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National Lectureships

1937 and 1938

SCIENCE IN PROGRESS

Science in Progress

By

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Preface

IN the present volume the Society of the Sigma Xi presents a group of notable contributions from ranking scientists who by their recent researches have blazed trails through new scientific realms; trails which are rapidly being widened and interconnected to form a network of important arterial highways over which will pass the men and materials destined to extend the frontiers of knowledge still farther.

The National Sigma Xi Lectureships, which are primarily responsible for the contributions presented here, originated from a suggestion of Professor L. J. Stadler at a meeting of the National Executive Committee in 1936 that the organization sponsor a series of lectures annually by recognized authorities; the lectures to be available to any Chapter desiring them. The 1937 and 1938 series have been given, and the material presented, somewhat augmented and revised in order to include even more recent data, forms the contents of the present volume. The great interest aroused in the participating Chapters, by the opportunity to obtain an authoritative report of the progress being made in various fields of scientific research directly from those largely responsible for the advances, has insured the continuation of the lectureships for the years immediately ahead. It is hoped that the response to this printed volume, *Science in Progress,* will be equally enthusiastic so that it will be possible to publish the manuscripts of the 1939 and 1940 series in a single volume, and other volumes still later. Only through publication will the vast audience of those interested in the extraordinary developments of present-day science be able to share in these riches.

It is a great pleasure for me, as editor of this volume, to ex-

press my appreciation to all of the contributing authors for their hearty coöperation and to the publishers who have endeavored in every way to present the printed material in the best possible manner. Acknowledgments are also due to Dean Edward Ellery of Union College and to Dean George B. Pegram of Columbia University for their invaluable assistance at all times; and to Professor Lorande L. Woodruff for suggesting as title, *science in progress*, which exactly fits.

GEORGE A. BAITSELL
National President,
Society of the Sigma Xi

New Haven, Connecticut,
March, 1939.

Foreword

AN encyclopedist of a century or so ago could gather into fruitful comprehension the facts and theories of all branches of science. A gifted individual was then able to know what conquests the techniques had achieved, into what fields of interpretation and imagination the current hypotheses were leading. But those days have gone. The capacity of the mind has not changed, while the activities and accomplishments of science have multiplied and become widely diverse. No longer is the botanist entirely conversant with the latest chemical theories, or the physiologist with galactic structure. And it is a pity. If this unity were still possible, philosophy would be the richer and the sciences would gain by the wider understanding.

The reader of the contributions to knowledge assembled in this book will find no synthesis of all the fields covered, but he will find, if attentive to detail, unification of another sort. He will note the employment of many of the same technical tools throughout the various branches of science and the general use of the most common instrument of all reasoned experimental science—the balanced alternation of guiding hypothesis and experimental test.

There is, however, another more subtle welding of the widespread sciences. For though we cannot hope to achieve, each for himself, a full and rounded view of the world of science, we can go far to envisage such a world and appreciate its variety. Whatever path the scientist chooses to travel, whether it be toward the minutest fragment of matter, or toward the wide stretch of the cosmos, or toward the still more mysterious activities of a human brain, he travels with one deep incentive—the satisfaction of intellectual curiosity, the understanding of some part of the world of nature.

This, then, is the attainable unity that lies behind science and furnishes the common ground on which all its aspects may be approached. We can, by looking even briefly at the progress in diverse fields, obtain some vision of the new explorations and some understanding of the temper of the sciences. We can, perhaps, sense where they are going, what kind of a world they will reveal, how they affect both present and future human growth.

Such a synthesis is achieved in partial measure by the present collection of essays. While the emphasis is on important phases of biological science, there is yet a range of subject that gives breadth to the volume. The results of researches into the nature of atoms, of studies of chemical and electrical reactions in living bodies, of explorations in the fields of biological processes, are gathered here and indications of future progress are drawn from them. Here is, indeed, one significant contribution to the panoramic view of science from which men must build their vision of the future.

HARLOW SHAPLEY

Cambridge, Massachusetts,
March, 1939.

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Science in Progress

Science in Progress

I

ATOMS, NEW AND OLD

By E. O. LAWRENCE

University of California, Berkeley, California

THE essential ideas of Rutherford and Bohr on the structure of matter are now firmly established. There is an abundance of evidence that an atom consists of a nebulous cloud of electrons whirling about a very dense, positively charged nucleus. Indeed, our assurance that this is so rivals our confidence that the planets revolve about the sun!

The number and configuration of the electrons determine the ordinary chemical and physical properties of the atom, and it might be thought that the nucleus is relatively unimportant, particularly as it is such a small region. But this is by no means the case; the nucleus really plays a dominant role in atomic structure.

THE NUCLEUS

The nucleus consists of a closely packed group of neutrons and protons, elementary building blocks of nature, which weigh something like two thousand times as much as the electrons. Thus, more than 99.99 per cent of the atom's mass is contained in the nucleus, and since mass is a manifestation of energy, it follows that practically all of the atom's energy also resides there. Moreover, the protons are positively charged particles, and the number of protons in the nucleus equals the number of electrons outside, for the atom as a whole is electrically neutral. Therefore, since it is the number of electrons outside that determines the chemical and physical properties of the atom, it follows that the nuclear charge determines the place of the atom

in the Periodic Table. On the other hand, the number of neutrons in the nucleus does not affect the number or configuration of the extranuclear electrons and hence does not have much to do with the chemistry of the atom; but the neutrons in the nucleus do contribute to the atom's weight, and hence we have isotopes of many of the elements—atoms of equal nuclear charge but differing in number of nuclear neutrons.

ATOMIC NUMBER	CHEMICAL ELEMENT	MASS	NUCLEAR STRUCTURE
1	HYDROGEN	1	1 PROTON
1	$HYDROGEN_2$	2	1 PROTON 1 NEUTRON
2	HELIUM	4	2 PROTONS 2 NEUTRONS } 1 ALPHA-PARTICLE
3	$LITHIUM_6$	6	3 PROTONS 3 NEUTRONS } 1 ALPHA-PARTICLE 1 PROTON 1 NEUTRON
3	$LITHIUM_7$	7	3 PROTONS 4 NEUTRONS } 1 ALPHA-PARTICLE 1 PROTON 2 NEUTRONS
4	BERYLLIUM	9	4 PROTONS 5 NEUTRONS } 2 ALPHA-PARTICLES 1 NEUTRON

FIG. 1. The structure of the nucleus of an atom determines its mass and its positive charge; hence determines the particular kind of a chemical element it forms.

These considerations are illustrated in Figure 1, which is a table of the isotopes of several of the light elements. First of all, there is hydrogen. Ordinary hydrogen has a nucleus consisting of only one proton, and the electron cloud outside consists correspondingly of only one electron, whirling around the nucleus, which, if it could be seen with the naked eye, would look like a diffuse spherical nebulosity. The nucleus next in simplicity is

the deuteron, the nucleus of heavy hydrogen or deuterium, discovered by Urey five years ago as described in the next chapter. This nucleus consists of a pair of nuclear particles, a neutron and a proton, and again, since there is but one proton around this nucleus, but one electron revolves, and hence the electron cloud is that of hydrogen. This nucleus containing two particles weighs twice as much, and therefore deuterium is approximately twice as heavy as ordinary hydrogen. There is some evidence for a triple-weight hydrogen having a nucleus containing two neutrons and one proton. Next there is the helium nucleus, consisting of two neutrons and two protons with, accordingly, two extranuclear electrons. Finally, let us consider lithium, which occupies the third place in the Periodic Table and therefore has three protons in its nucleus, with three electrons outside. It is well established that there are two isotopes of lithium. The lighter one is Li^6, which has a nucleus containing, accordingly, three neutrons and three protons; and the heavier is Li^7, which has an additional neutron. As we proceed up the Periodic Table, the number of isotopes of the elements varies from element to element; in some cases there are as many as ten, as, for example, with tin. The heaviest element we know of in nature is uranium. It has 92 protons in its nucleus and, accordingly, 92 electrons in the outside cloud. The heaviest isotope of uranium has 147 neutrons packed in with the protons, and hence weighs about 239 on the atomic scale.

It is well to say that the isotopes have weights "about" equal to the combined weight of the neutrons and protons in the nucleus, for they do not weigh exactly these amounts. In general, the close packing of the neutrons and protons together to form nuclear matter results in a small loss of mass, something less than one per cent. Although this is relatively small, it is, as we shall see later, extremely important.

Neutrons and protons are exceedingly small particles. At the present time we know so little about their properties that we are content to speak of them as elementary particles, spherical in

shape and having radii of the order of magnitude of 10^{-13} cm. As regards the structure of nuclei, we know little more than that they consist of neutrons and protons sticking together in much the same way as water molecules form a water droplet. Some day, doubtless, we shall have very definite and detailed ideas about nuclear structure, but for the present we know little more than that neutrons and protons can be put into nuclei and that they come out also. Whether the elementary particles fuse together to form a new form of nuclear matter or whether they retain their identity in the nucleus is a question that has no answer at this time. In any case, it is known that the nuclear particles are so closely aggregated that the volume of the nucleus is about what one would expect if the neutrons and protons were tiny spheres in contact. Thus, even for the heavier elements, the nuclei may be regarded as spheres with radii of approximately 10^{-12} cm.

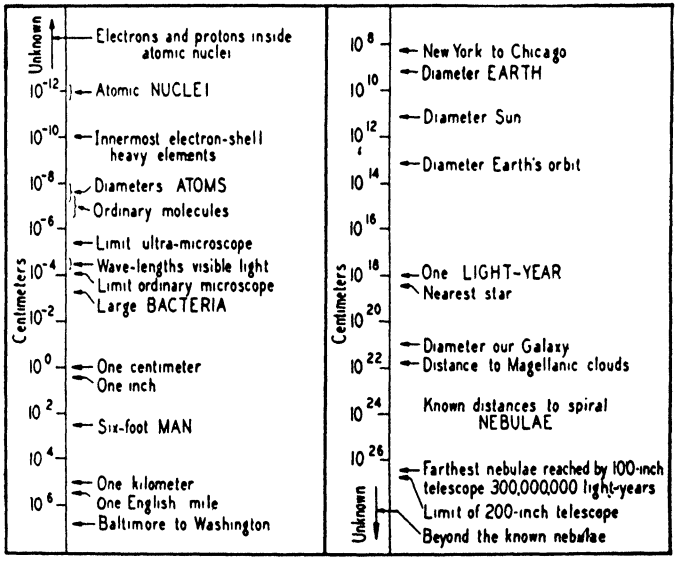


FIG. 2. Chart of known dimensions.

A conception of the size of the nuclear domain has been admirably illustrated by Dr. M. A. Tuve in Figure 2, where there are shown dimensions of various entities accessible to observation. With these dimensions plotted in centimeters in powers of ten as ordinates, we see that the atomic nucleus is at one end of the scale and the astronomer's universe at the other, while midway is the distance of one hundred kilometers; so that it may be said that, if we take one hundred kilometers as our yardstick, the nuclear domain is as small as the astronomer's universe is large. When one first becomes aware of the extreme smallness of the atomic nucleus, one is apt to despair of ever learning much about it. It would be natural to presume that it would be quite inaccessible to observation, but this has not turned out to be the case. In recent times the nucleus has been entered, and studies already have revealed it as a domain of incredible richness.

Transmutation

The Rutherford-Bohr theory of the atom reduced the age-old problem of alchemy—the transmutation of the elements—to very simple terms. For, since the number of protons in the nucleus determined the number of electrons outside and hence the place of the atom in the Periodic Table, it was clear that the conversion of one element into another was simply the problem of changing the number of protons in the atomic nucleus.

Rutherford considered how it might be possible to knock out one or more protons from the nucleus of an atom. He realized that it would be necessary to bombard the atomic nucleus with nuclear particles of great energy; for he had already established the fact that nuclear matter is held together by very strong forces and that the nucleus is surrounded by a great wall of electrical potential due to the nuclear charge. He had also shown that the alpha particles emitted from radium consist of swiftly moving nuclei of helium, and accordingly it was clear that they were promising projectiles for the nuclear bombardment. Just

before the Great War, in 1914, Rutherford carried out his first experiments on transmutation by alpha-particle bombardment and obtained some encouraging results, but war duties made it necessary to suspend the experiments until 1919. In that year he returned to the laboratory and was not long in establishing, beyond any doubt, the conversion of nitrogen into oxygen. He bombarded nitrogen gas with the alpha particles from a strong radioactive source and observed the emission of swiftly moving protons from the bombarded nitrogen. His observations led him to the logical conclusion that a small fraction of the alpha particles passing through the nitrogen gas collided with nitrogen nuclei, and, although they stuck to the nucleus, they generally collided with such energy as to knock out a proton with terrific energy. At that time, the existence of the neutron had not been recognized (the nucleus was thought to contain protons and electrons), but in recent years it has been established that often in such nuclear collisions neutrons as well as protons are knocked out. Rutherford's interpretation of his observations is

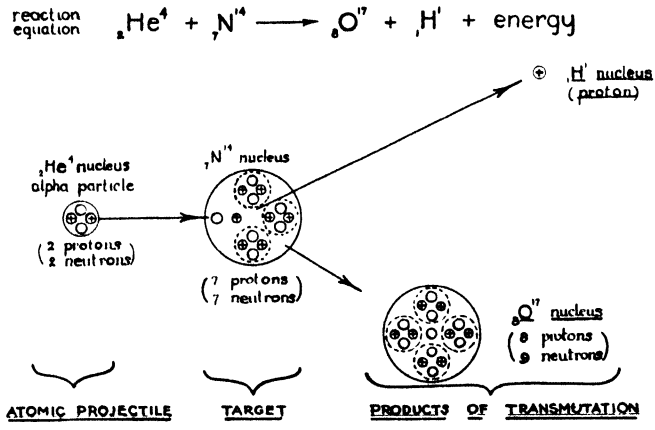


FIG. 3. Rutherford (1919) bombarded nitrogen with natural alpha particles and thus artificially produced disintegration and the transmutation of an element.

shown in Figure 3, which is the classical nuclear reaction, marking the beginning of a new science which may well be called nuclear chemistry, the science of the reactions of nuclei.

In discussing the reactions of nuclei, in general it is sufficient to describe the reacting nuclear particles by two numbers, one giving the total number of nuclear particles in the nucleus and the other the number of protons (i.e., the atomic number). Thus, in Figure 3, we see helium and nitrogen reacting to form oxygen and hydrogen. Helium has four particles in its nucleus, a fact which is denoted in the upper right-hand corner, and two protons, indicated in the lower left-hand corner, while the nitrogen isotope involved has fourteen nuclear particles and atomic number seven. These particles react to emit protons, hydrogen nuclei; and, assuming that the rest of the nuclear matter sticks together, it follows that the newly formed nucleus contains seventeen particles, eight of them being protons. That is to say, O^{17} is formed. At the time Rutherford postulated this nuclear reaction to explain his observation, the isotope O^{17} was not known in nature. Years later, however, its presence was detected in the atmosphere by spectroscopic methods, and in recent times many different lines of evidence have made both O^{17} and O^{18} familiar isotopes to both the chemist and the physicist. Following the transmutation of nitrogen, Rutherford and his collaborators in the Cavendish Laboratory succeeded in transforming in a similar way most of the elements in the Periodic Table up to potassium. Their epoch-making researches greatly extended our knowledge of the atomic nucleus.

Atomic Projectiles

Rutherford's experiments were clearly but a fragmentary glimpse into a region of the atom of inconceivable richness, and they brought to the forefront the need for atomic projectiles of various kinds and in vastly greater numbers for the further exploration of the nuclear domain. As a result, many laboratories over the world undertook the development of methods of ac-

celerating atoms[1] to very high speeds for purposes of nuclear research, and it was not long before atomic projectiles of many kinds were available to carry forward the attack.

From the moment the problem of accelerating atoms to high speeds was proposed, it was clear that the only practical way would be to form ions of the atoms in a rarefied gaseous atmosphere within a vacuum tube and cause them to be accelerated in the electric field between electrodes at a suitable difference of potential. Indeed, the kinetic energies of atomic projectiles have from the beginning been given in terms of the energy they would have if they were singly charged positive ions falling through a given potential difference. Thus we speak of a million-volt proton as one which has the kinetic energy that it would have as the result of passing through a vacuum tube from one electrode to another at a difference of potential of one million volts. The alpha particles emitted from radium have a kinetic energy of approximately eight million volts, and it was presumed from early experiments with these atomic projectiles that particles of energies of several million volts would be needed for nuclear investigations. Thus it became clear that a straightforward method of accelerating atoms to energies sufficient to bring about nuclear reactions would be to apply millions of volts to the electrodes of vacuum tubes and to cause the ions to pass from one electrode to the other. Accordingly, many laboratories undertook the development of means of generating very high voltages and vacuum tubes of sufficient ruggedness to withstand these great electrical potentials.

