collagen, depends on the functional state of the muscles. As soon as the functional stage of the skeletal muscles and myocardium is reduced (not accompanied by a decrease in the mass of muscles) protein turnover rate is decreased.

In skeletal muscle dystrophy caused by E-avitaminosis and hyperthyroidism, as well as in acute atrophies caused by tenotomy and sectioning of the motor nerve and accompanied by a considerable reduction in the muscle mass, the renewal rate of proteins is increased in those fibres which have not yet undergone any profound changes. The higher renewal rate of proteins is probably due to compensatory processes in the muscles that ensure preservation of their functions under adverse conditions.

The accepted idea that muscle dystrophy and atrophy are accompanied by a decrease in protein content of the muscle fibres should be supplemented by the notion that under such conditions there occurs a change in the dynamic state of the muscle proteins.

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Synthesis of 4:4'-Diaminodiphenylsulphone-S³⁵ (DDS) and its Use in Leprosy Research

By P. R. Saraiya, V. R. Khanolkar and A. R. Gopal-Ayengar,* India

The drug, 4:4'-diaminodiphenylsulphone (III) is extensively used in the treatment of leprosy. Under its action, marked improvement occurs in over 80% of the patients with this disease, even of long duration. However, in terms of the time-factor, effective therapy requires prolonged treatment over a period of 2-3 years. In a small proportion of cases, the lesions do not respond to the action of the drug. A proper evaluation of the course of the disease during therapy demands two considerations: (i) the determination of the bacteriologic index, and (ii) the recognition of gross and structural changes in the lesions themselves.

For a tracer study on the absorption of this drug by different tissues of the body, particularly the nervous tissues, in leprosy patients, it was necessary to prepare the sulphone bearing a "tagged" atom. The present paper deals with the synthesis of radioactive DDS and the application of the labelled drug in patients with frank lepromatous lesions.

SYNTHESIS OF LABELLED DDS

It was thought that acetylsulphanilyl chloride-S³⁵, which has been prepared by Klotz and Melchior,¹ would serve as the starting material for preparing the required radioactive sulphone (III). In trial experiments with unlabelled material it was however found that the Friedel-Craft reaction using the above compound with acetanilide gave poor yields of the expected diacetyl derivative (II); another method therefore had to be sought.

Thionyl chloride is the starting material in the following synthesis described by Sugawara and Sakurai.²

The scheme outlined above has now been adopted for the preparation of 4:4'-diaminodiphenylsulphone-S³⁵, using thionyl chloride-S³⁵. The over-all yield, based on the thionyl chloride, was about 45%; the experimental details of the synthesis are given below: (All mp's are uncorrected. The radioactivity was measured using an end-window type G-M tube with a mica window of density 2 mg/cm².)

4:4'-Diacetylaminodiphenylsulphoxide-S³⁵ (I)

A mixture of powdered anhydrous aluminum chloride (22 gm), acetanilide (10.8 gm) and dry carbon

disulphide (30 ml) was kept in a three-necked flask fitted with a mercury-seal stirrer, a dropping funnel, and a double-walled condenser with a calcium chloride guard-tube. The latter was connected to a water pump, and the entire assembly maintained at a slightly reduced pressure throughout the reaction. The flask was cooled in ice and a solution of thionyl chloride-S³⁵ (5 gm : 10 mc) in carbon disulphide (15 ml) was added drop by drop with vigorous stirring during 15-20 minutes. The reaction was continued below 5° for 1 hour and then at 10°-20°, 25°-30° and 60° for 1 hour at each of the temperature ranges. The mixture was then cooled, the carbon disulphide was recovered by decantation, and ice and acetic acid (50 ml) were added to decompose the residue. The crude sulphoxide (7.5 gm; activity: 5.09×10^6 cpm/gm) which separated out was collected, thoroughly washed with water and used in the next stage after drying. A sample crystallised from dilute acetic acid in transparent plates, mp 278°.

4:4'-Diacetylaminodiphenylsulphone-S³⁵ (II)

A suspension in acetic acid (75 ml) of the sulphoxide (I) described above was stirred and treated drop by drop with a solution of chromic anhydride (6 gm) in water (6 ml). During addition it was necessary to cool the mixture, as otherwise the reaction proceeded with considerable violence. The mixture was then heated at 60° for 1 hour and at 90° for a further period of 2 hours. The sulphone (II) (6.7 gm; activity: 5.02×10^6 cpm/gm) was obtained as a white powder on diluting the reaction mixture with water (100 ml) and cooling in ice. A sample on crystallisation from dilute acetic acid gave transparent rods, mp 281°.

4:4'-Diaminodiphenylsulphone-S³⁵ (III)

The sulphone (II) from the preceding experiment was hydrolysed by heating under reflux with hydrochloric acid (30 ml) and water (60 ml) for 2 hours. The hot solution was treated with activated charcoal and filtered, and the filtrate was neutralised with alkali after cooling. The sulphone (III) (5.0 gm; activity: 5.58×10^6 cpm/gm) was thus obtained as a white crystalline powder, mp 177°-178°. Recrystallisation from dilute ethanol (40% vol) gave transparent needles, mp 178° (4.4 gm; activity: 5.6×10^6 cpm/gm).

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Representative samples of the slides containing tissue sections fixed after various time-intervals after

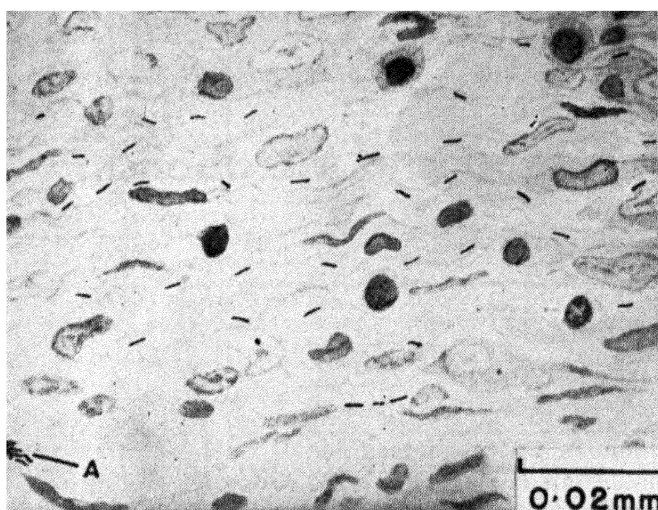


Figure 3. A longitudinal section of a nerve in the deeper portion of the dermis, from a prelepromatous lesion, showing large number of acid-fast bacilli lying in the nerve fibres and more or less following their wavy contours. A small blister in the perineural sheath is shown at (A) in which the bacilli are seen to be breaking through at the extreme left

the administration of the radioactive drug, have been developed and have so far revealed only very faint autoradiographs, especially in the tissues of the patients that had received over 400 mg of labelled DDS. No valid autoradiographs have been as yet observed in the slides of patients whose biopsies were taken

3 hours after the initial dose of 100 mg of DDS. It is proposed to allow an exposure time equivalent to $2\frac{1}{2}$ half-lives of the radioactive element (S^{35}) before processing the remaining slides.

While it is too premature to draw any inferences from these faint autoradiographs about the relative uptake of the radioactive substance, their appearance seems to suggest an absence of preferential incorporation by particular tissue systems. On the other hand, there are indications of a more or less generalised distribution of the radioactive material throughout tissues. Further work is in progress.

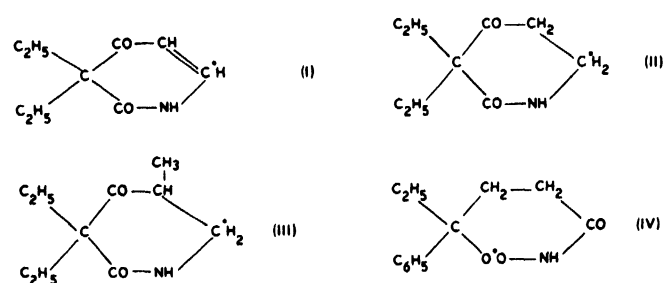
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Investigations on Distribution, Elimination and Metabolism of Some Hypnotic or Sedative Compounds

By Karl Bernhard and Georg Brubacher,* Switzerland

Because compounds with a pronounced physiological or pharmacological effect must be administered in very small doses, the investigation of their intermediate metabolism is normally rather difficult. It is easily understood that by the use of radioactive isotopes for labelling compounds, a favourable basis is provided for dealing with such problems. We have labelled tetrahydro-pyridine and -piperidine derivatives as well as a glutarimide, compounds that are effective as sedatives or hypnotic compounds, in an effort to investigate the distribution, elimination, and metabolism of these drugs after small dose application in rats.



The compounds are: (I) 2,4-diketo-3,3-diethyl-tetrahydro-pyridine, (II) 2,4-diketo-3,3-diethyl-piperidine, (III) 2,4-diketo-3,3-diethyl-5-methyl-piperidine, and (IV) α -phenyl- α -ethylglutarimide.

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Unlike the first three compounds that are readily soluble in water, glutarimide is only slightly water soluble but has the tendency to be soluble in fat.

Fifteen hours after all four compounds were administered orally to rats, dissection and investigation of the eight experimental animals revealed the following distribution of activity:

In the course of fifteen hours 45.8%, 59.0%, 55.8% and 37.0% of the administered activity was eliminated in the urine. The activities of the intestinal tract include those of the intestinal content. In a separate determination of the latter (II), (III) and (IV) showed the following values for the intestinal content: 3.91% and 3.83%, 17.7% and 12.6%, and 27.4% and 39.6% respectively. These findings allow one to assume that the bile was concerned to a considerable extent with the excretion of these compounds or their metabolites, i.e., detoxification or other chemical transformations to components eliminated by the bile occurred in the liver. Therefore, the labelled compounds were also fed to bile fistula rats. These animals excreted 42.0% of the activity administered with (I) in the course of 30 hours and 14.0% and 12.1% within a 25-hour interval with a reduced discharge of bile. The biliary excretion of (IV) was even higher showing 17.5% after 16 hours, 21.3% after 22 hours, 85.4% after 66 hours and 62.9% after 66 hours.

Table I

Animal no.	Compound fed							
	I		II		III		IV	
	1	2	3	4	5	6	7	8
Percentage uptake in:								
Liver	0.83	1.00	0.90	0.85	1.50	1.90	1.48	1.42
Kidney	0.16	0.23	0.23	0.16	0.13	0.23	0.38	0.19
Brain	0.03	0.05	0.21	0.24	0.06	0.10	0.09	0.10
Intestinal tract	28.7	26.9	6.37	6.04	23.5	15.8	35.4	51.5
Carcass	2.8	8.0	11.8	13.2	4.5	10.6	8.44	6.43
Respiratory carbon dioxide	0.3	0.3	0.11	0.14	1.1	0.3	1.3	1.2
Urine	43.2	48.3	59.2	58.8	52.6	59.3	40.9	33.0
Feces	0.8	1.7	0.34	—	4.3	4.9	3.0	0.2

By experiments of 198 hours it was possible to determine the half-lives of (I), (II) and (III) which are on an average 18.8, 19.3 and 13.3 hours respectively. These half-lives obviously depend on the dose given, as seen in Table II which refers to IV:

Table II

Dose (in mg/day/kg body wt)	Half-life (hours)
0.78	31
0.88	34
0.54	21
0.25	26
0.26	15

The brain, spinal cord and kidney, etc., show only very low uptakes. If one compares the relative specific activity of blood and depot fat, a certain accumulation of the oil soluble IV in the fatty depots can be observed (Table III):

Table III

	I	II	III	IV
Liver	33	35	58	11
Kidney	29	39	34	17
Brain—spinal cord	5	24	9	3
Blood	9	26	18	15
Fat depot	—	—	—	55

In further experiments, (I) was injected intravenously into rats which were killed after definite time intervals. The details of the distribution of activity in some organs are given in Table IV:

Table IV

Animal no.	Time, min	Percentage uptake in:			
		Brain	Spinal cord	Liver	Blood
1	5	0.06	0.01	0.5	1.5
2	10	0.9	0.29	7.0	10.0
3	20	0.6	0.26	5.1	9.6
4	30	0.7	0.16	4.5	6.7
5	43	0.5	0.16	3.3	4.5
6	60	0.6	0.18	4.4	6.1
7	88	0.6	0.19	4.3	5.3
8	130	0.5	0.19	4.5	5.2
9	263	0.7	0.21	4.7	7.4

An accumulation of the labelled compounds in the bone marrow did not take place. Experiments lasting 2 and 5 hours showed that the relative specific activity of the blood and the bone marrow are practically the same.

A part of the administered radioactive compounds was excreted unchanged in the urine. By the isotopic dilution method it was observed that 8.0% of (I), 55.0% of (II), 22.4% of (III), and 0.58% and 2.1% of (IV) appeared in the urine. The administered radioactive compounds were found also unaltered in the presence of conjugated and other bile products. However, the metabolism of the administered compounds cannot be very considerable since the activity of respiratory carbon dioxide during long duration experiments was not significant. (III) was transformed, through dehydration, into 2,4-diketo-3,3-diethyl-5-methyl-tetrahydro-pyridine, whereas (I) was paired with glucuronic acid.

Biochemical Changes of Experimental Precancer: Amino-Acid Metabolism in the Isolated Perfused Rat Liver Studied with the Aid of Lysine-6-C¹⁴ and Histidine-2-C¹⁴

By Leon L. Miller and William T. Burke,* USA

The liver's biochemically important role in intermediary amino-acid and protein metabolism taken together with the known ease with which it develops experimental cancer in the rat renders it uniquely attractive for study as an isolated perfused organ in connection with the biochemical changes of precancer. This is especially true because the simple technique used¹ permits it to be studied directly under conditions closely simulating its normal physiological activity.

This report describes how rat livers have been removed and perfused at varying times before the development of cancer as a result of feeding 3'-methyl butter yellow. In addition, because of the known association of hepatic cirrhosis and liver carcinoma in humans, livers from experimental cirrhotic rats have been similarly studied.

It will be seen that experimental cirrhosis or the feeding of the carcinogen leads to a remarkable decrease of the liver's normal ability to oxidatively destroy amino-acids, not only in terms of converting C¹⁴-labeled lysine or histidine to C¹⁴O₂ and urea but also in terms of failure to clear amino-acids rapidly from the perfusing blood. At the same time these experimentally disturbed livers maintain free tissue amino-acid concentrations significantly higher than normal, and may incorporate C¹⁴-labeled lysine and histidine into plasma and liver proteins to an extent as great as two to four times normal.

Adult male Wistar strain male rats of 225-300 grams initial body weight were maintained on one of the following dietary regimens:

(a) Normal control diet consisted of the commercial rat food known as Purina Checkers.

(b) The experimental cirrhotic diet essentially that of György and Goldblatt.² The animals were used for the liver perfusions from 90 to 150 days after being started on the diet. Characteristically, after a brief 2-3 weeks interval during which the animals lost weight, they gained weight slowly on this diet. The animals were used when, after 3 or 4 months they began to lose weight steadily, with their coats becoming unkempt, and they began to excrete bile

pigment in the urine; grossly and microscopically the experimental cirrhotic livers showed a variably severe picture with loss of normal architecture pseudobulbation, extensive fatty infiltration and degeneration, extensive scarring with fibrosis and some round cell infiltration, pigment deposits (presumably ceroid), and biliary duct proliferation.

(c) The 3'-methyl-dimethylaminoazobenzene (3' MeDAB) diet had the following composition: casein 180 grams, sucrose 650 grams, Mazola oil 120 grams, salt mixture USP XIV (Nutritional Biochemicals) 40 grams, cod liver oil 40 cm³, 3'MeDAB 600 mgs, thiamine 3 mg, riboflavin 2 mg, calcium pantothenate 7.0 mg, pyridoxine 2.5 mg, choline 500 mg to make 1 kilo of diet. Animals were on this diet from 5 to 8 weeks before being used as liver donors.

The 3'MeDAB livers were of a tan color, with an occasional tiny cyst visible in the capsular surface. Microscopically there was minimal periportal fibrosis, with some round cell infiltration and bile duct proliferation. The parenchymal cells appeared otherwise unremarkable.

The perfusion procedure and apparatus used was essentially as previously described.¹ However, instead of a finger stall pump, the stripping action pump of DeBroske³ was used. Further, the overflow return to the blood reservoir was connected with a 12-mm diameter Tygon tube directly to an outlet high on the blood reservoir side; this allowed oxygen to be passed into the air space of the reservoir, up through the overflow into the "lung" from which it was drawn with "expired" CO₂ through a sintered glass bubble trap containing 40% potassium hydroxide to remove the radioactive CO₂.

At the outset of the perfusion to the blood was added 500 mg glucose, and the same dose of amino-acids viz., 160 mg of a mixture containing the essential amino-acids (L-arginine HCl 800 mg, L-histidine HCl 400 mg, L-isoleucine 780 mg, L-leucine 1750 mg, L-lysine HCl 1230 mg, L-methionine 550 mg, L-phenylalanine 870 mg, L-theronine 740 mg, L-tryptophane 180 mg, L-valine 610 mg, and glycine 1000 mg), and 167 mg of a mixture of non-essential amino-acids (L-glutamic acid 1024 mg, L-proline 320 mg, L-hydroxyproline 128 mg, DL-alanine 480

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mg, DL-serine 488 mg, L-cysteine HCl 112 mg, L-tyrosine 272 mg, and DL-aspartic acid 520 mg), and L-lysine, 6- C^{14} , or DL-lysine-6- C^{14} , or L-histidine-2- C^{14} in amounts of 5 to 10 mg (6 to 12 microcuries of C^{14}) the same amounts being used in the normal controls as in the cirrhotic or 3'MeDAB experiments.

Chemical Methods

The procedure for sampling and processing the blood and the liver proteins in preparation for C^{14} assay were essentially as described elsewhere.¹ In addition, analyses for free amino-acid content were carried out on non-protein filtrates⁴ of the blood and of a 1:10 homogenate of the liver in ice cold distilled water. All filtrates were prepared within 10 or 15 minutes after homogenization. Aliquots of filtrate were analyzed for free α -amino-acid nitrogen by the ninhydrin procedure of Moore and Stein.⁵ The blood urea nitrogen content was measured by the ammonia micro-diffusion method of Conway.⁶

C^{14} assays were carried out by using the wet-ashing procedure of Van Slyke⁷ to convert organic material to carbon dioxide which was then introduced into an ionization chamber coupled with a dynamic vane electrometer according to Bale.⁸

RESULTS

Figure 1 presents results of blood amino-acid analysis and reveals that the normal liver rapidly removes amino-acids within the first 1½ to 2 hours of the perfusion, until the level falls to about 130 to 150 mg per cent (reckoned as leucine equivalents). In striking contrast to this is the comparative failure of the experimental cirrhotic liver to lower the circulating blood amino-acid level either as rapidly or as extensively as the normal. Although Fig. 1 shows only the curves obtained from liver perfusions with lysine-6- C^{14} , experiments with histidine-2- C^{14} yielded closely similar results.

The 3'MeDAB livers show a similarly impaired ability to clear amino acids from the blood but on the average the changes are not as severe as those seen in the experimental cirrhotics. Figure 2 presents

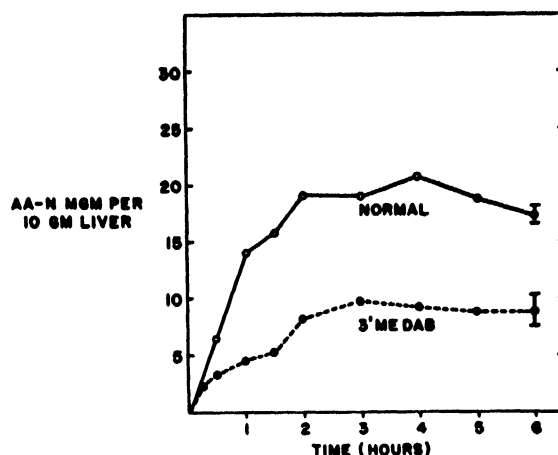


Figure 2. Disappearance of blood free amino-acid nitrogen in isolated rat liver perfusion

these findings with the total amount of free amino-acids disappearing expressed in terms of milligrams of free amino-acid nitrogen per ten grams of liver. The livers of the 3'MeDAB fed rats were not larger than the normals. Hence the differences cannot be explained except in terms of a real loss of amino-acid clearing ability in the former.

Figure 3 shows that the liver-free amino-acid nitrogen levels of the experimental cirrhotic and carcinogen treated rats are considerably higher than the normals under a variety of experimental conditions. This indicates that these livers have not in any sense lost their ability to concentrate amino acids against a gradient; in fact, to the extent to which a high intracellular free amino acid concentration favors protein synthesis, these livers might be expected to manifest enhanced protein synthesis. In this connection the same figure contains data obtained from partially hepatectomized rat livers (not referred to elsewhere in this report) which are known to be capable of rapid protein synthesis and cellular multiplication.

Figure 4 reveals data affording a comparison of the ability of the livers of these various experimental animals (5 normals, 5 3'MeDAB, and 5 cirrhotic) to oxidatively destroy the C^{14} -labeled lysine. Results

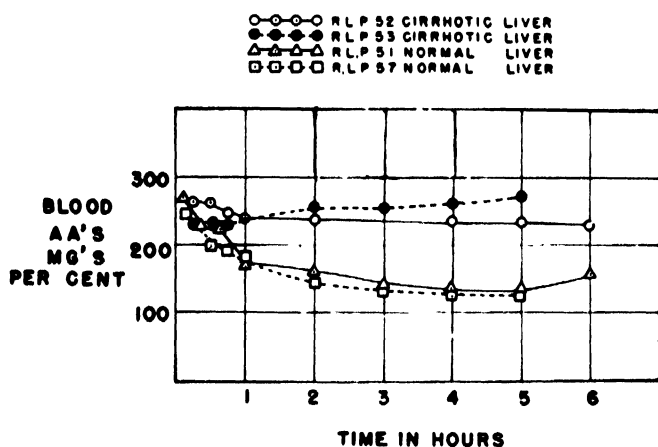


Figure 1. Impaired clearance of amino acids (lysine- C^{14}) by perfused cirrhotic liver

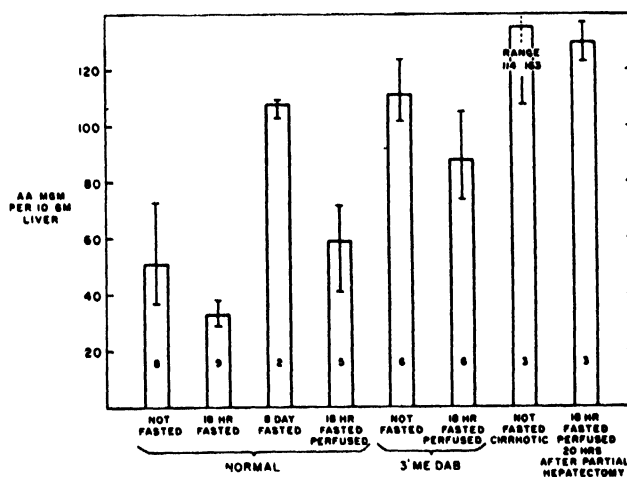


Figure 3. Concentration of free amino acids in liver

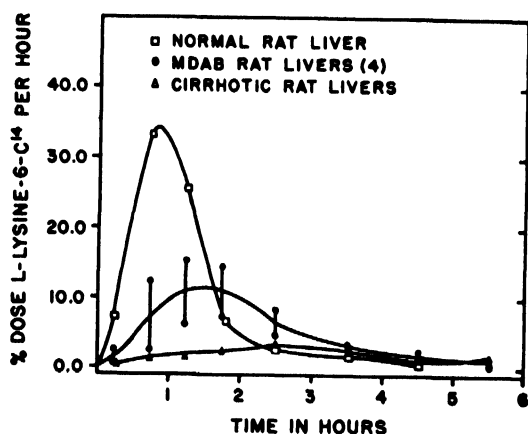


Figure 4. Depressed oxidation of L-lysine-6-C¹⁴ in dab fed and cirrhotic rats

with histidine were closely similar. The qualitative and quantitative differences are striking and might be anticipated from the known failure to clear amino-acids from the blood, an obvious necessary preliminary to oxidative breakdown of the carbon chain by the liver.

Figure 5 reflects the comparative failure of oxidative deamination of amino acids and portrays the diminished urea output of the 3'MeDAB livers. Although the data are not shown here, in two experiments the cirrhotic livers show even larger deviation from the normal, with greatly decreased urea production.

Figure 6 compares the incorporation of C¹⁴-labeled lysine into the plasma proteins by the isolated perfused cirrhotic livers. A glance suffices to reveal that the incorporation of the C¹⁴-labeled amino acids may proceed at a rate somewhat less than normal, however the final extent of incorporation is equal to normal or greater in the experimental cirrhotics. The livers of the 3'MeDAB fed rats were similar in that they retained apparently greater than normal ability to incorporate C¹⁴-labeled lysine into the plasma proteins as revealed in Fig. 7.

The extent of incorporation of the C¹⁴-labeled lysine into the liver proteins of five 3'MeDAB rats is even more remarkable as shown in Fig. 8. The differences are significant whether the results are

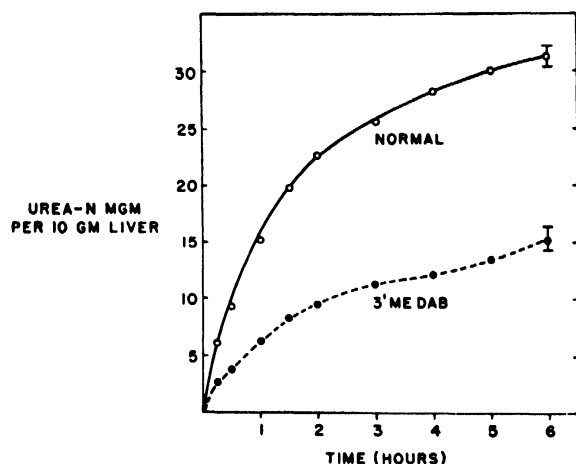


Figure 5. Production of urea-nitrogen by isolated perfused rat liver

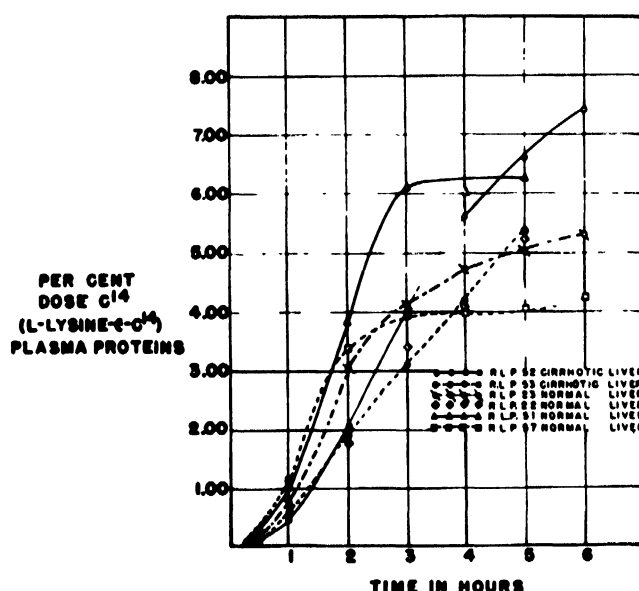


Figure 6. Isolated perfused liver plasma protein synthesis unimpaired by experimental cirrhosis

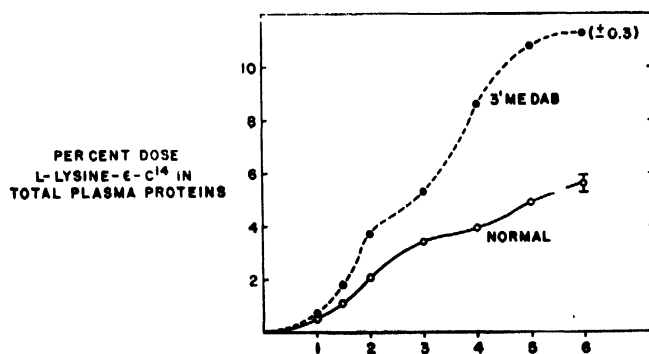


Figure 7. Per cent of dose of L-lysine-6-C¹⁴ incorporated into total plasma proteins

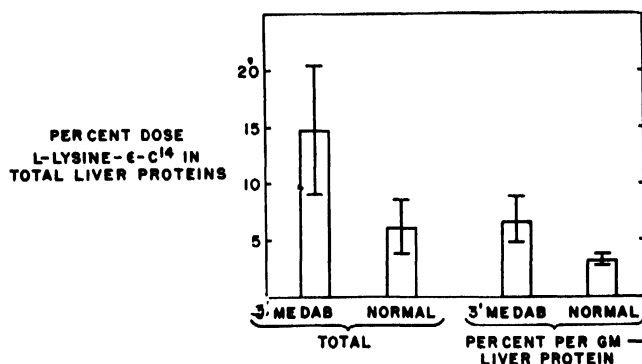


Figure 8. Per cent of dose of L-lysine-6-C¹⁴ incorporated into total liver proteins

expressed on a total liver protein basis or on the basis of per gram liver protein. Similarly (but not shown in the figure) the cirrhotic livers incorporated C¹⁴-labeled lysine or histidine to an extent of 11.0 per cent to 25.0 per cent of the C¹⁴ dose used. This corresponds to two to four times the normal incorporation.

DISCUSSION

An understanding of the relationship between net protein synthesis and cellular growth and multiplication at the mammalian level is the ultimate objec-

tive of much inquiry. In the last analysis it is a minimum necessary (if not sufficient) condition for net protein synthesis that all the constituent amino acids become available to the protein synthesizing mechanisms at effective concentrations. An obvious corollary of this truism implies that a cell may limit net protein synthesis if it possesses a mechanism for maintaining the concentration of at least one amino acid below a level effective for net protein synthesis. Many observations of unicellular organisms are in harmony with the view that the availability of at least the amino acid substrate necessary for net protein synthesis will elicit cellular growth and multiplication. One may thus legitimately inquire into the mechanisms which cells of more highly organized animals possess for biochemically discriminating individual amino acids and for limiting their intracellular concentrations. Such biochemical discrimination may exist at the cell surface in the form of enzymatic mechanisms specifically effective in concentrating the various amino acids within the cell. On the other hand normal cells may possess enzymes which rapidly destroy absorbed amino acid substrate if only to the extent of oxidatively deaminating them. Nutritional or genetic alterations of a cell or an organism could conceivably affect these biochemical mechanisms to a point where the presence of a nutritionally complete amino acid substrate in effective concentration is sufficient to promote net protein synthesis. One may then inquire how far net protein synthesis may proceed in a given cell before the mechanisms of cell division are invoked.

The experimental observations recorded here are in keeping with the above-described hypothetical notions concerning the control and the possible function of the qualitative and quantitative pattern of intracellular amino acids in regulating net protein synthesis.

A more precise definition of the enzymatic mechanisms of amino acid catabolism in mammals is restricted by our limited factual knowledge. The L-glutamic dehydrogenase system may through concerted action with various transaminases⁹ represent the major general pathway for oxidative deamination of amino acids. At this point it is desirable to learn more about the possibility of a decrease in the activity of this enzyme system under conditions where the organism normally or abnormally is called on either to conserve or destroy amino acids. Our observations on changes in rat liver enzymes with inanition or protein depletion¹⁰ have been greatly extended by others, yet data bearing on dietary changes in enzymes potentially involved in the early phases of amino acid catabolism have not to our knowledge been described. One might reasonably conjecture that protein deprivation *per se* could be associated with a diminution of the enzyme proteins

involved in amino acid catabolism. Further, that with increasing availability of dietary amino acids there is normally a rapid resynthesis of L-glutamic dehydrogenase (and other catabolic enzymes) which in effect may interpose a biochemical barrier between excessive amino acids and protein synthetic mechanisms. Under conditions where such normal protective mechanisms are wanting inadequately regulated protein synthesis may occur and manifest itself in the form of cellular proliferation.

SUMMARY

1. The metabolism of amino acids including lysine-6-C¹⁴ and histidine-2-C¹⁴ has been studied in the isolated perfused livers of normal, experimental cirrhotic and 3'-methyl-dimethylaminoazobenzene fed rats.

2. The experimental cirrhotic and carcinogen fed rat livers show marked impairment of their normal ability to oxidatively destroy amino acids. Despite this, the free amino acid concentration in these livers is greater than normal.

3. Both liver and plasma protein synthesis as measured by the incorporation of C¹⁴-labeled amino acids are greatly enhanced in the experimental cirrhotic and carcinogen fed rat livers.

4. These changes occur before experimental cancer is histologically demonstrable.

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Radioisotopic Studies of Human Serum Lipoproteins*

By M. E. Morton† and W. H. Florsheim,‡ USA

In recent years a large amount of work has been published concerning the nature and function of the protein-lipid complexes found in human plasma. Since most of this work has centered upon the role of the lipoproteins in atherogenesis, primary emphasis has been placed upon one constituent of these complexes: cholesterol. The protein component has received scant attention, and the phospholipids least of all. Yet, the latter two constituents of the lipoprotein molecule must account for its surface charges and for the solubility of the cholesterol and neutral fats in the blood.¹

The nature of the lipoprotein complex and its stability are poorly understood. Recently Barr and his associates² have concluded from the differing responses of alpha and beta lipoproteins to hormone therapy that the two types of lipoproteins might be elaborated and metabolized by different physiological mechanisms. Gofman, Lindgren and Elliott³ also expressed the opinion that lipoproteins are reasonably stable compounds and not merely labile complexes. However, much evidence argues against this point of view. Nikkila⁴ has described a rapid acceleration of the electrophoretic mobility of beta-lipoprotein to that characteristic of an alpha-lipoprotein under the influence of heparin. Gould and his associates⁵ have calculated regeneration times for the cholesterol and protein moieties of dog lipoproteins, and they found that the protein was turned over almost twice as fast as the cholesterol. In man, also, one can calculate from the data of Moser⁶ and of Volwiler and collaborators⁷ that the serum phospholipids are turned over more than twice as fast as the proteins of Cohn's fractions I + III, which contain the protein moiety of the beta-lipoprotein molecule. The turnover time of serum cholesterol has not yet been measured satisfactorily, but appears to be even longer than that of the Cohn fraction I + III protein.^{8,9} Finally, an extreme position is represented by Maurer,¹⁰ who concluded on the basis of electrophoretic

retic behavior of lipoproteins in buffers containing added albumin, that lipid-protein bonds of lipoproteins are of a strength comparable to hydration bonds, and that the nature of the protein component is determined largely by the mass-action principle.

The experiments described here were performed in order to elucidate the forces holding together the lipoprotein complex. We have studied the turnover of the phospholipid moiety of lipoproteins isolated by two different methods from human serum, and we studied the exchange of phospholipids between alpha and beta lipoproteins *in vitro*. We also have repeated Maurer's experiments, using Kunkel's¹¹ improved electrophoretic procedures.

METHODS

Six serum specimens from a patient who had received a total of 30 mc of radiophosphate orally for the alleviation of pain due to a carcinoma with widespread metastases were used in the study. The sera were taken 10, 30, 66, 91, 115 and 211 hours after the administration of the last dose of the radioisotope. Enough of all but the first and last specimens was available to permit ultra-centrifugal and electrophoretic fractionation as well as the determination of the total serum phospholipids.

Serum lipid phosphorus was determined by the method of Zilversmit.¹² Total serum phospholipids and serum sphingomyelin and lecithin-cephalin were analyzed according to Petersen¹³ with the sphingomyelin being determined directly by ashing the TCA-insoluble residue remaining after the hydrolysis of lecithin and cephalin by *N* KOH.

In the electrophoretic studies duplicate 1.0 cm³ serum samples were applied to 5 superimposed strips of Whatman 3mm paper and separated by Kunkel and Slater's¹¹ procedure for 24 hours using a current of 12 ma and 270v. The buffer was barbital of ionic strength 0.1 at pH 8.8. The strips were cut into segments representing the albumin and α_1 , α_2 , β - and γ -globulin zones, using the bromphenol blue-stained top strip as a template. Lipids were extracted from the paper by two changes of acetone-alcohol and were ashed with nitric and perchloric acid prior to assay for radioactivity and total phosphorus. In the experiments repeating and extending Maurer's work rat, rabbit and human sera labeled biosynthetically with radiophosphorus were used. Electrophoresis was carried out by the "starch block" technique of Kunkel and Slater, since this gave a better separation of the

* After completion of this manuscript a paper by H. G. Kunkel and A. G. Bearn was published, ("Phospholipid Studies of Different Serum Lipoproteins Employing P³²", Proc. Soc. Exp. Biol. and Med. 86: 887, August-September 1954) which demonstrated by means of electrophoretic techniques the similarity of phospholipid specific activity between β , α_2 , and α_1 lipoproteins. An *in vitro* shift of phospholipids from β to α_1 lipoprotein was also shown.

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α -lipoproteins than the "paper sandwich" method, particularly with rat serum. When human serum albumin was added to the buffer (at a level of 2.5 gm%), the position of the albumins in the electrophorograms was marked by means of I^{131} iodinated human albumin,¹⁴ which proved to be an adequate marker for animal albumins also. The starch blocks were cut up and eluted as described by Kunkel and Slater. Analysis for protein was performed by the biuret method, and for cholesterol by the method of Zlatkis, Zak and Boyle¹⁵ on the chloroform extract of an aliquot.

Ultracentrifugal β - and total lipoprotein fractions were prepared by the methods of Gofman¹⁶ and Page¹⁷ using a Spinco preparative ultracentrifuge. Centrifugation was carried out for sixteen hours at 30,000 rpm (80,000 gm). The top layer containing the floated lipids and lipoproteins was removed and designated the "lipid fraction". It amounted to 16% of the total solution. Lipid extracts were prepared from both fractions by means of alcohol-acetone, and the lipids were fractionated into sphingomyelin and lecithin-cephalin as before and ashed.

All radioactive measurements were corrected for decay back to the time of isotope injection.

In the experiment studying exchange of phospholipids between α - and β -lipoproteins *in vitro*, total lipoproteins were prepared from two sera by ultracentrifugation at density 1.21. One of the sera was from a polycythemic patient who had received radio-phosphate two days prior to phlebotomy. The other serum was from an untreated patient. Both total lipoprotein fractions were dialyzed against several changes of saline for four hours and then re-centrifuged at density 1.063. The opalescent top layer amounting to 1/9 of the solution was designated the β -lipoprotein, and the rest α -lipoprotein. The solutions were kept in the ice box prior to use. 1.0 cc of β -lipoprotein solution was then mixed with 1.0 cc of α -lipoprotein and 5.0 cc of saline of density 1.063. The mixture was incubated either at 37° or at 4° for 2 to 24 hours and then re-ultracentrifuged for 16 hours. The β -layer was separated from the α -layer and phospholipid and cholesterol determined. Cholesterol: lecithin ratios were calculated for all samples, and they remained reasonably constant throughout the experiment, being 1.52 ± 0.31 for the β -lipoprotein fractions and 0.54 ± 0.11 for the α -lipoproteins. No attempt was made to correct the results for phospholipid exchange occurring during the final ultracentrifugation, during which labeled and control fractions were in contact for 16 hours at a temperature averaging 8°C. Analysis of a sample of Control β -lipoprotein on a Spinco Model E analytical ultracentrifuge showed no change in its S_r pattern after 8 hours incubation at 37°.

RESULTS

Figure 1 shows the specific activity curves for the serum total phospholipid phosphorus and its sphingomyelin and lecithin-cephalin subfractions. The

similarity between our total lipid phosphorus curve and that published by Balfour¹⁸ indicates that the patient studied here was normal with respect to phospholipid synthesis and catabolism. This is confirmed by comparison with a large number of phospholipid turnover studies carried out in this laboratory.¹⁹ During the nine days of observation the serum phospholipids remained within the range of $78.6 \pm 1.3 \gamma \text{ P/cm}^3$ and the composition of the phospholipids remained within the normal range given by Petersen, with an average of 76% lecithin-cephalin. Since the cephalin level was low we did not attempt to determine its specific activity.

Our data indicate that while lecithin and sphingomyelin are not equally labeled, their specific activity vs time curves are similar and they have similar turnover times, as Zilversmit's²⁰ calculations have also indicated.

Table I shows the result of our electrophoretic experiments. By cutting our strips into segments determined by the protein distribution patterns we obtained considerable overlapping of the lipoprotein bands with our protein-determined segments. This was done in order to detect possible variations of the phospholipid specific activity within the broad α - and β -lipoprotein bands. One major cause for the variations observed in our experiments should be mentioned: Extraction of lipid material from the electrophoretic strips by Bloor's solvent or acetone-alcohol was not complete, as Kunkel and Slater had already shown.¹¹ Contrary to these authors' statements, however, solvent retention by the paper was not at fault, since repeated extraction yielded no further phospholipids. It seems likely that the recoveries could have been increased by use of Delsal's²¹ extraction technique. Distribution of serum between the 5 strips was approximately equal. The recovery of lipid was not significantly influenced by the age of the serum or by the length of the drying period prior to the extraction of the strips. Within the limits of our technique the specific activities of the lipid fractions eluted from the five regions of the electrophoretic pattern were not significantly different from each other. This was true for all points on the time scale investigated.

The same conclusion is reached from a consideration of the results of the ultracentrifugal fractionations presented in Fig. 2. The same curves fit the

Table I. Electrophoretic Separation of Serum Phospholipids

Fraction	Specific activity, c/s/ γP			
	30 hr	66 hr	91 hr	115 hr
Total phospholipid	11.2	19.4	18.3	16.1
Extracted from paper	13.8	21.2	17.3	16.2
Albumin	17.0	18.8	23.3	16.1
α_1 -globulin	13.5	20.1	19.9	15.7
α_2 -globulin	12.3	21.8	15.7	10.2
β -globulin	12.8	22.3	18.2	17.4
γ -globulin	13.7	20.9	17.2	18.8

phospholipid components of β - and α -lipoproteins at all times.

Figures 3 and 4 show the results of the *in vitro* phospholipid exchange between labeled and control lipoproteins. Since no mechanism exists in this system for the synthesis of new phospholipids, the results clearly indicate that exchange has taken place far beyond the possibility of contamination introduced by the procedure of separation.

Figure 5 presents typical electrophoretic patterns of human and animal sera in the presence and absence of added albumin. Whereas Maurer¹⁰ showed a shift of phospholipids from the globulins to albumin due to albumin added to the buffer, no such shift could be demonstrated when the conditions specified above were employed. Cholesterol as well as phospholipid distribution remained unaltered.

DISCUSSION

In the preceding section data were presented to the effect that all lipoprotein fractions of human sera obtained by electrophoretic or by ultracentrifugal methods contain phospholipids of the same specific activities. This remained true during the entire period covered by the experiment, while the specific activity of lipid phosphorus increased to a maximum at about 75 hours after isotope injection, and for two days thereafter. This result would be quite unexpected if α - and β -lipoproteins were elaborated by the liver and then released into the blood to pursue their individual metabolic fates. The second experiment presented here shows in fact that the lipoproteins do exchange component parts even *in vitro*. Thus it appears that lipoproteins are indeed loosely bound complexes, and that the exchange of phospho-

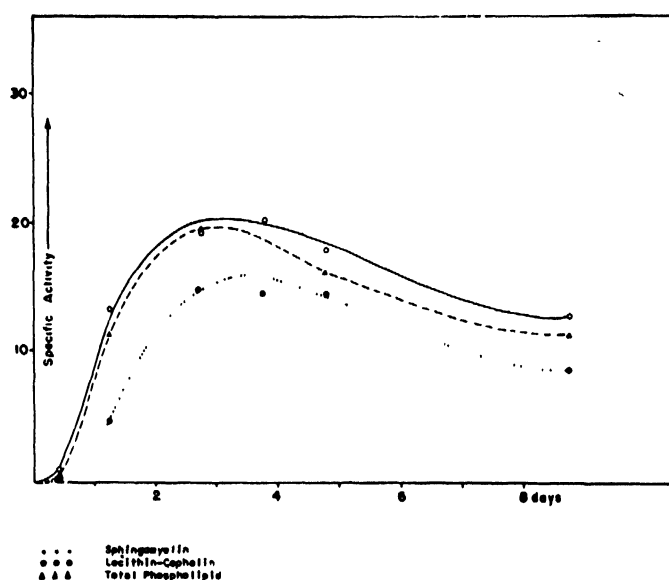


Figure 1. Specific activities of serum phospholipid fractions as a function of time

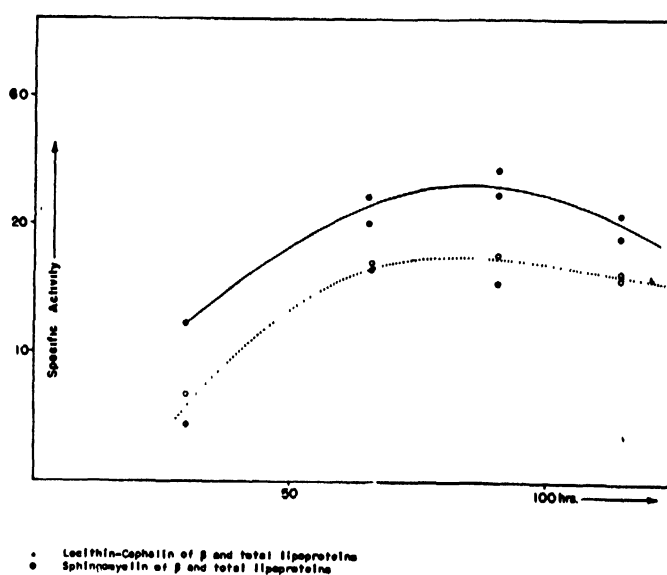


Figure 2. Specific activities of the phospholipid fractions of ultracentrifugally isolated serum lipoproteins as a function of time

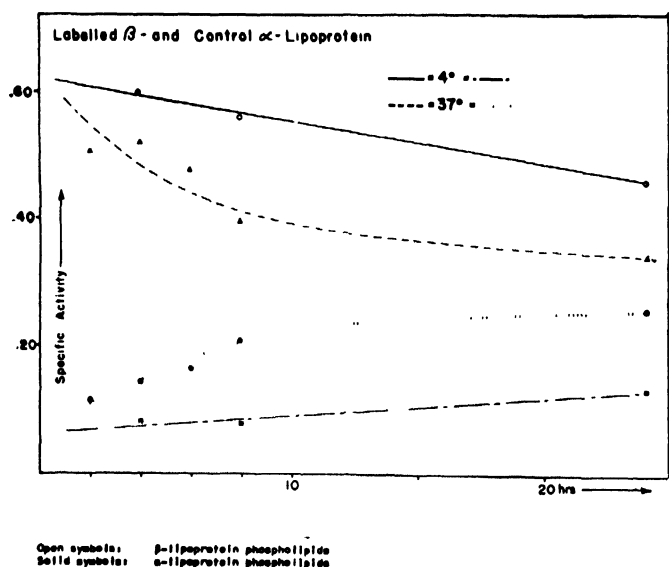


Figure 3. Time course of phospholipid specific activities of α and β lipoproteins after mixing P^{32} -labelled β -lipoprotein with unlabelled α -lipoprotein at 37° and at 4°

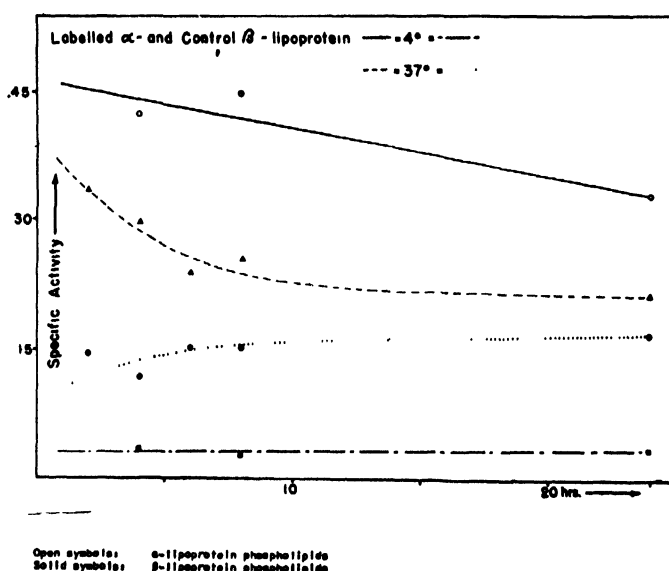
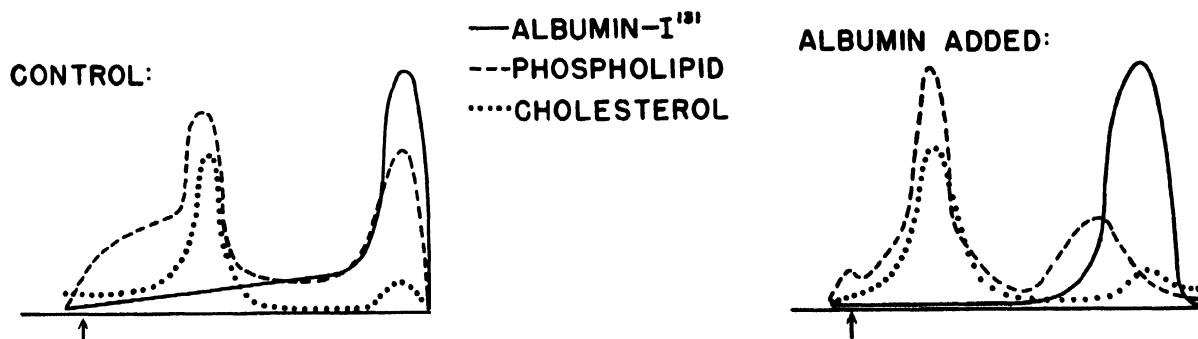
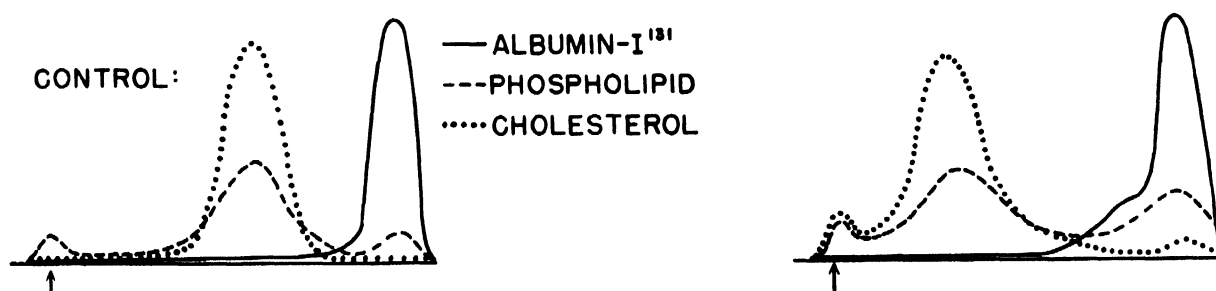


Figure 4. Time course of phospholipid specific activities of α and β lipoproteins after mixing P^{32} -labelled α -lipoprotein with unlabelled β -lipoprotein at 37° and 4°

HUMAN SERUM FROM LEUKEMIC PATIENT



HYPERCHOLESTEROLEMIC RABBIT SERUM



HYPERCHOLESTEROLEMIC RAT SERUM

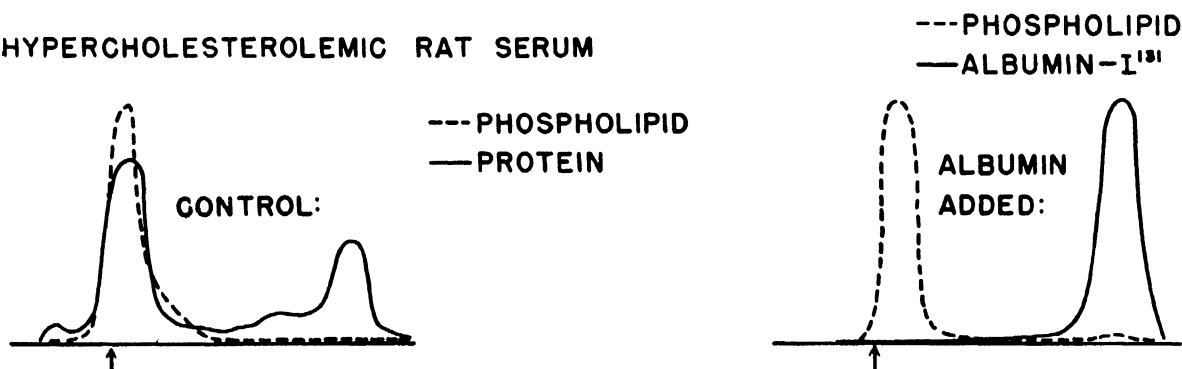


Figure 5. Typical electrophoretic patterns of human and animal sera in the presence and absence of added albumin

lipids between lipoprotein complexes is rapid compared to the metabolic "half-life" of the phospholipid molecule.

A reasonable "model" which satisfies these criteria and explains many other properties of lipoproteins pictures the lipoprotein complex as an ionic association between protein and a mixed micelle consisting of non-ionic cholesterol and its esters and neutral fats, and polar phospholipids and fatty acids. A mixed micelle satisfies Batchellor's²² findings of highly variable cholesterol: phospholipid ratios in individual plasmas, and such a model offers a possible explanation of the work which has been done on the denaturation of lipoproteins.²³ Substances which destroy the micelle structure, such as alcohol and detergents, liberate both cholesterol and phospholipids simultaneously. Some compounds struc-

turally related to cholesterol, such as saponin, may be able to substitute for cholesterol without destroying the micelle integrity, thus explaining the liberation of cholesterol only. The model also explains the non-extractability of the lipid components of the complex by ether,²⁴ since this solvent does not extract lipids from micelles dispersed in water until the micelle has been broken up, as for example by freeze-drying. Our lipoprotein model, however, cannot explain the differences between α - and β -lipoproteins, except by ascribing them entirely to differences in the nature of the protein moiety. This emphasis on the protein conflicts with the theories of Maurer,¹⁰ who postulated only the weakest association between phospholipids and a non-specific carrier protein. We were unable to confirm Maurer's results using more reliable techniques. It is likely

that denaturation of the sensitive lipoproteins is the basis of Maurer's results. This is indicated by the large amounts of phospholipids remaining at the origin of his electrophorograms and the diffuse distribution of the portion which migrated in the absence of added albumin. Denaturation is also indicated by Maurer's inability to detect the α -lipoprotein peak in rabbit serum, which, according to Lewis²⁵ accounts for at least a third of the serum lipids in this species. We have observed that the addition of albumin to the buffer greatly increases the amount of protein-free phospholipids prepared from human plasma which will migrate with rat serum in paper electrophoresis. It therefore appears safe to discount Maurer's results in assessing the specificity of lipid protein association in the lipoprotein complex. The work of Ruhenstroth-Bauer²⁶ clearly indicates that, in man, the carrier protein is specific and cannot be replaced by either albumin or γ -globulin.

Recent findings indicate that in addition to the ionic binding of mixed lipid micelles to protein, other types of bonds may play a part. Gordon²⁷ has shown recently that free fatty acids can be adsorbed on lipoprotein molecules, and that the adsorbed polar groups can alter the electrophoretic mobility of the complex. Delsal and collaborators²⁸ have shown that a portion of the cholesterol of normal serum is extractable by dry ether without concomitant extraction of phospholipids. We have found that only about 7% of serum cholesterol can be extracted in this manner after removal of the chylomicra, but Lever's group²⁹ found that a much higher percentage could be extracted from certain pathological sera. In "normal" lipoproteins, however, only small amounts of such unstabilized lipid material exist. The dominant lipoprotein type in human serum appears to have its lipids held in a micelle. However, our results require that lipid components must be freely exchangeable between the different types of micelles. Our data indicate that exchange of phospholipids between lipoproteins occurs at a sufficiently rapid rate to keep all the serum phospholipids associated with the various lipoproteins at the same specific activity. The mechanism of this exchange and the question of whether cholesterol and other serum lipids are similarly exchangeable needs clarification.

SUMMARY

The specific activity of lipid phosphorus in lipoproteins obtained from labeled human serum by electrophoresis and ultracentrifugation was the same within experimental error. This remained true for 8 days after tracer administration. From this it is concluded that the exchange of phospholipids between lipoprotein "molecules" must be rapid compared to the metabolic half-life of the phospholipid molecules. Such an exchange was demonstrated to occur even *in vitro*. These data indicate that lipoproteins may consist of a specific carrier protein and mixed micelles of lipids held in loose ionic association.