There is not space here to describe adequately the various methods of generating high voltage; but it is of interest to show pictures of the apparatus developed by Cockcroft and Walton, Figure 4, and that of Van de Graaff, Figure 5. Cockcroft and

1. Perhaps it should be remarked that, although it is the high-speed nuclei of various sorts, such as protons, deuterons, and alpha particles, that are required for nuclear research, swiftly moving atoms serve just as well, as in nuclear collisions the diffuse electron clouds surrounding the nuclei play no significant role.

Walton devised a very good method of multiplying the voltage of a transformer by means of a combination of condensers and rectifiers, and the apparatus here shown was capable of delivering to a vacuum tube a steady potential of about one million volts. This photograph of their apparatus is of particular historical interest, for it shows not only their voltage generator but

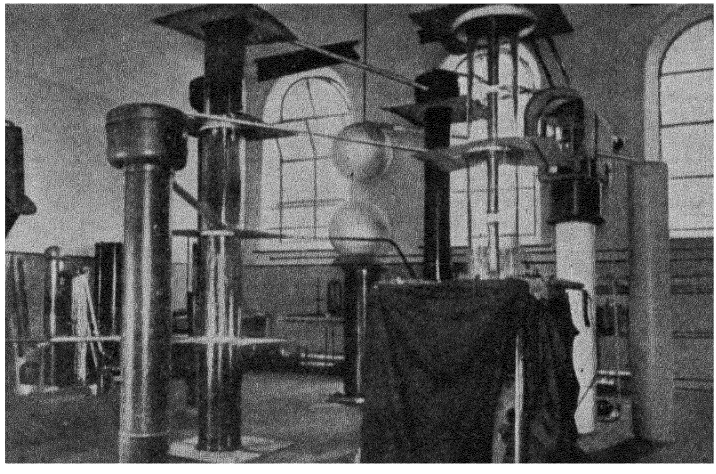


FIG. 4. The apparatus of Cockcroft and Walton in the Cavendish Laboratory with which lithium and other light elements were for the first time disintegrated by proton bombardment.

also the attached vacuum tube capable of accelerating protons to energies of about eight hundred thousand volts, with which they carried out the pioneer experiments on the disintegration of the light elements with artificially accelerated particles.

Cockcroft and Walton endeavored to reach higher voltages but encountered difficulties which can be appreciated from the photograph of the Van de Graaff generator in operation; for Figure 5 gives a good idea of the behavior of millions of volts. The Van de Graaff generator, which has proven to be very use-

ful for nuclear research,[2] consists of a large metal sphere, mounted on a gigantic insulator within which there is a revolving belt which carries electrical charges from the ground to the

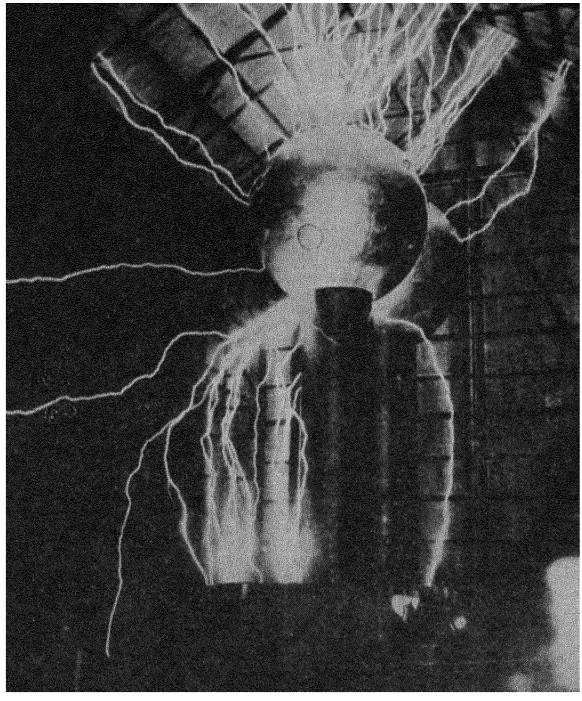


FIG. 5. The Van de Graaff electrostatic high-voltage generator in operation. The spark discharges, many feet in length, resulted from voltages above 2.5 million.

sphere. With the generator operating in air, it is necessary to build it very large and to house it in a large room, for the intense electric fields produced by high voltages in the sphere give rise to corona discharges and electric sparks. In this picture,

2. For example, Dr. M. A. Tuve and associates, of the Department of Terrestrial Magnetism, Carnegie Institution of Washington, have used the Van de Graaff generator with notable success in many important nuclear investigations.

the Van de Graaff generator is running with the sphere at a potential of about two and a half million volts, with the result that electric sparks are seen jumping from the sphere to the ground and to the rafters of the airship hangar housing the machine, distances of approximately twenty feet. If the machine were to generate voltages of twenty-five million, the machine would have to be ten times larger, and sparks would jump ten times farther, and these would indeed be bolts of lightning.[3]

The Cyclotron

Another method of accelerating particles might be termed the method of resonance or of multiple acceleration. A child in a swing knows that a high swing velocity can be achieved by one big push, corresponding to the single acceleration of ions by application of high voltage, or by a succession of small pushes, properly timed with the swing action, corresponding to the resonance method of accelerating ions by repeated application of low voltages. One type of apparatus that uses this resonance principle involves both a magnetic field and an oscillating electric field; and, because the ions spiral around as they are accelerated, the apparatus has come to be called the "cyclotron."

The most prominent feature of the cyclotron is a giant electromagnet. The one in the Radiation Laboratory at the University of California, shown in Figure 6, weighs about eighty tons. With this apparatus, deuterons have been accelerated to energies of nearly eight million volts and alpha particles to nearly sixteen million volts. A much larger installation in an

3. Recently, important progress in combating the necessity of large apparatus dimensions for the direct generation of high voltage has been achieved by placing the apparatus in air tanks under high pressure. At the University of Wisconsin, Herb, Parkinson, and Kerst have made particularly noteworthy progress in this direction with a Van de Graaff generator actuating a vacuum tube at two million volts. Much larger apparatus of this type is now under construction at the Department of Terrestrial Magnetism, Carnegie Institution of Washington, at the Westinghouse Company in Pittsburgh, and at the University of Minnesota. These new installations are designed for voltages of above five million.

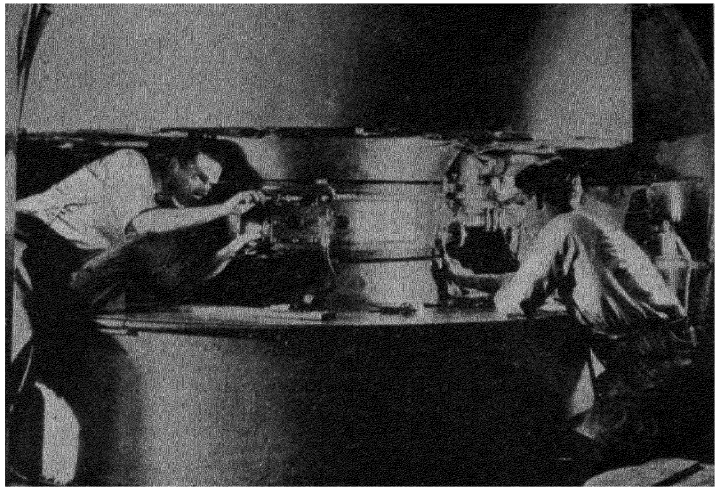


FIG. 6. General view of the University of California cyclotron.

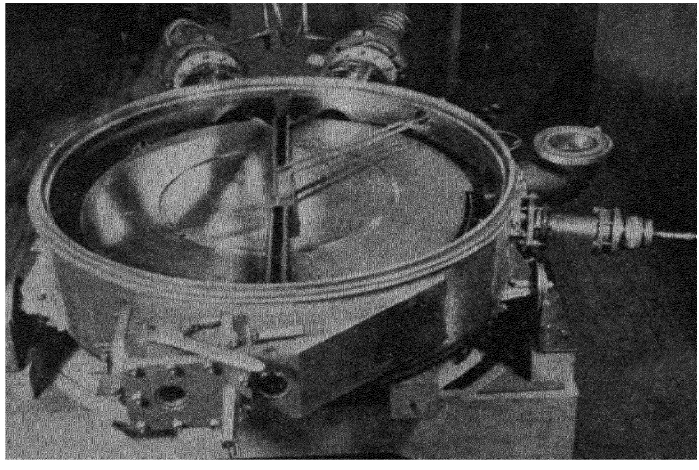


FIG. 7. The cyclotron vacuum chamber with cover removed.

adjoining laboratory, having a magnet weighing over two hundred tons, is now being made, and it is expected that the larger apparatus will be capable of generating ions at much higher voltages.

It is perhaps well to indicate briefly the principle of the cyclotron. The ions are accelerated in the vacuum chamber between the poles of the magnet. The function of the magnetic field is to cause the ions to spiral around with constant angular velocity. Within the chamber there are two semicircular hollow electrodes, between which is applied a high frequency potential difference. The pillbox-shaped chamber, with cover removed, exposing the accelerating electrodes, called "dees," is shown in Figure 7. The ions circulate around from within one dee to within the other, and, as they cross the diametrical region, they gain increments of kinetic energy corresponding to the potential difference. Inasmuch as the angular velocity of the ions is determined by the magnetic field alone, they can be made to spiral around in synchronism with the oscillating electric field, thus gaining successive increments of velocity. As they are speeded up, the ions travel on ever widening spirals, finally emerging at the periphery of the apparatus, where they may be withdrawn by a deflecting electrostatic field and directed to a target for bombarding purposes.

It is sometimes desired to bring the swiftly moving ions out of the vacuum into the air; and this can be readily done, as they have enough energy to penetrate a thin metal window, usually of platinum. A beam of deuterons emerging through such a window passes for some distance through the air before losing its energy, and excites the molecules of the air to the emission of visible light, appearing as a bright lavender-colored glow. This is shown in the photograph of Figure 8, where a six-million-volt beam of deuterons is emerging from the window, producing a luminosity through the air for a distance of about thirty centimeters. For most purposes, it is not necessary to bring the accelerated ions out of the vacuum chamber, since the

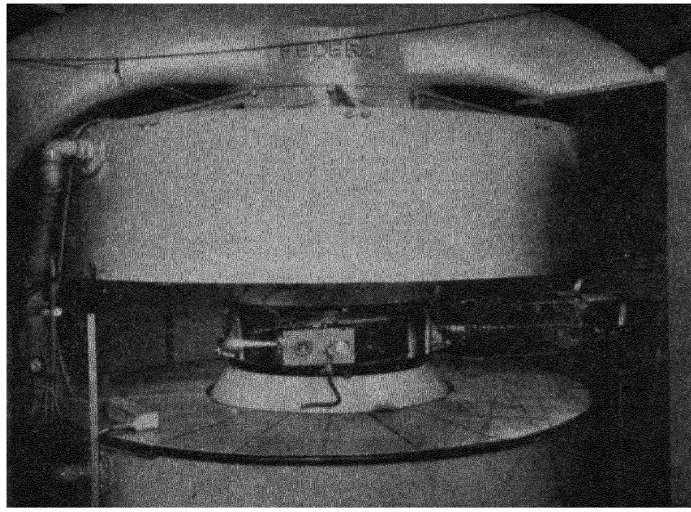


FIG. 8. The Berkeley cyclotron in operation. A beam of six-million-volt deuterons is shown emerging into the air.

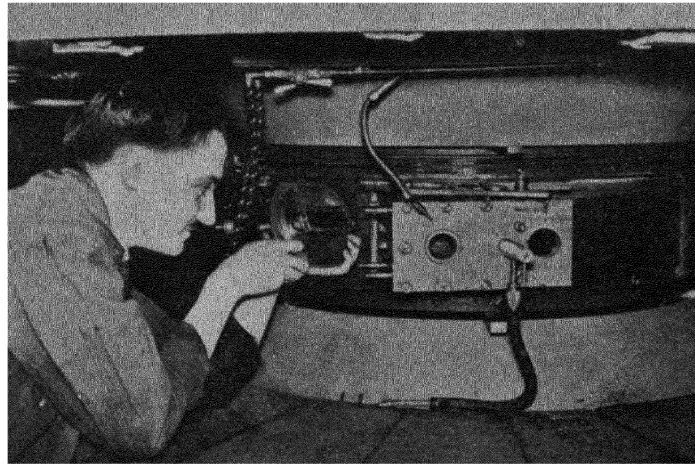


FIG. 9. Placing a water-cooled beryllium target in position in the cyclotron for bombardment as a neutron source.

targets to be bombarded can be placed conveniently within the vacuum (Fig. 9). On the other hand, it is sometimes more convenient to have the beam come out into the air; for example, when it is desired to render a salt crystal radioactive. This can be done simply by holding the salt crystal in the beam in the air for a short while. For some purposes, it is desirable to withdraw the beam entirely away from the vacuum chamber, and this can be done by attaching a suitable vacuum-tube extension. Figure 10 is a photograph of a five-million-volt beam of deu-

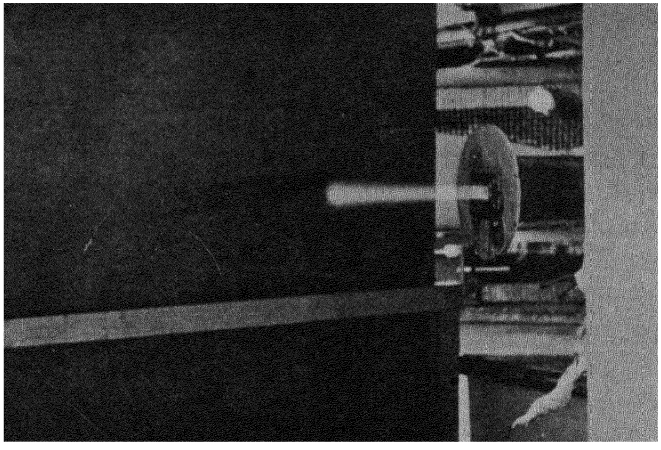


FIG. 10. A beam of five-million-volt deuterons emerging into the air at the end of a vacuum-tube extension six feet from the main cyclotron chamber.

terons emerging from the window at the end of such a vacuum tube, extending entirely out of the field of the magnet to a distance of about six feet from the pillbox vacuum chamber.

THE EQUIVALENCE OF MASS AND ENERGY

One of the consequences of Einstein's theory of relativity, first enunciated in 1905, was the discovery that matter is one form of energy. As the relativity theory grew in favor, the validity of the mass-energy relation became more apparent, but

until direct experimental verification was forthcoming Einstein's great deduction could not be regarded as an established law of nature.

The pioneering experiments of Cockcroft and Walton on the disintegration of lithium by accelerated protons constituted the first direct evidence of the truth of this great principle. They observed that when a lithium target was bombarded with protons of energy of about a half-million volts, 8.5-million-volt alpha particles were emitted from the lithium in great numbers.

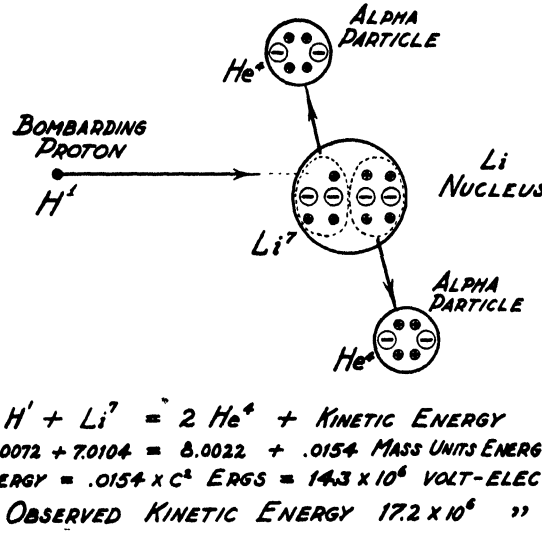


FIG. 11. Bombardment of lithium with accelerated protons (Cockcroft and Walton) resulting in its artificial disintegration.

The obvious interpretation of their observations was that the protons, on colliding with lithium nuclei, were reacting to form helium nuclei according to the equation shown in Figure 11. Here, the fact that the alpha particles came off with a great deal more kinetic energy than the bombarding protons had means that the reaction is highly exoergic, that a great deal of energy is released in the reaction.