ACKNOWLEDGEMENTS

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Use of Radioactive Metabolites in Studying Modes of Action of Anticancer Agents and Searches for Exploitable Biochemical Differences Between Normal and Cancer Cells

By H. E. Skipper,* USA

Approximately 25–30% of all cancer is curable today by means of surgery and ionizing radiation. The remaining 70–75% is now incurable by these procedures because the neoplastic cells are widely disseminated throughout the body when detected. It has been estimated that if good early detection techniques could be developed (for all cancer), and if means could be found for continual monitoring of a population, then perhaps the cure rate could be increased to 50% by employing early surgery and radiation therapy.

On further analysis of the cancer problem there come to mind a number of obviously worthwhile approaches which are receiving attention from scientists who have dedicated their efforts to seeking means for control of human cancer. These are: (a) prevention of the original carcinogenic event; (b) early detection and conventional therapy; (c) radiologic control of disseminated cancer; and (d) chemotherapy of disseminated cancer.

The present brief discussion will be limited to certain phases of cancer chemotherapy, with particular reference to research designed to shed light on exploitable biochemical differences between normal and neoplastic cells.

As has been the case in other fields of chemotherapy, the progress in cancer chemotherapy has resulted in no small degree from the efforts of practitioners of the "enlightened empirical" approach who have been willing to "suffer the slings and arrows" sometimes directed by purists who feel that nothing very profound will come from trial and error research.

The unprecedented successes achieved in the field of bacterial chemotherapy have come largely from screening programs. In retrospect, it has been established that sulfonamides which cure certain widely disseminated bacterial diseases in man do so because they inhibit chemical events vital to the bacteria but unimportant to human body cells. This combination of applied and fundamental approaches which supplied our useful antibacterial agents and our present concepts of the mechanisms of specificity is now being applied to the cancer problem.

There is in progress considerable research effort entailing the synthesis and screening of candidate cancer inhibiting agents, studies of the modes of action of active agents, and searches for exploitable biochemical differences between normal and neoplastic cells. In principle, the objective of much of the biochemical work associated with the cancer chemotherapy effort is to discover a series of chemical events vital to the cancer cell but unimportant to the existence or function of normal cells. If such information were at hand a concerted effort to synthesize compounds as potential antagonists of intermediates on this exploitable biochemical pathway could be considered a rational and most promising approach to control of cancer.

There can be little doubt that chemical differences do exist within the chemical assembly lines of normal and cancer cells. How else can one explain the differences in appearance and growth potential of cancer cells and their normal cells of origin? The difficulty in finding the underlying biochemical lesions responsible for uncontrolled growth in cancer cells stems from the fact that all cells are somewhat analogous to small chemical factories carrying out thousands of simultaneous chemical reactions, many of which are interdependent, and most of which are catalyzed by complicated chemicals, enzymes. The difficulty in picking out the basic causes of cancer is largely the difficulty of bracketing the biochemical area in which to search, and developing means for obtaining meaningful results in the maze of dynamic chemical reactions in progress in all cells.

Of all the worthwhile returns from the large peacetime application of atomic knowledge, I would predict that the application of radioactive isotopes to the discovery of the chemical processes of the living cell will in the long run prove to be one of the most beneficial to mankind. It would appear for instance that radioactive carbon-14 is an almost ideal tool for searching for the so-called exploitable biochemical difference between normal and cancer cells. Two approaches are rather obvious. If one can discover the sites of action of tumor-inhibiting agents within the biochemical events of a cell, then such knowledge can be employed to suggest areas of difference between normal and cancer cells. A more

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direct approach is to map the chemical events leading to biosynthesis of metabolites in normal and cancer cells employing appropriately labeled precursors. In such an effort widely useful fundamental information can be gathered as well as immediately applicable leads for the guiding synthesis of candidate chemotherapeutic agents.

It is yet to be established unequivocally just where in the multitude of simultaneous chemical reactions within a given cell there occurs a change (a carcinogenic change) which results in the birth of a cancer cell. Many working in the cancer field believe that cancer is a disease of mutation having to do with genes (nucleoprotein), and the processes controlling their biosynthesis, degradation, or their biochemical function. This concept, plus the fact that a number of agents which temporarily inhibit tumor growth act on nucleotide synthesis,¹ has lead us to concentrate our basic studies in this area.

Within the limited scope of this paper a few examples of studies on the mechanisms of action of certain anticancer agents will be presented as well as attempts to apply such information to the problem of cancer chemotherapy. A brief summary of certain efforts to find differences between cancer and normal cells within the area of nucleotide and nuclei acid synthesis will be presented.

PURINE NUCLEOTIDE SYNTHESIS

The work of Buchanan,² Shive,³ Hartman,⁴ Love,⁵ Bennett,⁶ and others has suggested that purine nucleotide synthesis in a variety of cells may follow a pathway somewhat similar to the one presented in Fig. 1. This metabolic map is not the complete picture, nor is it necessarily accurate in all detail, but it is almost surely the precursor of an accurate map. It is now well established that the carbon atoms of the purine skeleton of purine nucleotides come from the precursors indicated in Fig. 2.

With such information as is presented in Figs. 1 and 2, it is now possible to carry out *in vivo* experiments designed to shed light on the effects of compounds on nucleotide and polynucleotide biosynthesis.

FOLIC ACID ANTAGONISTS

Aminopterin (4-aminopteroylglutamic acid) was first shown to provide temporary remissions of acute leukemia in children by Farber *et al.*⁷ This compound or A-methopterin (4-amino-N-10-methyl-pteroylglutamic acid) increases the life span of mice with lymphoid leukemia with amazing regularity (Table I).

The inhibition of *E. coli* with Aminopterin has been shown to cause the accumulation of 4-amino-5-imidazole carboxamide⁸ indicating a block due to antagonism of a formylating coenzyme (citrovorum factor), and failure of the cell to close the purine ring (Fig. 1).

We have carried out studies which demonstrate that A-methopterin profoundly inhibits the incorporation of C¹⁴-formate into the nucleic acid purines and thymine of leukemic cells (Table II).

Table I. Effects of A-methopterin on the Life Span of Mice with Leukemia L-4946*

Treatment	Dosage mg/kg	Avg. life span	
		Days	% above controls
None	—	14.1	—
A-meth	3.0	31.0	119
A-meth	1.5	24.9	77

* Ten mice per group. Treatment begun 24 hours after leukemic inoculation and continued every other day for 10 injections.

Table II. The Effect of A-methopterin on Formate Incorporation into Nucleic Acid Moieties of Mice*

Tissue	Treatment	Specific activities ($\mu\text{C}/\text{mole C}$)					
		DNA			RNA		
		Gu	Ad	Thy	Gu	Ad	
L-1210 Leuk.	None	206	227	100	309	308	
L-1210 Leuk.	A-meth	34	39	10	54	112	
Viscera	None	171	280	—	199	248	
Viscera	A-meth	26	16	4	70	90	

* Lymphoid tumor masses were employed. A-methopterin (3 mg/kg) was administered immediately prior to injection of C¹⁴-formate; all mice were sacrificed for nucleic acid isolations at 6 hours following the injection of C¹⁴-formate.

These results and others suggest that one of the sites of action of A-methopterin is in the *de novo* synthesis of purine nucleotides and another is in the synthesis of DNA thymine. At equal doses A-methopterin failed to inhibit nuclei acid synthesis in A-methopterin-resistant leukemic cells.

AZASERINE (O-DIAZOACETYL-L-SERINE)

The isolation and proof of structure of azaserine has been described by Fusari *et al.*^{9,10} The marked inhibitory effect of this compound upon Sarcoma 180 was discovered by Stock, *et al.*¹¹ Clarke, *et al.*¹² first observed the striking potentiation of the tumor-inhibiting action of azaserine and 6-mercaptopurine.

In view of previous observations showing that A-methopterin, an inhibitor of *de novo* purine synthesis, and 8-azaguanine or 6-mercaptopurine were potentiating with regard to antileukemic activity^{13,14,15} it was considered possible that azaserine was also an inhibitor of *de novo* nuclei acid synthesis. That this was the case was demonstrated by tracer studies in animals,¹⁶ and confirmed and extended by studies in isolated enzyme systems.⁴ The more recent results of the efforts to pinpoint the inhibitory effects of azaserine on nuclei acid synthesis are summarized in Table III.

The data presented in Table III indicate that azaserine blocks *de novo* nucleic acid synthesis in the early step prior to formation of 4-amino-5-imidazole-carboxamide (Fig. 1). Hartman, *et al.*,⁴ employing enzyme systems from pigeon liver, observed that azaserine effected a considerable increase in the accumulation of glycine amide ribotide and formyl-glycine amide ribotide, and blocked the conversion

Table III. Effects of Azaserine on Nucleic Acid Metabolism*

Expt. No.	Labeled compound	Specific activities (% of untreated control)											
		Tumor				Intestine				Liver			
		DNA		RNA		DNA		RNA		DNA		RNA	
		Ad	Gu	Ad	Gu	Ad	Gu	Ad	Gu	Ad	Gu	Ad	Gu
1	C ¹⁴ -Formate	11	14	20	20	10	10	10	10	—	—	—	—
2	C ¹⁴ -Formate	< 21	< 28	< 15	< 15	< 19	< 17	< 11	< 11	—	—	—	—
3	Glycine-1-C ¹⁴	< 8	< 9	< 7	9	< 19	< 25	13	16	—	—	—	—
4	4-Amino-5-imidazole carboxamide-4-C ¹⁴	71	81	92	96	40	90	82	132	—	—	—	—
5	Adenine-8-C ¹⁴	100	—	119	—	70	—	104	—	78	—	82	—

* Sarcoma 180-bearing mice were used in these experiments. The polynucleotide purines from pooled tissues obtained from six untreated control and six mice treated with

azaserine (20 mg/kg) were compared. All experiments were terminated at six hours following the injection of the labeled compound indicated.

of these acyclic ribotides to inosinic acid. Tomisek¹⁷ has observed the accumulation of radioactive formylglycine amide riboside in azaserine-treated *E. coli* following administration of C¹⁴-formate or C¹⁴-glycine.

With the availability of these results it is possible to postulate that the potentiation of the anticancer activity of azaserine by 6-mercaptopurine may result from a dual or sequential block in biochemical events leading to purine nucleotide synthesis. Azaserine blocks early stages of *de novo* synthesis whereas 6-mercaptopurine is an antagonist of preformed purine nucleotides.

The results presented in Table IV demonstrate the degree of potentiation obtained by this pair of chemotherapeutic agents.

Table IV. Effects of Azaserine plus 6-Mercaptopurine on Life Span of Mice with L-1210 Leukemia

Treatment*	Average life span	
	Days	% above controls
Controls	6.6	
X	10.5	59
Y	10.6	61
X + Y	20.4	209
X/2 + Y/2	21.4	224
X/4 + Y/4	15.2	128

* X = Azaserine, 8.0 mg/kg $\times$ 10; Y = 6-Mercaptopurine, 40 mg/kg $\times$ 10.

Table V. Effects of 6-Mercaptopurine on Incorporation of Labeled Compounds into Nucleic Acids*

Labeled compound	Treatment	Tumor	Incorporation into combined nucleic acids (% of control)	
			Tumor	Intestine
Formate	6-MP	Sarcoma 180	39	27
	6-MP	Adenoca. 755	43	34
Adenine-8-C ¹⁴	6-MP	Sarcoma 180	88	85
	6-MP	Adenoca. 755	93	76

* 6-Mercaptopurine was injected (40 mg/kg) immediately prior to administration of labeled compounds. Pooled tissues from 12 mice were employed in each experiment. All experiments were terminated at 6 hours.

EFFECTS OF OTHER ANTICANCER AGENTS ON NUCLEIC ACID SYNTHESIS

6-Mercaptopurine synthesized by the Burroughs-Wellcome group,¹⁸ and shown to inhibit acute leukemia in children by Burchenal, *et al.*¹⁹ appears to inhibit *de novo* nucleic acid synthesis while failing to inhibit significantly adenine incorporation into polynucleotides,²⁰ Table V.

Using *in vitro* systems LePage and Greenlees found that 6-mercaptopurine inhibited glycine incorporation into acites tumor cells,²¹ and Heidelberger and Keller²² observed that 6-mercaptopurine inhibited formate incorporation into nucleic acid adenine and guanine of Flexnor-Jobling carcinoma.

Our group and Goldthwait²³ have observed that nitrogen mustard inhibits DNA synthesis *in vivo* (Table VI).

The data which are available to date suggest that certain of the known temporary anticancer agents inhibit nucleic acid metabolism in the areas, Fig. 3.

The fact that azaserine plus 6-mercaptopurine or 8-azaguanine, and Λ -methopterin plus 6-mercaptopurine or 8-azaguanine are potentiating with regard to antileukemic activity suggests that further studies on dual blocking within this area of chemical events may prove of practical value, and to a degree, rational. With the further development of knowledge on the sites and modes of action of anticancer agents, rational applications will most certainly be made.

Table VI. The Effects of Nitrogen Mustard on Incorporation of Labeled Compounds into Nucleic Acids*

Expt. No.	Labeled compound	Treatment	Specific activity of intestinal Ag purines (μ c/mole C)	
			DNA	RNA
1	Formate	None	154	312
2	Formate	Nitrogen mustard	60	317
3	Adenine	None	667	1137
4	Adenine	Nitrogen mustard	287	948

* Groups of three rats were used in each experiment. Nitrogen mustard was injected prior to administration of labeled compounds. All experiments were terminated at 24 hours.

Table VII. Relative Incorporation of C¹⁴-Labeled Formate, Guanine, 2, 6-Diaminopurine and Adenine by Animal Neoplasms and Intestine*

Tumor	Host	Strain	Ratio of specific activities (CNA),			
			tumor: intestine			
			Guanine	2, 6-DAP	Formate	Adenine
Sarcoma 180 (SRI)	Mouse	Swiss	0.04	2.0	0.91	0.58
Adenocarcinoma 755	Mouse	C57	0.19	1.7	0.55	0.33
Ehrlich carcinoma	Mouse	Swiss	0.07	0.4	0.26	0.13
Wagner osteogenic sarcoma	Mouse	AKR	0.02	1.5	0.28	0.23
Gardner lymphosarcoma	Mouse	C3H	0.07	1.7	0.24	0.11
Ridgeway osteogenic sarcoma	Mouse	AKR	0.09	1.0	0.25	0.14
Mecca lymphosarcoma	Mouse	AKR	0.03	1.5	0.25	0.13
Sarcoma Ma 387	Mouse	AKR	0.11	0.8	—	0.16
Bashford carcinoma 63	Mouse	Swiss	0.08	2.7	0.24	0.17
Lewis sarcoma T241	Mouse	C57	0.06	1.1	0.21	0.13
Carcinoma 1025	Mouse	AKR	0.03	1.5	0.42	0.13
Miyono adenocarcinoma	Mouse	AKR	0.10	2.3	0.32	0.25
Harding-Passey melanoma	Mouse	Swiss	0.004	—	0.17	—
R. C. carcinoma	Mouse	dba	0.13	—	—	—
Patterson lymphosarcoma	Mouse	AKR	0.002	—	—	—
Sarcoma 180 (SKI)	Mouse	Swiss	—	2.8	—	0.25
Adenocarcinoma Eo 771	Mouse	C57	—	1.7	0.49	—
L1210 leukemia	Mouse	dba	0.17	1.6	0.57	—
Jensen rat sarcoma	Rat	Sherman	0.03	0.9	0.63	0.10
Murphy-Sturm rat lymphosarcoma	Rat	Sherman	0.11	0.4	0.33	0.22
Flexner-Jobling carcinoma	Rat	Sherman	0.08	0.4	0.23	—
Walker carcinosarcoma 256	Rat	Sherman	0.10	1.3	0.56	0.07
Average			0.07	1.4	0.37	0.20

* Comparisons were made on CNA isolated from pooled tissues of groups of 5-10 animals each. All experiments were terminated at six hours after injection of the labeled compound.

Table VII-A. Relative Incorporation of C¹⁴ Labeled Formate, Guanine, 2, 6-Diaminopurine and Adenine by Animal Neoplasms and Liver*

Tumor	Host	Strain	Ratio of specific activities (CNA),			
			tumor: liver			
			Guanine	2, 6-DAP	Formate	Adenine
Sarcoma 180 (SRI)	Mouse	Swiss	0.03	2.6	15	—
Adenocarcinoma 755	Mouse	C57	0.24	1.7	14	0.33
Ehrlich carcinoma	Mouse	Swiss	0.07	0.6	6	0.11
Wagner osteogenic sarcoma	Mouse	AKR	0.03	2.2	5	0.13
Gardner lymphosarcoma	Mouse	C3H	0.05	3.2	5	0.08
Ridgeway osteogenic sarcoma	Mouse	AKR	0.02	1.2	5	0.17
Mecca lymphosarcoma	Mouse	AKR	0.03	—	6	0.20
Sarcoma Ma 387	Mouse	AKR	0.13	1.6	—	0.14
Bashford carcinoma 63	Mouse	Swiss	0.11	5.3	9	0.43
Lewis sarcoma T241	Mouse	C57	0.07	2.2	8	0.10
Carcinoma 1025	Mouse	AKR	0.03	1.8	8	0.10
Miyono adenocarcinoma	Mouse	AKR	0.17	—	4	0.80
Harding-Passey melanoma	Mouse	Swiss	0.009	—	4	—
R. C. carcinoma	Mouse	dba	0.21	—	—	—
Patterson lymphosarcoma	Mouse	AKR	0.003	—	—	—
Sarcoma 180 (SKI)	Mouse	Swiss	—	3.8	—	0.25
Adenocarcinoma Eo 771	Mouse	C57	—	1.5	9	—
L1210 leukemia	Mouse	dba	0.10	0.8	5	—
Jensen rat sarcoma	Rat	Sherman	0.02	0.9	6	0.06
Murphy-Sturm rat lymphosarcoma	Rat	Sherman	0.06	0.5	—	0.30
Flexner-Jobling carcinoma	Rat	Sherman	0.02	0.3	3	—
Walker carcinosarcoma 256	Rat	Sherman	0.07	1.3	9	0.05
Average			0.07	1.9	7	0.22

* Comparisons were made on CNA isolated from pooled tissues of groups of 5-10 animals each. All experiments were terminated at six hours after injection of the labeled compound.

DIRECT SEARCHES FOR EXPLOITABLE BIOCHEMICAL DIFFERENCES BETWEEN NORMAL AND NEOPLASTIC CELLS

The fact that a number of the known inhibitors of neoplastic growth affect nucleotide metabolism suggests that the cytotoxic specificity exhibited by these agents may result from quantitative or qualitative differences between normal and neoplastic cells having to do with polynucleotide synthesis. With this concept in view, studies have been carried out on the uptake of certain labeled precursors by a large variety of animal tumors and certain normal tissues.²⁴ To date tracer studies have been carried out in animals bearing some twenty-five different neoplasms. The labeled compounds employed were C¹⁴-formate, C¹⁴-2,6-diaminopurine, C¹⁴-adenine, and C¹⁴-guanine. Relative to normal tissues all tumors incorporated guanine poorly.

Most tumors utilize 2,6-diaminopurine as a polynucleotide guanine source as well as or better than intestine or liver. The specific activities of intestinal polynucleotides six hours after administration of labeled formate or adenine were somewhat higher than most tumors. These studies have now been extended to human tumors growing in the rat, and it is quite apparent that the two human tumors studied to date incorporate labeled formate, glycine, 4-amino-5-imidazolecarboxamide, 2,6-diaminopurine or adenine reasonably efficiently, but these human tumors are inefficient in utilizing C¹⁴-guanine or C¹⁴-hypoxanthine for polynucleotide synthesis when compared to the normal tissues of the rat.

This failure of tumor tissue to incorporate guanine or hypoxanthine into its polynucleotides at levels even approaching that observed in many normal tissues raises the question as to whether this rather profound quantitative difference is associated with the primary biochemical lesion of neoplasia. Further studies on the relative utilization of nucleosides and tides for nucleic acid synthesis in normal and cancer cells, as well as turnover studies, will be required before the significance of these observations can be ascertained.

SUMMARY

1. It appears to be fairly well established that a number of agents known to temporarily inhibit the growth of neoplastic cells in animals and man effectively block or inhibit chemical events leading to nucleotide synthesis. These include the folic acid antagonists, azaserine, the purine antagonists, nitrogen mustard and ionizing radiation.

2. Certain combinations of agents have been shown to be potentiating with regard to anticancer activity. Information on the modes of action of these agents suggests that they may be blocking sequentially in biochemical events leading to nucleotide synthesis.

3. Tracer studies designed to uncover exploitable biochemical differences between normal and cancer cells have demonstrated one rather profound difference between such cells—the poor incorporation of labeled guanine into tumor polynucleotides.

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Studies on the Metabolism of Tubercle Bacilli, and Their Fate After Phagocytosis

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This report deals with two phases of a continuing program aimed at providing knowledge concerning the metabolic patterns of the tubercle bacillus and its metabolic relationship to the host which it infects.¹ On the one hand tubercle bacilli, which are extraordinary in their ability to biosynthesize rather complex organic substances from simple substrates,² have been cultured on C¹⁴-substrates. The bacilli and products of growth and autolysis were recovered and analyzed. On the other hand, the fate of the bacillus and of the host cell after phagocytosis has been explored by exposing radioactive bacilli to leucocytes from guinea pigs. The experimental conditions permitted measurement of oxygen consumption, of C¹⁴O₂ production, and of cleavage of the bacillus to small molecules.

THE UTILIZATION OF C¹⁴-LABELLED SUBSTRATES BY THE TUBERCLE BACILLUS

Outline of Experimental Methods

A human strain of *M. tuberculosis* (H₃₇Rv) was grown for 6 weeks on Long's medium,³ containing C¹⁴-glycerol,⁴ under conditions which allowed the continuous collection of C¹⁴O₂ produced by the culture. At the end of the incubation period the bacilli were removed by filtration, and thoroughly washed. The bacilli were then heat-killed and dried over calcium chloride *in vacuo*. The filtrate and washings were combined and filtered through a Seitz filter. Trichloroacetic acid was added to a concentration of

10%, and any CO₂ evolved was collected. The precipitated protein of the medium was centrifuged off, washed and dried. The polysaccharide of the medium was obtained by filtering the deproteinized medium through an 11% collodion membrane, and washing the material which did not pass through the filter. The filtrate contained the small molecule fraction.

In experiments where C¹⁴O₂ was used in the presence of unlabelled glycerol, the experiment was carried out in a closed system, and the C¹⁴O₂ was generated from BaC¹⁴O₃ and perchloric acid.

All fractions obtained were analyzed for C¹⁴ by standard methods.⁵ The amount of residual glycerol was determined.⁶

Distribution of Carbon-14 in the Fractions Recovered

It is of interest to note that glycerol carbon comprises 84% of the total carbon of Long's medium, which contains, in addition to glycerol, such organic substances as asparagine and citrate. The disposition of C¹⁴ from the substrate (α - or β -C¹⁴-glycerol, or C¹⁴O₂) in the metabolic products of the bacillus is summarized in Table I. The carbon of the labelled glycerol appears to a significant degree in all the fractions. A rather larger proportion of the activity appeared in the bacterial bodies in the case of experiments using β -labelled glycerol than in those with α -labelled glycerol. A considerable amount of activity originally contained in glycerol carbon appeared in the small molecules (non-glycerol) of the ultrafiltrate fraction. The exact distribution of activity in this fraction (which contains amino acids, nucleotides, etc.) remains to be determined. It should be

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Table I. Disposition of C¹⁴ After Growth of Tubercle Bacilli on Various C¹⁴-Substrates

Fraction	Substrate					
	α -C ¹⁴ -glycerol*		β -C ¹⁴ -glycerol*		C ¹⁴ O ₂ †	
	(1)	(2)	(1)	(2)	(1)‡	(2)§
Recovery of C ¹⁴	100.3	70.0	60.0	98.3	—	—
Respiratory CO ₂	13.9	21.9	27.0	7.3	—	—
Bacteria	13.2	24.5	50.5	35.3	2.2	28.4
Protein of medium	2.0	4.5	5.0	1.5	0.8	19.0
Polysaccharide of medium	3.7	4.8	2.0	2.1	0.7	1.5
Ultrafiltrate of medium	67.5	45.0	15.7	54.0	96.4	50.9

* Results for each fraction as percentage of recovered C¹⁴.

† Results for each fraction as percentage of fixed C¹⁴.

‡ C¹⁴O₂ introduced into system after 4 weeks of growth.

§ C¹⁴O₂ introduced into system after 1 week of growth.

borne in mind that products of both growth and of autolysis were present in the medium after the long period of incubation.

In the case of experiments in which the organisms were grown in a closed system in an atmosphere containing $C^{14}O_2$, but on unlabelled glycerol, it was found that the $C^{14}O_2$ was fixed to an extraordinary degree (50 and 20% respectively of the total $C^{14}O_2$ recovered) and it is noteworthy that the greater part of the fixed C^{14} (i.e. C^{14} as organic compounds) appeared in the small molecule fraction.

In Table II are reported the relative specific activities of the carbon of the various fractions. It is very interesting that in the case of the bacteria obtained after growth on β - C^{14} -glycerol the specific activity of the carbon was higher than that of the substrate glycerol. This fact is in accord with the chemical nature of the organism in that it contains a great proportion of lipide.⁷ If the fatty acids of this lipide were biosynthesized by usual routes from two-carbon fragments, it may be envisaged that the situation described could indeed exist. In its passage to a two-carbon unit glycerol must lose one of its terminal carbons. Thus, on the average, two molecules of α - C^{14} -glycerol give rise to two two-carbon fragments, only one of which is labelled. On the other hand, every two-carbon unit derived from β - C^{14} -glycerol is labelled. Thus it may be calculated that a fatty acid derived entirely from α - C^{14} -glycerol could have a maximum carbon specific activity of 75% of that of the original glycerol, while fatty acids derived entirely from β - C^{14} -glycerol could have a maximum carbon specific activity of 150% of that of the original glycerol. The specific activity values of 123.0 and 129.9 for the carbon of the bacteria grown on the β - C^{14} -glycerol are thus a reflection of the large number of two-carbon units that have gone into its makeup.⁸ In the case of $C^{14}O_2$ experiments the differences between the two experiments reflect the time at which the C^{14} was made available to the metabolizing organism.

It is now of importance to carry out further fractionation of this material and to isolate pure metabolites which may be degraded to give the location of activity in the individual carbon atoms of each

compound isolated. Thus various lipide components of the bacterium, amino acids obtained from the tuberculin protein, and different sugars from the polysaccharide are under study, as well as components of the small-molecule fraction. In addition, C^{14} -labelled products of the bacillus, such as tuberculin protein, and the lesion-inducing lipopolysaccharide, could be made available for biological studies.

THE PHAGOCYTOSIS OF TUBERCLE BACILLI

The availability of dead C^{14} -labelled tubercle bacilli from the above experiments provided an impetus for studies on the fate of the organism after phagocytosis by guinea pig leucocytes. Later, when live organisms were required the experience previously obtained was useful in growing C^{14} -labelled tubercle bacilli of strain R₁Rv in small batches.

The experiments were designed to take advantage of labelling one of the two biological systems involved in phagocytosis experiments. Thus, the bacilli were labelled but not the leucocytes. The respiration and $C^{14}O_2$ production of labelled bacteria, alone, was to be compared with that in the presence of leucocytes (i.e., after phagocytosis). $C^{14}O_2$ liberated from bacilli alone represented the CO_2 of endogenous substrates oxidized whereas $C^{14}O_2$ liberated from phagocytized bacilli could have represented $C^{14}O_2$ from endogenous substrates of the bacilli plus $C^{14}O_2$ produced by leucocytic degradation and ultimate oxidation of bacillary material. To distinguish between these sources of $C^{14}O_2$, experiments were also carried out using heat-killed bacteria with very low endogenous respiration and $C^{14}O_2$ production.