That the two helium nuclei resulting from the union of a proton and a Li^7 nucleus should fly apart with more than eight million volts of energy was quite expected on the basis of the principle of the equivalence of mass and energy. The masses of many of the isotopes of the elements had been measured by Aston, Bainbridge, Dempster, and others, and the best mass values of H^1, Li^7, and He^4 were those indicated in Figure 11. Thus, the accepted weights of these nuclei indicated that two helium nuclei have a combined mass of 0.0154 mass units less than that of H^1 plus Li^7, so that in the formation of two helium nuclei out of hydrogen plus lithium, there is a loss of this amount of matter, which would be expected to appear in the form of an equivalent amount of kinetic energy of the particles flying apart. As shown in the figure, the then accepted mass values indicated that the energy released by the loss of mass in this reaction was 14.3 million volts, while actually the observations of the kinetic energies of the alpha particles indicated that they flew apart with the combined energy of 17.2 million volts. Thus, there seemed to be at the time a slight discrepancy, as though the proportionality factor between mass in grams and equivalent energy in ergs was not exactly that given by the Einstein theory. It was not long, however, before this apparent discrepancy was eliminated. The masses of the atoms involved were remeasured with greater precision, and in the end it was found that the difference in mass of the reacting particles and the products was just the amount that one would calculate from the observed energy release in the reaction according to the Einstein principle. Following this first definite and direct experimental proof of the equivalence of mass and energy, many other nuclear reactions were studied in rapid succession, providing additional proof; at the present time this great principle has as firm an experimental foundation as any of our laws of nature.

Now that it is an established fact that matter can be destroyed and converted into energy, let us consider for a moment what

this means. At the outset one is impressed with the tremendous store of energy tied up in the form of matter. For example, a simple calculation according to the relativity theory shows that a glass of water, if completely destroyed and converted into useful energy, would yield more than a billion kilowatt hours, enough energy to supply a city with light and power for quite a time. It is a highly practical question, therefore, to inquire whether it is possible to tap this almost inexhaustible supply of fuel.

The source of the sun's energy has long been a great mystery, for there is good evidence that it has been blazing at its present brilliance for billions of years. Fuel for this eternal fire could be of no ordinary sort, and astronomers and physicists now believe that conditions within the sun are such that nuclear reactions are taking place on an extensive scale with the destruction of matter and conversion into radiant energy. Thus, the sun is gradually through the ages losing its mass. Slowly its very substance is radiating into space.

But whether it will be possible to release subatomic energy on a practical and profitable basis for industrial purposes, whether perhaps it will be possible to realize conditions on the earth similar to those in the sun, are questions which, of course, interest the engineer. Indeed, they are questions of interest to everyone, and accordingly this has been a subject for much popular discussion and speculation. The fact is, at this time, that although we now know that matter can be converted into energy, we are aware of no greater prospect of destroying nuclear matter for power purposes than of cooling the ocean to freezing temperatures and extracting the heat for profitable work. Certain considerations bearing on the second law of thermodynamics appear to govern the availability of energy in the hearts of atoms as in the Atlantic Ocean itself. The establishment of the great principle of mass-energy equivalence is, however, a keystone in the development of physical theory.

NEUTRONS

The story of the discovery of the neutron by Chadwick is an exceedingly interesting one. Following his discovery of the emission of these electrically neutral particles from various substances under alpha-particle bombardment, many other nuclear reactions were observed wherein neutrons are emitted, and it was such observations that led to the conception of the nucleus as consisting of groups of neutrons and protons rather than protons and electrons as had been previously thought. One of the most prolific sources of neutrons was found to be a beryllium target under deuteron bombardment, the reaction being as follows:

$$_4Be^9 + _1H^2 \rightarrow _5B^{10} + _0n^1$$

In other words, it was found that when a beryllium target is bombarded with swiftly moving deuterons, one of the nuclear reactions occurring is that in which a neutron is emitted and the light isotope of boron is formed. This reaction occurs to a detectable extent at bombarding voltages of only a few hundred thousand volts, but the neutron emission from the beryllium target increases very rapidly with the voltage of the bombarding particles. For example, six-million-volt deuterons give a neutron yield which is about a hundred times that at one million volts.

The cyclotron in the Radiation Laboratory at the University of California produces more than a hundred microamperes of five-million-volt deuterons, and these, directed against a beryllium target, give rise to an emission of neutrons equivalent to the yield from a similar target bombarded with alpha rays from a hundred kilograms of radium. The neutron emission from the cyclotron is such that it produces in the surrounding air an amount of ionization of the same order of magnitude as that obtained from an X-ray machine.

Of course, to the physicist, neutrons are of great interest be-

cause they are elementary particles of matter, and especially because they have no electrical charge and hence penetrate the nuclear domain without hindrance of the electrical barrier. Being electrically neutral, they pass right through the electron clouds of atoms and are only impeded by direct collisions with atomic nuclei. Sometimes, in hitting an atomic nucleus, a neutron will bounce off with only a slight loss of kinetic energy, but in many cases the neutron sticks and shares its energy with the other nuclear particles. In the latter event, it usually happens that one (or more) of the nuclear particles of the nucleus so "heated up" is "evaporated off," resulting in the formation of a new isotope. Thus, neutrons are very effective agents for the transmutation of the elements and are playing an important role in the development of our knowledge of the atomic nucleus.

BIOLOGICAL ACTION OF NEUTRON RAYS

Neutron rays are also of great immediate interest to the biologist because of the remarkable way they are absorbed in matter. They are more readily absorbed in light substances rich in hydrogen, such as biological tissues, than in denser substances like iron or lead. If one should use a fluoroscope and look through the body with neutron rays, one would find that the bones would appear relatively transparent and the flesh perhaps somewhat darker. Neutron rays are also unique in the manner in which they produce ionization. X rays produce ionization by liberating high-speed electrons from atoms, while neutrons, being tiny, dense particles of neutral matter, ionize only by making intimate collisions with atomic nuclei.

The great difference in the mode of ionization by neutrons and X rays can actually be photographed, thanks to the ingenious cloud-chamber method of C. T. R. Wilson, which makes use of the fact that fog droplets form on ions in an atmosphere of supersaturated water vapor. Ionization in the cloud chamber thus can be seen in detail in the sense that each ion can be made to manifest itself as a visible fog droplet. Figure 12 is a cloud-

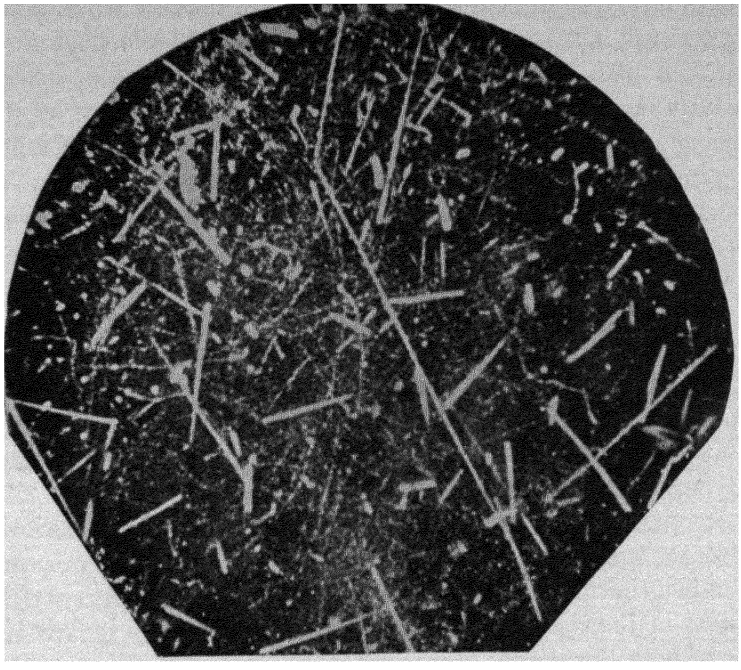


FIG. 12. Wilson cloud-chamber photograph of ionization in a mixture of air, hydrogen, and water vapor produced by neutron rays and gamma rays from the cyclotron. The thin tracks of ions were produced by secondary electrons liberated by the gamma rays, while the thick and very dense tracks were produced by the recoil protons resulting from collisions of neutrons with the hydrogen atomic nuclei. This picture gives a general impression of the great difference in the distribution of ionization in tissues produced by neutron rays and X rays. In comparison to X-ray ionization, neutron ionization is very much more localized and intense.

chamber photograph of the ionization produced in a chamber filled with a mixture of hydrogen, oxygen, nitrogen, and water vapor by a mixture of gamma rays and neutron rays from the cyclotron. The very thin lines of fog droplets were caused by the high-speed electrons liberated by the gamma rays passing through the chamber, and these formed only a few ions per centimeter of path. This is the sort of ionization produced by X rays. The much thicker and much more dense ionization

tracks were due to neutrons. At one end of a dense track, a neutron struck a hydrogen nucleus, a proton, and the proton was knocked with tremendous energy. The recoil proton, being a heavily charged particle, rapidly dissipated its energy by producing very intense ionization over a few centimeters—before coming to rest and picking up an electron, thus becoming an ordinary hydrogen atom again. The heavy fog tracks, several inches long, were produced by recoil protons having more than a million volts of energy. Electrons of such energies would make ionizing tracks a hundred times longer. The ionization produced by a recoil proton is something like a hundred times more dense than that produced by an X-ray secondary electron, and so we see that neutron ionization in comparison with X-ray ionization is very much more localized and very much more intense where it occurs.

In view of this great difference in the physical behavior of neutrons and X rays, the biologist is at once led to inquire whether the two forms of ionizing radiation are also very different in their biological action. The questions here involved are of both theoretical and practical interest. According to some theories of the biological processes induced by ionization, it is the total amount of ionization and not the distribution in a biological system that is important, a point of view which would indicate that neutron rays would parallel X rays completely in their biological action. Observations of differences in biological effects produced by the two radiations would therefore contribute significantly to our understanding of biological effects due to ionization. With this in mind, as well as the possibility that neutron rays might have valuable practical applications, some experiments on the comparative biological effects of neutrons and X rays have been carried out in the Radiation Laboratory at Berkeley with very interesting results, for they have already established beyond any question that neutron rays and X rays do not parallel each other in their biological action. Perhaps it should be remarked here that these experiments were

originally undertaken for the immediate practical purpose of obtaining information for the protection of workers in the laboratory. There was a sincere desire not to repeat the unfortunate experiences of many of the early roentgenologists.

The first experiments were undertaken by Dr. John H. Lawrence, then of the Yale University School of Medicine. By placing some rats near the cycloton neutron source, he found immediately that the neutron rays are lethal, that a rat is killed by a few minutes' exposure. These first observations were followed by a succession of investigations by Dr. Lawrence, Dr. R. E. Zirkle, and Mr. P. C. Aebersold on the comparative effects of neutrons and X rays on various biological objects, including both normal and neoplastic tissues.

Zirkle and Aebersold studied the growth-inhibiting effects of neutron rays and X rays on wheat seedlings and found that 120 r of neutrons produced as much effect as 600 r of X rays.[4] In other words, in inhibiting the growth of wheat seedlings neutrons were found to be five times as effective as X rays. In the case of fern spores, neutron rays were found to be 2.5 times as effective as X rays; while in killing Drosophila eggs they appeared to be only 2.1 times as effective. Thus the experiments of Zirkle, Aebersold, and Dempster established definitely that neutrons in comparison with X rays have a different selective biological action.

This result immediately raised the very important question of the action of neutron rays on tumor tissue, whether the neutrons have a greater selective action than X rays. A series of experiments were carried out by Lawrence and Aebersold on a

4. The neutron dosage was measured by the same instrument as used for the X-ray dosage measurements, i.e., a Victoreen meter, and hence the neutron dosage is given in arbitrary units, the indications of the meter. A discussion of neutron dosage measurements here would be too long, but it perhaps is well to mention that experiments have been carried out indicating that the neutron dosage measurements obtained with the Victoreen meter are approximate indications of the ionization in ordinary biological tissues. The question of absolute ionization in tissue in relation to the dosage measurements is here not essential, as we are interested at this time only in dosage ratios and can regard the dosage measurements as in arbitrary units.

mouse tumor, a mammary carcinoma, and they found that the neutrons, as exhibited in Figure 13, were about 5.1 times as effective in killing this tumor tissue. They also studied the comparative effect of neutrons and X rays in killing mice, and in this case found the neutrons to be but 3.8 times as effective. The experiments accordingly indicated that in terms of the dose that could be given a mouse without killing it, the neutrons had a greater effect on tumors *in vitro* than the X rays. If these indications of a greater selective action of the neutron rays on tumor tissue prove to be true for carcinoma *in vivo*, it is a very important practical matter. The neutron may prove to be a very valuable therapeutical agent.

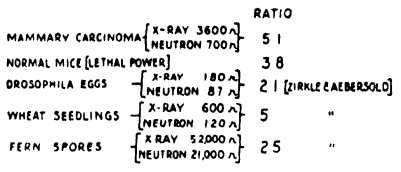


FIG. 13. Summary of comparative effects of X rays and neutron rays on five biological objects, showing that the ratio of the doses of the two forms of radiation required to produce the same biological action in the several instances varies from 2.1 to 5.1. These results show that neutrons have a selective action on biological substances which in general is different from that of X rays.

ARTIFICIAL RADIOACTIVITY

In 1932, Curie and Joliot discovered that boron under alpha-particle bombardment gives off neutrons and also positrons, the electrons of positive charge discovered that same year by Anderson. They concluded that when boron is bombarded with alpha particles alternative reactions may occur. Either a proton may be emitted, as observed by Rutherford and Chadwick many years earlier, or a neutron and positive electron may come off together—as though a proton is not itself a simple particle but is made of a neutron and a positive electron and that sometimes the process of expulsion in the reaction is so violent that the proton is broken up into its constituent parts. One day, more than six months after they had noticed the emission of positrons from boron bombarded by alpha particles, Curie and Joliot accidentally observed that the positron emission continued after the alpha-

particle bombardment had been stopped—they happened to remove the radium from the vicinity of the boron. With this observation, they had discovered that a new kind of radioactivity is stimulated in boron by alpha-particle bombardment, which they called artificial radioactivity.

This discovery made it evident that the neutron and positron do not come off simultaneously, but that the neutron in the reaction is emitted first, leaving a nucleus having a charge of seven (hence a nitrogen nucleus) and a weight of thirteen, which is lighter than ordinary nitrogen and presumably unstable. They proved chemically that the radioactive substance formed is in fact an isotope of nitrogen and accordingly christened it "radio-nitrogen." They concluded that the radio-nitrogen nucleus, by emitting a positive electron, thereby losing one positive charge, is converted into the heavy isotope of carbon, C^{13}, which is known in nature. Thus the nuclear reactions involved are written:

$$_5B^{10} + {}_2He^4 \rightarrow {}_7N^{13} + {}_0n^1$$

$$_7N^{13} \rightarrow {}_6C^{13} + e^+$$

In those pioneer experiments, they produced two other radioactive substances, radio-magnesium and radio-silicon; and, since that time, other synthetic radioactive substances, new unstable atoms not known in nature, resulting from alpha-particle bombardment, have been discovered by various investigators.

In their first discussion of their important discovery, Curie and Joliot suggested that such new radioactive isotopes might well be produced by bombardment with other atomic projectiles, especially protons, neutrons, and deuterons; and it was not long before experiments in many laboratories proved this to be the case. One interesting example of the formation of a radioactive isotope is shown in Figure 14. When sodium is bombarded with deuterons, several nuclear reactions occur, and one of them is that in which the neutron of the deuteron sticks to

the sodium nucleus. The neutron added to the sodium, increasing its weight to twenty-four, renders it unstable, and in the course of time the unstable nucleus releases its excess energy by emitting a negative electron, thereby increasing the positive charge on the nucleus by one and creating Mg^{24}, next higher in the Periodic Table. This radioactive form of sodium has a half life of 14.5 hours. That is to say, such an unstable nucleus has a 50 per cent likelihood of emitting an electron in that time.

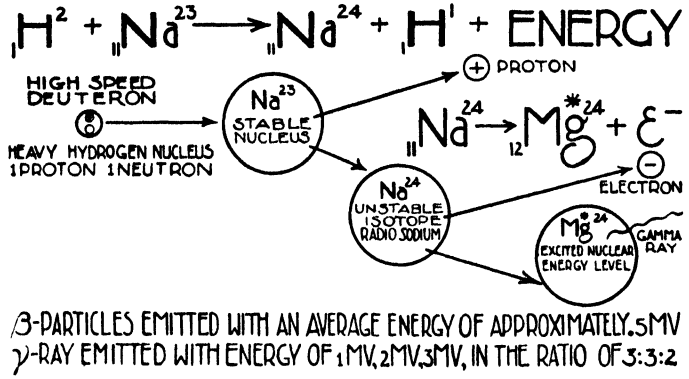


FIG. 14. The production of radio-sodium as the result of a bombardment of sodium with deuterons.

Another unstable isotope of sodium can be produced by knocking out a neutron from the Na^{23} nucleus, forming Na^{22}. This radioactive form emits positive electrons and thereby turns into the isotope Ne^{22}, and, instead of having a half life of about 14.5 hours, has a half life of approximately three years.

Although artificial radioactivity was discovered but five years ago, at the present time about two hundred radioactive isotopes of the common elements are known. They have been produced in various ways: bombardment with alpha particles, protons, neutrons, and deuterons. Bombardment of various substances with deuterons from the cyclotron has thus far been found to be the most prolific source of these new radioactive atoms. In fact,

every element thus far bombarded with five-million-volt deuterons has been rendered radioactive, and in many cases several radioactive isotopes of the same element have resulted. In some cases, especially in the heavier elements, the yields have been very small, hardly more than measurable; and in other cases, especially among the lighter elements, it has been possible to produce artificial radioactivity in intensity comparable with that of a gram of radium. Indeed, it is already customary to express the artificial radioactivity induced in a substance by deuteron bombardment in terms of millicuries.[5]

The large number of new isotopes of the elements that have been revealed in the past few years are shown in the isotope chart in Figure 15. The isotopes in the squares are the stable isotopes in nature, while those in the circles are the radioactive forms. A casual inspection shows that already there are about as many new synthetic unstable isotopes as there are natural stable ones.

LABELED ATOMS

From what has been said, it is clear that the radioactive isotopes of the elements have the same chemical, physical, and biological properties as the ordinary elements, for the former are distinguished from the latter only by having a greater or smaller number of neutrons in the nucleus, and these do not affect the disposition of the electron cloud outside, which determines the chemical nature of the atom. It is only when a radioactive atom disintegrates with the emission of an electron and perhaps a gamma ray that it is evidently a different atom from its stable neighbor. In most cases, the radiations given off by the radioactive isotopes in the course of disintegration are very energetic and consequently can be detected with extraordinary ease. Indeed, with the Geiger counter—an inexpensive apparatus which is to be found nowadays in most physical laboratories

5. One millicurie is defined as radioactivity in which 3.7×10^4 electrons are emitted per second.

—and also with the cloud chamber, it is possible to observe the individual electrons emitted from radioactive substances. In consequence, a very small number of radioactive atoms can be detected without the slightest difficulty. This circumstance means that the chemist and the biologist now have at their disposal a new powerful technique for labeling atoms and tracing them with complete certainty through a complicated chemical or biological system.

A recent investigation by Dr. J. M. Hamilton, in which sodium was traced through the human body, illustrates the simplicity and power of the technique. The experiment was as follows: Ordinary salt was rendered radioactive by a short deuteron bombardment and then dissolved in water. The bombardment of the salt produced both radio-chlorine and radio-sodium, but since the radio-chlorine is a relatively short-lived substance, having a half life of thirty-nine minutes, it was necessary only to allow less than a day for the radioactivity of the chlorine to die away to an inappreciable magnitude, leaving in the solution an adequate amount of radio-sodium. In Hamilton's experiments it was customary to use about one-half millicurie of radio-sodium (an amount of radio-sodium having a gamma-ray activity equivalent to that of 0.0005 gram of radium). Although the number of radioactive atoms of sodium in the solution was an extremely small fraction of the total sodium, nevertheless the radioactive radiations were so strong as to make it possible to detect one millionth of the total radioactivity. In the first experiment, a subject drank the salt solution containing radio-sodium, with his hand touching the tube of a Geiger counter and protected by a thick lead box. The purpose of the lead box was to prevent the radioactivity in most parts of the body from reaching the Geiger counter so that its response would be an indication of the presence of radio-sodium atoms in the hand near the counter tube. The arrangement of the subject for the experiment is shown in Figure 16. The counter responded in about two minutes after the drink of radioactive salt

solution, which meant that some of the labeled sodium ions were absorbed into the blood stream and reached the finger tips in that short period of time. In succeeding minutes, the radioactivity in the hand steadily rose, reaching a maximum in about

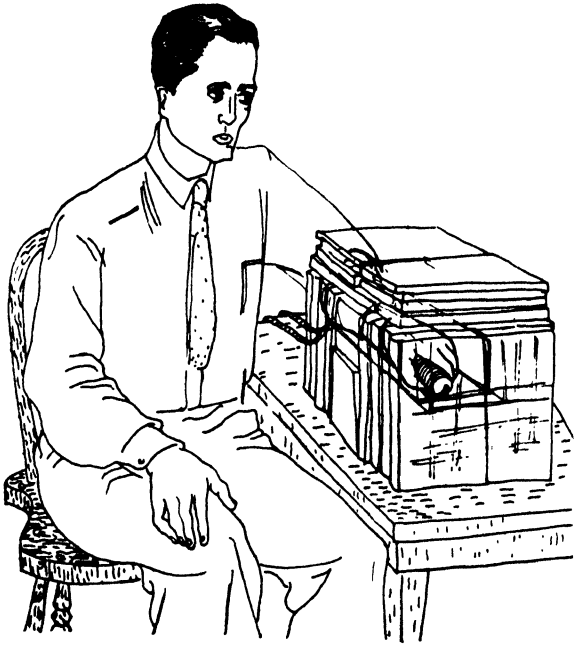


FIG. 16. Determination of the presence of radio-sodium atoms in the hand of the experimenter by means of the Geiger counter, following the drinking of a salt solution containing radio-sodium. The result is shown in Figure 17.

three hours, as shown in Figure 17. Thus it was established that an equilibrium distribution of sodium in the blood stream was reached in that period of time; and here again it cannot be emphasized too strongly that the radioactive sodium atoms behaved exactly as the normal sodium atoms and hence were everywhere present in like proportion, so that the radioactivity was always an accurate measure of the fraction of the original sodium solution.

In another experiment, Dr. R. S. Stone and Dr. Hamilton gave a considerably larger amount of radio-sodium—about 130 millicuries—to a patient in the University Hospital suffering from a malignancy. With this large amount of radioactive sodium in the patient, it was possible to make the precise observations shown in Figure 18. By placing an electroscope at some

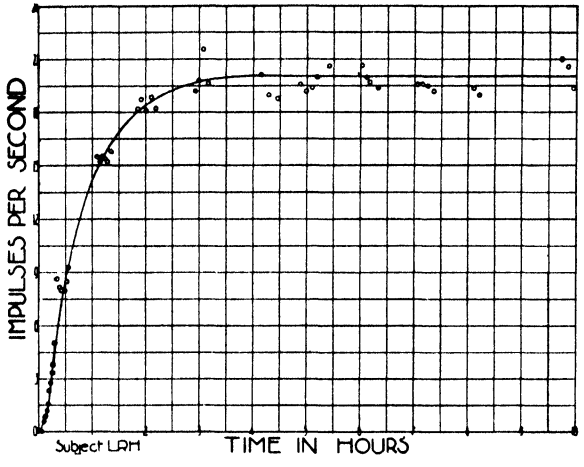


FIG. 17. The rate of absorption of sodium in a normal human subject. The ordinates represent the observed radioactivity in the hand at various times, given by the abscissas, after drinking a salt solution containing radio-sodium.

distance from the patient, something like ten feet away, the decay of the radioactivity in the patient could be followed for several days after the intake of the radio-sodium. Since radio-sodium has a half life of 14.5 hours, had the patient not excreted any of the sodium, the observed decay of the activity would have followed the upper dotted curve, marked "Theoretical." The activity, however, was observed to be a little less, as indicated by the experimental curve, marked "Measured," showing that a small part of the sodium was excreted. Although the difference between the theoretical decay and that measured was very small, nevertheless it was quite real, as the measurements

were made with considerable accuracy. By measuring the radioactivity in the bedclothes, the amount of the sodium excreted in the sweat was readily determined (as shown in the lower part of the figure) and that, coupled with the radioactivity observed in the urine at various times, accounted almost entirely for the difference between the theoretical and measured decay. Thus the experiment was quantitative in accounting for what happened to the sodium that was taken into the body originally.

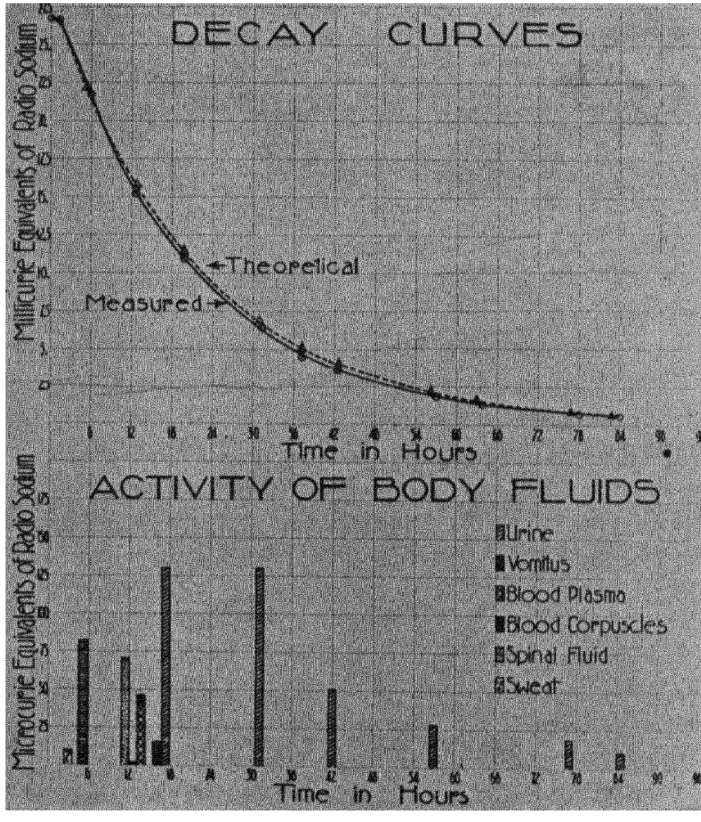


FIG. 18. The decay of radioactivity in a hospital patient following the intake of a large amount of radioactive sodium.

The sodium getting into the spinal fluid and blood was also determined by taking samples at various times and measuring the radioactivity, with results shown in the figure. It was of particular interest that so much of the sodium appeared in the spinal fluid and the blood plasma, with so little penetrating into the blood corpuscles.

Another interesting example is that of radio-phosphorus. The radioactive isotope P^{32} has a half life of two weeks, which is a convenient time for many biological investigations, and, moreover, radio-phosphorus is biologically an important element. Important work with this labeled element has been carried out by Professors Hevesy and Krogh in Copenhagen, Artom and Segré in Palermo, and others; but for the purposes of the present illustrations, it is sufficient to mention briefly here some experiments recently carried out at the University of California.

Tissue	Per Cent. of Total Phosphorus		Per Gram Tissue		Per Gram Phosphorus	
	4 Days	60 Days	4 Days	60 Days	4 Days	60 Days
Spleen	.194	.03	.0066	.0015	3.1	0.6
Bone Marrow	.07	.01	.0017	.0016	0.6	1.0
Brain	.13	.05	.0016	.0014	1.0	0.8
Skel. Muscle	60.00	13.00	.0071	.0010	9.3	0.3
Liver	2.00	.10	.0066	.0009	2.3	0.3
Kidney	.40	.08	.0047	.0009	2.0	0.4
Heart	.24	.10	.0027	.0009	1.5	0.7
Testes		.20		.0007		0.4
Lung	.31	.05	.0037	.0006	0.8	0.3
Intestine	3.30	.17	.0028	.0004	1.0	0.2
Gizzard				.0003		0.2
Blood	.36	.20	.0008	.0002	0.8	0.8
Bone	32.00	84.00	.1700	.0600	1.0	0.5
Total in Bird	3.70	0.62				

FIG. 19. Deposition of radio-phosphorus in tissues, measured in microcuries.

In the first place, Scott and Cook observed the deposition of radio-phosphorus in tissues of chickens. Radio-phosphorus was administered to a group of chickens in the form of phosphates in their food. Some of them were killed four days after intake of the phosphorus, while another group was killed sixty days afterward. After killing, the various tissues of the animals were weighed and ashed, and the radioactivity in the ashed material was measured, with the results shown in Figure 19. Thus, at the end of sixty days, 84 per cent of the phosphorus was observed to be deposited in the bones, a result consistent with the fact that calcium phosphate is an important constituent of the bone tissue.

Having established that radio-phosphorus is deposited largely in the bones, it was next of interest to determine the effect of the

radioactive material on blood. Blood counts on a number of chickens revealed that the radioactivity produced a decrease in the number of polymorphonuclear leucocytes without appreciably affecting the lymphocytes—a result quite in contrast with usual experience; for the lymphocytes are always found to be more "radio-sensitive" to X rays than the other white blood cells. This selective action on the polymorphonuclear cells is quite understandable since it is known that the bone marrow has to do with the production of these cells and it is in the bones that the radioactivity was localized. We have here perhaps the first definite demonstration of a selective effect of radioactive materials on cells resulting from selective deposition and it seems not unlikely that many other examples will come to light in the future. The important practical therapeutical implications are self-evident.

Recently, Lawrence and Scott administered radio-phosphorus to leukemic mice with the thought that since X rays have a temporary beneficial effect on leukemia, and since the disease involves the bone marrow, it seemed possible that introducing the radioactivity selectively into the bone structure by deposition of radio-phosphorus might have a desirable result. In the course of the investigation, as a routine matter, they ashed various tissues of the diseased animals and measured the radioactivity therein. To their surprise, they found not nearly as much radio-phosphorus deposited in the bones of leukemic animals as in normal ones, a matter which they have investigated further and established beyond any question. They have shown definitely that there is something wrong with the phosphorus metabolism of a leukemic animal, that the phosphorus does not deposit in the bones as much as in a normal animal, and, on the other hand, that in the blood and the lymphatic system there is a marked increase in the phosphorus present. At this time, of course, it is premature to hazard a guess as to whether the disturbance of the phosphorus metabolism is responsible for the leukemia or whether the disease produced the disorder, or in-

deed whether both are manifestations of a more fundamental condition. However it may be, the use of the radio-phosphorus made it possible to detect the disturbance of the phosphorus metabolism, and it is difficult to conceive of any other way this could have been found. The use of the radio-phosphorus as a tracer has given a significant lead in the study of leukemia which will doubtless contribute to important advances in knowledge of this disease.

The experiments of Chaikoff and his collaborators may be taken as an example of the power of the radioactive tracer technique in chemistry, in this instance in answering important biochemical questions on the formation of phospholipides. First of all, rats were fed inorganic radioactive phosphorus and later the phospholipides were extracted from the various body tissues. Radio-phosphorus was definitely found in the extracted tissue phospholipides, thereby indicating the synthesis of the phospholipides in the body. This same group of workers proved the more fundamental fact that cells themselves outside the body are able to synthesize the phospholipides from inorganic phosphorus; they mixed the labeled phosphorus with tissue slices in suitable tissue culture-solutions and observed radioactivity in phospholipides extracted subsequently from the tissue culture. Here again, a simple experiment resulted in important information which could not have been obtained in any other way.

Thus we see that the recent advances in our knowledge of the atomic nucleus are already of importance in presumably diverse fields—physics, chemistry, biology, and medicine; but this is not surprising, for all these domains of natural science are indeed concerned with atoms—new and old.