Outline of Experimental Methods

Tubercle bacilli of strain R₁Rv were grown in submerged culture.⁹ The radioactive carbon sources were C^{14} -glycerol, C^{14} -acetate, or $C^{14}O_2$. The bacilli were harvested after about ten days by centrifugation, and were thoroughly washed to remove adherent radioactive contamination. Leucocytes were obtained from guinea pigs by intraperitoneal injection of 12% caseinate to obtain polymorphonuclear leucocytes, or a 1 mg per cent solution of glycogen to obtain monocytes. The peritoneal cavity was

Table II. Relative Specific Activity of Carbon of Different Fractions After Growth of Tubercle Bacilli on Various C^{14} -Substrates

Fraction	Substrate*					
	α - C^{14} -glycerol		β - C^{14} -glycerol		$C^{14}O_2$	
	(1)	(2)	(1)	(2)	(1)†	(2)‡
Carbon dioxide	91.8	100.0	102.1	78.6	—	—
Bacteria	99.0	95.6	123.0	129.0	2.2	5.5
Protein of medium	87.2	79.9	107.4	107.1	4.1	15.0
Polysaccharide of medium	104.0	84.5	65.0	85.8	0.5	0.4
Ultrafiltrate of medium	—	67.2	—	53.5	4.2	0.2

* The specific activity of substrate glycerol carbon is taken as 100; in the case of $C^{14}O_2$ fixation experiments that of the $C^{14}O_2$ recovered at the end of the experiment is taken as 100.

† $C^{14}O_2$ added to system after 4 weeks of growth.

‡ $C^{14}O_2$ added to system after 1 week of growth.

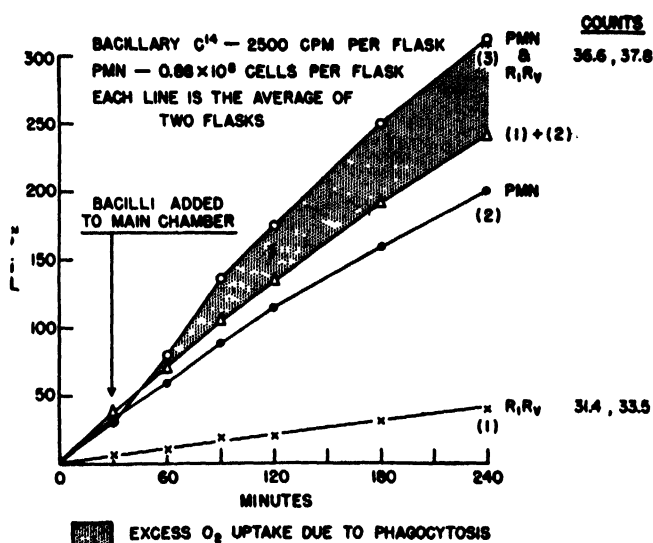


Figure 1. Respiration and $C^{14}O_2$ production of R_1R_v alone, and in the presence of PMN

washed after 16 hours in the former case or 3 to 5 days in the latter, and the cells were centrifuged and suspended in Krebs-Ringer phosphate buffer containing 30% serum. The polymorphonuclear leucocyte (PMN) preparation was found to contain $79.7 \pm 2.9\%$ PMN, the rest being mononuclear cells. The monocyte (MN) preparations contained 0–10% PMN. The preparations had the following oxygen uptake: PMN 36.5 ± 1.9 , and MN $57.2 \pm 2.7 \mu l O_2$ per hour and $100 \mu g$ cellular phosphorus.

Phagocytosis experiments were conducted as follows: Warburg flasks were set up to contain leucocytes in the main compartment and living or heat-killed bacilli ($100^\circ C$ for 15 min) in the sidearm. The center-well contained 10% KOH solution to absorb CO_2 . Some flasks contained leucocytes alone or bacilli alone. Experiments were also conducted using sonically disintegrated tubercle bacilli. After equilibration and preliminary measurements, bacilli and leucocytes were mixed with each other. Throughout the incubation measurements of respiration were continued. After four hours, the experiments were concluded by addition of acid to the main compartment. Total carbonate was then recovered from the center-well and the radioactivity counted. PMN were used for all radioactive experiments reported here.

Results

Figure 1 records a typical experiment using live tubercle bacilli. Several points may be noted:

(i) Upon addition of the bacilli from the sidearm, there was an increase in the rate of respiration of PMN. That this increase is due to a response of the leucocyte may be inferred from experiments with dead tubercle bacilli. The total oxygen uptake by the combined system after four hours is greater than the sum of the oxygen uptakes of the separate systems. Mean data on the respiration of leucocytes before, during, and after phagocytosis are in Fig. 2.

(ii) The total oxygen uptake of PMN and live R_1R_v together is greater than that observed for

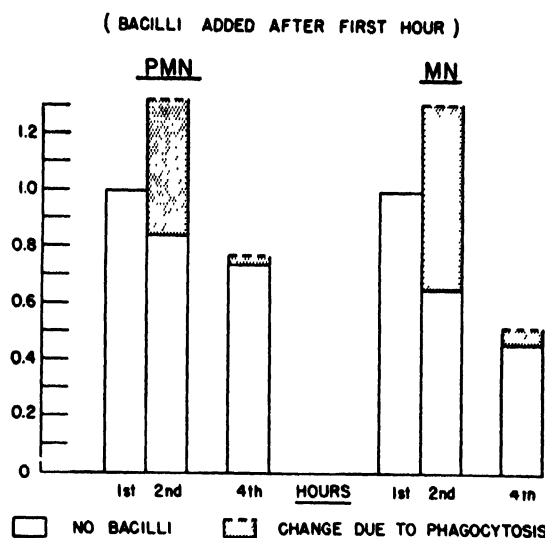


Figure 2. Influence of phagocytosis on the relative oxygen uptake of leucocytes

PMN and dead R_1R_v , indicating that live R_1R_v continue to respire inside the leucocyte.

(iii) The radioactive counts recovered from live R_1R_v alone are only slightly different from those recovered from the combined system. This is consistent with (ii) above.

(iv) Where examinations were carried out at the end of the experiments, about 90% of the bacilli were found to have been phagocytized.

The counting data for the carbon dioxide trapped in the centerwells are given in Tables III–VI.

In Table III data obtained with live tubercle bacilli, from experiments similar to that of Fig. 1, are shown. There is little difference in $C^{14}O_2$ production by bacilli alone or from phagocytized bacilli. The ratios of $C^{14}O_2$ production from tubercle bacilli *inside* leucocytes to $C^{14}O_2$ production by tubercle bacilli *outside* leucocytes is, in general, somewhat below unity. Since, as shown in Table IV, there is practically no $C^{14}O_2$ produced by the combined system of PMN and *dead* R_1R_v , it may be inferred that the $C^{14}O_2$ recovered from the combined system with *living* R_1R_v is exclusively due to the metabolic activity of the latter. Except for the case of two experiments with C^{14} -glycerol-grown organisms, where $C^{14}O_2$ evolution both with and without leucocytes is unaccountably high, it may be said that there is no significant breakdown of bacillary material to $C^{14}O_2$ by the leucocytes. The case is quite different for the three other organisms mentioned in Table IV where a considerable production of $C^{14}O_2$ by leucocytes from bacillary material occurs.

When C^{14} -labelled tubercle bacilli were sonically disrupted and exposed to the action of leucocytes, there was some oxidation by the latter of bacillary material to $C^{14}O_2$ as is shown in Table V.

A very interesting phenomenon was found with respect to tubercle bacilli killed at $56^\circ C$ for 60 minutes which are incapable of multiplying on ordinary media. These organisms alone have a very low residual oxygen uptake and $C^{14}O_2$ production. When

Table III. $C^{14}O_2$ Production from Live Tubercle Bacilli Alone, and After Phagocytosis

C^{14} -substrate for growth of bacilli	Counts per minute*		Number of experiments	Ratio† (i + n) (i)
	R_1R_2 alone (i)	R_1R_2 plus PMN† (n)		
Glycerol	75.33 ± 10.2	— 14.3	13	0.811 ± 0.061
CO ₂	48.46 ± 3.4	+ 0.94	16	1.019 ± 0.052
Acetate	52.50 ± 1.8	— 7.9	2	0.850 ± 0.014

* Results expressed as cpm/hour, 10^4 counts used and 10^8 PMN. Standard deviations of the means are given.

† The change in the amount of $C^{14}O_2$ produced is given.

‡ Ratio of $C^{14}O_2$ production from phagocytized bacilli to that from bacilli alone.

Table IV. $C^{14}O_2$ Production from Heat-Killed (100°C) Bacteria by PMN

Bacterium	C^{14} -substrate for growth	Counts per minute*	
		Bacteria alone	Plus PMN†
<i>M. tuberc.</i>	Glycerol	35.00 ± 1.60 (2)	+ 22.25 ± 2.80 (2)
<i>M. tuberc.</i>	CO ₂	0.36 ± 0.08 (18)	+ 0.97 ± 0.21 (28)
<i>M. tuberc.</i>	Acetate	2.80 ± 1.90 (2)	+ 0.03 ± 0.02 (2)
<i>M. phlei</i>	Glucose	0.95 ± 0.13 (3)	+ 7.20 ± 1.25 (3)
<i>M. pyogenes</i>	Glucose	0.00 (4)	+ 10.40 ± 0.67 (4)
<i>B. subtilis</i>	Glycerol	0.62 ± 0.56 (4)	+ 14.16 ± 0.33 (4)

* Results expressed as cpm/hour, 10^4 counts used and 10^8 PMN. Standard deviations of the means are given. The number of experiments performed is indicated in parentheses.

† The change in the amount of $C^{14}O_2$ produced is given.

Table V. $C^{14}O_2$ Production from Sonically Disrupted Tubercle Bacilli by PMN

C^{14} -substrate for growth	Counts per minute*		
	Disrupted bacteria alone	Plus PMN†	No. of experiments
Glycerol	3.5 ± 2.5	+ 7.1 ± 5.03	2
CO ₂	9.4 ± 2.5	+ 8.7 ± 1.19	14
Acetate	2.5 ± 1.8	+ 23.5 ± 0.35	2

* Results expressed as cpm/hour, 10^4 counts used, and 10^8 PMN. Standard deviations of the means are given.

† The change in the amount of $C^{14}O_2$ produced is given.

Table VI. $C^{14}O_2$ Production by Heat-treated (56°C) Tubercle Bacilli, Alone and After Phagocytosis

C^{14} -substrate for growth	Counts per minute*		No. of experiments performed
	Bacilli alone	Plus PMN†	
Glycerol	4.53 ± 2.35	+ 28.1 ± 4.65	4
CO ₂	6.32 ± 2.27	+ 17.8 ± 3.60	5

* Results expressed as cpm/hour, 10^4 counts used and 10^8 PMN. Standard deviations of the means are given.

† The change in the amount of $C^{14}O_2$ produced is given.

phagocytized, however, $C^{14}O_2$ production increases greatly as is shown in Table VI. That this effect is not due to breakdown by leucocytes of bacillary material is suggested by the experiments with bacilli killed at 100°C. The great increase in $C^{14}O_2$ production on phagocytosis of the organisms killed at 56°C is thought to be due to activation of the damaged organisms inside the phagocyte. In addition, an actual increase in the respiration of the phagocytized bacilli may be detected. The stimulatory effect may also be obtained by adding sonically disintegrated leucocytes to the heat-treated bacilli. This phenomenon could be due to the provision by the phagocyte of an enzyme, coenzyme, or substrate effectively lost to the bacillus on heat treatment. The significance of such a phenomenon in the host-parasite relationship remains to be explored.

The information provided by these experiments may be summarized as follows: (1) The live tubercle bacillus continues to consume O_2 and produce CO_2

even when phagocytized, and its carbon is apparently not oxidized by the leucocyte. It is of note that only small amounts of radioactive material capable of passing a bacterial filter were detected after phagocytosis of live or dead labelled tubercle bacilli. (2) The heat-killed tubercle bacillus, in contrast to *M. phlei*, *Micrococcus pyogenes* and *B. subtilis*, is also not vigorously attacked by the phagocyte to yield $C^{14}O_2$. (3) The bacillary material of sonically disrupted tubercle bacilli is, however, subject to attack by the leucocyte and part of the tubercle carbon is converted to $C^{14}O_2$.

The intact cell-surface of the tubercle bacillus would thus appear to be an essential factor in the resistance of the bacillus to enzymatic attack by the leucocyte, at least within the period of the present experiments. It is realized that the experiments described, and the inferences drawn from them, represent only a first step in the analysis of metabolic interrelationships between host-cells and parasites in a bacterial infection.

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Biosynthesis of Abnormal Proteins in Multiple Myeloma

By F. W. Putnam,* USA

The profuse synthesis and the diverse nature of the abnormal proteins elaborated by different patients with multiple myeloma represent one of the most profound alterations in protein metabolism yet encountered. This disease is a tumor of the plasma cells that are implicated in globulin synthesis and in antibody production. It may result in the formation of abnormal serum proteins, the excretion of characteristic urinary (Bence-Jones) proteins, or in both aberrations. By physical and chemical study, we have previously demonstrated that the abnormal globulins are almost individually specific for the patient.¹⁻³ Similarly, the Bence-Jones proteins have proved to be almost individually characteristic.^{4,5} The origin of Bence-Jones protein is uncertain, but has been ascribed to renal cleavage of the myeloma serum globulins or to degradation of the tissue proteins associated with wasting in the disease. This paper describes the application of radioactive amino acids to the study of the rate of synthesis and excretion of Bence-Jones proteins in several patients. The results indicate that Bence-Jones protein is synthesized *de novo* from free amino acids rather than from preformed body proteins. The Bence-Jones protein is both rapidly synthesized and rapidly excreted.

EXPERIMENTAL

Subjects

Patient HH was a 67-year-old male who was admitted to the Argonne Cancer Research Hospital for terminal care and study. A bone survey revealed numerous osteolytic lesions. A sternal marrow aspirate was compatible with the diagnosis of multiple myeloma. The patient was anemic, and the urine contained Bence-Jones protein. Total serum proteins were 8.1 gm per cent, with 4.8 gm per cent globulins. Several transfusions were given and a brief course of urethane. Radiation therapy was given to his thoracic spine between 10-12-54 and 10-29-54. The patient exhibited renal insufficiency. Urea clearance, determined by the square root method, was 6 and later fell to 2 (normal 40 to 65). His hospital course was progressively downhill. He died of uremia on his 107th hospital day. Autopsy revealed a classical myeloma kidney but no amyloidosis or paramyloi-

dosis. The morphology of the plasma cells in the bone marrow was not uniform, and it was possible to distinguish at least three types of cells. A tumor was isolated from the sternum and from the vertebrae.

Patient MB was a 64-year-old female with a 5 year history of severe anemia. She was admitted to the Argonne Cancer Research Hospital for study and terminal care after Bence-Jones protein was discovered in the urine. The marrow contained large numbers of myeloma cells and was diagnostic of multiple myeloma. Roentgenographs revealed marked degenerative changes in the thoracic spine. Because of nausea from urethane, she was treated only by transfusions and with sodium salicylate. Periodic transfusions were needed to sustain the hemoglobin level above 7.4 gm, the initial value. Serum proteins were at first normal but later fell to a total protein of 4.8 gm per cent with 1.7 gm per cent globulins. There were a number of signs of renal insufficiency; e.g., the urea clearance, determined by the square root method, was 24. The patient died two months after admission. Postmortem examination revealed severe amyloidosis involving the entire gastro-intestinal tract. There was an amyloid nephrosis. The marrow changes were diffuse.

Serum and Urinary Proteins

Patient HH was on a low N intake but excreted daily 3 to 7 gm of Bence-Jones protein. The first sample that was isolated had a sedimentation constant (s_{20}) of 2.25 Svedberg units (S), but later samples obtained after isotope administration yielded a diffuse sedimenting boundary containing one component with a lower s_{20} . This protein was chemically heterogeneous as indicated by the presence of fractional quantities of several N-terminal groups. Electrophoretic analysis of the serum in pH 8.6 veronal buffer revealed the presence of a sharp elevated peak in the gamma region (mobility of -1.3 units). This component comprised about 35 per cent of the total protein, accounting for the inverted A/G ratio. The isolated myeloma globulin had an s_{20} of 6.6 S, the same as that of normal gamma globulin. However, it could be distinguished from the normal protein by immunologic analysis.

On the other hand, patient MB was hypoproteinemic and had a significantly diminished gamma globulin, with a relative but not an absolute increase in alpha and beta globulins. The patient daily excreted from 10 to 15 gm of a Bence-Jones protein

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that was very homogeneous in electrophoresis and ultracentrifugation. The sedimentation constant was 3.4 S, the value most often found for Bence-Jones protein. After precipitation with ammonium sulfate and prolonged dialysis against distilled water, this protein crystallized as needles. No N-terminal amino acid was detected.

Methods and Procedures

The serum and urinary proteins were characterized by analysis in the Tiselius type electrophoresis apparatus and with the analytical ultracentrifuge (Spinco, Model E). Although separation by starch zone electrophoresis was employed in some instances, most specimens for isotopic analysis were prepared by salt fractionation in the cold. The gamma-type myeloma globulin of patient HH was isolated by precipitation with 1.3M ammonium sulfate at pH 6.5. The precipitates were dialyzed against 0.9 per cent NaCl until free of sulfate ion, then against pH 8.6 veronal buffer, and stored in the frozen state. The urinary proteins of both patients were precipitated with 3M ammonium sulfate at pH 4.8. The precipitates were dissolved and dialyzed against 0.9 per cent NaCl until free of sulfate ion, then against distilled water until free of chloride ion, and finally lyophilized. Representative samples of these preparations were submitted to electrophoretic and ultracentrifugal analysis. N-terminal amino acids were investigated using the fluorodinitrobenzene method.

The radioactive amino-acids administered were obtained from commercial sources: DL-glutamic acid-1-C¹⁴ from Isotopes Specialties Corp. and L-lysine, uniformly labeled with C¹⁴, from Nuclear Instrument and Chemical Corp. The compounds were tested for toxicity and sterility prior to administration. For determinations of radioactivity, the protein samples were deposited on oxidized copper planchets as a dried film, both in the presence and absence of salt. The planchets were counted with a flow-gas counter (Atomic Instrument Co.) Counts were made to a minimum of 1000 over background and were corrected for self-absorption by the samples. Respiratory CO₂ was also collected, and the specific activity and cumulative activity were kindly measured by Dr. George Okita. In order to obtain an estimate of the excretion of labeled amino acids, the urine samples were decarboxylated by reaction with ninhydrin. The CO₂ was trapped with Ba(OH)₂ and counted as BaCO₃. The residual radioactivity was also measured. The specific activity of the urinary urea CO₂ is being determined separately by Dr. Okita, who is using the urease reaction.

Protocol

Indwelling venous and urethral catheters were inserted into HH to allow frequent withdrawal of blood and urine samples. Five-hundred μ c of DL-glutamic acid-1-C¹⁴ were administered by intravenous catheter. A total of 20 blood samples were taken up to the fourth day. On the first day, 23 urine

samples were obtained by catheter. A complete urine collection was maintained for 2 weeks. The first specimens were taken 15 minutes apart. After some weeks, aliquots of the more active samples of the lyophilized Bence-Jones proteins were pooled and dissolved in Ringer's solution. After sensitivity and sterility tests were made, a solution containing about 1 gm of labeled Bence-Jones protein was given by intravenous infusion. Urine specimens were collected as above.

Since MB was very anemic and hypoproteinemic, only urine samples were collected by catheterization. In this case, 300 μ c of L-lysine, uniformly labeled with C¹⁴, was injected intravenously. Thirteen urine specimens were obtained over a period of 64 hours.

RESULTS

Subject HH

The labeled carbon of L-glutamic acid-1-C¹⁴ is rapidly lost via the citric acid cycle. Hence, in this patient, the specific activity of the expiratory CO₂ attained a maximum within 25 minutes and then fell off with a half-time of 85 minutes (see Fig. 1). At 8 hours, the curve for the cumulative activity had reached a plateau. Similarly, the specific activity of the CO₂ liberated from urinary amino acids by ninhydrin was high at 25 minutes, reached a maximum at about 2.5 hours, and was greatly diminished at 8 hours. The respiratory activity is attributed to the L-isomer and the urinary activity to the D-isomer, for D-glutamic acid is not utilized by the rat.⁶ These results indicate that the tracer dose of amino acid was rapidly metabolized or incorporated into body proteins (L form) or rapidly eliminated (D form) so that the specific activity of circulating amino acid must have been low after the first hour or two.

As shown in Fig. 2, the samples of Bence-Jones protein obtained up to 35 minutes had negligible radioactivity (although the urinary amino acids had a high activity at this time). A similar lag has been reported for the formation of labeled serum proteins in the rabbit.⁷ The maximum activity in the excreted protein is attained at 5 to 6 hours, followed by a sharp decline. However, the Bence-Jones protein remains labeled for a longer period than might have been expected from the initial rapid decline.

Evidence derived from electrophoretic and ultracentrifugal analysis, and from solubility and isotopic data indicated that HH excreted at least two different Bence-Jones proteins. When the ammonium sulfate precipitate of the urinary protein was dialyzed against distilled water, each sample yielded an insoluble precipitate and a supernatant solution containing protein. As indicated in Fig. 2, the protein of the precipitate and of the supernatant solution had different levels of radioactivity, the former being more active. Electrophoretic analysis of the whole urine samples gave evidence that the composition of the Bence-Jones protein was changing throughout the experiment. All the protein excreted was of low molecular weight (s_{20} from 1 to 2 S).

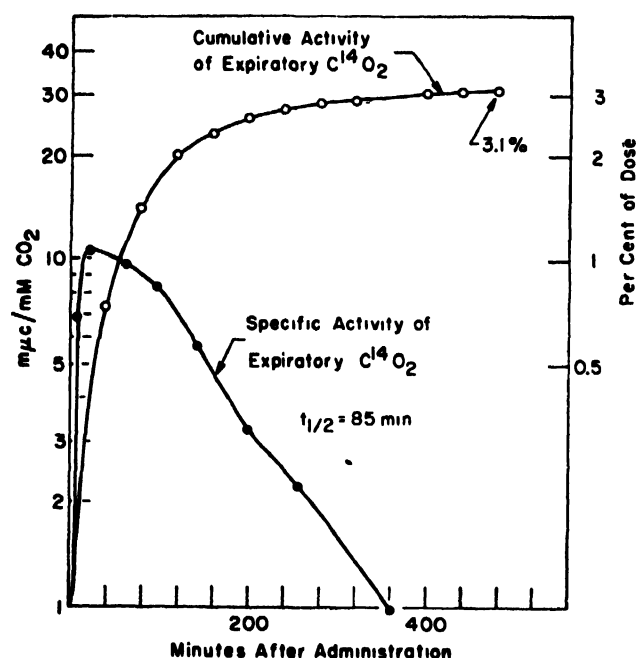


Figure 1. Specific activity and cumulative activity of expiratory CO_2 after injection of DL-glutamic acid-1- C^{14} into patient HH

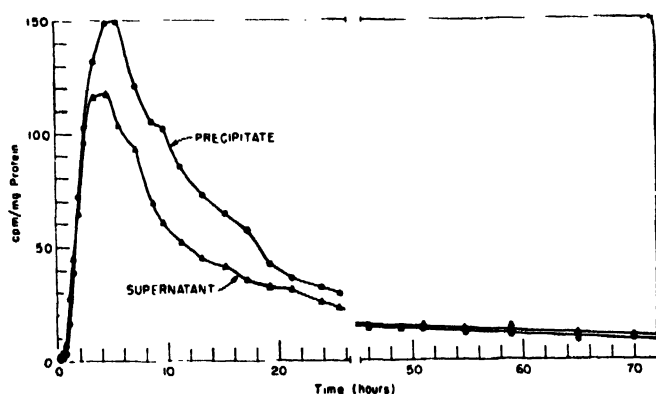


Figure 2. Activity of Bence-Jones protein from subject HH after injection of DL-glutamic acid-1- C^{14} . Supernatant refers to protein soluble in distilled, precipitate to the insoluble protein

Fractionation and isotopic analysis of the serum proteins have not yet been completed, but results on a few samples indicate that at comparable times, the gamma globulin had a much lower radioactivity than did the Bence-Jones protein. The urinary protein excreted after injection of the patient's labeled Bence-Jones protein also had a low activity, and it will be necessary to resort to enzymatic decarboxylation to obtain samples suitable for accurate counting.

Subject MB

A very similar course of events was observed in this patient after injection of 300 μc of C^{14} -lysine. However, owing to the slower metabolism of the carbon chain of this essential amino acid, the maximum in specific activity of the expiratory C^{14}O_2 occurred at about 2.5 hours. The specific activity fell off with a half-time of about 250 minutes, and the cumulative activity curve was still rising at 24 hours. As in the above experiment, the specimen of Bence-Jones protein obtained at 30 minutes was devoid of

radioactivity. The activity of the excreted protein rose rapidly to a maximum at about 9 hours. Thereafter, the activity fell sharply for the first day and then somewhat more slowly (see Fig. 3). The higher activity of the Bence-Jones protein of MB indicates that lysine is more efficiently incorporated than is glutamic acid.

The close similarity in the uptake and decline of isotopic amino acid in the urinary proteins of the two patients is illustrated by the semilogarithmic plot of Fig. 4. After 30 minutes, the labeled Bence-Jones protein first appears in the bladder. The maxima in the activity of the Bence-Jones proteins in the two patients are displaced by about the time difference between the maxima in the specific activity of the respiratory CO_2 . In both cases the decline in radioactivity of the Bence-Jones protein is not truly logarithmic. This curve has two components, the first with a half-time of about 8 hours, the second, with a half-time of about 20 hours. The latter segment appears to be analogous to the curves reported in our previous experiments in which large doses of amino acids labeled with N^{15} or C^{13} were given orally

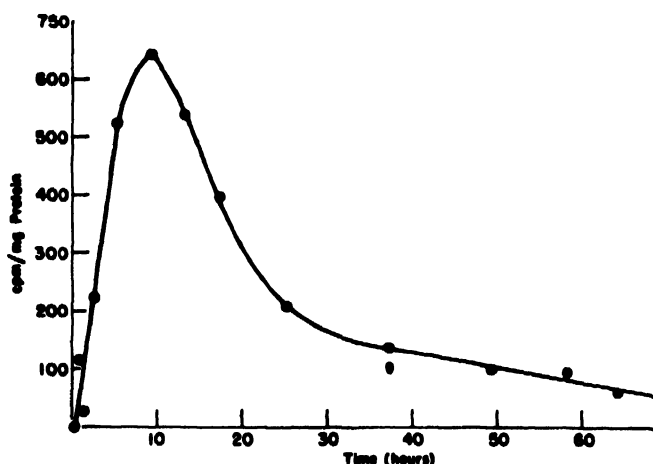


Figure 3. Activity of Bence-Jones protein from subject MB after injection of L-lysine- C^{14}

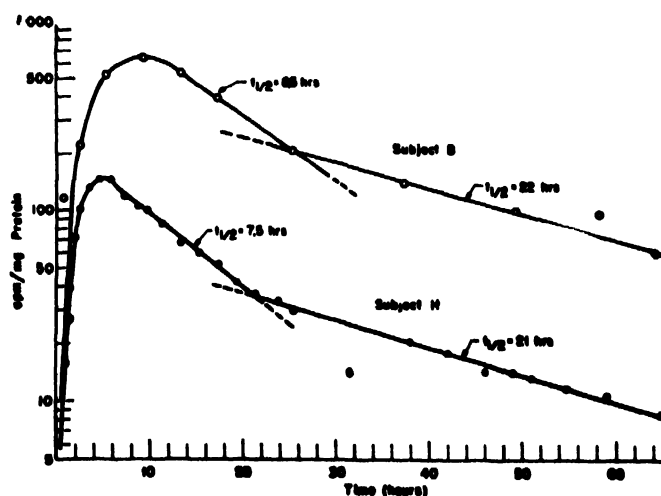


Figure 4. Comparison on a semilogarithmic basis of the activity vs time curves for the Bence-Jones proteins of patients HH and MB

to patients and urine collections were made only at 24 hour intervals. In these cases, the maximum in activity appeared on the first or second day.^{8,9}

Suitable tests have been established that the Bence-Jones proteins are not spuriously labeled by adsorption of C¹⁴-amino acid rather than by true incorporation. These tests include dialysis of labeled protein against unlabeled amino acid, dialysis of unlabeled protein in the presence of labeled amino acid, and incubation with specific amino-acid decarboxylases. Most significant is the finding that inactive Bence-Jones protein was found in the bladder at 25 minutes in patient HH although the urinary amino acids were extremely active at this time and respiratory C¹⁴O₂ was at a maximum specific activity.

DISCUSSION

Oral administration of amino acids labeled with N¹⁵ or with C¹³, followed by isolation of the abnormal serum and urinary proteins, had previously led us to conclude that the urinary protein is not derived by cleavage of circulating serum proteins nor by degradation of serum proteins.^{8,9} In fact, no evidence for any type of precursor relationship between the two types of abnormal proteins was found. These experiments also suggested that the Bence-Jones protein is rapidly formed *de novo* from free amino acids and is excreted rapidly. However, the heavy isotopes proved unsuitable for the precise study of the rate of synthesis and excretion of Bence-Jones protein, for they are too insensitive for intravenous administration at tracer levels and for direct measurement of the isotopic concentration of the protein. Thus, for the present investigation radioactive amino acids were chosen to facilitate study of the biosynthesis and fate of Bence-Jones proteins.

In this investigation, two subjects were studied who excreted different Bence-Jones proteins and whose serum protein patterns differed upon electrophoresis. The two patients were also given different tracer amino acids—in one case, a rapidly metabolized compound, glutamic acid, in the other, an essential amino acid, lysine. However, the specific activity-time curves for the two Bence-Jones proteins were almost identical. Only about 30 minutes are required for the formation of Bence-Jones protein and its excretion into the bladder. The specific activity attains a maximum in a few hours and then declines very rapidly. The half-time for the excretion of the labeled protein is comparable to that for the removal of labeled urea. As discussed previously, the conclusion is drawn that the urinary proteins are not derived by breakdown of serum or tissue proteins. This is based on the fact that all body proteins that

have been investigated have longer turnover times than could be reconciled with the isotopic data found for Bence-Jones protein. This is supported by the preliminary observations that the myeloma serum globulin of patient HH had a much lower activity than did his Bence-Jones protein on the first day of the experiment. The results of all the experiments are in keeping with the conclusion that the Bence-Jones proteins are rapidly synthesized *de novo* from free amino acids rather than from preformed proteins.

These experiments shed no light either on the site of synthesis or the possible physiologic function of Bence-Jones proteins. Study is now under way on material from both patients obtained after autopsy. The results thus far are compatible with the view that the plasma cell is the site of synthesis. Further studies of abnormal protein metabolism in multiple myeloma should aid in the understanding of this disease and possibly may further the elucidation of the mechanism for biosynthesis of normal serum proteins.

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Utilization of Nuclear Energy in Public Health Problems on the Epidemiology of Communicable Diseases

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In the field of epidemiology of communicable diseases, when significant progress is made, the results are measured in human lives and comfort. Nuclear energy has provided a new tool which has permitted real progress in solving epidemiological problems that in the past have been insoluble or difficult of attack by conventional methods. Use of radioactive isotopes has resulted in many important advances in studying public health problems relating to dissemination of communicable diseases by arthropod vectors, by animal reservoirs, and by water borne and air borne routes. Radioisotopes have been of great value in studying the ecology and natural control of insect vectors of disease. This is extremely important since DDT and other new insecticides become less useful year by year as more insects acquire resistance to them.

This paper is an attempt to review the progress in this rapidly advancing field, and to suggest new uses and techniques for making radioisotopes of greater value in studying the epidemiology of communicable diseases.

EPIDEMIOLOGICAL STUDIES

The exact role of arthropods in the epidemiology of certain diseases is still obscure, and use of radioisotopes provides a technique to help elucidate some of these relationships. Labeling of medically important arthropods with radioisotopes was initiated in 1949 by Hassett and Jenkins¹ and Bugher and Taylor.² Since that time extensive studies have been carried out using this technique on a variety of arthropods. Radioisotope tagging is an efficient method since it permits positive marking and identification of large numbers of arthropods with a minimum of labor and cost. It is of importance to determine dispersal range of these arthropods to enable control methods to be efficient and effective, and for understanding the role of the vectors in an epidemic. Various species of mosquitoes have been labeled by growing the larvae in solutions of P^{32} , Sr^{89} , and thorium, and releasing the radioactive marked adults in large numbers. The results of studying the dispersal habits and flight range of these mosquitoes using this technique are shown in Table I.

Dispersal and flight range of houseflies have been subjects of study for 50 years, and a variety of

marking techniques have been used. Most of these studies showed effective dispersal of 2 km or less. The effective dispersal and maximum flight ranges of houseflies and blowflies marked with radioisotopes are shown in Table II. Various species of *Phaenicia* blowflies have been made radioactive and found to disperse widely. Flight dispersal studies have been carried out by the US Public Health Service on the secondary screw worm *Callitroga macellaria* (16.5 km) and eye gnats, *Hippelates*. The primary screw worm *Callitroga hominivorax* was made radioactive by Radeleff *et al.*⁹ by rearing larvae in a meat medium containing P^{32} , or in wounds of living sheep or goats injected with P^{32} . The emerged adults were well marked and 10,000 were released for field studies. Blackfly pupae of *Simulium venustum* were tagged with P^{32} by Jenkins in 1950¹⁰ at Churchill, Manitoba by moistening them with a solution containing 0.3 μ c P^{32} /ml. Five species of blackflies were marked by Fredeen *et al.* in 1953 in Saskatchewan using P^{32} . Colonies of radioactive larvae and pupae were found over 0.5 km downstream from the point of release, and an adult was recaptured 100 m from the stream.

About 6500 American cockroaches *Periplaneta americana* were labeled with P^{32} and released in sewer manholes at Phoenix, Arizona, by Schoof and Siverly¹⁹. The 906 recaptured radioactive roaches indicated no movement in the sewerage system in 8.5 weeks, but one tagged roach was trapped in a yard 20 m away from a release site.

Efficient tagging and ease of detection using radioisotopes make possible accurate studies on determination of the abundance and size of natural populations. This is done by releasing a known number of radioactive labeled insects in an area, collecting, trapping, and then calculating the per cent of marked recaptures to unmarked naturally occurring insects. By knowing the original number of released tagged insects, and the daily mortality rate, the natural population can be calculated.

The longevity and mortality rates of various insects have been ascertained with radioisotopes in studies reported in Tables I and II. The timing and size of different broods and generations can be verified by tagging as shown in the studies on *Aedes taeniorhynchus* by Provost in 1952.⁵

Many other ecological studies are made possible or facilitated by using radioisotopes. The habitat and

* National Research Council, Washington, D. C.

Table I. Dispersal and Flight Range of Radioactive Marked Mosquitoes

Species	Locality	Number mos- quitoes released $\times 10^3$	% recovery	Effective dispersal, km	Maximum flight range, km	Reference
<i>Aedes aegypti</i>	Nigeria	276	0.11	--	1.2	Bugher and Taylor ²
<i>Aedes communis</i>	Churchill	3000	0.005	0.5	1.6	Jenkins and Hassett ³
<i>Aedes nigromaculis</i>	California	400	0.12	1.6	3.0	Thurman and Husbands ⁴
<i>Culex tarsalis</i>	California	10	—	—	1.6	Thurman and Husbands ⁴
<i>Aedes taeniorhynchus</i>	Florida	1000	0.03	16.0	32.0	Provost ⁵
<i>Aedes</i> sp. & <i>flavescens</i>	Saskatchewan	470	0.006	—	0.9	Shemanchuck <i>et al.</i> ⁶
<i>Psorophora confinnis</i>	Arkansas	50	0.09	3.4	10.0	Quarterman <i>et al.</i> ⁷
& <i>P. discolor</i>		—	—	—	2.5	
<i>Anopheles</i> (<i>Kerteszia</i>) spp.	Brazil	—	—	—	0.9	Aragao ⁸

feeding habits of *Aedes communis* in the Hudson Bay area were discovered during studies on dispersal by Jenkins and Hassett.³ Tagging this species proved that it was not an important biting pest of man, as had been assumed. Flower feeding habits of northern *Aedes* mosquitoes were confirmed experimentally by West and Jenkins.²⁰ Arctic flowers were made radioactive with P³² and adult mosquitoes ingested radioactive nectar or plant sap.

Studies of the epidemiological role of arthropods using radioisotope marking during epidemic outbreaks have not been made, but several preliminary experiments of this type have been carried out. In a city in the Crimea, Shura-Bura²¹ studied houseflies in their relation to carrying diseases from outdoor privies. Human fecal material was saturated with a solution of P³² containing 2×10^6 cpm/ml. Radioactive flies were collected in traps in kitchens in two houses 25 to 30 m from the outdoor privy. It was determined that flies kept in a laboratory after feeding on the feces remained radioactive for 11 days. In Texas, *Drosophila* fruit flies were found to be radioactive after visiting P³² baits in privy pits, and marked flies were recovered from 8 of 10 nearby houses.²² In other tests 4000 P³² marked fruit flies

were released in privy pits and were recovered in houses and other privies. Marked *D. melanogaster* were found up to 160 m and *D. repleta* up to 320 m from the release points. This indicated that a major portion of the *Drosophila* which were found in houses had at one time or another frequented privies. These results should be borne in mind in relation to outbreaks of dysentery and poliomyelitis.

DISEASE TRANSMISSION BY VECTORS

A very promising and fertile but unexplored field awaits investigators using radioisotopes in studying disease transmission by arthropod vectors. If the pathogenic organisms can be tagged, many experiments are possible in tracing the organisms through the arthropod and determining the sites and numbers of disease organisms disseminated. A large number of viruses, bacteria, protozoa, and nematodes have been labeled with various radioisotopes as shown by examples in Table III.

In an attempt to study the persistence and multiplication of *Escherichia coli* in the housefly, Jenkins *et al.*³⁴ fed flies P³²-labeled bacteria in 0.5M sucrose-milk. The flies were mounted on paraffin blocks on sticks and suspended over saline blanks as described

Table II. Dispersal and Flight Range of Radioactive Marked Flies

Locality	Habitat	Number flies released $\times 10^3$	% recovery	Effective dispersal, km	Maximum flight range, km	Reference
Housefly <i>Musca domestica</i>						
Oregon	Rural	36	4.6	1.6	20.0	Lindquist <i>et al.</i> ¹²
Oregon	Rural	54	2.78	6.8	32.0	Yates <i>et al.</i> ¹³
Arizona	City	31	0.73	3.4	5.0	Schoof <i>et al.</i> ¹⁴
Arizona	City	56	0.51	8.0	8.0	Schoof <i>et al.</i> ¹⁴
Georgia	Rural	13.5	8.0	13.6	16.5	Quarterman <i>et al.</i> ¹⁵
Georgia	City	40	0.24	6.8	12.6	Quarterman <i>et al.</i> ¹⁶
Arizona	City	342	1.8	3.9	14.1	Schoof & Siverly ¹⁷
Blowfly <i>Phormia regina</i>						
Oregon	City	1.2	14.0	6.8	13.6	Lindquist <i>et al.</i> ¹²
Oregon	City	60	2.64	24.0	45.6	Yates <i>et al.</i> ¹³
W. Va.	City	16	0.06	8.0	16.5	Schoof and Mail ¹⁸
Georgia	City	—	—	3.4	—	Quarterman <i>et al.</i> ¹⁶

Table III. Disease Organisms Labeled with Radioisotopes

Organism	Radioisotope	Reference
Influenza virus	P ³²	Graham & McClelland ²³
Virus bacteriophage	P ³²	Lesley <i>et al.</i> ²⁴
<i>Pasteurella pestis</i>	P ³²	Goldberg & Leif ²⁵
<i>Escherichia coli</i>	I ¹³¹	Schweinberg <i>et al.</i> ²⁶
<i>Mycobacterium tuberculosis</i>	P ³²	Strom & Rudback ²⁷
<i>Bacillus subtilis</i>	P ³²	Harper & Morton ²⁸
<i>Trypanosoma equiperdum</i>	P ³²	Moraczewski & Kelsey ²⁹
<i>Plasmodium vivax</i>	Fe ⁵⁵	Ferrebee <i>et al.</i> ³⁰
<i>Plasmodium lophurae</i>	Fe ⁵⁵ , Fe ⁵⁹	Thompson <i>et al.</i> ³¹
<i>Onchocerca volvulus</i>	Sb ¹²⁴	Barter <i>et al.</i> ³²
<i>Trichinella spiralis</i>	P ³²	McCoy <i>et al.</i> ³³

by Hawley *et al.*³⁵ and the radioactivity and bacterial counts of the saline were determined daily. This technique provides a method of developing a quantitative method of determining the growth and persistence of bacteria in the fly. In other experiments *E. coli* was labeled with P³² and fed to houseflies together with inert carmine. The time and amount of regurgitation and excretion of the bacteria was quantitatively checked by plating bacteria, counting the radioactivity and determining the presence of carmine. During the first few days of the experiment there was good correlation between the radioactivity and bacterial counts, but the flies then accumulated P³² indicating that the bacteria were probably not molecularly tagged. Di-isopropylfluorophosphate-P³² was reacted with certain bacteria and P³² was chemically bound; I₂¹³¹ was reacted and chemically bound in bacteria but not in sufficient amounts to carry out transmission experiments. These experiments serve to illustrate the type of studies on arthropod transmission of diseases which are possible using radioisotopes.

Use of radioisotopes offers a technique for studying the dissemination, vector ability, feeding habits and the numbers of organisms or size of infective dose of organisms introduced by medically important arthropods.

RELATION OF ANIMAL HOSTS AND PARASITES

The habits, life histories, and epidemiological role of many important ectoparasites of vertebrate reservoir and host animals are inadequately known. Ticks, mites, fleas, lice, and other medically important ectoparasites require more detailed ecological studies. None of these has been made radioactive and this is a most promising field of investigation. Use of this technique should provide needed epidemiological information on the length of time ectoparasites remain attached to their hosts under natural conditions, whether they change to other hosts, where they are located while unattached, longevity, dispersal, and population size. Tagging of these ectoparasites is quite simple since the host animal can be made radioactive as desired. White rats and rabbits were injected with P³² by Jenkins and

Hassett³⁶ so that female *Aedes aegypti* would take up 760–1073 cpm of P³² per blood meal. Similar results were obtained by Yates *et al.*³⁷ with the mosquitoes *Aedes sticticus* and *A. vexans*.

It is of epidemiological importance to know the bionomics of the vertebrate host and reservoirs of disease. In 1954 at Hudson Bay, Jenkins³⁸ made a female lemming radioactive by feeding 250 µc of P³². The radioactivities of the lemming and of its excretory products were measured at intervals for one month. After 24 hours the lemming measured 12 × 10⁶ cpm at 1 cm and deposited 8.8 × 10⁶ cpm of radioactivity in feces. Using this marking technique it is possible to establish the home range, activity periods, feeding sites, middens, and resting places of the host, which facilitates collection and study of its ectoparasites. The lemming was located by a field Geiger counter at 2 m. After the period of study the lemming gave birth to a normal offspring which was radioactive. In 1953 in Wisconsin Miller injected a *Microtus pennsylvanicus* with 200 µc of P³² and followed its movements with a geiger counter in a marsh. In Alaska, Griffin⁴⁰ put a Zn⁶⁵ band on a semipalmated plover to study returning of the bird at the nest, and in England, Godfrey⁴¹ used Co⁶⁰ rings on field voles *Microtus agrestis*. The band method is of course less suited to study of the arthropod parasites.

Internal parasites have been made radioactive and their relationships with their host animals have been elucidated. The parasite of benign tertian malaria was shown by Ferrebee *et al.*³⁰ to selectively invade young erythrocytes by using Fe⁵⁵. Many other valuable studies of this nature have been carried out using radioisotopes with protozoa, bacteria, and viruses. Several studies on helminth parasitism directly relate the parasite-host relationships. Use of P³² demonstrated that larvae of *Trichinella spiralis* in the encysted stage exchange ions with the host through the cyst wall and probably carry on active metabolism.³³ Radioiron labeled red blood cells in dogs were shown to be completely lost to hookworms *Ancylostoma caninum* in 16–27 days.⁴² The adult heartworm *Dirofilaria immitis* was shown to concentrate and localize radioantimony, indicating a specific affinity for this material which had been injected into parasitized dogs by Brady *et al.*⁶⁰ It appears possible to trace the path of internal parasites through the animal body by the use of gamma-emitting radioisotopes.

INSECTICIDE AND NATURAL CONTROL OF VECTORS

Control of arthropod vectors provides a constant challenge since almost every effort by human beings results in a counter move by the arthropods. The widespread use of newer residual insecticides has resulted in the development of resistance which requires use of greater quantities of more effective insecticides. When the main control effort is expended in applying insecticides, other controls such

Table IV. Insecticides Labeled with Radioisotopes

Insecticide	Radioisotope
DDT	C ¹⁴ , Br ⁸² , I ¹³¹
BHC	Cl ³⁵ , Deuterium
DFP, HETP, TEPP, TiPPP, TnPPP, TnBPP, OMPA, diethyl phosphoric acid, parathion, paraoxon	P ³²
HETP	C ¹⁴
Parathion	S ³⁵ , & P ³²
HCN	C ¹⁴
Sulfur dioxide	S ³⁵
Carbon disulfide	S ³⁵
Methyl bromide	C ¹⁴ , Br ⁸²
Methyl iodide	C ¹⁴
Nicotine	C ¹⁴
Pyrethrins	C ¹⁴
Elemental sulfur	S ³⁵
Elemental arsenic, arsenic trioxide, arsenious acid	As ⁷⁶
Lead arsenate	As ⁷⁴ , ThB ²¹²
Disodium arsenate	As ⁷⁴
Basic calcium arsenate	As ⁷⁴

as natural parasites and predators, sanitation, drainage, and filling, control of animal reservoir hosts, etc., usually suffer, and the control results are not always predictable. Radioisotopes provide a research tool for furthering basic research on control of vectors and disease organisms.

Radioisotopes have already been of great use in studying the site of entry, mode and site of physiological action, and fate of insecticides in arthropods. Numerous insecticides have been labeled with radioisotopes and these have been summarized in Table IV from reviews by Dahm.^{43,44} Radioisotopes have been used in studying arthropod resistance to insecticides. Use of labeled insecticides also permits study of the distribution of insecticide on treated surfaces.⁴⁵

Assessments of the dissemination of airplane sprays have been made by Ladd *et al.*⁴⁶ using Mn⁵⁴ and by Jenkins and Davis⁴⁷ using radiogold Au¹⁹⁸. This radioisotope technique has several advantages over dye tracers in assessing the results of airplane spraying of insecticides. These are greater accuracy, shorter time for assessment, fewer personnel required, and ability to measure depositions on vegetation and uneven surfaces without the use of panels. This technique should be utilized in assessing fog and smoke generators and other methods of insecticide dispersal.

Field observations on predation of mosquitoes, flies, and other medically important arthropods are only occasionally made and few quantitative data are available. Predation of radioactive arthropods presents a definite record in the predator. Preying mantids became highly radioactive when they fed on adult *A. aegypti* mosquitoes labeled with P^{32/36} and large dytiscid beetles showed 25,500 cpm after feeding on radioactive larval mosquitoes *A. pullatus* and *A. excrucians*.⁴⁸ Thurman and Husbands⁴ report that beetles and water bugs of several species showed over 20,000 cpm after consuming radioactive mos-

quito larvae, and spiders and frogs became radioactive from eating radioactive adult *A. nigromaculis* mosquitoes. Jenkins³⁸ put radioactive mosquito larvae with various predators. After 48 hours dytiscid and gyrinid beetles, dragonfly, damselfly, caddis fly, and chaoborine larvae all showed large amounts of radioactivity. After three million radioactive adult *A. communis* mosquitoes had been released, seven species of seven genera of nocturnal spiders in the area became quite radioactive.

Radioisotopes can also be used to determine parasitic versus commensal habits of suspected parasites. The mite *Pimeliaphilus podapolipophagus* was proven by Cunliffe⁴⁹ to be parasitic on cockroaches. The mites became quite radioactive after feeding on roaches which had been fed radioactive NaCl. Jenkins³⁸ while studying mermithid nematode parasites (*Hydromermis*) of mosquitoes found that nematodes emerging from radioactive *Aedes communis* had an average radioactivity of 459 (range 300–800) cpm/nematode. This radioactive marking made it possible to identify specific nematode worms under field conditions.

RADIATION CONTROL OF VECTORS AND DISEASES

Radiation from radioisotopes and X-rays is proving valuable in preventing spread of diseases due to use in cold sterilization of foods, and pharmaceutical products such as antibiotics; and also in controlling or killing vectors, disease organisms, and parasites. Large stores of highly radioactive reactor wastes may be made available in the future which have great potential value. If the radiation from these radioisotope wastes can be made economically useable, cold sterilization of food will become important in replacing refrigeration, steam sterilization, and vacuum packing. Food sterilization by beta, gamma, and X-rays is effective at about $1.5\text{--}3.0 \times 10^6$ rep.^{50,51} Certain problems such as preventing off flavor and destruction of food values must be studied before commercial exploitation is practical.

Elimination of trichinella in pork appears to be feasible by exposure to 750,000 to 10^6 rep.⁵² About 20,000 rep of Co⁶⁰ gamma radiation prevents maturation of the worm larvae to adults and 12,000 rep completely sterilizes the female worms. Either of these will control trichinosis without high radiation cost or deleterious flavor changes. In fission product tests, complete sterilization occurred at 10,000 r. Pasteurization achieved by 60,000 r increases the refrigerator shelf life of meat by approximately 100%.

Radiation from radioisotopes may prove to be of value in controlling or killing disease vectors. Eradication of the screw worm fly *C. hominivorax* may be possible in the southeastern United States by releasing large numbers of irradiated sterile males to prevent egg-laying by the small overwintering population in southern Florida. Bushland and Hopkins⁵³ found that about 5000 r of either gamma radiation from Co⁶⁰ or X-rays resulted in sterilization

of the males. Hallberstaedter *et al.*⁵⁴ found that *Calliphora* larvae X-irradiated with 4000–5000 r did not develop into adults, and that in *Culex* mosquito larvae 12,000 r resulted in death. The younger the stage of insect irradiated, the less radiation is required to kill. Many papers have appeared on X-irradiation of *Drosophila* fruit flies and other insects. The radiation from Ta¹⁸² and Co⁶⁰ required to kill six species of insects was 64,000 r.⁵⁵ Irradiation of adults with 16,000–32,000 r was necessary to inhibit reproduction. An X-ray dosage of 30,000–40,000 r killed 100% of *Aedes aegypti* adults in 12–21 days.⁵⁶

Radioisotope radiation offers a possible means of killing or arresting development of disease organisms in the animal host body due perhaps to the large uptake of metabolically active elements by these organisms.

Ingestion of P³² by the yellow fever mosquito *Aedes aegypti* during the period of extrinsic incubation arrested development of malaria *Plasmodium gallinaceum* during the oocyst stage.⁵⁷ Dissection of the salivary glands showed no sporozoites in 93 mosquitoes given P³², whereas 11 of 15 controls showed these forms. *A. aegypti* X-irradiated with 5000–30,000 r before infection with *Plasmodium gallinaceum*⁵⁸ caused decrease in the number of oocysts produced in the mosquito. Erythrocytes parasitized by *Plasmodium gallinaceum* were shown by Clarke⁵⁸ to take up more P³² than normal cells, probably due to a more active rate of metabolism. Radioiron Fe⁵⁵ and Fe⁵⁹ were administered by Thompson *et al.*^{31,59} to Pekin ducks and canaries to study radiation effects on *Plasmodium lophurae* and *P. cathemerium*. At levels of radioactivity of 0.1 and up to 1.0 μ C/ml of blood there were no effects on initial infection, morphology, staining reaction, or ability to produce an acute parasitemia.

Hallberstaedter showed in 1914 that the protozoans causing sleeping sickness *Trypanosoma brucei* when treated with beta radiation from radium became non-infective but remained viable and motile. *T. gambiense* was killed by 600,000 r and made non-infectious for mice by 12,000 r of X-rays.⁶¹ *T. cruzi* was rendered non-infectious for mice by X-ray dosages between 51,000 r and 100,000 r.⁶²

Numerous studies have been carried out in attempts to control or reduce infections due to a variety of bacterial and viral diseases as reviewed in 1946 and 1947 by Lea.^{63,64} This field holds great promise, but the results in many cases have been variable and more research is required.

Radiation from radioisotopes has been used to attempt control of worm parasite infections. Polonium-210 and selenium-75 were administered to mice infected with the worm form of *Schistosoma mansoni*, but control was not obtained.⁶⁵ Filariasis infection (heartworm, *Dirofilaria immitis*) in dogs was treated with Sb¹²⁴ by Brady *et al.*⁶⁶ The radioactive antimony became concentrated and localized in the worms. A human infection of the blackfly trans-

mitted worm *Onchocerca volvulus* was treated by Barter *et al.*⁸² using radioactive trivalent antimony. A specific uptake of the radioantimony by adult *Onchocerca* parasites was indicated in one nodule.

STUDIES ON AIR-BORNE AND WATER-BORNE DISEASES

Labeling of communicable disease organisms has resulted in valuable aerosol studies on intake and retention of organisms in the body of hosts, and on effect of particle sizes. Examples of some of these studies include use of P³² labeled plague bacteria *Pasteurella pestis* which showed that when mice inhaled an aerosol, they retained over 80% of the calculated dose. The respiratory retention was about 30%, and the gastrointestinal about 70% of the retained organisms.²⁵ Particle size studies have been carried out by exposing guinea pigs to P³²-labeled *Bacillus subtilis* spores. Aerosols of larger particle sizes generated from a spinning disc, and aerosols with single organisms from an apparatus used with a Collison spray were used. The test guinea pig tissues were assessed for spores by a geiger counter.⁶⁶ Use of marked organisms and particles has also helped in studying and improving air filters.

Radioisotopes have been used in research on water-borne diseases involving drinking water analysis, as a tracer in studies of sewage oxidation ponds, and in a variety of sanitary engineering research problems. Results of these studies are contributing to the prevention of spread of enteric and other bacterial and viral diseases.

Use of nuclear energy in studying the epidemiology of communicable diseases offers an extremely promising and fertile field to epidemiologists and other investigators. Additional discoveries of major importance in the field of public health can be expected in the near future. These discoveries will be of definite value for the benefit of mankind.

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A New Radioactive Method for Marking Mosquitos and Its Application[‡]

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THE METHOD

A method used in the study of animal migrations consists in marking a determined number of specimens, releasing them and by means of subsequent captures, evaluating the distance they travel. For obtaining the range of mosquito flight two techniques were previously employed. In the first, insects, which were taken in large numbers from nature, were dusted with powder (the type used for producing metallic colors), freed at a certain point and then captured at different distances, in order to determine the range of the migration.¹ In the second, mosquitos were hatched in a solution of radioactive isotopes, such as phosphorus, and the identification was made using counters.² Lattes³ suggested the possibility of identifying the radioactive mosquitos by means of nuclear emulsion. This method, which is of much higher sensitivity than that of counters, permits the use, even in small quantities, of long-lived radioactive elements like thorium and uranium for marking the mosquitos.

This method was tested in the present work, in which two techniques were employed: the use of prepared plates, and the use of liquid emulsion for the preparation of the plates containing the material to be studied. The second technique has the advantage of permitting simultaneous observation of the activated material and of the tracks of alpha particles emitted by it. In the first case, the material was put on a glass slide and placed in contact with the emulsion side of the plate. In the second case, the material was glued to a glass slide and then covered with the liquid emulsion. In both cases, the entire set up was kept for several days in the refrigerator. After exposure, the plates were developed and fixed.

The prepared plates used were type G-5 (Ilford), 50 μ thick, and the liquid emulsion was type G-5. For the detection of alpha particles, as in these experiments, less sensitive emulsions could have been used.

Results

When the mosquito was left whole, no tracks of alpha particles appeared in the emulsion. This would indicate that the thorium localizes internally, and that the alphas are absorbed before reaching the emulsion. Therefore the exoskeleton was removed, that is, the mosquito dissected, in order to obtain tracks of alpha particles. It was observed, in the plates covered with liquid emulsion, that the thorium was not evenly distributed throughout the material. In plates where the various organs of the same mosquito were separated, it was verified that the thorium was located in the Malpighian tubes. As observed by the technique of prepared plates, the ashes of five adult mosquitos, two months old, which had been hatched in a solution of $\frac{1}{100,000}$ thorium nitrate, still contained thorium. Control mosquitos, taken both from nature and cultivated in the laboratory, did not produce any alpha particle tracks in the emulsion.