II

THE SEPARATION OF ISOTOPES AND THEIR USE IN CHEMISTRY AND BIOLOGY

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WHEN Soddy first suggested that the atoms of an element were not identical but differed in atomic weight, the experience of chemists furnished overwhelming evidence that these isotopes of the elements were chemically identical. In the many fractionation and other separation processes, no variation of atomic weights or other properties had been observed apart from those which could be reasonably ascribed to the presence of impurities in varying amounts. The early attempts to separate isotopes were based on methods which were not commonly used by chemists for the purification of substances and which at the same time on the basis of well-established theory should give some separation. These methods were diffusion of gases and evaporation of liquids or solids at low pressures under conditions which prevented the establishing of an equilibrium between the vapor and the condensed phase. Both the rate of diffusion and the rate of evaporation under these conditions should be inversely proportional to the square root of the molecular weight and hence some separation should be possible by these methods. Aston and Harkins showed that the atomic weights of neon and chlorine could be changed by the diffusion of gases, and Hevesy, using the evaporation method, changed the atomic weight of mercury by measurable amounts. These researches demonstrated that the atomic weights of the elements could be varied

and thus confirmed the mass spectrographic proof of the existence of isotopes; but these methods were not suitable for the effective separation of isotopes in the pure state nor for their concentration by amounts sufficient for chemical and physical research. Lindermann further considered the problem of separation by distillation both theoretically and experimentally. He showed that no difference in the vapor pressures of the lead isotopes could be expected if zero-point energy existed in the condensed phase, i.e., if an amount of energy equal to $\frac{1}{2} h\nu$ (h being Planck's constant and ν the vibrational frequency in the solid state) per degree of freedom is retained by the solid at absolute zero. Since no separation was secured in his experiments, they constituted evidence that this zero-point energy existed.

At present we know that differences in the chemical and physical properties of isotopic substances exist, but that these differences are so small in most cases that they cannot be demonstrated except by very careful experiments by a persistent investigator. In other cases, elements consist of one predominantly abundant isotope and one or more rare ones, so that even a considerable fractionation would change the atomic weight only by amounts within the experimental error of measurement. Thus, if the natural abundance of the hydrogen isotope of atomic weight 2, deuterium, were comparable with that of the lighter isotope, variations in the atomic weight of various samples of hydrogen prepared in the laboratory would have been so large that they could not have been overlooked. Also, variations in different samples of oxygen, nitrogen, and carbon would undoubtedly have been observed had their rarer isotopes been more abundant. Again, it happens that elements whose isotopes are present in comparable amounts, as, for example, lithium and boron, have properties which make the measurement of small variations in their physical properties difficult. Exact atomic weight determinations of these elements cannot be secured; the melting points or boiling points lie at such high temperatures

that precise measurements are impossible. In this connection, we now know that differences in physical and chemical properties of isotopic compounds decrease with increasing temperature and atomic weight. Hence, the conclusion that isotopes are identical was a natural one and was due to the mixtures occurring in nature being nearly a pure isotope of one mass or to difficulties in detecting differences in atomic weights or other properties. The discovery of these differences dates from the discovery of the isotope of hydrogen in 1931, and the experimental proofs that the physical and chemical properties of protium and deuterium and their compounds differ markedly. This led naturally to the careful investigation of the properties of other isotopic mixtures.

THE SEPARATION OF THE HYDROGEN ISOTOPES

The vapor pressures of the three hydrogen molecules, H_2 (hydrogen or protium), HD (protium deuteride), and D_2 (deuterium), are very different. (We neglect the very rare tritium, if it exists.) Recent measurements give for the vapor pressures of the liquids at the triple point of protium, 13.92° K: 54.0 mm. of mercury for H_2, 19.6 mm. for HD, and 8.1 mm. for D_2. Knowing these, it is possible to calculate from the Rayleigh distillation formula the increased concentration to be expected in a simple distillation. This formula is

$$\left(\frac{1-N_0}{1-N}\right)^{\frac{1}{\alpha-1}} \left(\frac{N}{N_0}\right)^{\alpha-1} = \frac{W_0}{W}, \qquad (1)$$

where N and $1-N$ are the fractions of the heavy and light constituents after a distillation has reduced the original volume, W_0, to the final volume, W, if N_0 and $1-N_0$ are the initial fractions of the constituents and α is the ratio of the vapor pressures of the pure light constituent to that of the pure heavy constituent. Natural hydrogen consists of approximately 99.96 per cent

of H_2 and 0.04 per cent of HD and a negligible amount of D_2. Hence, if four liters of natural hydrogen are evaporated to

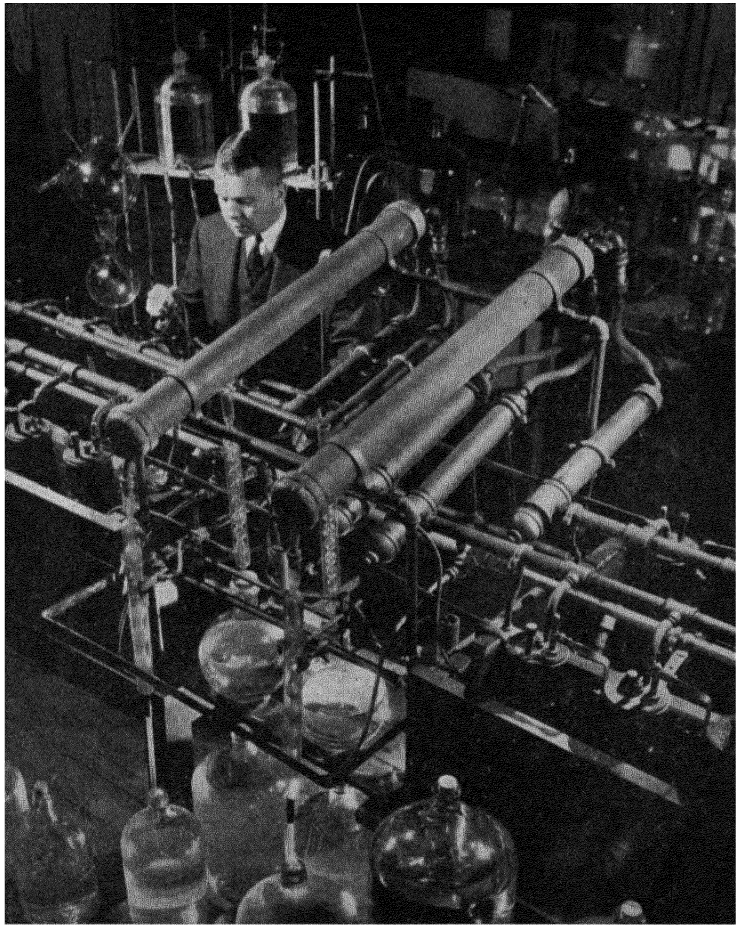


FIG. 20. The electrolytic heavy water plant at Columbia University. This was used to produce large quantities of heavy hydrogen water before the water was available commercially at reasonable prices. The electrolytic cells consist of round steel tubes mounted in a water-cooling bath. The gases escaped and were burned in the large pipes supported near the middle of the photograph. (Photograph by New York *Times*.)

1 cc., the residue should be 8 per cent HD as calculated from equation (1), using α equal to 2.755, secured from the vapor pressures given above. This amounts to a two-hundredfold increase in the concentration of deuterium. Urey, Brickwedde, and Murphy carried out this experiment and secured a sample of hydrogen in which the deuterium was increased fivefold over that in natural hydrogen. The difference between the calculated and observed increases is partly due to the fact that electrolytic hydrogen from fresh cells was used, and we have learned subsequently that such hydrogen contains less deuterium than the water from which it is produced because of the fractionation of the hydrogen isotopes in this process. The experiment was not carried out exactly as postulated by equation (1), and the residue was probably greater than one cubic centimeter. Things of this sort contribute to the discrepancy between calculation and observation.

This method of separation has been used by Scott and Brickwedde to produce nearly pure HD, even though it is not useful for the preparation of pure deuterium, D_2, because of the greater convenience and speed of the electrolytic method. As is well known, the efficiency of distillation methods can be greatly increased by the use of distillation columns. Though they vary greatly in structure, such columns are arranged to provide a counter current flow of liquid and vapor in such a way that very close contact between the two phases results. If liquid is boiled at the bottom and vapor condensed at the top, the vapor at the beginning will contain more of the lower boiling constituent than the liquid and hence there is a net transport of the lower boiling liquid (having the higher vapor pressure) toward the top and of the higher boiling liquid to the bottom of the column. This results in a difference in concentration of the constituents at the two ends of the column and immediately mixing processes—convection currents and diffusion—oppose this process of separation until finally no further change is produced. Experiment shows that the concentrations at the top and bot-

tom, N_t and N_b, respectively, are related by the equation,

$$\left(\frac{N_t}{1-N_t}\right) / \left(\frac{N_b}{1-N_b}\right) = \alpha^k, \qquad (2)$$

where α is the ratio of vapor pressures of the pure constituents and k is a number called "the number of theoretical plates." In the case of the columns we shall consider, k is approximately proportional to the length of the column. If α is large, k may be small and yet a large change in concentration may be secured. However, if α is nearly unity, i.e., the vapor pressures of the constituents are nearly equal, k must be large in order to secure a large change in concentration. In the case of a simple distillation, the value of k equals one. No condensation or evaporation of liquid should occur in the column for the best operation, though the temperature varies along the column since the mixture at the top is enriched in the lower boiling compound while that at the bottom is richer in the higher boiling one. If some of the vapor is removed from the top of the column, i.e., if there is a "forward flow," equation (2) still holds, though k is less than when no vapor is removed. These relations are stated for the case of ideal solutions, for which the vapor pressure of a constituent over the solution is proportional to the mole fraction of that constituent in the solution, but solutions of isotopic substances are the most nearly ideal of any that we know and hence these statements should hold exactly. Such distillation columns are one of the most effective methods for the partial separation of isotopes and they will be discussed in greater detail later.

The value of α in the case of protium, H_2, and the protium deuteride, HD, is 2.755 and thus k may be rather small. The difficulties of this separation reside in the low temperatures necessary for the distillation. Scott and Brickwedde used a glass distillation column consisting of a spiral glass tube surrounded by two evacuated glass tubes to prevent the flow of heat to it.

Condensation at the top was effected by liquid hydrogen boiling under reduced pressure, and boiling at the bottom was easily accomplished and indeed difficult to prevent at liquid hydrogen



FIG. 21. The apparatus for the separation of the nitrogen isotopes at Columbia University. Dr. Roberts at the left is adjusting one of the pumps which pumps liquid from the bottom of one of the sections to the top of the next. Dr. Thode at the right is adjusting a feed pump supplying the third section from the concentrated material produced by the second section. (Photograph by *Newsweek*.)

temperatures. The result was nearly pure HD in the flask at the bottom. The vapor pressure of this substance given above was determined by the use of the sample so prepared.

Though the concentration of deuterium by distillation at low

temperatures was very interesting at the time it was done, the electrolytic method of separating these isotopes discovered by Washburn is faster, more convenient, and less expensive and has made possible the preparation of deuterium on a commercial scale. Washburn found that the water in an electrolytic cell used for the manufacture of hydrogen and oxygen contained several times as much deuterium as natural water. This meant that protium was preferentially discharged in such cells, and subsequent experiments by many workers show that the ratio of the protium to deuterium in the gas escaping from the cell is from three to more than ten times the ratio of these isotopes in the water of the cell, depending on the electrodes, current densities, and, probably, other factors. Now, if a quantity of water is electrolyzed down to a small residue, equation (1) holds for the process, except that α is the ratio of the two isotopes in the gas discharged divided by this ratio of the isotopes in the water, and provided that α does not change with the concentration of deuterium in the water or of the electrolyte, which is usually sodium hydroxide. This appears to be true, and, taking α equal to 6 (a common value), the values of W_0/W for various values of N, the fractions of deuterium in the residue, calculated from equation (1), for a number of values of W_0/W, are:

TABLE I

N	W_0/W
.01	24.4
.10	393.5
.50	3054.0
.90	8531.0
.999	24280.0

Lewis first used this method to prepare nearly pure D_2O and it has since been used to prepare comparatively large quantities of this most interesting substance. It is interesting that one of the rarest of known isotopes should be the first to be separated

in large quantities and in fact to be sold as an article of commerce. (A tariff of 25 per cent ad valorem is levied on heavy water imported into the United States, and thus it is even taxed.)

The separation of the hydrogen isotopes by electrolysis is due partly to differences in the thermodynamic properties of protium and deuterium and their compounds and partly to differences in the velocity of the reactions involving the isotopic compounds. If equilibrium is established between water containing both protium and deuterium oxides and hydrogen gas containing its two isotopes, the ratio of the protium to the deuterium in the gas is greater by a factor of three than the ratio of the isotopes in the water, so that a fractionation of the two isotopes will occur even if the electrolysis is conducted under equilibrium conditions, that is, very slowly, and the α for the process is equal to three. Larger values of α are observed when the electrolysis is carried out rapidly and these larger values are due to the greater velocity of the formation of protium than of deuterium from their respective waters in this case.

The successful separation of the hydrogen isotopes by electrolysis immediately raises the question of separating other isotopes by the same method. This is not possible, for the values of α are too small. Oxygen of atomic weight eighteen, O^{18}, is discharged less rapidly than oxygen of atomic weight sixteen, O^{16}, and a similar difference is observed in the case of the isotopes of lithium. In the latter case, the value of α is approximately 1.02 and the use of this value in equation (1) will convince the reader that no appreciable separation of the lithium isotopes by electrolysis is possible. Chemical differences of this kind decrease with increasing atomic weight and thus no separation of other isotopes by this method can be expected.

THE HERTZ DIFFUSION METHOD

Large changes in the concentrations of certain isotopes have been effected by Hertz, using an ingenious arrangement of

many diffusion units. The individual units consist of a material through which the gases diffuse. This may be clay tubes or may be a moving stream of vapor of mercury or other substance. The diffused gas is pumped from the nth unit to the $n-1$th unit while the gas which has not passed through the diffuser flows to the $n+1$th unit. In this way, the effect of a single unit is multiplied many times. The fractionation produced by a unit may be defined as the ratio of the ratios of the isotopes in the diffused gas and in the gas not diffused. This ratio depends on the fraction of the total gas diffused, but in practice is of the order of magnitude of the ratio of the square roots of the molecular weights, e.g., in the case of the neon isotopes, Ne^{20} and Ne^{22}, about 1.05, or somewhat larger, depending on the fraction of gas diffused. Calling this ratio α, the total change in concentration is given by equation (2), if N_t and N_b refer to the fractions of one isotope at the ends of the series of diffusers and k is the number of units in the series. The arrangement is shown

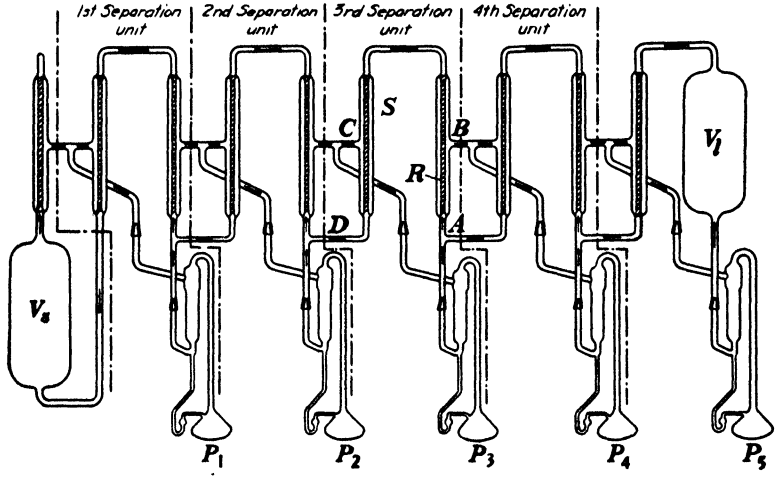


FIG. 22. The Hertz diffusion apparatus. Large changes in the concentrations of certain isotopes have been effected by Hertz, using an ingenious arrangement of many diffusion units. The individual units consist of a material through which the gases diffuse.

diagrammatically in Figure 22; the heavier fractions flow to the left, and the lighter fractions to the right in this diffusion apparatus.

This apparatus has been used by Hertz and his co-workers to produce large changes in the relative abundances of the isotopes of hydrogen, oxygen, and neon, and others have used essentially the same method to partially separate the isotopes of nitrogen and carbon. The concentrations secured are listed in Table II, together with references to the original literature:

TABLE II

Element	*Isotopes*	*Natural Percentage of Heavier Isotope*	*Maximum Percentage Secured*	*Reference*
Hydrogen	1, 2	0.017	~100 from 1	Harmsen, Hertz, and Schütze
Carbon	12, 13	1.08	16	Wooldridge and Smythe
			32	Capron, Delfosse, de Hemptinne and Taylor
Nitrogen	14, 15	0.38	3	Wooldridge and Jenkins; Wooldridge and Smythe
Oxygen	16, 17, 18	0.20	0.30	Sherr and Bleakney
Neon	20, 21, 22	10	~100	Harmsen, Hertz, and Schütze

The concentrations of C^{13} and Ne^{20} and Ne^{22} produced by this method are the highest obtained so far by any method. Pure deuterium has been produced also by the electrolytic method and in very large quantities. Higher concentrations of O^{18} (0.85 per cent) have been produced by the distillation method.