Figure 1 shows part of a smear of the Malpighian tubes and of the genital apparatus, covered with liquid emulsion. The paths of the alpha particles are clearly visible. The exposure time was 17 days. The mosquito had 12 days of life in the adult phase and had been grown in a $\frac{1}{20,000}$ solution of thorium nitrate.

Figure 2 shows a microscope field which contains part of the Malpighian tubes of a female *Anopheles (Kerteszia) spp.* It shows tracks of alpha particles which originated in this organ. The mosquito was enclosed in paraffin and sections, 3 μ thick, were glued to the slide. To eliminate the paraffin, the slide was submerged for 10 minutes in xylol and then for 10 minutes in 100% alcohol. After drying, the plates were covered with liquid emulsion, developed, fixed and dyed with hematoxylin which permeated the emulsion. The exposure time was 29 days. The mosquito had 20 days of life in the adult phase, and had been hatched in a $\frac{1}{20,000}$ solution of thorium nitrate.

Cultivation of the Mosquitos

The same experiment was done using larvae obtained from puddles on the ground and also from the bromelia water. The latter developed well even in a solution of $\frac{1}{1000}$, while the former could not stand a concentration higher than $\frac{1}{500,000}$. The salt used was thorium nitrate. The solution in which the larvae

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were cultivated was prepared with water of the same nature as that from which the larvae were taken. As supplementary nourishment, the larvae received corn meal and granulated Fleischman yeast. The adults were kept in wire cages, covered with damp towels, and fed honey and human blood.

Preparation of Plates Covered with Liquid Emulsion

The slides with mosquitos were arranged on a level plane to ensure even distribution of the emulsion, which was poured over them at 51°C. In order that the emulsion should reach the edge of the slides, the operation was furthered with a stainless steel spatula, in accordance with the maker's instructions. For a slide with a surface of 18 cm², approximately 1.6 gm of emulsion were used.

Development and Fixation of Plates

The following developer was used:⁴ distilled water, 1000 cm³; boric acid, 35 gm; sodium sulphite (anhydrous), 18 gm; potassium bromide (10% solution), 8 cm³; amidol, 4.5 gm.

The developing time was 20 minutes, at a temperature of 28°C. The stop bath was a 0.2% acetic acid solution. The plates were put into the stop bath at an initial temperature of 28°C, which was gradually lowered to 5°C, and kept there for one hour.

The fixing bath contained: water, 1000 cm³; sodium hyposulphite, 400 gm.

After fixing, the plates were washed for two hours in water at 10°C, which was renewed each half-hour.

THE APPLICATION

By means of a preliminary experiment, verification of the adequacy of this method for the study of the dispersion of *Anopheles (Kerteszia) spp* inside the forest was made by one of the authors (Aragão). The experiment was conducted at Mata do Malucher (Fig. 3) in Brusque, Santa Catarina, Brazil.

Cultivation of the Mosquitos

As a protection for the culture of the larvae, a hut 3 m × 4 m in size (Fig. 3) was constructed by the stream that crosses the forest. The larvae of *Anopheles (Kerteszia) spp*. were obtained from the water of several species of bromeliac. From March 5th, 1952 to March 28th, 1952, 11,400 larvae were collected. The larvae were grown in a 1/10,000 solution of thorium nitrate, made by mixing a 1/100 distilled water solution of thorium nitrate with bromelia water, which had been filtered through cotton. As containers, flasks of 500 ml capacity were used.

In each flask containing the 1/10,000 thorium nitrate solution, 100 larvae were placed. During the

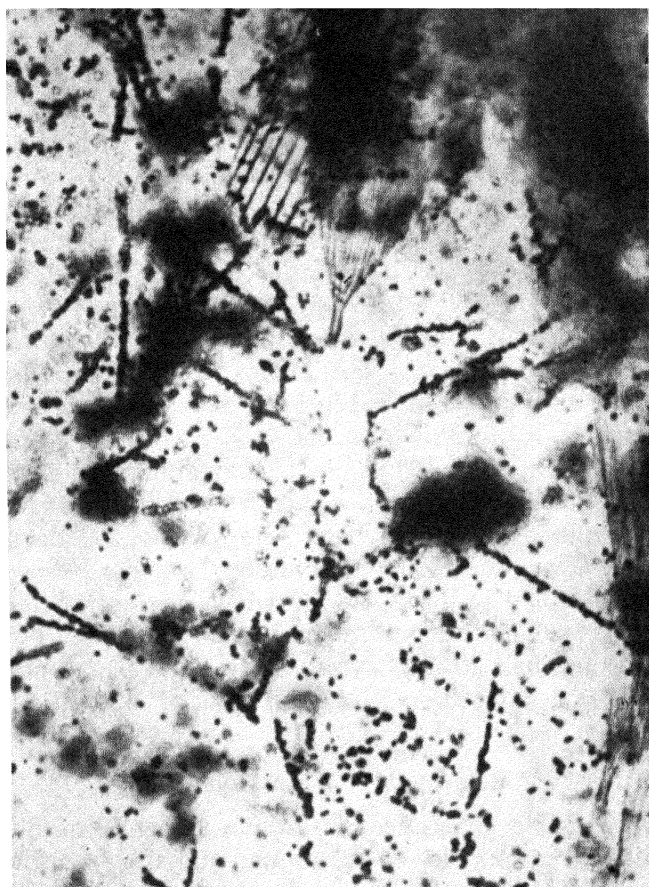


Figure 1. Part of a smear of the Malpighian tubes and of the genital apparatus, covered with liquid emulsion. The paths of the alpha particles are visible

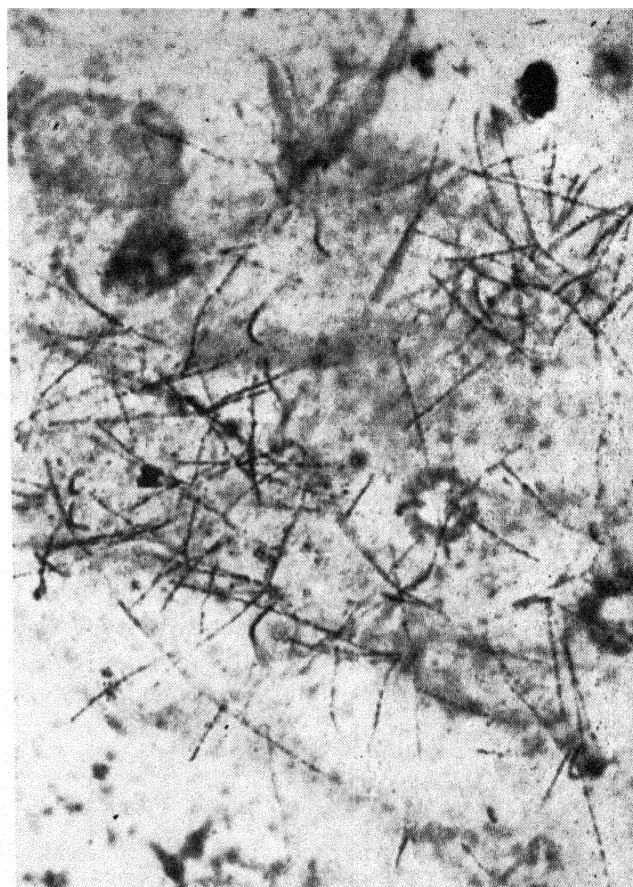


Figure 2. A microscope field which contains part of the Malpighian tubes of a female *Anopheles (Kerteszia) spp*. It shows tracks of alpha particles which originated in this organ

3 months in which the cultures were maintained, the level of the solution in the flasks was kept constant by addition of bromelia water. At least twice a week it was necessary to remove spider threads that had accumulated on the mouths of the flasks. At such times the pupae were counted and a small amount of corn flour was put in the flasks. Twice a month half a dozen of grains of Fleischman yeast were also placed in each of the flasks. The formation of a surface film, which commonly appears on laboratory cultures and destroys the larvae, was not observed in any of the flasks. From the counts of the pupae and the number of days the insect spend in the pupal stage, the number of hatched adults was evaluated to be approximately 3000.

The Collecting Procedure

Forty days after the cultures were started, mosquitos were caught in the area of the housing at collecting stations situated at 200 m intervals, along 4 different directions (Fig. 3). Men were posted at these points from 4:30 p.m. to 6:30 p.m. in order to catch the mosquitos that tried to bite them. As there were only six men available, collections were made each day at the stations where there were few mosquitos, but only every other day at those stations where they were more abundant. A total of 395 *Anopheles (Kerteszia) spp.* were caught during 45 days.

Incineration of the Mosquitos

The mosquitos that were caught during the first 15 days from April 15th–30th) were grouped accord-

ing to the collecting station from which they came. The mosquitos collected from May 1st–31st constituted different groups which were also distinguished by the locality of origin. When a group was large, it was divided into smaller groups, each of 20 to 30 mosquitos. Each group was placed in a porcelain evaporating dish and heated over an alcohol lamp until incinerated. The ashes from each dish were transferred to a glass slide 2.6 cm $\times$ 7.7 cm by means of a platinum wire wet by saliva. Saliva is very effective for making the ashes stick to the glass slide.

Identification of the Marked Mosquitos: Results

For detection of the radioactivity in the different samples of ashes, Ilford G-5 plates were used. A stainless steel plate 1 mm thick, provided with holes, was interposed between the emulsion and the slide with ashes. The holes corresponded to the location of the ashes on the slide (Fig. 4). This plate was used in

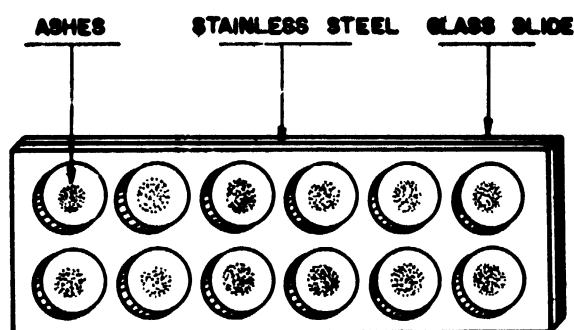


Figure 4. Stainless steel plate 1 mm thick, provided with holes, interposed between the emulsion and the slide with ashes

order to avoid direct contact of the ashes with the emulsion and also to keep each radioactive region well defined. The exposure was 30 days.

Figure 5 shows a microscopic field of the region of the emulsion covered by the steel plate. Figure 6 shows the region of the emulsion over which the ashes of the mosquitos caught at the 800 m station have been placed. The alpha particle tracks can be seen in Fig. 6.

Most of these emulsion regions which were exposed to the ashes of mosquitos caught in April failed to show a significant number of alpha particle tracks. In contrast, all the emulsion regions exposed to the ashes of mosquitos caught in May showed a great number of alpha particle tracks. Since the farthest collection station from the culture housing was at a distance of 800 m, it can be concluded that the maximum range of flight of the mosquitos was at least 800 m.

ACKNOWLEDGEMENTS

Grateful acknowledgment is made to Professor G. M. de Oliveira Castro for helpful discussions; to zoologist Rudolf Barth for preparing the sections, and to Mr. Luiz Machado de Lima for the photographs.

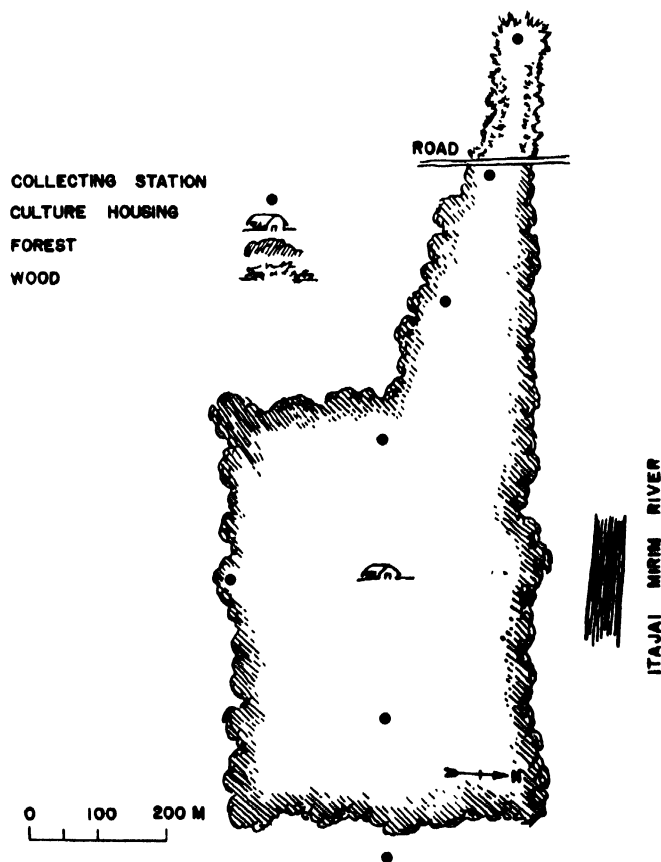


Figure 3. Woods of Malucher

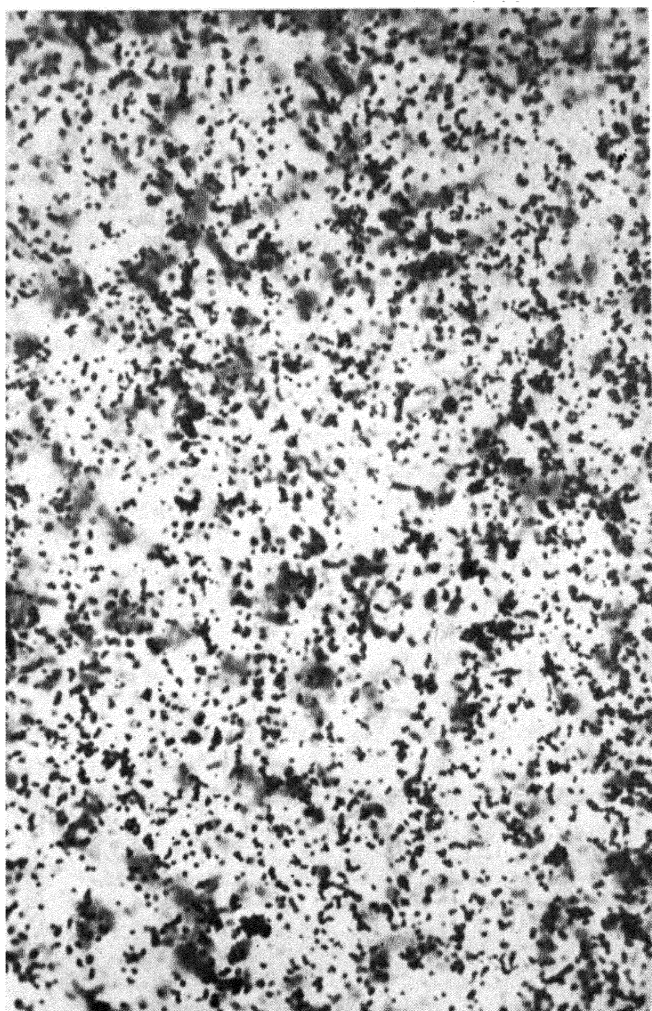


Figure 5. A microscopic field of the region of the emulsion covered by the steel plate

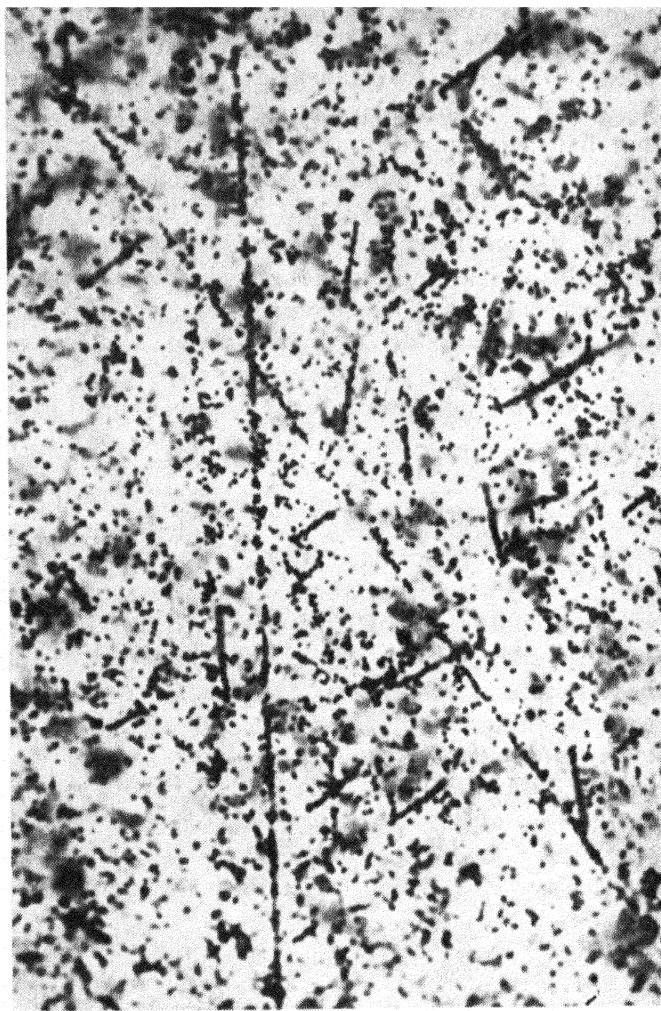


Figure 6. A region of the emulsion over which the ashes of the mosquitoes caught at the 800 m station have been placed. The short tracks are produced by alpha particles. The two long tracks have no significance for the present paper

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The Use of Radioactively Labeled Compounds in Study of Metabolic Inter-relationships of Host and Parasitic Helminths

By Richard D. Stoner and Lawrence V. Hankes,* USA

Radioactive isotopes have been used to advantage in medical entomology for studying the ecology and the epidemiological role of various insects and arthropods in disease transmission. Jenkins¹ has reviewed recent advances in this field gained through the use of radioisotopes. Isotope-labeled amino acids have been employed extensively for *in vivo* and *in vitro* incorporation studies in vertebrate tissues. A discussion of this literature may be found in the review by Borsook.² There are no reports in the literature concerning the use of carbon-14-labeled amino acids to study the metabolism of parasitic helminths and the effect of parasitism upon the metabolism of the host.

In the present study, *Trichinella*-infected mice were fed C¹⁴-labeled amino acid diets to test the ability of the nematode, *Trichinella spiralis*, to incorporate labeled compounds from the tissues of the host. It is well-known that encysted *Trichinella* larvae remain viable in the skeletal muscles of a host for long periods of time. McCoy, Downing and Van Voorhis³ fed *Trichinella*-infected rats radioactive phosphorus by stomach tube and demonstrated an exchange of phosphate ions through the cyst wall surrounding the larvae. Their work suggests an active metabolism by the encysted larvae. Our experiments indicate that encysted muscle larvae and migrating larvae prior to encystment in skeletal muscle incorporate C¹⁴ from their hosts which were fed diets containing the following C¹⁴-labeled amino acids: glycine-1-C-14, glycine-2-C-14, DL-alanine-1-C-14 and DL-alanine-2-C-14.

MATERIALS AND METHODS

Animals and *Trichinella* Infections

Swiss mice, 4- to 6-weeks-old females, from our own colony were used in these experiments. They were raised on a complete well-mixed diet supplemented with fresh carrots and lettuce. All animals used in these experiments were given an infective dose of 450 ± 15 *Trichinella* larvae on the same day. This infective dose produces a heavy larval invasion of the skeletal muscles and is usually sublethal in our mice. The procedure used in giving the experimental infections has been described in a previous report by Stoner and Hale.⁴

Feeding and Activity of Diets

Trichinella-infected mice and noninfected controls were given an initial 3-day feeding period of the mixed diet before being placed on the C¹⁴-labeled diets. The diets were supplemented with fresh carrots and lettuce and water was unrestricted. Carbon-14-labeled amino acids were added to the mixed diet as shown in Table I. Unlabeled amino acids were used to dilute the labeled amino acids to assure a uniform distribution in the whole diet which was mixed in a ball mill crock for 8 hours. Weighed amounts of the diet were fed in porcelain feeding cups in the morning and in the evening. Spilled food was weighed and deducted to approximate the amounts actually consumed. The average daily intake for 56 *Trichinella*-infected mice and 24 noninfected mice was 2.1 and 2.7 gm, respectively.

Preparation of Larvae for Analysis

Infected animals were killed, skinned, eviscerated and the carcass passed through a meat grinder. The *Trichinella* larvae were freed from their cysts by pepsin digest. The ground meat was digested for 3 hours at 37.5°C in a tap water solution of 1% pepsin, adjusted to pH 2.5 with hydrochloric acid. After digestion the larvae were separated from bone fragments and other undigested material by passing

Table I. Composition of C¹⁴-Labeled Diets

Components	Grams	Activity in μC^*
Mixed diet	2175.0	None
Glycine	25.3	None
Glycine-2-C ¹⁴	0.0211	11.36/mg
Total glycine diet	2200.3211	10.336/100 gm
Mixed diet	2159.6	None
DL-alanine	40.4	None
DL-alanine-2-C ¹⁴	0.02999	4.08/mg
Total alanine diet	2200.02999	5.553/100 gm
Mixed diet	2175.0	None
Glycine	25.3	None
Glycine-1-C ¹⁴	0.0228	17.544/mg
Total glycine diet	2200.3228	18.183/100 gm
Mixed diet	2159.6	None
DL-alanine	40.4	None
DL-alanine-1-C ¹⁴	0.0445	8.989/mg
Total alanine diet	2200.0445	18.182/100 gm

* To convert above millimicrocuries to counts per minute, multiply by 2.22×10^6 .

* Brookhaven National Laboratory

Table II. C^{14} Activity of *Trichinella* Larvae Isolated from Infected Mice

Experiment I						
Amino acid C^{14} -labeled diet	No. mice	Day of infection mice placed on C^{14} diet	Days fed C^{14} diet	Age of larvae when isolated by pepsin digest	C^{14} activity of larvae	
					carbon muc/mg	counts/min/mg carbon
Glycine-2- C^{14}	28	14*	7	21	0.130	289.49
Glycine-2- C^{14}	26	56	7	63	0.080	176.93
DL-alanine-2- C^{14}	29	14*	7	21	0.042	94.13
DL-alanine-2- C^{14}	26	56	6	62	0.024	52.17
Experiment II						
Glycine-1- C^{14}	26	180	6	186	0.139	307.47
DL-alanine-1- C^{14}	26	180	6	186	0.020	46.84

* Immature larvae recovered before they normally become encysted in skeletal muscle.

the digest solution through a U. S. Standard No. 50 metal sieve. The larvae were suspended in 3 liters of tap water and allowed to settle out. The supernatant was drawn off by aspiration. This procedure was repeated four times to remove soluble digestion products. The larvae were then washed five times by suspension in 50 ml of distilled water. The final suspension of larvae was centrifuged at 2500 rpm in 12 ml conical centrifuge tubes and supernatant removed by aspiration. The larvae in the tubes were frozen, dried *in vacuo*, and stored at -10°C . Carbon-14 analysis was carried out on weighed samples of the dry larvae using the method of Van Slyke, Steele and Plazin.⁵ The gas phase counting procedure was used for C^{14} analysis. Stannard *et al*⁶ reported the average dry weight of 10,000 larvae to be 3.5 mg. Therefore, the 4 to 6 mg weighed samples of lyophilized larvae analyzed for C^{14} contained approximately 11,000 to 17,000 larvae. In the present study the total carbon content of the lyophilized larvae based on twelve determinations was 46.9% by weight, with but little difference among samples.

Muscle Preparation Procedure

A 5 gm sample of muscle tissue with 10 ml of 0.9% saline was homogenized with a modified Elvehjem-Potter homogenizer. The homogenate was divided into two parts, A and B. Part A was mixed with 45 ml of 0.9% saline solution and centrifuged for 10 minutes at 2800 rpm. The supernatant was poured off and the residue resuspended in 45 ml saline and centrifuged. This procedure was repeated and the samples dried in a high vacuum oven at 30°C . These samples represent insoluble muscle protein separated from soluble proteins and other organic compounds.

Using a modification of Folin's tungstic acid procedure for total protein precipitation,⁷ part B homogenate was mixed with 4 ml of 10% sodium tungstate plus 35 ml of $\frac{1}{12}$ N sulfuric acid and centrifuged for 10 minutes at 2800 rpm. After decanting off the supernatant, the residue was resuspended in 4 ml of sodium tungstate, redissolving the protein. The protein was precipitated again by the addition of

35 ml of $\frac{1}{12}$ N sulfuric acid and centrifuged down. The resuspension, precipitation, centrifugation and decantation was repeated once more and the precipitate vacuum oven dried at 30°C .

RESULTS AND DISCUSSION

In the first experiment, the animals were placed on glycine-2- C^{14} and DL-alanine-2- C^{14} diets on day 14 and day 56 from the time they were given *Trichinella* infections and were fed the diets for periods indicated in the tables. The mice were then killed and the larvae were separated from the muscle by the pepsin digestion method. Noninfected control mice were also fed the C^{14} -labeled diets under the same conditions used with the infected animals. In the groups placed on the diets 14 days from the time of infection, the immature larvae were isolated before they normally become encapsulated in skeletal muscle. The groups infected for 56 days before being fed the C^{14} -labeled diets showed well-defined cysts surrounding the larvae at the time the mice were sacrificed.

In the second experiment, glycine-1- C^{14} and DL-alanine-1- C^{14} -labeled diets were fed to mice infected 180 days previously with *Trichinella* larvae. It was thought that an infection of 6 months' duration would be a more valid test of the permeability of the cyst wall and of the ability of the encysted larvae to incorporate C^{14} from the host.

The specific activity of the glycine-1- C^{14} and DL-alanine-1- C^{14} diets was much higher than the glycine 2- C^{14} and DL-alanine-2- C^{14} diets of Experiment I (see Table I). The purpose of this procedure was to compensate for losses of C^{14} activity by decarboxylation and, thus, assure an adequate level of C^{14} activity in the host for the experiment.

The C^{14} activity of the lyophilized *Trichinella* larvae expressed in millimicrocuries per milligram of carbon is summarized in Table II. From the C^{14} analysis of the isolated larvae, it is evidence that the amount of C^{14} found in the immature larvae (14-day mice) was almost double that found in the encysted larvae. The C^{14} content of the muscle samples is shown in Table III. The same proportion of C^{14} was incorporated into the muscles whether they were

Table III. Incorporation of C¹⁴-Labeled Amino Acids into Leg Muscle Protein

Experiment I						
Amino-acids C ¹⁴ -labeled diet	Days fed C ¹⁴ diet	C ¹⁴ activity of samples				
		Noninfected control* m μ c/mg carbon		Trichinella-infected m μ c/mg carbon		
		Insoluble protein	Total protein	Duration of infection in days	Insoluble protein	Total protein
Glycine-2-C ¹⁴	7	0.075	0.074	56	0.073	0.088
DL-alanine-2-C ¹⁴	6	0.011	0.014	56	0.012	0.012
Experiment II						
Glycine-1-C ¹⁴	6	0.041	0.055	180	0.032	0.054
DL-alanine-1-C ¹⁴	6	0.006	0.008	180	0.009	0.006

* Control groups consisted of 12 noninfected mice of the same age as the *Trichinella*-infected mice. One millimicrocurie = 2220 counts per minute, observed in the gas counter and corrected for about 10% loss by geometry (Van Slyke, Steele and Plazin, 1951).

normal or contained encysted muscle larvae 56 days after infection. As shown in Table II, the C¹⁴ content of the larvae isolated from the mice infected 56 days prior to glycine-2-C-14 feeding was not significantly higher than the C¹⁴ level of the muscles in which the parasites live. However, as shown in Tables II and III, in the group infected 56 days prior to DL-alanine-2-C-14 feeding, the amount of C¹⁴ found in the larvae was almost double that observed in muscle. It should be stated again that muscle protein from infected mice and controls showed about the same incorporation of C¹⁴.

The amount of C¹⁴ found in the larvae isolated from mice with 180-day infections is shown in Table II. These data show that the more firmly encysted larvae are able to exchange metabolites with the host. Because of the difference in the position of the C¹⁴ label in the amino acids used in the two experiments, it is not valid to compare the rates of incorporation by the 56- and 180-day infected larvae. However, experiments underway with glycine-1-C-14 and alanine-1-C-14 fed to 56-day infected mice, and glycine-2-C-14 and alanine-2-C-14 fed to 180-day mice should provide the necessary data for such a comparison. It should be emphasized that the C¹⁴ found in the samples analyzed may be present in various organic compounds and amino acids other than in the specifically labeled amino acid fed in the diet. The observed changes of the metabolism of glycine-2-C-14 and DL-alanine-2-C-14 may indicate a change in the metabolism of the larvae as they become more firmly encapsulated with duration of infection. The lower proportional incorporation of the labeled amino acids by the 180-day larvae may possibly be explained by a lower transmission of metabolites across a thicker cyst wall or by a loss of activity by the decarboxylation of the glycine-1-C-14 and DL-alanine-1-C-14. Part of the C¹⁴ activity from the alanine diets may be present in the larvae in the form of labeled alanine, aspartic acid or glutamic acid. Lien and Greenberg⁸

injected rats with alpha-labeled alanine and found C¹⁴ activity in alanine, glutamic acid and aspartic acid fractions of protein-free liver extracts.

A lower level of activity was shown in muscle samples from mice fed DL-alanine-1-C-14 compared to muscle samples from mice fed DL-alanine-2-C-14 (see Table III). From present knowledge of amino-acid metabolism, the reduced incorporation of C¹⁴ activity from carboxyl-labeled alanine cannot be explained by direct decarboxylation of the carboxyl-labeled alanine. Although numerous amino-acid decarboxylases are known, there is no evidence for an alanine decarboxylase. Littlefield and Sanadi⁹ and Schweet and Cheslock¹⁰ have shown the decarboxylation of pyruvate by enzymes extracted from pigeon heart muscle. Thus, a loss of C¹⁴ activity may occur through decarboxylation or pyruvate formed from the deamination of alanine.¹¹ A portion of the labeled carbon dioxide formed by this reaction may combine with oxalosuccinic acid to form ketoglutaric acid which is then aminated to form glutamic acid. The labeled carbon dioxide may also combine with pyruvate to form oxalacetic acid which may be aminated to form aspartic acid. Zamecnik and Frantz¹² incubated liver slices with C¹⁴-carboxyl-labeled DL-alanine and found activity in alanine, glutamic acid, aspartic acid, glycine, serine and proline fractions of acid hydrolysates of the liver protein.

The C¹⁴ activity in millimicrocuries per milligram of carbon of 0.1 ml samples of pooled serum is shown in Table IV. The amount of C¹⁴ in the pooled serum was several times greater than the amount of C¹⁴ found in the isolated muscle larvae and muscle samples. The pooled serum from the 56-day *Trichinella*-infected mice fed glycine-2-C-14 or DL-alanine-2-C-14-labeled diets contained a lower amount of C¹⁴ than the serum obtained from noninfected animals fed the same diets. However, in mice with *Trichinella* infections of 180 days' duration and fed glycine-1-C-14 or DL-alanine-1-C-14 diets, the pooled serum

Table IV. Incorporation of C¹⁴-Labeled Amino Acids into Serum by *Trichinella*-infected and Noninfected Mice

Experiment I					
Labeled diet	No. mice	Days fed C ¹⁴ diet	Duration of infection in days	C ¹⁴ activity of samples of pooled serum	
				Trichinella-infected muc/mg carbon	Noninfected control* muc/mg carbon
Glycine-2-C ¹⁴	26	7	56	0.408	0.508
DL-alanine-2-C ¹⁴	26	6	56	0.041	0.078
Experiment II					
Glycine-1-C ¹⁴	26	6	180	0.428	0.364
DL-alanine-1-C ¹⁴ *	26	6	180	0.062	0.046

* Control groups consisted of 12 noninfected mice of same age as the *Trichinella*-infected mice.

from the infected animals contained more C¹⁴ than the serum from the noninfected animals.

The C¹⁴ in the *Trichinella* larvae may be present in components other than in the labeled amino acid fed in the diet. Furthermore, it should not be assumed that these metabolites are entirely preformed in the metabolic processes of the host. Aldrich, Chandler and Dougherty¹³ studied transaminase activity of the tapeworm, *Hymenolepis diminuta*, in the rat. They obtained evidence for four transaminase systems in the tissues of the parasite: (1) alanine → glutamate; (2) aspartate → glutamate; (3) glutamate → alanine; and (4) glutamate → aspartate.

The data summarized in Table III indicate that the presence of encysted muscle larvae does not significantly alter the incorporation of C¹⁴ by muscle tissue. Although the specific activity of the diets in the second experiment was higher than the level used in the first experiment, the C¹⁴ content of muscle samples from animals fed the two-position C¹⁴-labeled amino acids was greater than that found in the samples from animals fed the one-position C¹⁴-labeled amino acids. The lower level of incorporation of the carboxyl-labeled amino acids may be explained on the basis of the known metabolic pathways of amino acids. The level of activity of the carboxyl-labeled amino acids in the body is rapidly lowered by the process of decarboxylation and the formed carbon dioxide is passed from the body by the respiratory system. The loss of activity through decarboxylation may account for the lowered amount of C¹⁴ in samples from animals fed the one-position C¹⁴-labeled amino acids. Although the amino acids labeled in the two-position are decarboxylated as rapidly as those labeled in the one-position, the labeled portion of the latter has more possibilities of being combined with other organic fragments and synthesized into other amino acids for incorporation into protein.

SUMMARY

1. Carbon-14-labeled amino acids were fed to mice infected with *Trichinella spiralis* to test the ability

of immature larvae and encysted larvae to incorporate C¹⁴ from the host. The C¹⁴ amino acids were fed beginning 14, 56 or 180 days after infection.

2. The presence of encysted *Trichinella* larvae in the muscles of infected mice did not alter the incorporation of C¹⁴ activity into muscle protein.

3. Immature larvae (14 days) incorporated higher levels of C¹⁴ activity (C¹⁴ per gram of dry tissue) from glycine-2-C-14 and DL-alanine-2-C-14 than were incorporated in the muscle tissues.

4. When the 7-day period of C¹⁴ feeding began 56 days after infection, the encysted muscle larvae incorporated C¹⁴, indicating that they were exchanging metabolites with the host. The specific activity of the carbon in the larvae exceeded that found in the muscles of the host. Although the larvae were encapsulated in the 56-day infections, the cyst wall was not as well-developed as in the 180-day infections.

5. The encysted larvae in the 180-day infected mice fed glycine-1-C-14 or DL-alanine-1-C-14 incorporated significant levels of C¹⁴ activity, showing an active metabolism by well-encapsulated muscle larvae.

ACKNOWLEDGEMENTS

We are indebted to Dr. Donald D. Van Slyke for his critical review of this manuscript. We wish to extend our appreciation to Peter N. Casey and Elizabeth N. Winkel for their technical assistance.

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Record of Proceedings of Session 10C

FRIDAY AFTERNOON, 12 AUGUST 1955

Chairman: Mr. M. Tsuzuki (Japan)

Vice-Chairman: Mr. C. Nuñez (Argentina)

Scientific Secretaries: Messrs. C. Polvani, I. D. Rojanski and L. F. Lamerton

PROGRAMME

- P/178 The use of isotopes in biochemical and medical research . . . A. B. Hastings
- P/840 The use of isotopes in analysis of metabolic disorders DeW. Stetten
- P/180 The use of radiosodium and radiopotassium tracer studies in man B. A. Burrows and J. F. Ross
- P/447 The absorption of vitamin B₁₂ and the pathogenesis of vitamin B₁₂ deficiency D. L. Mollin and E. Lester-Smith
- P/711 The application of radioactive isotopes to the study of the biochemistry of muscles D. L. Ferdman
- P/921 Synthesis of 4:4'-diaminodiphenylsulphone-S³⁵ (DDS) and its use in leprosy research . . . P. R. Saraiya *et al.*
- DISCUSSION
- P/224 Utilization of nuclear energy in public health problems on the epidemiology of communicable diseases . . . D. W. Jenkins
- P/140 A new radioactive method for marking mosquitos and its application M. B. Aragao *et al.*
- DISCUSSION

The CHAIRMAN: We now open the afternoon session. It is unnecessary to say that radioactive isotopes are a new tool which will enable us to open up a new field in our biological studies. This afternoon seven papers will be presented by our colleagues, and I hope that we shall all have a very profitable time.

Mr. A. B. HASTINGS (USA) presented papers P/178 and P/840.

Mr. B. A. BURROWS (USA) presented paper P/180, as follows:

The acute loss of sodium or potassium from the body, as with vomiting or diarrhoea, may be as injurious, if uncorrected, as lack of food or water. In such conditions the prompt replacement of sodium or potassium may be life-saving. Such serious losses of sodium or potassium may be detected by measurement of the serum concentration of these elements, which usually is low with acute losses of any importance.

We have been interested in the less obvious but perhaps equally important changes in the body content of sodium or potassium that may occur in chronic illness. Heretofore such changes have been studied by the difficult techniques of measuring the dietary intake and excretory loss of sodium and

potassium. Such studies are possible only in patients who are hospitalized, and are accurate only over relatively short periods of time. The negative or positive balances of sodium or potassium which appear are small in comparison with the daily intake, and after a few days the inaccuracies of even careful metabolic studies render them of little value.

However, the long-term changes in body sodium or potassium can be followed with tracer studies using radioactive sodium or potassium, which can be repeated at intervals of months or years. Also, important information as to the initial body content of sodium or potassium can be obtained only from these tracer measurements. Further information concerning the distribution and rates of mixing of sodium and potassium in the body can also be obtained from tracer studies.

I would like to refer first to radiosodium distribution.

Sodium is an important constituent of the extracellular fluids, which must be maintained if the circulatory, renal and other body functions are to be adequate. Sodium is also present in cells and other tissues, so that changes in body sodium may be due to changes in either the extracellular or non-extracellular portions. The portion of body sodium which is in extracellular fluid can be estimated if a com-

parison is made between the rates of distribution of tracer doses of radiosodium and radiosulphate. Radiosulphate has been shown to mix soon after injection with the extracellular fluid.

In Slide 1 (Fig. 1 of P/180) the early distribution of the tracer radiosodium and radiosulphate following rapid intravenous injection is shown by the sharp drop in the serum concentration, expressed as per cent of the dose per liter. The disappearance of the radioisotopes from the serum then becomes slower and is presumably due to their loss from the extracellular fluid. At the time of this change in slope, at around eighteen minutes following the injection, the volume of fluid with which the radioisotopes have mixed can be calculated. In this case, if between six and seven per cent of the dose is in one liter, the apparent volume of distribution of the entire dose is in approximately fifteen liters, which can be taken as the extracellular fluid volume.

These curves were obtained in a patient undergoing a surgical operation to determine if the rates of distribution of the radioisotopes would be influenced by the reaction to surgical trauma. There was no apparent change before and after the operation.

Further distribution of radiosodium is shown in Slide 2 (Fig. 2 of P/180), in which the time is shown in hours. At twenty-four hours the radiosodium had mixed with an apparent volume of distribution somewhat larger than at eighteen minutes, indicating that approximately one-fourth of the "exchangeable," or metabolically active, sodium at this time was not in the extracellular fluid. Again there was no apparent difference in these curves before and after operation.

I will go now to radiopotassium distribution. In contrast to sodium, potassium is chiefly intracellular, so that the partition of potassium between extracellular fluid and cells and other non-extracellular sites is of minor importance. However, the rate of distribution of a tracer dose of radiopotassium with the potassium in the body is of considerable interest.

In Slide 3 (Fig. 3 of P/180) the ratio of radioactive potassium to naturally occurring non-radioactive potassium observed in the urine at various time intervals after the dose shows the extent to which the injected radioisotope has been diluted by the potassium in the body. Distribution of the radioactive K^{42} , expressed as per cent of the dose, in the non-radioactive K^{39} , shown in milliequivalents, is virtually complete at twenty-four hours in the patients shown here. These patients had varying amounts of body potassium, indicated by final potassium specific activities from 0.03 to 0.1 per cent of the dose per milliequivalent, and varying serum concentrations as shown by the symbols on the slide, without obvious differences in the completeness of distribution at twenty-four hours. Here the "exchangeable" potassium, or the potassium in which the radioisotope has mixed, can be estimated from the specific activity at twenty-four hours. If there is 0.03 per cent of

the dose of K^{42} per milliequivalent of K^{39} , the entire dose of K^{42} has mixed with 3300 milliequivalents of K^{39} .

To determine if the figure for "exchangeable" potassium represents the metabolically active body potassium, we have compared the values obtained from tracer studies to the values derived from conventional balance studies in patients in whom there were acute changes either in some metabolic abnormality or in the body content of potassium. Slide 4 (Fig. 4 of P/180).

The values for "exchangeable" potassium are shown by the columns in milliequivalents, the changes in potassium balance by the dotted lines, also in milliequivalents. In the first patient, with severe renal disease, acidosis was corrected by extracorporeal dialysis with the artificial kidney, as shown by the return of the total carbon dioxide concentration to normal. There was little change in the serum potassium concentration, and slight change in the value for exchangeable potassium, matched by a slightly positive potassium balance. The second patient had severe potassium deficiency due to chronic diarrhoea, with an initial serum concentration of potassium approximately one-half normal. With potassium replacement there was an increase in the "exchangeable" potassium value shown by the columns which coincided very well with the positive potassium balance which was observed, indicated by the distance between the dotted lines, together with a restoration to normal of the serum potassium concentration. Thus it seems reasonable to assume that changes in body potassium measured by the isotope dilution technique are a valid measurement of the metabolically active potassium in the body, also involved in the balance studies.

The values obtained by this measurement are related in Slide 5 (Fig. 5 of P/180) to body weight in a variety of patients with chronic illness. The normal values fall between 40 and 50 milliequivalents of potassium per kg of body weight. With marked obesity there is an expected drop in this value due to increased body fat, which does not contain potassium. In malnutrition the potassium-containing tissues are disproportionately wasted. The lowest values were obtained in patients with overactive adrenal glands and Cushing's syndrome.

Repeated measurements on individual patients are connected by lines. In patients with severe liver disease marked clinical improvement sometimes occurred without marked changes in body potassium, but restoration to near normal values was observed only in patients who made almost complete recoveries. The two patients with uncomplicated potassium depletion showed a marked rise in this value with treatment. Except in this latter group, changes in the serum concentration of potassium were not reliable indications of changes in body potassium, as is shown by the symbols for each determination. Many of the patients with low "exchangeable" po-

tassium values had normal or high serum potassium concentrations.

I would now like to refer to the simultaneous measurement of body sodium and potassium.

In certain metabolic abnormalities changes in the ratio of body sodium and body potassium may be more informative than changes in either value taken by itself. Because of the short and quite similar half-lives of the radioisotopes of sodium and potassium, their simultaneous measurement depends on either chemical separation or differential absorption of their radioactive emanations.

Currently we are using scintillation counting of serum samples which is made possible by the introduction of well-type beta-sensitive plastic phosphors in addition to the sodium iodide well counters with which you are all familiar. The relative sensitivity of these detection devices for Na^{24} and K^{42} is indicated in Slide 6 (Fig. 6 of P/180), together with the two well counters—on the left is the plastic and on the right the sodium iodide—a steel absorber which is used with the sodium iodide well counter to eliminate the energetic K^{42} beta particles, and the plastic tubes which contain the three milliliter serum samples.

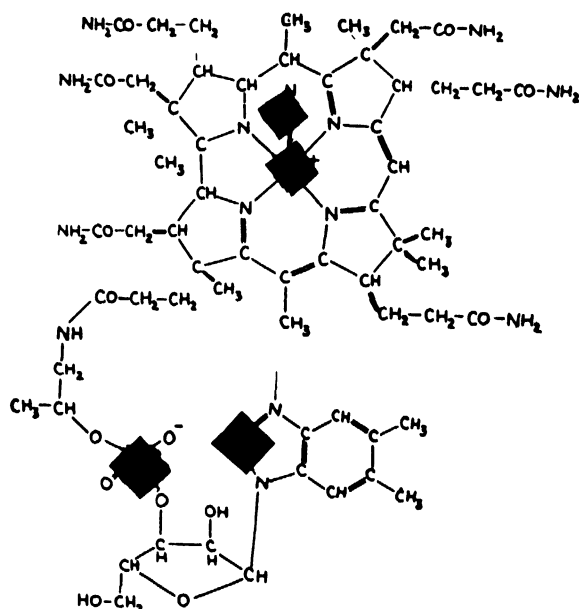
I might say, parenthetically, that the sodium counting is favored by the sodium iodide by a ratio of 3 to 1 because the sodium gammas are not stopped as well by the plastic, and on the other hand the plastic favors potassium counting by about 10 to 1 because the potassium beta particles are effectively absorbed by the plastic.

With such equipment it is possible to obtain multiple serum samples with permissible tracer doses in human subjects for circulatory and other studies as well as those reported here.

In summary, tracer studies in man using radio-sodium and radiopotassium show (1) the amount and distribution of metabolically active sodium and potassium in the body, (2) changes in body sodium and potassium content in various illnesses, and (3) the rates of mixing of administered sodium and potassium with that already present in the body. Such information is essential not only in understanding disease processes, but also in the diagnosis and treatment of patients with a variety of chronic diseases.

The CHAIRMAN: Since there are no questions or comments on the preceding papers, we will go on with the next paper.

Mr. E. LESTER-SMITH (UK) presented paper P/447, as follows:



Slide 7

Vitamin B_{12} deficiency gives rise to a characteristic macrocytic anaemia; this is frequently accompanied by neurological lesions. The deficiency may occur in association with intestinal malabsorption syndromes; it can also be caused by dietary deficiency. The precise incidence of the deficiency is uncertain, partly because it may be present for several years without clinical manifestation, and partly because its presence may be masked by other deficiencies.

The introduction of vitamin B_{12} labelled with the isotopes of cobalt has considerably improved the methods of diagnosing these various B_{12} deficient states; in addition the absorption and metabolism of the vitamin can be adequately studied in both health and disease with the help of this radioactive B_{12} .

We wish to describe some of the ways in which the radioactive vitamin has been used to investigate the metabolism of B_{12} and the pathogenesis of the B_{12} deficient states.

Coming to the preparation of the vitamin, Slide 7 is the complete structural formula of vitamin B_{12} , disclosed a few weeks ago in England. The dark patches indicate the atoms that can be labelled isotopically. One can replace the carbon in the cyano group with C^{14} , and can put C^{14} in the iminazole biosynthetically, P^{32} can be introduced biosynthetically, and one can put radioactive cobalt in the centre of the molecule in the same fashion; but it is only the cobalt-labelled vitamins B_{12} that are useful in medical research on account of the low specific activities of the other substances.

I will deal with four ways of studying absorption.

Table I. Cobalt Isotopes

Isotope	Reaction	Method	Half-life	Radiations
Cobalt-60	$\text{Co}^{59} (n, \gamma)$	Pile	5.3 yr	β^- 0.5, γ 1.3
Cobalt-58	$\text{Ni}^{58} (n, p)$	Pile	72 d	β^- 0.47, γ 0.8
Cobalt-57	$\text{Fe}^{57} (p, \gamma)$	Cyclotron	270 d	β^+ 0.26, γ 0.11-0.21
Cobalt-56	$\text{Fe}^{56} (p, n)$	Cyclotron	72 d	β^+ 1.5, γ 0.84-3.25

Table I on page 536 shows the isotopes that can be employed. Cobalt-60 has been widely used in Great Britain and America. It has, however, a great disadvantage in respect of its long half-life of 5.3 years, so that the amount that can be used for any one patient is limited to about 1 microcurie. We have therefore turned to cobalt-58 made from nickel in the pile and most of the work here reported was done with cobalt-58 B_{12} . We now prefer cobalt-57 and cobalt-56 which are made jointly by cyclotron bombardment of iron. The relative proportions appear to differ with the energy of the proton beam, but cobalt-57 in particular has this very useful half-life of 270 days and is relatively safe so that amounts up to about 5 microcuries can be used with a single patient, who may thus serve as his own control in a series of experiments. The labeled vitamins are made biosynthetically by small-scale fermentations in an almost cobalt-free medium. We have had some difficulties with the extraction, purification and crystallization of preparations of very high specific activity when the amounts available finally were of the order of 0.1 to 3.0 mg; at the best we have achieved specific activities up to 13 mc/mg of vitamin B_{12} , and that is almost too hot for most clinical uses.

Slide 8 indicates extremely diagrammatically the behaviour of vitamin B_{12} in the body and the four ways we have of studying its absorption. Just to refresh your memories, an injected dose goes straight into the circulation and is eliminated in the urine, hardly at all in the feces. The amount eliminated depends entirely on the dose, being very small with a small dose, and increasing steeply with large doses. Some goes into the serum and some to the liver.

I want to deal almost exclusively with oral administration, and here the behaviour is different. A small oral dose depends on the activity of an enzyme-like substance, the intrinsic factor, for its absorption. In normals something like 50 per cent to 95 per cent—a rather variable proportion—is absorbed through the gut wall. In pernicious anaemia cases, where the intrinsic factor is lacking, the absorption is nil to some 30 per cent. What is not absorbed goes straight

out into the feces, and the counting of the feces is the first method by which we can measure by a difference technique the amount absorbed. Normally, the excretion in the urine from this very small dose is negligible, but if along with, or shortly after, the oral dose you give a very large flushing dose by injecting something of the order of 1 mg of B_{12} , that washes out, as it were, a large proportion of the vitamin that has been absorbed into the urine, and you can then count the urine as a rough measurement of the amount absorbed—a technique which has come to be known as the Schilling test. In addition, a large part of the absorbed vitamin goes into the liver, where it can be measured approximately by directional γ -counters; and finally some of the radioactive B_{12} passes into the serum, so that with this very hot B_{12} you can measure serum radioactivity with a small dose, and this gives a fourth method.

Thus there are these four methods: counting feces, urine, liver, or serum.

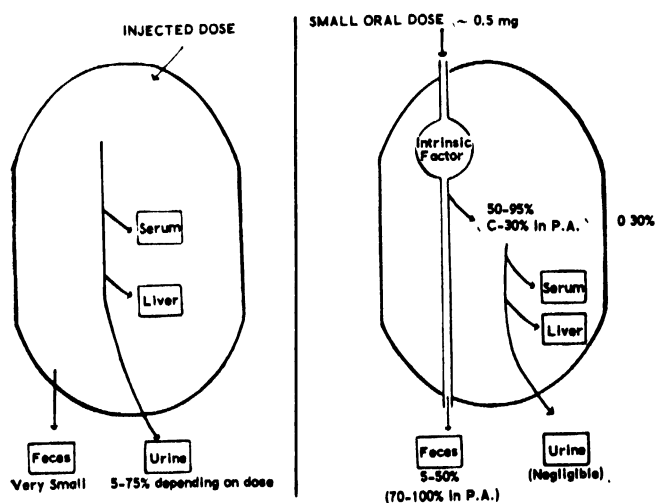
The fecal method, the first method, is the most suitable for the quantitative study of B_{12} absorption. Most workers give doses of a half to 2.0 mg of radioactive B_{12} by mouth as the test dose, and the amount excreted can be measured in two ways, by counting aliquots of numerous fecal samples in a scintillation counter, or more simply by a single measurement, in a ring counter of Geiger-Müller tubes in parallel, of the radioactivity in the total 7-day excreta. The latter is of course very much simplified. The difference between the amount administered and the amount excreted is considered to be the amount of B_{12} absorbed.

Slide 9 (Table I of P/447) summarizes the result in a group of patients with pernicious anaemia and a group of control subjects. There were over thirty subjects in each group. The controls absorbed more than 0.3 mg of the 0.6 mg given, while the pernicious anaemia cases absorbed from nothing to 0.21 mg, so there is not even any overlap between these groups. To prove that the subject has pernicious anaemia it is also necessary to show that although ordinarily he cannot absorb the vitamin yet can absorb it when given along with a potent source of intrinsic factor (I.F.).

Slide 10 (Table II of P/447), "Fecal Elimination with I.F.," shows what happens with the intrinsic factor. The amount of B_{12} absorbed is increased in every case—the bottom line indicates the increase due to the addition of intrinsic factor—so that pernicious anaemia patients can be differentiated from others by the combination of these tests.

So much for pernicious anaemia. Slide 11 (Table III of P/447) illustrates the way in which the test may be used to investigate the pathogenesis of other B_{12} deficiency states.

All these patients suffered from B_{12} deficiency. All were anaemic. Low serum B_{12} concentrations responded to treatment with B_{12} . Each patient was given the test dose of B_{12} alone, and then with I.F.



Slide 8. Excretion of vitamin B_{12}

The five patients with B₁₂ deficiency associated with partial gastrectomy behaved like patients with P.A. B₁₂ was absorbed only when it was given with I.F. This suggests that the B₁₂ deficiency in these patients was also due to inadequate intrinsic factor secretion.

Patients with nutritional macrocytic anaemia absorbed B₁₂ normally even without I.F.; on the other hand the patients with steatorrhoea and those with loop syndrome (i.e., patients in whom B₁₂ deficiency was associated with blind intestinal diverticuli or loops) failed to absorb B₁₂ even if I.F. was given. The deficiency in these patients was presumably due to failure of the intestine to absorb the B₁₂-I.F. complex. The cause of this is uncertain, but it is interesting that if patients with loop syndrome are given antibiotics which nearly sterilize their gut, they then do absorb B₁₂ (Table II on this page).

Before the administration of antibiotics all failed to absorb B₁₂; after antibiotics only patients with steatorrhoea failed to absorb B₁₂. In one case of tropical sprue the absorption was increased from almost nothing to a considerable level with antibiotics.

These results make it clear that radioactive B₁₂ is very valuable for both the diagnosis of the megaloblastic anaemias and for the study of the pathogenesis of B₁₂ deficiency.

The next few slides illustrate the way in which this material may be used to study the physiology of B₁₂ absorption.

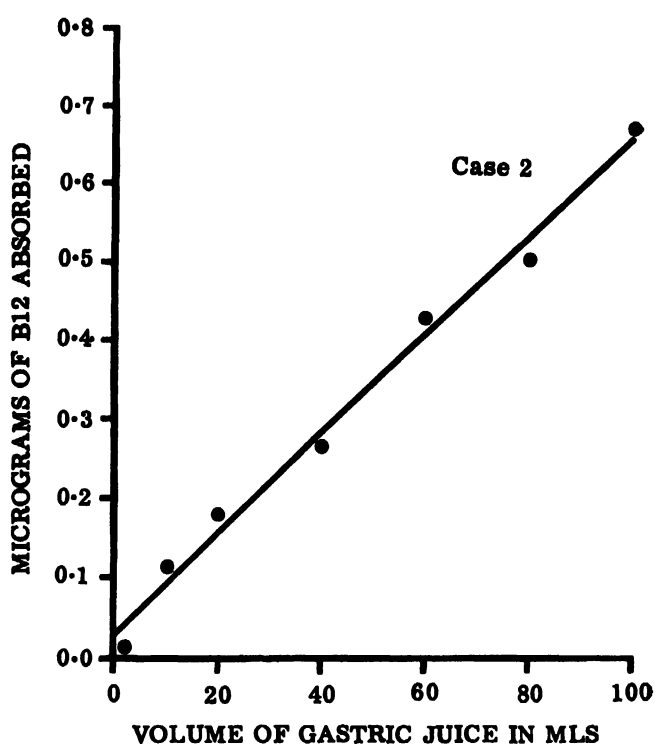
All the observations were made on patients with pernicious anaemia in remission, not relapse; in other words on subjects who secrete little or no I.F.

Table II. Effect of Antibiotics on the Absorption of Co⁵⁸B₁₂ in Megaloblastic Anaemias

Condition	No. of subjects	Co ⁵⁸ B ₁₂ absorbed (μg)	
		Before antibiotics	After antibiotics
Idiopathic			
steatorrhoea	2	0.00-0.08	0.04-0.06
Loop syndrome	5	0.00-0.12	0.33-0.45
Tropical sprue	1	0.03	0.37

Slide 12 shows the amount of B₁₂ absorbed when 0.8 μg radioactive B₁₂ was given with varied amounts of pooled gastric juice. Up to a point there is a nice straight-line relationship. As you increase the amount of gastric juice so the number of micrograms absorbed increases, so that in the end you have 0.7 out of the total dose of 0.8 absorbed. These measurements are all done by the fecal excretion technique.