The amounts of concentrated materials produced by this method are rather small. Amounts of the order of magnitude of one cubic centimeter of the less abundant isotope of carbon, for example, in the form of methane gas at atmospheric pressure

can be transferred from the natural mixture to the more concentrated mixture during a 24-hour period. Formulae for this transport of material will be given later for another process and, though the processes differ considerably, it is true that this transport is roughly proportional to the concentration in the low-concentration material. Though this method will probably be replaced by others for the initial concentration stages, it will probably remain as one of the most effective methods for producing the most concentrated samples of pure isotopes of those elements of lower atomic weight having gaseous compounds suitable for use in such apparatus.

DISTILLATION AS A GENERAL METHOD OF ISOTOPE SEPARATION

Before the work on the fractionation of the hydrogen isotopes by distillation, Keesom and Van Dijk, following a suggestion made by O. Stern, first investigated the differences in vapor pressures of isotopic substances using the monatomic gas neon. The theory of the solid state developed by Debye predicts a difference in the vapor pressures of isotopic monatomic substances as first shown by Lindermann, and, in fact, predicts that the lighter substance should be more volatile. The difference in vapor pressure of Ne^{20} and Ne^{22} at the triple point should be 3 or 4 per cent. Their experiments were made with a distillation column designed to work at low pressures. In their first experiments, only slight changes in isotopic composition were secured. This work has been continued, and samples of neon of atomic weight 20.043 and 21.157 have been secured. This is a really remarkable change in the concentration of these isotopes.

The concentration of rare isotopes of elements other than those of hydrogen can be accomplished in some cases by the fractional distillation of their compounds. Lewis and Washburn showed that the boiling points of the protium and deuterium oxides were not the same and further, Lewis showed that the vapor pressures of H_2O^{16} and H_2O^{18} were slightly different.

The distillation of water as a method for the separation of the two hydrogens cannot compete with the electrolytic method unless perhaps some extensive industrial use for deuterium should develop. However, the concentration of the heavier isotope of oxygen in fair amounts by distillation is feasible since the electrolytic method cannot be used in this case.

The ratios of the vapor pressures of the H_2O^{16} and H_2O^{18} waters were determined by Wahl and Urey by a simple distillation. The value of α which will be the ratio of vapor pressures of the pure constituents, the initial and final values of the concentrations, and the initial and final volumes are related to each other in accordance with equation (1). Hence it is only necessary to determine the latter quantities and solve for α. The results are shown in Table III. The precision of the results is not high, but some idea of the magnitude of k (equation 2) required for partial separation can be seen from Table IV, in which the ratio of isotopes at one end of a fractionation column divided by this ratio at the other end is given as a function of k and α. At 60° to 70° C., α has the value 1.006, and thus, as can be seen from the table, k must be large. Distillation columns used in industrial processes have values of k up to approximately 50, and hence the development of more efficient columns is necessary for the concentration of isotopes.

TABLE III

$T°$ C.	$p_{H_2O^{16}}/p_{H_2O^{18}}$
11.25	1.013
23.00	1.008_8
35.60	1.008_2
46.35	1.007_7

TABLE IV

α	$k=100$	$k=200$	$k=300$	$k=400$	$k=500$	$k=600$
1.003	1.35	1.85	2.46	3.31	4.47	6.03
1.006	1.82	3.31	6.02	10.90	19.90	36.20
1.007	2.00	4.04	8.11	16.30	32.80	65.80

Fractionation columns with large values of k, i.e., large numbers of theoretical plates, have been developed by Fenske, by Stedman, and by Pegram, Huffman, and Urey. All these frac-

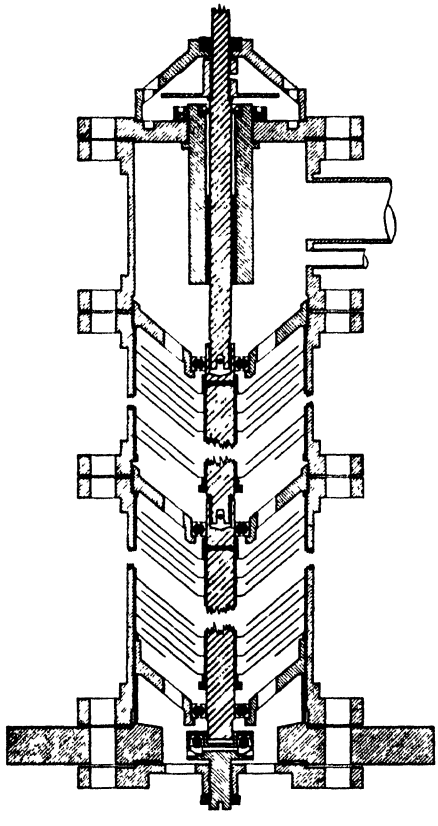


FIG. 23. The Pegram Column. The apparatus consists of alternate rotating and stationary cones; the stationary cones are attached to a six-inch tube at the outer edge, while the rotating cones are attached to a rotating shaft.

tionation columns depend for their efficiency on arrangements for bringing gas and liquid into intimate contact as the liquid flows downward and the gas upward through the column. Fenske has used various types of packing material, rings, chains,

carding teeth, etc., in pipes. Stedman uses a packing made of a regularly crumpled gauze, each layer placed horizontal in the tube and touching the one above and the one below in such a way that liquid drains from one layer to the next. The liquid is held in the mesh. Holes are cut in the mesh in such a way that the gas travels upward by a zigzag path. These columns have large numbers of theoretical plates, from ten to forty per foot, depending on the diameter of the tube and the size of the packing. The Pegram column consists of alternate rotating and stationary cones; the stationary cones are attached to a six-inch tube at the outer edge, while the rotating cones are attached to a rotating shaft. The arrangement is shown in Figure 23. Liquid flows down the stationary cone, drains to the rotating one and is thrown off by the centrifugal force, and then flows down the next stationary cone. The gas flows in the opposite direction. This column gives between seven and twelve theoretical plates per foot, depending on the speed of operation.

There are several factors which must be considered in the use of such distillation columns for the concentration of isotopes. It is easily seen that the column will contain considerable amounts of the concentrated material and an important question is the length of time required for the column to reach a steady state where the distribution in the column follows the formula

$$\left(\frac{N}{1-N}\right) / \left(\frac{N_0}{1-N_0}\right) = \alpha^{Kx}, \tag{3}$$

where K is the number of theoretical plates per unit length, and x is the length of the column.

This steady-state distribution is attained quickly in this case of the usual processes of the chemical industry where the value of α differs considerably from unity, but in the processes under consideration here the time may be a matter of days, months, or even years. It is therefore of importance to secure formulæ which will show how rapidly this steady state is attained. No

exact theory for such columns has been given, but useful formulæ have been derived by Urey and Huffman using certain simplifying assumptions in regard to the process. In their distillation process, water was pumped into the top of the column at a constant rate of speed. It drained to the bottom of the column and was boiled by a flash boiler so that there was no accumulation of water at the bottom. The vapor rose through the column, was condensed at the top and discarded. For this process they found that the rate of transport of the heavy oxygen isotope to the bottom of the column was given by the formula $T=wN(\alpha-1)$. The fundamental assumption underlying this formula is that the net flow of heavy oxygen water to the bottom of the column is equal to the total rate of flow in the column, w, multiplied by the difference of the concentration in the liquid and vapor phases. Also, they derived a formula showing how the maximum concentration of O^{18} water at the bottom of the column increased with the time of operation. This formula is

$$N ln \frac{N}{N_0} + (1-N) ln \frac{1-N}{1-N_0} = \frac{wN(\alpha-1)K ln \alpha}{H} t, \quad (4)$$

where N is the mole fraction of the O^{18} isotope in natural water; N_0, its mole fraction in the concentrated water at the bottom of the column; H, the holdup in moles per unit length of the column; and t, the time, and other symbols having meanings previously assigned to them.

The experiments of these men lead to the following values of the constants in this equation—$K/H=10$, $\alpha=1.006$, $w=33$ cc. per minute. The equation for the transport, T, shows that about 0.67 cc. of liquid H_2O^{18} will be transported in a 24-hour period. Also, the value of Kx was found by them to be about 430. This shows that the final concentration to be expected is about 2.5 per cent O^{18} water, and that the time required for the final steady state to be obtained is approximately 70 days.

From equation (4) we can see what the best conditions are

for rapid attainment of the final steady state of the column. It is desirable that the quantity

$$\frac{wN(\alpha-1)K\ln\alpha}{H}$$

shall be as large as possible, and this means that we wish to have a large total flow, w, a large number of theoretical plates per unit length, K, α as far from unity as possible, and a high concentration of the isotope in natural water, and, finally, that the holdup per unit length, H, shall be as small as possible. The values of the natural concentration of the isotope cannot be adjusted except by working on another element than oxygen. A maximum rate of flow and a maximum number of plates per unit length proved to be incompatible conditions, since the number of plates decreases with increasing rates of flow. The value of α can be increased by decreasing the temperature of boiling by working under partial vacuum, but the pressure cannot be

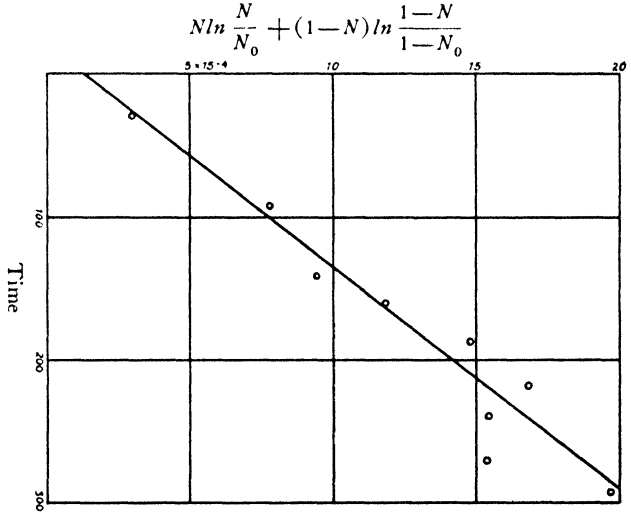


FIG. 24. Curve showing results of the most satisfactory experiment of Huffman and Urey. The run was made at 60° C. and the rate of flow was 33 cc. per minute.

decreased too far since the vapor velocity increases rapidly and causes "flooding" of the column, i.e., the liquid will not run down against the rapid stream of vapor and the column fills with liquid. The practical conditions can only be determined by experiment. It should be noted that a high rate of flow and a high value of α increase the transport of H_2O^{18}.

The results of the most satisfactory experiment of Huffman and Urey are shown in Table V and in Figure 24. The run was made at 60° C. and the rate of flow was 33 cc. per minute. The column is 35 feet long, contains 1,240 cones as described above, and has a total holdup of about 4,300 cc. of water. The maximum concentration of H_2O^{18} secured was about 5 times that of natural water, which would require some 250 theoretical plates. However, the slope of the curve with the known values of α, w, and Hx indicates that the column should contain a total of about 428 theoretical plates and after 50 days of operation should produce about 2.5 per cent H_2O^{18}, or a thirteen-fold increase in concentration, and should then produce about 25 cc. of water of this concentration per day.

TABLE V

Time (*Hr.*)	*Mole fraction* H_2O^{18}
0	0.00194
29	0.00405
91.5	0.00587
141	0.00642
162	0.00723
188.25	0.00811
218	0.00872
240	0.00830
269	0.00828
293.25	0.00955
307	(0.00872)

Stedman has approximately doubled the concentration of H_2O^{18} by the use of his column packed with gauze. His column

is certainly a very efficient one. Only further experiments will be able to decide whether his or the Pegram column is the better. It has the advantage that there are no moving parts, but this feature of the Pegram column has never given difficulty in the author's work except for the initial difficulties in selecting proper bearings.

The use of distillation for the concentration of other isotopes may be feasible in some cases, but at present it is difficult to see to what isotopes the method could be applied. The hydrogens can be separated by the distillation of water, but the transport will be rather small because of the low natural concentration of deuterium. A careful analysis of costs for large-scale production might show that distillation would be more economical than the electrolytic method. Aten and Urey showed that there is a slight difference in the vapor pressures of the ammonias, $N^{14}H_3$ and $N^{15}H_3$. However, fractional distillation at the boiling point of ammonia is inconvenient, though of course not impossible. The fractionation factor of 1.003 to 1.005 is not very favorable, and hence a large column is necessary, together with all the difficulties of maintaining low temperatures. Differences in vapor pressures of the liquid oxygens have been reported, but this is a difficult region for experimentation. The distillation of a substance boiling at temperatures much above that of water is almost certainly doomed to failure because these differences in vapor pressures decrease in general with increasing temperature. The distillation of such substances as methyl alcohol, formic acid, and hydrogen cyanide should be tried. Experiments made in the Columbia laboratories on methyl alcohol with respect to the fractionation of the oxygen isotopes showed that there is a difference in the vapor pressures of $CH_3O^{16}H$ and $CH_3O^{18}H$, but the difference was not sufficient to make the distillation of this compound preferable to that of water. On the whole, other methods appear to be necessary for the concentration of other isotopes.

THE EXCHANGE REACTION METHOD

The separation of the hydrogen isotopes led to experiments in regard to the equilibrium constants of exchange reactions. The theory of such reactions involving diatomic, triatomic, and some polyatomic molecules could have been given before the discovery of deuterium and was in the case of one reaction, $H_2+2DI=D_2+2HI$, before deuterium was secured in concentrated form. The work on the equilibrium constants of exchange reactions involving deuterium led naturally to the calculation of equilibrium constants of exchange reactions for other isotopes, and this work points the way to the exchange reaction method for the separation of isotopes as outlined by Urey and Greif.

In order to discuss the theory of this method, we will take a specific reaction as an example, namely,

$$\underset{A}{2H_2O^{18}}+\underset{B}{CO_2{}^{16}}=\underset{C}{2H_2O^{16}}+\underset{D}{CO_2{}^{18}}. \qquad (I)$$

Under equilibrium conditions, $CO_2{}^{16}$ and $CO_2{}^{18}$ will be in equilibrium with $CO^{16}O^{18}$ according to the reaction,

$$\underset{B}{CO_2{}^{16}}+\underset{D}{CO_2{}^{18}}=\underset{E}{2CO^{16}O^{18}}. \qquad (II)$$

We shall use the letters placed below the chemical symbols in these equations to indicate concentrations of the respective substances. The equilibrium constants for these reactions are,

$$\frac{C^2D}{A^2B}=K_1, \text{ and } \frac{E^2}{BD}=K_2.$$

The value of K_2 is very nearly 4, and K_1 is slightly different from 1. The ratio of O^{18} to O^{16} in the CO_2, divided by the ratio of O^{18} to O^{16} in the water, is easily seen to be

$$\frac{2D+E}{2B+E} \cdot \frac{C}{A},$$

and making use of the equations for $K_2(=4)$ and K_1, this is easily shown to be equal to $K_1^{\frac{1}{2}}$, and hence the ratio of the isotopes in CO_2 will not be the same as that in H_2O, if K_1 differs from unity. The value of $K_1^{\frac{1}{2}}$ is 1.04, and thus a slight difference in concentration of the oxygen isotopes is secured when water and carbon dioxide are mixed under conditions which permit the exchange reaction to take place, and then separated. This difference can be detected by allowing the carbon dioxide to react with hydrogen to produce water and methane and then comparing this water with that with which the carbon dioxide was equilibrated. The two waters differ in density by ten parts per million, which is exactly that required if $K_1^{\frac{1}{2}}=1.04$. This could be repeated by bringing carbon dioxide enriched in O^{18} by being equilibrated with water into equilibrium with water enriched in O^{18} and repeating the process much as is done in fractional crystallization. Simple calculations show that no great increase in concentration can be secured except by the use of a prohibitive amount of work and time. It is necessary to use some method of multiplying the effect of the simple process and this method of multiplication limits the types of exchange reactions which can be used and in fact eliminates the particular reaction which we have considered.