This relationship was only true over a limited range. When larger amounts of I.F. are given a point is reached when no further absorption of B₁₂ occurs [Slide 13 (Fig. 3 of P/447)]. In the case of two patients with P.A., as before the amount of B₁₂ ab-



Slide 12

sorbed (from 1.5 μg by mouth) increased with increase in the amount of I.F. given, but beyond this level no more B₁₂ was absorbed. In spite of increasing the amount of I.F., an intestinal blocking mechanism limits the total B₁₂ absorbed from a dose. You have first to get the B₁₂ combined with the intrinsic factor in order to get it through the gut wall. After that there seems to be a blocking mechanism, perhaps similar to that observed with iron absorption.

I now want to pass to our second method, the urinary method. Though the fecal method is probably the most accurate method of estimating the absorption of radioactive B₁₂, these tests are time-consuming, involving as they do seven-day stool collections, and they demand considerable patience and co-operation from the patient.

A much simpler method for demonstrating the absorption of radioactive B₁₂ was introduced by Schilling. This is our "second way." He showed that if a small oral dose of radioactive B₁₂ is given to a normal subject no radioactivity appears in the urine. If, however, a large injection of unlabeled B₁₂ is given with, or shortly after, the oral test dose of radioactive B₁₂, then radioactivity appears in the urine of normal subjects. Little or none appears in the urine of patients with P.A. unless a potent source of intrinsic factor is given with the dose. These observations have been confirmed and extended by several other writers.

Slide 14 (Table IV of P/447) shows the results of this test on normal subjects, on patients with pernicious anaemia, and on patients with B₁₂ deficiency associated with steatorrhoea. Each subject was given a test dose of 0.6 μg Co⁵⁸-B₁₂ and an injection of 1000 μg of non-radioactive B₁₂ was given at the same

time. There is an over-all correlation between the amount of B_{12} absorbed and the amount excreted in the urine—approximately $\frac{1}{3}$ of the absorbed dose being excreted. This ratio, however, unfortunately varies widely in repeated tests in the same patient and in different patients.

Thus the Schilling test is rapid, sensitive and convenient for clinical purposes; for the accurate study of the physiology of B_{12} absorption, however, this test is less satisfactory; moreover, large parenteral injections of non-radioactive B_{12} may interfere to a variable extent with the absorption of radioactive B_{12} . For precise quantitative studies, therefore, the fecal excretion method is to be preferred.

There are still two other methods. Our third way of measuring absorption, by counting liver radioactivity, will not be considered. So finally, we pass on to the fourth way, namely serum radioactivity.

If radioactive B_{12} with the very high specific activity (4 to 6 $\mu\text{C}/\mu\text{g}$) is given in oral doses of 0.5 to 1.0 μg to normal subjects, significant changes occur in serum radioactivity. The pattern of these changes is shown in Slide 15 (Fig. 4 of P/447). There is no count for three hours, then it rises steadily and plateaus at about eight hours.

In pernicious anaemia cases there is no activity in the serum, as shown by case B at the bottom, but the picture becomes similar to normal if I.F. is given. Not much work has been done on this serum radioactivity measurement, but we have good hopes that it will prove useful.

In conclusion, we think that the radioactive vitamin B_{12} has already proved to be an extremely valuable tool for studying the absorption of B_{12} and the pathogenesis of the vitamin deficiency. For the first time it has been possible to study the absorption of B_{12} in subjects other than patients with severe B_{12} deficiency. We would end by emphasizing that in this talk we have only been able to indicate a few of the ways in which radioactive B_{12} can help in the study of metabolism of B_{12} . Its possible usefulness extends beyond the field of absorption studies.

Mr. R. E. KAVETSKY (Ukrainian SSR) presented paper P/711.

Mr. V. R. KHANOLKAR (India) presented paper P/921, as follows:

After the excellent papers presented during the last three sessions, it is not necessary for me to refer to the new possibilities opened up in biological and chemical research with the introduction of tracer techniques. A new possibility consists in the study of drugs incorporating a tagged atom, with a view to determining the mechanism and site of their action in the human body. Leprosy presents a serious problem in many parts of the world, particularly South-East Asia, Africa, Central and South America. It has been estimated that about 2 million people are affected with the disease in India.

The causative agent of the disease is an acid-fast mycobacterium (1–8 μ in length and about 0.1–0.5 μ

in width). It shows considerable pleomorphism. The disease manifests itself in the form of a chronic malady extending over several years and shows two distinct clinical types, the malignant or lepromatous variety and a more benign form, the tuberculoid variety. The lesions in the lepromatous variety are characterised by chronic granulomatous lesions involving mainly the skin, the upper respiratory tract, the peripheral nervous system and lymph nodes.

Although it is over 80 years since Hansen first described the human leprosy bacillus, the organism has eluded all attempts at growing it in artificial culture media or transmitting it to experimental animals. In consequence our knowledge of the modes of transmission, epidemiology and dissemination of disease in man has remained in an unsatisfactory state. Despite these limitations, it has been possible with certain refinements in techniques to obtain an understanding of the causal factors at work in the evolution of the disease and its prophylaxis.

The prognosis in the lepromatous variety when it is well established has been grim and until the last decade very ineffective. Some twenty years ago some compounds of sulphones known under commercial names as promine, sulphatome, etc., were tried on human patients after some hopeful observations in other chronic infective diseases. The results were very striking and one expected that finally a potent remedy was at hand against a dreadful malady. As was to be expected, some people waxed eloquent, even lyrical, before the advantages and limitations of those drugs had been carefully assessed.

Later experience has now shown that (1) the active substance in all these preparations is 4:4'-diaminodiphenyl-sulphone; (2) that it takes a long time to effect a clinical and, what is more important, a bacteriological improvement, usually not less than 2–3 years of regular continuous treatment; (3) about a quarter of the patients do not react favourably to the administration of the drug and the disease follows its relentless course; and finally, that in a certain proportion of patients who had benefited greatly from the treatment with sulphones, there is a recurrence of the disease after a lapse of some 4–5 years.

We have been interesting ourselves with these problems for the past seven years in an attempt to discover the reasons for the extremely slow action of the sulphones on leprosy lesions and the causes of the recurrences in some patients.

I might refer to one striking feature of the evolution of the disease which was disclosed as a result of our work. The leprosy bacillus migrates from the site of its entrance in the human body (almost always the skin) along and in the substance of the peripheral sensory and autonomic nerve fibres.

The proliferating micro-organisms later break out from the intercalated spaces into the dermal connective tissue [Slides 16 and 17 (Figs. 1 and 2 of P/921)]. They are then carried along the tissue spaces, with the lymphatic fluid and circulating blood.

As is well known, the proliferation of leprosy bacilli in the dermis assumes astounding proportions. In lepromatous nodules millions of bacilli are easily demonstrable in a minute drop of exuded serum.

After treatment with sulphones there follows a slow but steady clinical improvement of the patient's condition and a gradual diminution of bacillary content in the dermis. We were able to show, however, that even after a complete disappearance of bacilli from the skin, it is possible to demonstrate foci of bacillary agglomerations in the nerves. We therefore felt that for any drug to be really effective it is necessary for it to gain selective accumulation in the axoplasm of nerve fibres. The present study was designed to ascertain if the sulphones gain entrance to the nerve tissue. For a tracer study of the absorption of this drug by the different tissues of the body, particularly the nerve tissue, in leprosy patients, it was necessary to prepare a sulphone bearing a "tagged atom."

It was thought that acetylsulphanilyl chloride- S^{35} , which has been prepared by Klotz and Melchior, would serve as the starting material for preparing the required radioactive sulphone. In trial experiments with unlabelled material it was, however, found that the Friedel-Craft reaction using the above compound with acetanilide gave poor yields of the expected diacetyl derivative; another method therefore had to be sought.

Thionyl chloride is the starting material in the synthesis described by Sugawara and Sakurai.

The scheme outlined in the paper has now been adopted for the preparation of 4:4'-diaminodiphenylsulphone- S^{35} using thionyl chloride- S^{35} . The over-all yield, based on the thionyl chloride, was about 45 per cent; the experimental details of the synthesis have been described in the duplicated paper which has been circulated. I will not attempt to describe it, because I am afraid some chemists may ask me questions which I might not be able to answer.

Labelled DDS (as prepared and described in paper P/921) was administered orally to 6 leprosy patients in 100 mg doses per day on successive days. In order to ensure a certain uniformity, all the 6 cases were selected so as to conform to approximately the same ages (30-35 years) and to include the same types of lesions (lepromatous); two biopsies were taken from each patient at the following time-intervals after the commencement of the treatment: 3, 6, 24, 48, 96 and 120 hours. The time-schedule, though selected arbitrarily, covered a fairly representative range.

The material was fixed in 20 per cent neutral formalin and processed rapidly through dehydration, clearing and embedding procedures. Sections were cut at 10-15 μ and mounted on previously subbed slides. After dewaxing, the slides were brought into water in the usual manner, covered with stripping emulsion from Kodak autoradiographic plates in the dark room and stored in the dark. The technique of

autoradiography was essentially similar to that developed by Doniach and Pelc.

Representative samples of the slides containing tissue sections fixed after various time-intervals after the administration of the radioactive drug have been developed and have so far revealed only very faint autoradiographs, especially in the tissues of the patients that had received over 400 mg of labelled DDS. No valid autoradiographs have been as yet observed in the slides of patients whose biopsies were taken 3 hours after the initial dose of 100 mg of DDS.

It is proposed to allow an exposure time equivalent to $2\frac{1}{2}$ half-lives of the radioactive element (S^{35}) before processing the remaining slides.

While it is too premature to draw any inferences from these faint autoradiographs about the relative uptake of the radioactive substance, their appearance seems to suggest an absence of preferential incorporation by particular tissue systems. On the other hand, there are indications of a more or less generalized distribution of the radioactive material throughout tissues.

We therefore venture to suggest that for an effective treatment of the disease it may still be necessary to discover a bacteriocidal or bacteriostatic agent which would find preferential entrance to the nerve tissue.

DISCUSSION OF P/447, P/711, AND P/921

The CHAIRMAN: The first question is presented by Mr. D. E. Clark of the USA and refers to paper P/447. The question is, "Would you please define loop syndromes?" Perhaps Mr. Lester-Smith will answer.

MR. LESTER-SMITH (UK): I am not the medical partner in this association, I just make the radioactive B_{12} , but I can say something about it. A loop syndrome is a sort of circuit in the gut made at operation where you have a portion that has been joined in to make a sort of re-entrant loop. I think that is what it means. The point is, you can get a stagnant condition in the contents of this loop.

MR. P. CHEOSKUL (Thailand): I have a question for Mr. Lester-Smith concerning the nature of the vitamin B_{12} in the feces. He has measured the activity of cobalt-60 in the feces. I wonder whether the biological activity runs parallel with the activity of the cobalt-60 which he measured, or whether there is a metabolic change, that is the cobalt-60-labelled vitamin B_{12} has undergone metabolic change. I wonder if he has any information concerning the metabolic product.

MR. LESTER-SMITH (UK): That sounds a simple question to answer, but in fact it is not. The trouble is there is a great deal of synthesis of vitamin B_{12} going on in the gut of normal and indeed of pernicious anaemia patients, so that what is excreted comes partly from the labelled B_{12} administered and

partly from this much larger quantity of gut-synthesised B₁₂, and they cannot of course be separated. I suppose one could attempt to fractionate the radioactivity by methods of separation of the B₁₂ from its degradation products, but we have not done much along this line. I seem to remember in the literature some evidence that vitamin B₁₂ is in fact degraded in the gut, but there is not much knowledge about this. We did do a small experiment to check the nature of the B₁₂ excreted in the urine after giving a large oral dose of material labelled with both cobalt and phosphorus, and I can say there that the proportions of cobalt and phosphorus excreted were much the same as in the dose, so that if the material was degraded it was not very much changed because the product contained both the cobalt and the phosphorus which are normally very easily separated by chemical degradation.

Mr. J. F. ROSS (USA): On the basis of clinical response it has been reported by Monto that vitamin B₁₂ is absorbed directly across the respiratory epithelium in patients with pernicious anaemia even in the absence of intrinsic factor. This is very interesting if it is true, and I should like to ask Mr. Lester-Smith if he has made any such determinations employing a labelled cobalamin, and if so has he found that there is absorption across the respiratory epithelium in the absence of intrinsic factor. If he has such observations it will be most interesting if he could comment on vitamin B₁₂ absorption without the intrinsic factor being present.

Mr. LESTER-SMITH (UK): We have not made any direct experiments with labelled B₁₂ to study its absorption in this manner, I am afraid. I think you refer to the work where B₁₂ is given in a kind of snuff to take through the nose. I do know about that, but we have not studied it. I think it is perhaps not unreasonable to expect that the vitamin would be absorbed in this fashion. After all other drugs are given in this way, or are given sublingually, to stimulate the effects of injection, and it might well be that B₁₂ is actually absorbed in this fashion.

Moreover, one might perhaps mention something which I had no time to speak of in giving the main paper. All this work we described was with the use of very small oral doses of B₁₂, of the order of magnitude of what you might get in an ordinary meal; but if large doses of B₁₂ are given orally there seems to be another mechanism of absorption acting, a kind of mass action effect—call it what you like. It is a fact that if several milligrams of B₁₂ are given in one dose one can get perhaps 100 mg—as much as that—into the circulation.

In my country one or two people, and in particular Dr. Marshall Chalmers, have been maintaining pernicious anaemia cases over a period of years on quantities of 50 µg of B₁₂ daily, given on a fasting stomach, without any I.F. being used, and it has been shown that no I.F. is in fact secreted by these subjects.

Mr. C. A. MAWSON (Canada): I was a little unhappy at the use which Mr. Kavetsky made of the term "turnover rate." This is a term which is discussed a great deal in biochemical circles at the present time. It seemed to me that what Mr. Kavetsky's colleagues have determined was the ratio between the radioactivity per gram of muscle in normal muscle and in dystrophic muscle. You cannot determine a turnover rate by the estimation of a single point; it is possible by putting the time at which you do the experiment at a certain point to get one result, and then by taking a different time at which you do the experiment to get a different result, because the turnover curves cross one another. I should like some comments from Mr. Kavetsky on that, because I feel that his results may be very sound, provided a series of experiments was done. It may be that he has only reported the experiments at one time interval, but I feel that just the one time interval cannot give us very well-defined results.

Mr. KAVETSKY (Ukrainian SSR): I am sorry but I did not quite follow the question. I am also in some difficulty, as I am not a co-author of the paper, but merely presented it. Perhaps it would be better if we had a talk after the meeting.

Mr. C. NUÑEZ (Vice-Chairman) then took the chair.

Mr. D. W. JENKINS (USA) presented paper P/224.

Mr. J. COSTA-RIBEIRO (Brazil) presented paper P/140 as follows:

As I am not a biologist, I am a little embarrassed to be speaking to you on a subject which is of special concern to epidemiology, as the previous speaker has just shown us so convincingly.

My subject is a piece of research carried out by Dr. Mario B. Aragão of the Institute of Malariology of the Brazilian National Malaria Service, by my assistant, Mrs. Elisa Frota-Pessôa and by Miss Neusa Margem of the physics department of the Faculty of Sciences of the University of Brazil and of the Brazilian Centre for Physical Research, who have specially evolved the technique employed in this method of study.

There are several methods of studying animal migrations all of which in one way or another involve tagging a certain number of specimens, setting them at large and then determining the distance traveled on the basis of subsequent recaptures.

Two principal methods have hitherto been used to flight range of mosquitos (a highly important question in the epidemiology of malaria). The first is to dust insects, taken from nature, with coloured dust (of the type used to produce metallic colours) to release them at a clearly defined point and to recapture them at varying distances and in different directions from the point of release in order to determine the range of their migration. This is the method used by Majid in the West Indies.

The second method is to hatch mosquito larvae in a solution of a radioactive element such as radiophosphorus, so that the adult insect retains sufficient radioactivity for a period of time to permit its identification after capture by means of a Geiger-Mueller counter. This is the method developed by Hasset and Jenkins in the United States which has just been described by Mr. Jenkins in his paper.

The procedure outlined in this paper is similar to the two methods I have mentioned, but differs from them in two respects.

In the first place, the larvae are grown in a solution of thorium nitrate, a radioelement with a very long half-life. A very weak radioactive solution can thus be used without any substantial decline in the intensity of radiation with the passage of time.

In the second place, nuclear emulsions are used to detect radioactivity after the capture of the insects. As this nuclear emulsion method is highly sensitive, permitting the detection of alpha particles from the disintegration of a single atom, the presence of insects tagged with extremely small quantities of thorium nitrate can be detected with certainty, even after a considerable period of time.

As an indication of the sensitivity of this procedure, suffice it to say that it has enabled us to detect the presence of thorium in the ashes of two-month old mosquitos, whose larvae had been grown in an extremely weak solution of thorium nitrate (1:100,000).

The use of a very weak solution probably ensures that the presence of a very small quantity of the radioactive element in an insect's body will have no appreciable detrimental effect on its physiological state, normal behaviour or average life span. Preliminary experiments seem to bear out these assumptions, but it would clearly be extremely useful to make a comparative study of the conditions of life and mobility of mosquitos reared in solutions of radiophosphorus, in very dilute solutions of thorium nitrate and in normal conditions, free of all radioactivity. To the best of our knowledge, no such study has so far been made.

Two different techniques were used to detect radioactivity by the authors of this paper.

The first method involved the use of ordinary nuclear emulsions mounted on glass plates. The plates used were type G-5 (Ilford), with emulsion 50 microns thick. But, as the purpose was the detection of alpha particles, much less sensitive emulsions might also have been used.

The different groups of captured mosquitos were incinerated in porcelain evaporating dishes, each group consisting of specimens from the same collection station.

A stainless steel plate (26 mm by 77 mm and 1 mm thick with 12 circular holes 4 mm in diameter) was superimposed on a rectangular glass slide of identical size.

The ashes of each group of mosquitos from the same collection station were then removed from the porcelain evaporating dishes (by means of a previously dampened platinum wire) and placed in the circular holes at the base of which was the glass slide.

The sensitive plate with the nuclear emulsion was then placed over the stainless steel plate with the emulsion facing downwards, i.e., towards the holes containing the radioactive ashes.

These precautions serve to demarcate and define the areas of the nuclear emulsion exposed to alpha particles emitted by the ashes of the different groups of captured mosquitos, while also preventing direct contact with the emulsion.

Details of this technique are shown in Slide 18 (Fig. 6 of P/140) and Slide 19 (Fig. 5 of P/140).

Tracks due to the presence of alpha particles are visible on Slide 18, which is a microphotograph of the emulsion after exposure and normal development. These tracks are short. The two long tracks running across the slide have no connexion with alpha particles and are probably due to cosmic radiation. This was a plate on which the emulsion was fairly old.

Slide 19 shows the same plate, but a part of it not exposed to the action of alpha radiation from the mosquito ashes. You will note the absence of any of the small tracks characteristic of alpha radiation which were visible on the previous slide.

The second technique involves the use of liquid nuclear emulsion. This method has the advantage of permitting a detailed study of the localization of the radioactive material in the different organs of the insect. Adult mosquitos from larvae grown in a 1:10,000 solution of thorium nitrate were mounted on a paraffin block according to the usual procedure. Sections 3 microns thick were obtained with a microtome and glued to a glass slide. The slide was immersed in xylol for ten minutes and for a further ten minutes in 100 per cent alcohol in order to eliminate the paraffin. After being dried, the slides were coated with liquid nuclear emulsion, care being taken to hold it perfectly horizontal during this process in order to ensure that the emulsion was of uniform thickness.

The slide was then placed in the refrigerator and, after the appropriate period of exposure, was developed, fixed and coloured with hematoxylin.

This method enabled us to establish that the radioactive material is localized in the Malpighian tubes of the adult mosquito—see Slide 20 (Fig. 1 of P/140) and Slide 21 (Fig. 2 of P/140). These microphotographs show part of the Malpighian tubes and of the genital apparatus of a female *Anopheles (kerteszia)* and enable us to observe the tracks of alpha particles emanating from that part of the insect's body. The continuous tracks you see are due to the presence of alpha particles in this area.

Slide 21 shows particularly clearly the points at which the tracks made by alpha particles originate.

The use of this method revealed the reason why in preliminary experiments made with liquid emulsion, in which the body of the insect was kept intact, alpha particle tracks were not always observed. The latter were absorbed by the tissues enveloping the internal organs, in which the radioactive element was localized. This drawback can be overcome by the incineration technique.

A check of this method has given very favourable results. Mr. Aragão carried out experiments with *Anopheles (kerteszia)* mosquitos in a forest in Southern Brazil known as "Mata do Malucher" and at Brusque in the State of Santa Catarina.

Eleven thousand four hundred larvae were collected from water accumulated in different species of "Bromeliae." The larvae were grown in solutions of thorium nitrate (1:10,000). The number of adult insects obtained was estimated at approximately 3000.

The total number of insects caught was 395. Collections were made at eight stations roughly 200 metres apart and situated along four lines roughly at right angles to one another. The experiment lasted 45 days.

As radioactive mosquitos were captured even at the most distant station from the point of origin, which was 800 metres away from it, it may be concluded that the flight range of the type of *Anopheles* used is 800 metres or more.

But the most important result of the experiment was to prove the practicability and convenience of this method and the reliability of the results obtained.

DISCUSSION OF P/224 AND P/140

Mr. J. C. BUGHER (USA): Effective control of insect-borne diseases usually requires precise quantitative knowledge of the vector's biologic relationship, including the population characteristics. Could Mr. Jenkins comment further concerning the applications of these methods in tsetse fly control for *trypanosomiasis* and *Anopheles* control for malaria?

Mr. JENKINS (USA): Mr. Bugher has asked whether there might be applications of the use of radioisotopes for studying tsetse fly control in Africa. There are several ways in which this could be used very effectively. One is in studying the flight dispersal of the tsetse fly, which is extremely important in regard to control by both eliminating vegetation and insecticide treatment. This is very expensive, and to determine the exact flight range of the tsetse fly from their breeding habitat would be a valuable contribution. It would also be valuable to determine the fly population sizes. This could be done by labelling a certain known number of flies and then releasing them among the entire population. By calculating the percentage of recaptures it is then possible to state how many tsetse flies occur in the area. By releasing tagged tsetse flies it is also possible to determine the entire range and the size of the broods.

The next question was, what value would the radioisotopes have in regard to *Anopheles* control in regard to malaria. One of the most important aspects of malaria control is determining the exact flight range of the mosquito population. It is necessary to know how far the mosquitos fly in order to carry out effective control. For example, if a city were located half a mile away from a breeding pond where the *Anopheles* mosquito occurred and you knew the flight range was not half a mile, it would not be necessary to insecticide that pond. In other words, the information gained by using radioisotopes would be valuable in guiding control operations. The comments on tsetse flies also apply to determination of population size and length of life of *Anopheles*.

Mr. V. L. TROITSKI (USSR): I was extremely interested in Mr. Jenkins' paper.

I should like to ask him a question about the labelling of bacteria and the use of this method for epidemiological purposes. It is a well-known fact that the bacteria of dysentery and bubonic plague are very rapidly destroyed in the human body, as are the bacteria of dysentery in that of the fly. I should therefore like to know whether Mr. Jenkins considers it practicable to use labelled bacteria of these types for the solution of epidemiological problems. I should be very grateful for his views on this point.

Mr. JENKINS (USA): This question covers a very large area so that it is not possible to answer it adequately in a brief time. However, a number of bacteria have been labelled and a number of studies carried out. It would be very difficult to attempt to give a resumé now of all of this literature. Some of the examples I have already mentioned in regard to some of the insect dissemination. The literature is very large in regard to studies with bacteria, for example, taking in of the aerosols into the lung using the radioactive label. A number of studies have been carried out in connection with dysentery organisms, and there is a great deal of information along this line which is extremely promising. I feel I cannot adequately answer this question in a very short period.

One other pertinent piece of data which comes to mind is that the entrance of bacteria into the human body through the skin has been studied in some cases. There is a fair amount of work with the tubercle bacillus—I think by Strom. It is cited in the paper in the Proceedings.

If there is anything specific that I can add I will be very glad to do so.

Mr. A. R. GOPAL-AYENGAR (India): While congratulating Mr. Jenkins on his comprehensive review of a really extensive subject, I should like to ask him if he has any information, or if any work has been done, on the following questions.

The first is about the dissemination and spread of allergens like the pollen of ragweed and other

plants causing hay fever, studied by the use of radioactive tracers.

Secondly, the infectivity and spread of the causal agent of filariasis.

Thirdly, whether any work has been done *in vitro* concerning the metabolism of tubercle bacillus using tracer substances.

I should like to ask another question. Mr. Jenkins mentioned some work being done on the entry of bacteria through the surface of the skin. I would be very grateful to him if he could suggest how this question could be applied in the problem of the entry and dissemination of the leprosy bacillus, of which Mr. Khanolkar spoke earlier.

Just one more question. Since you are using aerosol sprays over fairly wide areas in the field, how much radiation hazard would there be to the carriers to these insects, and grazing animals?

Mr. JENKINS (USA): In reply to the first question in regard to the labelling of plant spores causing hay fever and other allergies, I can only quote that some work has been carried out in labelling a number of plants. This is done by growing plants in solutions of appropriate radioisotopes. Some of the work which has been done, and it is quite extensive, was done in the Agricultural Experimental Station at Wooster, Ohio. There they have done a great deal of work in studying the dissemination of spores of certain plants, and also of corn pollen. A great deal of work has been done on the dissemination of spores, rust spores from wheat. I believe some of this work was done in California. I do not know of any work done on ragweed or goldenrod or some of the more common spores causing hay fever.

In regard to filariasis, there are about 20 different papers on the subject, and about 8 or 10 are referred to in the text of the paper I presented.

Another question was whether any work has been done *in vitro* concerning the metabolism of the tubercle bacillus. I would like to refer Mr. Gopal-Ayengar to the study by Strom and Rettbach in 1949. This was a paper on tubercle bacteria labelled with radioactive phosphorus, and that paper is also cited in the full text of my paper.

In regard to the last question—how much radiation hazard is there in aerosol sprays scattered in areas in the field? I know of no studies of this type which have been carried out. Aerosol spray studies have been carried out in small containers. This work is also cited in the text.

The CHAIRMAN: There are a number of questions to be answered on paper P/224. I suggest that the questioners, particularly Mr. Reifer and Mr. Siwa-

bessy, get into touch with Mr. Jenkins directly with a view to discussing these problems after the session.

Mr. JENKINS (USA): I would like to ask Mr. Costa-Ribeiro what plans he has for continuing the study of the dispersal of *Anopheles* mosquitos.

Mr. COSTA-RIBEIRO (Brazil): I am not able to give you a positive answer because these plans have been worked out by the surveys of malaria of the Ministry of Health in Brazil and I work in a very different field. I have only presented a paper by Dr. Aragão and Mme. Frota-Pessôa, because Mme. Neusa Margem is my assistant as Professor of Physics in the University. I can, however, give some information about the development of a similar technique in the study of the dispersion of insects responsible for the transmission of Shaya's disease. There have been some interesting experiments on marking with thorium nitrate, and the two types of method have been used, one consisting in injecting the solution directly in the nymphs, and the second to get the nymphs to suck the blood of pigeons to which a previous injection of radioactive salt had been given. In both cases the nuclear emulsion technique permitted the detection of the presence of thorium in the insects.

So far as epidemiological control is concerned, I am unfortunately unable to give any information.

I also have a question to ask Mr. Jenkins. I would like to know if any experiments have been carried out on the possible influence of the radioactive elements on the physiological condition of the mosquitos as compared with other mosquitos not marked with radioactive elements.

Mr. JENKINS (USA): In answer to this question I would like to present a reprint which covers this in detail, and curves are given for each of the concentrations used. In general we can say that at about 20,000 cpm in the adult mosquito there was no damage to the mosquito. In practice we use something like 5000 cpm per mosquito, which will carry it through about five half-lives, which is sufficient for all the studies, and we will be able to locate them with the field Geiger counters at the end of the period. We use something in the neighborhood of about 0.05 microcuries of P^{32} per millimeter in the water for producing mosquitos, with approximately 5000 cpm, and they are completely normal, reproduce, and lay eggs.

The CHAIRMAN: Before closing the session, I should like to congratulate those who have spoken on the excellence of the papers presented and those who have taken part in the debate on the high level of discussion. We are grateful for their interesting contribution to this International Conference.

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