The method of increasing the effect of the simple process is precisely the same as that used in distillation. A reaction must be chosen which involves a gaseous and a liquid phase (or in general two phases), and equilibrium of the exchange reaction must be established rapidly. A distillation column can be used to establish the equilibrium many times and the theory is the same as that for the distillation process. At the top some process which converts the gaseous compound to the liquid compound must be used to replace the condensation of the vapor, and at the bottom some process converting the liquid to the gaseous

compound is required to take the place of the boiling process in distillation. The use of the carbon dioxide water reaction discussed above is eliminated because no satisfactory process for the conversion of carbon dioxide to water exists, and in addition approximately two or three hours are required for carbon dioxide to come to equilibrium with water with respect to the oxygen isotope exchange.

Three exchange reactions are worthy of serious consideration in connection with the concentration of the isotopes of carbon, nitrogen, and oxygen:

$$HC^{12}O_3^- + C^{13}O_2 = HC^{13}O_3^- + C^{12}O_2, \qquad \text{(III)}$$

$$N^{14}H_4^+ + N^{15}H_3 = N^{15}H_4^+ + N^{14}H_3, \qquad \text{(IV)}$$

and

$$\tfrac{1}{2}SO_2{}^{16} + H_2O^{18}(l) = \tfrac{1}{2}SO_2{}^{18} + H_2O^{16}(l). \qquad \text{(V)}$$

The calculated equilibrium constant for the last reaction is 1.014, so that O^{18} would be concentrated in the sulfur dioxide and thus at the top of the column. It is necessary to convert the oxygen of the sulfur dioxide to water at the top of the column. This could be done by the reaction of sulfur dioxide with hydrogen sulfide to form water and sulfur. It would appear that the process would be feasible, but would require the separation of large amounts of sulfur from the water formed. This would not be easy to do, and hence the distillation of water is probably a better way to accomplish the concentration of the heavier isotopes of oxygen.

The carbon isotope of mass 13 has been concentrated slightly by the use of the first reaction given above by Urey, Aten, and Keston. In this case, the heavier isotope concentrates at the bottom of the distillation column. Potassium bicarbonate solution is pumped into the top of the column at a constant rate, it runs to the bottom where sulfuric acid is added, and the carbon dioxide removed from the solution by boiling. The carbon dioxide then flows upward through the column and escapes at the

top. In this way, carbon containing about 1.4 per cent C^{13} instead of 1.06 per cent C^{13}, as in natural carbon, has been produced. The increase is not large. The exchange reaction is slow but is catalyzed by carbonic anhydrase, an enzyme of the red blood corpuscles of mammalian blood. The extraction of this enzyme on a large scale is difficult and the proteins present in the enzyme extract cause serious frothing in the vessels used for boiling out the carbon dioxide from the solution at the bottom of the column. No certain solution of these difficulties has been secured, but unpublished results of Dr. H. G. Thode and the writer indicate that the operation of the column is greatly improved by using partial vacuum and increasing the temperature of the column.

The ammonia-ammonium ion exchange reaction has been used successfully by Urey, Huffman, Thode, and Fox to increase the concentration of N^{15} from 0.38 per cent to 2.5 per cent and produce very substantial amounts of nitrogen containing more than the natural concentration of N^{15}. In this case, ammonium sulfate solution is pumped into the top of the column at a constant rate, and ammonia is liberated at the bottom by the addition of sodium hydroxide and fractional distillation. The ammonia flows up the column and is absorbed in water at the top. The heavy nitrogen accumulates at the bottom. In this case, there appears to be no serious difficulty to overcome. The column used was the same as that used by Huffman and Urey for the distillation of water, but with a flow of liquid only half as great and a flow of gas one-twenty-fifth as great; the number of theoretical plates was only one-fifth as great in the case of the ammonia-ammonium ion exchange as in the case of the distillation of water. The process has been tried also with a Stedman column and the indications are that the column gives fewer plates than it does for distillation processes. It appears that the velocity of exchange of ammonia with solutions of ammonium salts is definitely slower than the rate of exchange of water vapor with liquid water.

Lewis and MacDonald have used an exchange reaction for the concentration of the rarer isotope of lithium and have used a counter-current method for increasing the effect of the simple process fractionation factor. However, their two-phase system consists of two liquids instead of a liquid and a gas. The exchange reaction used by them may be written,

$$\begin{aligned} &Li^6(\text{amalgam})+Li^{7+}(\text{alcohol solution})= \\ &Li^7(\text{amalgam})+Li^{6+}(\text{alcohol solution}). \end{aligned} \qquad \text{(VI)}$$

A vertical tube eighteen meters long was filled with a solution of lithium chloride in anhydrous ethyl alcohol. A lithium amalgam flowed in small droplets down through this solution. At the bottom the lithium of the amalgam was converted to lithium chloride and dissolved in alcohol which was then fed into the tube at the bottom.

Preliminary results on an exchange reaction using the counter-current method in which solid and liquid phases are used have been reported by Taylor and Urey. Their experiments cover the isotopes of lithium, nitrogen (ammonium ion), and potassium. As is well known, the ions of one alkali or alkaline earth or ammonium in a zeolite are replaced by those of another alkali, etc., more or less readily. A typical exchange reaction of this type is

$$\begin{aligned} &Na^+(\text{aqueous solution})+KZ= \\ &K^+(\text{aqueous solution})+NaZ, \end{aligned} \qquad \text{(VII)}$$

where KZ and NaZ are the potassium and sodium zeolites, K_2O, Al_2O_3,$6SiO_2$,nH_2O and Na_2O,Al_2O_3,$6SiO_2$,nH_2O, respectively. When apparent equilibrium is attained, the sodium is mostly in the solution and potassium mostly in the zeolite. The ions may be arranged in the order in which they are preferentially "adsorbed" on the zeolite as follows:

$$Cs^+,Rb^+,K^+,NH_4^+,Na^+,Li^+.$$

Thus, if it is desired to displace Li^+ completely from the zeolite, any of the other ions will do this readily, Cs^+ being the most effective.

The use of zeolites for the partial separation of the isotopes was suggested by the chance discovery that two samples of lithium compounds, bought commercially, differed in isotopic composition by about 10 per cent in the ratio of isotopes. The origin of this difference is unknown, but lithium is often extracted from its complex aluminium silicate ores by heating with potassium sulfate, giving a zeolite type of compound, and it occurred to them that the replacement of one ion by another in zeolites might result in a fractionation of the isotopes. Such proves to be the case. When a lithium zeolite is shaken with successive small amounts of a sodium chloride solution, the lithium is extracted from the zeolite. However Li^{7+} is extracted less readily than Li^{6+}, and hence concentrates in the residue.

A strictly solid-liquid counter-current arrangement analogous to the gas-liquid and the liquid-liquid systems described above might be devised, but the methods of chromatographic analysis are considerably more convenient. These methods have been developed particularly for the separation of biochemical compounds. Their adaptation to the concentration of isotopes is fairly simple. A tube about nine meters long is filled with a zeolite of sodium, for example. A potassium chloride solution is then run through the tube replacing the sodium in the zeolite. If one isotope travels more rapidly than the other, that is, is adsorbed less strongly than another, the first potassium coming through should have a different isotopic composition than ordinary potassium. After the tube is filled with potassium chloride, sodium chloride solution may be run through the tube and then the trailing sample should have a different isotopic composition. If the first sample has an increased concentration of one isotope, the other should have a decreased concentration of the same isotope.

Taylor and Urey showed that the leading sample of lithium

when lithium chloride replaces sodium ion from the zeolite is enriched in the heavier isotope, and that the leading sample of nitrogen when ammonium chloride replaces sodium chloride is enriched in the lighter isotope. Also, the trailing sample of potassium when potassium chloride is replaced by sodium chloride is enriched in the heavier isotope. Thus, the heavier ion is concentrated in the zeolite in the case of the potassium and ammonium ions, but in the solution in the case of the lithium ion. The changes in concentrations secured were small in all cases, but the method is likely to be useful in securing considerably increased concentrations of rarer alkali metal or alkaline earth isotopes, even though anything approaching complete separation is improbable.

THE MASS SPECTROGRAPH METHOD

During recent years, the separation by the action of magnetic and electric fields on charged ions originally used to detect isotopes has been used to separate small amounts of lithium isotopes. Alkali metal ions are emitted from hot filaments which have been covered with alkali salts, and thus a satisfactory source of ions is available. Such separations have been made by Oliphant, Shire, and Crowther and by Rumbaugh, and these authors have used the separated isotopes to study their transmutation reactions. The amounts that can be separated in this way are small, of the order of one milligram in twenty-four hours.

THE CENTRIFUGAL METHOD OF SEPARATION

Mulliken suggested a centrifugal method for the separation of isotopes and presented much of the theory of this method. High velocities of rotation are needed. These have been developed by Beams, using an air-driven centrifuge. The theoretical fractionation factor, i.e., the ratio of the isotopes at the center divided by the ratio at the periphery is given by the equation

$$\alpha = e^{\frac{v^2(M_2-M_1)}{2RT}}, \qquad (5)$$

where v is the peripheral velocity of the centrifuge, M_2 and M_1 are the molecular weights of the two constituents, R is the gas constant, and T, the absolute temperature. Beams and Haynes have secured peripheral velocities of 8×10^4 cm. per sec. Using this velocity, very favorable values of α can be secured which depend only on the differences in molecular weights, as can be seen from the formulae. Table VI shows the calculated values of α as a function of M_2-M_1 and the temperature.

TABLE VI

M_2-M_1	$T=$ 300	200	80	20	°K
1	1.13	1.21	1.62	6.8	
2	1.29	1.47	2.61	47.0	
3	1.47	1.78	4.23	300.0	
4	1.67	2.16	6.90	2,000.0	

Appreciable changes in the ratio of the chlorine isotopes have been effected by evaporating a liquid from the centrifuge, the vapor coming from the middle and differing in composition from the liquid at the periphery in accordance with equation (1). Altogether, this method appears to be one of the most promising for the separation of isotopes in appreciable amounts. It has the great advantage that the separation secured depends only on the difference in atomic weights of the isotopes and not on their total atomic weights. Thus the isotopes of lead, using tetramethyl lead for example, can be separated almost as readily as the isotopes of oxygen, using water as the distilling liquid. This is not true of any other method described.

USES OF SEPARATED ISOTOPES

In the preceding paragraphs I have reviewed the methods available for the separation of isotopes, paying particular atten-

tion to elements other than hydrogen, since the electrolytic method for the separation of the hydrogen isotopes has been so extensively studied and reported in other places. However, when one turns to the possible uses of stable isotopes, it is necessary to give considerable attention to the use of the hydrogen isotopes, since these have been available for a sufficient length of time to make such studies possible, while the isotopes of oxygen and nitrogen have not been available until recently. The comparative ease with which the deuterium is separated from ordinary hydrogen is due to the fact that there are very appreciable differences in the chemical properties of hydrogen and deuterium, while the great difficulty of separating the isotopes of other elements is due to the great similarity of the chemical properties of their isotopes. For this reason, two types of studies on deuterium have been carried out. In the first place, there are the differences in the physical and chemical properties of hydrogen and deuterium and their compounds, and, in the second place, deuterium has been used as a tracer in following the course of chemical reactions. In the case of the isotopes of other elements, however, the differences in chemical properties are so slight that perhaps no very extensive studies can be made on the differences in these properties, and also these isotopes will be more useful as tracers than is deuterium. The more similar the chemical properties, the more useful a tracer can be, for it then reacts in the same way as the more abundant isotope, and, particularly, is handled by living organisms in very much the same way.

Studies on deuterium which have been made since it was discovered about six years ago are so very numerous that it would be impossible for me to give any adequate review of the field in a short space. I wish to give only a few examples of the uses to which this isotope has been put as illustrating something of what may be expected when other isotopes are available in larger quantities. The first concentration of the hydrogen isotopes was made by the distillation of liquid hydrogen, thus ef-

fecting a partial separation of H_2 and HD. The use of the electrolytic method has prepared pure samples of D_2, and recently Brickwedde, by using the distillation method, has prepared nearly pure samples of HD. We can expect the most marked differences in the chemical and physical properties in the case of the isotopes of hydrogen because the percentage difference in the masses of these atoms is larger than in any other element. Table VII shows some interesting properties of these three varieties of hydrogen. The heats of vaporization, sublimation, and fusion are markedly different, as are also the boiling points and melting points and molecular volumes. These differences in the hydrogens are partially understandable with the aid of the Debye theory of the solid state, though interesting deviations from this theory occur.

TABLE VII

Properties of the Hydrogens

	D_2	HD	H_2
Heat of vaporization	302.3 cal.	257 cal.	219.7 cal.
Heat of sublimation	274.0 cal.	219 cal.	183.4 cal.
Heat of fusion	47.0 cal.	38.1 cal.	28.0 cal.
θ value C_p	89°		91°
θ value C_v	97°		105°
Boiling point	23.6°	22.1°	20.38°
Melting point	18.65°	16.60°	13.95°
Volume of liquid	23.14 cm^3		26.15 cm^3
Volume of solid	20.48 cm^3		23.21 cm^3
Compressibility	$(3.3 \pm 0.7) \cdot 10^{-4}$		$(5.0 \pm 0.5) \cdot 10^{-4}$
Expansion coefficient	0.08		0.12
Zero point energy	215 cal.	260 cal.	305 cal.

In the case of the compounds of hydrogen and deuterium, considerable differences in the physical properties have been observed as are shown by Table VIII. Thus there are considerable differences in melting points, boiling points, and heat of vaporization, though the differences are not nearly so great as in the case of H_2, HD, and D_2. There appears to be no marked regu-

larity in these differences, and no satisfactory explanation of the differences in these properties is known at present. It is interesting to note that Trouton's constant is not the same for the last five pairs of compounds in the table; in fact, the variation between these compounds is of the same order of magnitude as the variation between the pairs of isotopic compounds. This is a rather surprising result, for, after all, Trouton's rule is a fairly good description of the ratio of heats of evaporation to boiling points. Of course, in the case of ammonia and water, the Trouton's constants given do not agree with those of the other compounds, as is well known.

TABLE VIII

Properties of Hydrogen and Deuterium Compounds

	M.P.	B.P.	Heat of Vaporization	Trouton's Constant	Critical Temp.
NH_3	195.3	239.8	5797	24.18	
ND_3	199.6	242.1	5990	24.72	
H_2O	273.2	373.2	10735	28.74	374.2
D_2O	277.0	374.6	11109	29.32	371.5
HCN	259	298.5	6600	22.17	
DCN	261	299.1	6500	21.73	
H_2F_2		293.1	6023	20.55	
D_2F_2		291.8	5772	19.78	
HCl	162.2	188.1	4081	21.70	51.0
DCl	158.2	191.6	4151	21.67	50.3
HBr	186.2	206.3	4257	20.40	89.9
DBr	185.7	206.3	4258	20.63	88.8
HI	222.3	237.5	4724 (?)	19.89	150.7
DI	221.5	237.0	4713 (?)	19.91	148.6

Other thermodynamic properties of the compounds of the hydrogens show measurable differences between the isotopic compounds. Thus the free energies, entropies, heat contents, etc., are all different for such pairs. Perhaps these differences may best be illustrated by a consideration of the exchange equilibria by which one pair of compounds exchange their isotopes

to form a second pair of isotopic substances. This is well illustrated by the chemical reaction $H_2+2DI=D_2+2HI$. If there were no differences between the free energies of the substances on one side of this equation and those on the other, the equilibrium constant for this reaction would be 1, whereas it differs decidedly from 1 and varies with the temperature. Equilibrium constants of this kind are known which vary from the neighborhood of unity to as much as 10, depending upon the chemical reaction involved, so that rather marked differences in the chemical and thermodynamic properties of hydrogen and its compounds are known in many cases. Other examples of such differences are well known. The solubility of salts and other compounds are different in H_2O and D_2O. The heats of solution are not the same. The mobilities of the ions involved differ, and in this case the difference is mostly a matter of viscosity differences between the hydrogen and deuterium oxide solvents. The equilibrium constants of chemical reactions in solution also differ markedly from unity so that these chemical differences are characteristics of these compounds in all states of aggregation. Most of these effects have not been described by exact theory.

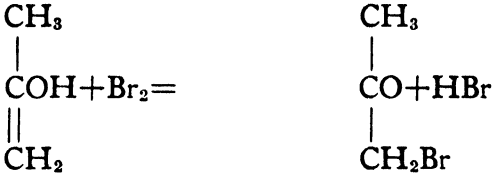
In recent years, the velocities of chemical reactions have been studied very extensively and very successfully, and in this study the isotopes of hydrogen have proved to be of very great interest. The mechanisms of many chemical reactions can be elucidated by their aid. Particularly is this true of reactions involving organic compounds where the transfer of hydrogen ions plays such an important part in these reactions. I can illustrate the use of the isotopes of hydrogen and of oxygen perhaps best by the discussion of the velocity of reactions involving acetone. It has been known for many years that acetone forms what we call both a keto and an enol form. In the first of these, the oxygen is attached to carbon by means of a double bond, while in the second the oxygen is attached to one carbon atom by a

single bond, and to a hydrogen atom by a single bond. These relations are shown as follows:



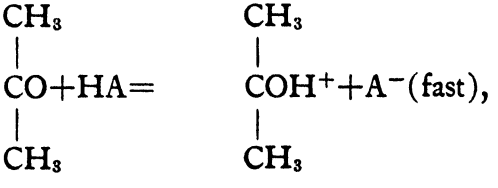
Keto form Enol form

It is found that if bromine is added to a water solution of acetone, the bromine adds to one of the carbons. Also, it is found that iodine acts in a similar way and that the velocity of addition is precisely the same in the two cases. These facts are explained by the postulate that as soon as the enol form is produced it reacts instantly with the bromine (or iodine) as follows:



and the measured reaction rate is the rate of production of the enol form.

A reasonable postulate for the way in which the reaction proceeds has been given by Petersen in the following reactions consisting, first, of a donation of a proton to the acetone at the oxygen atom, and, second, of a removal of a proton from one carbon atom:

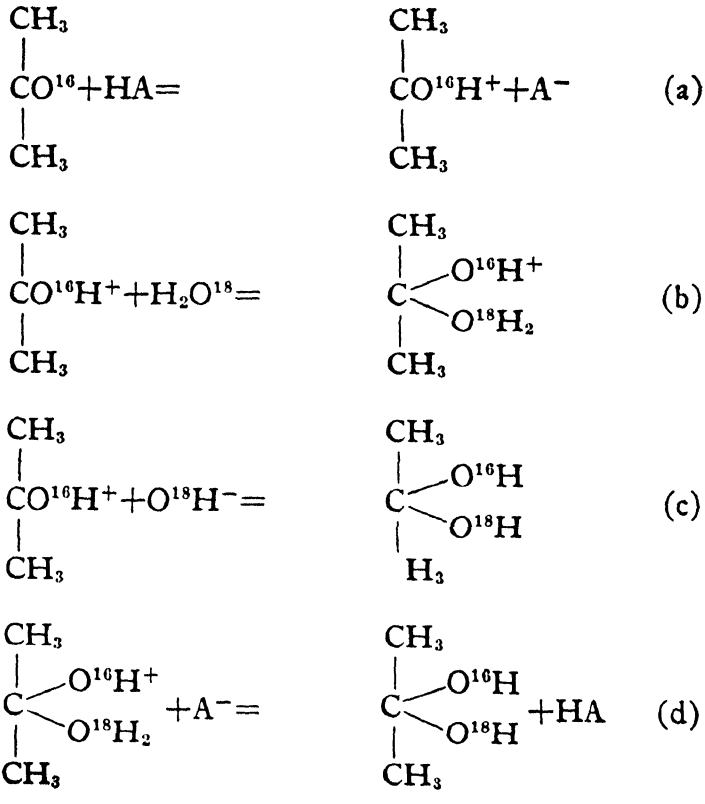


$$\begin{array}{l} CH_3 \\ | \\ COH^+ + A^- = \\ | \\ CH_3 \end{array} \qquad \begin{array}{l} CH_3 \\ | \\ COH + HA \text{(slow)}. \\ \| \\ CH_2 \end{array}$$

The dependence of the reaction velocity on the concentration of the acid, HA, and of the base, A^-, shows that the second reaction must be the rate-determining step and hence this is the reaction that determines the rate of formation of the enol form of acetone. The first reaction above must be much faster than the second.

If acetone is dissolved in deuterium oxide and an acid added, the reactions indicated above would proceed except that the enol form would be $CH_3-C(OD)=CH_2$. This could then react with DA to form $CH_3-C(OD)-CH_2D$, and this last compound with A^- to form $CH_3-CO-CH_2D$, and thus deuterium may be substituted for hydrogen in acetone by the same intermediate reactions as those involved in the bromine or iodine reactions. If this is true, the rate of exchange of hydrogen and deuterium should proceed at the same velocity as the other reactions. This is approximately the case, and the reaction is catalyzed by acids and bases in the same way as the reactions discussed above. The velocities are not precisely the same, due, undoubtedly, to the small but unmistakable differences in properties of hydrogen and deuterium compounds.

In some work that was done in our laboratories by Dr. Mildred Cohn, heavy oxygen was used for further study of the course of this reaction. It was found that the oxygen atom of acetone will exchange with the oxygen atoms of water. This reaction is catalyzed by acids and by hydroxide ion. The detailed reactions which it seems reasonable to postulate in this case are as follows:



Either reactions (a), (b), and (d) or reactions (a) and (c) would provide a mechanism for the exchange of O^{16} and O^{18} between water and acetone.

The observed velocity of this reaction is about five hundred times as fast as the velocity of deuterium exchange with acetone. Thus it appears that another rate-determining step other than that involved in the enolization or bromination must be postulated. The kinetics of the reaction do not permit us to distinguish between reactions (a) and (d) as the rate-determining step. Reaction (a) is the same as the first reaction postulated in the exchange of deuterium with acetone.

This work on the exchange of oxygen between water and organic compounds has been studied further in our laboratories by Dr. Irving Roberts. Up to this time it had been supposed generally that protons could be added and subtracted from organic molecules with considerable ease in reactions involving hydrolysis and general organic rearrangements, but until Dr. Cohn showed that the oxygen atoms could be moved about also, no one suspected this property. Since her work on acetone was completed, Dr. Roberts has shown that oxygen atoms in organic acids exchange in the presence of strong acids. Thus acetic acid exchanges both of its oxygens in one month if the concentration of hydrochloric acid is tenth normal.

The use of deuterium as a tracer for chemical reactions has been particularly important in the field of biochemistry. It has been found as a result of careful investigations, particularly by Trelease and by Schoenheimer, that heavy hydrogen produced no measurable effect upon the physiological processes in the usual experimental animals, and that deuterium in its various compounds is handled in much the same way by mammalian organisms as is hydrogen. I might mention in this connection particularly, the beautiful work of Schoenheimer and his coworkers on tracing the fats through mice. Before Schoenheimer did this work, it was generally known that the fat depots of mammals exchange their contents with the blood stream at least over considerable periods of time, and there was also considerable evidence to indicate that one variety of fat might be converted by mammals into other varieties, but the clear demonstration of these possibilities could only be done by the use of fats containing deuterium or perhaps the heavier isotope of carbon. Schoenheimer introduced deuterium into linseed oil, thus producing a saturated fat which contained deuterium as one of its constituents. When this fat was fed to experimental animals it would be handled in much the same way as in other fatty substances in the food of the mouse. By killing groups of mice at regular intervals after they were fed with this material, and ex-

tracting the fat from their bodies, it was possible to show how rapidly this deuterated food was taken up by the fat depots of the body, and, moreover, by following the concentration of deuterium in the water of the body, an estimate could be made of the fraction of the fat that was burned for fuel. The surprising result of these studies was that the fat of the depots exchanged with the fat of the blood in comparatively short lengths of time, the period being of the order of magnitude of a few days in the case of mice. A new theory of the function of these depots results. The animal converts part of its carbohydrates into glycogen, which serves as a reservoir of food for those periods when it is not actively digesting food. But this is insufficient for its needs. The fat which is eaten is also stored in the fatty tissues during the time of the digestion of food, and then is withdrawn again at the times between active digestion as the supply of fuel for muscular activity. This results in a constant exchange of the fat of the depots with the newly digested fats.

In addition to this, Schoenheimer was able to show that so-called saturated fatty acids could be converted to unsaturated acids, and vice versa. There are three principal varieties of fats in the mammalian animals. These are composed of glycerine as one constituent, combined with one of three acids known as stearic acid containing eighteen carbon atoms, palmitic acid containing sixteen carbon atoms, and oleic acid containing eighteen carbon atoms but two less hydrogens than stearic acid. The stearin and palmitin are the fats containing stearic and palmitic acids, respectively, and are solid at body temperatures and give the fat a hard character. Olein, the fat containing oleic acid, is liquid and imparts an oily character to fats. Each particular species of animal stores a mixture of these three and small amounts of other varieties of fats, and this mixture is characteristic of that particular species. Thus an animal eats food containing one set of proportions of these fats and stores in its body a fat of other proportions. Of course, it could do this by burning up fractions of the fat which it eats, leaving a fat of the par-

ticular composition which it stores. On the other hand, the same thing might be accomplished if the animal were able to convert one of these varieties of fats into another. In order to test this hypothesis, it is only necessary to feed stearic acid containing deuterium instead of hydrogen and then extract olein from its fatty tissues and analyze for the presence of deuterium. Very appreciable amounts of deuterium were found in the olein of mice; in particular when they were fed saturated fats containing 8.66 per cent of deuterium, the oleic acid extracted contained 1.16 per cent of deuterium. The natural abundance of deuterium in these compounds is less than .02 per cent, so that there could be no doubt of the ability of mice to convert the saturated acids into the unsaturated ones. The reverse was also tried by feeding other mice the oleic acids secured from the bodies of the first mice. In this case it was shown that saturated stearic acid was secured. In a similar way stearic acid, which contained 7.13 per cent deuterium, was fed to the mice, and it was found that the palmitic acid extracted from the fat of the mouse contained .33 per cent of deuterium, again showing that part of the stearic acid had been converted into palmitic acid.

Schoenheimer concludes that the average lifetime of fatty acids in mice is less than three days. Of course, it will probably be considerably longer than this in the case of larger animals. In a similar way he has been able to investigate the half-time of cholesterol in mice, and finds that it probably lies between fifteen and twenty-five days. It is interesting indeed that animals are able to keep such permanent physical characteristics and yet have all of the compounds of which their bodies are composed apparently completely destroyed and replaced in a short period of time.

Experiments on the use of other stable isotopes, for exchange reactions of this kind, are still in the developmental stage. Some experiments have been made using the heavy isotope of nitrogen in the case of biochemical experiments, but these are of a minor character and serve more to demonstrate the usefulness

of the method than anything else. It would be interesting indeed if some good indicator for carbon could be secured, since this is such an important constituent of all living things.

USES OF ARTIFICIALLY RADIOACTIVE SUBSTANCES

The discovery by F. Joliot and I. Curie, of artificial radioactivity has made possible the use of these artificially radioactive substances as tracers for many purposes, just as the natural radioactive substances have been used in the past. These radioactive isotopes are produced now in a variety of ways. By means of the high-voltage machines which have been developed in this country and abroad, it is possible to bombard ordinary elements by high-speed particles of various kinds. The three particles which are commonly used are protons, deuterons, and helium nuclei. All three of these are able to induce radioactivity when used to bombard these substances because the nuclei of the hydrogen atom and the deuterium atom and of the helium atom are brought very close to the nuclei of other atoms. In addition to the use of these charged particles in high-voltage machines, it is found that neutrons—particles carrying no charge and having masses about the same as that of the hydrogen atom—will react with the nuclei of atoms and produce artificially radioactive substances. These neutrons themselves are produced by other transmutation reactions, particularly by bombarding heavy hydrogen with the heavy-hydrogen nucleus, or deuteron. This method furnishes the most intense source of neutrons which are known. The first method used by Joliot and Curie consisted of a mixture of radon gas and beryllium metal. The alpha particles from the radon gas collided with the beryllium nuclei and produced carbon and neutrons. This method is very convenient because it is possible to transport the sources without difficulty. It is impossible for me to discuss any great number of these reactions, and it is only my purpose to mention a few of those which illustrate them and the application to tracer studies.

TABLE IX

Radioactive Indicators

Z	*Element*	*Indicator*	*Life*
81	Thallium	RaC″	1.32 m.
		ThC″	3.20 m.
		AcC″	4.76 m.
82	Lead	RaB	26.8 m.
		RaD	16.0 y.
		ThB	10.6 h.
		AcC	36.0 m.
83	Bismuth	RaC	19.5 m.
		RaE	4.85 d.
		ThC	60.5 m.
		AcC	2.16 m.
84	Polonium (136 d.)	RaA	3.05 m.
85	——		
86	Radon (3.825 d.)	Tn	54.5 s.
		An	3.92 s.
87	——		
88	Radium (1580 y.)	ThX	3.64 d.
		AcX	11.2 d.
89	Actinium (20 y.)	$MsTh_2$	5.92 h.
90	Thorium 2.2×10^{10} y.	Io	9×10^4 y.
		RdTh	1.90 y.
		RdAc	18.9 d.
		UX_1	23.8 d.
91	Protactinium 1.2×10^4 y.	UX_2	1.19 m.
92	Uranium 4.5×10^9 y.	——	——

These artificially radioactive substances have a great advantage over the natural ones due to the fact that radioactive varieties of atoms of almost every atom of every element can be secured. In order to make a satisfactory tracer it is necessary that the radioactive isotopes shall have certain properties. In the first place, it is necessary for most purposes that the atom shall have a reasonably long lifetime. It is possible to do some experiments

if the lifetime is as long as, say, about twenty minutes, but many experiments can be done better providing the lifetime is a matter of days at least. Unless the lifetime is reasonably long the radioactivity disappears before the experiment can be completed unless very high radioactivities exist in the initial substance and

TABLE X

*Artificial Radioactive Indicators**

Z	Element	Particle	Half Life	Energy Millions Electron-Volts
4	Be^{10}	e^-	>10 y.	<0.3
6	C^{11}	e^+	20.5 m.	1.15
	C^{14}	e^-	3 mo.	0.3 (?)
9	F^{18}	e^+	112 m.	
11	Na^{24}	e^-	14.8 h.	1.7
14	Si^{31}	e^-	170 m.	1.8
15	P^{32}	e^-	14.5 d.	1.69
16	S^{31}	e^+	26 m.	
	S^{35}	e^-	80 d.	
17	Cl^{34}	e^+	33 m.	1.8 (?)
	Cl^{38}	e^-	37 m.	4.8
18	A^{41}	e^-	110 m.	2.7
19	K^{42}	e^-	12.2 h.	3.5
20	Ca^{45}	e^-	2.4 h.	1.9 (?)

* For a more complete list of artificially radioactive isotopes see Livingston and Bethe, *Reviews of Modern Physics*, IX (1937), 359.

the method of detection is very sensitive. In the second place, the radiations that are given off by these radioactive substances must be sufficiently penetrating for them to escape from the preparations in which they are held. Thus, in some cases, the emitted particles move with such great velocities that they penetrate through considerable thicknesses of matter without difficulty. In this way, if the sample is not quite pure and has an appreciable thickness, while another sample is purer and can be gotten in a thinner layer for investigation, the radiations from

the two samples will nevertheless be approximately proportional to the amount of radioactive material present. On the other hand, if the radiations have a low penetrating power, the greatest care must be exercised in getting the samples in precisely comparable conditions before a test of the radioactivity is made. There are many of these artificially radioactive substances known at the present time, but only a fraction of these have any possibility of being useful. The elements of the periodic system of the atoms lying above silicon in atomic number, mostly have artificially radioactive isotopes which can be prepared by bombardment with neutrons, protons, or deuterons, which are satisfactory for tracer-reaction studies. Of those below silicon, however, only beryllium, carbon, fluorine, and sodium appear to have isotopes which can be used at all. In the case of carbon, there is a radioactive isotope of mass 11, which has a half life of 20.5 minutes. This is somewhat too short for the investigation of physiological processes, since these cannot be followed in less than a matter of days, and in many cases suitable food compounds containing the radioactive carbon cannot be prepared in less than months. Hence this isotope, though useful in some respects, can hardly be used in biochemical studies. On the other hand, though the carbon isotope of mass 14 has a half life time of many years, the radiation of this isotope is very soft and has low penetrating power, thus making it very difficult to investigate the radioactivity of different samples under comparable conditions. Accordingly, it appears that no radioactive isotopes of carbon are known which are suitable for these important studies of biochemistry. The isotope of sodium of mass 24, however, has a half life of 14.8 hours. It is easy to apply it to many problems because its compounds are so simple and so easily prepared. Moreover, its radiation, which consists of negative electrons, is very penetrating.

The only extensive biochemical research which has been made by the use of artificially radioactive elements is that of Hevesy and his co-workers, using radioactive phosphorus for the study

of the metabolism of phosphorus in animals. They have made extensive studies covering the absorption of phosphorus by bones and by teeth, both in man and experimental animals, particularly rats. Hevesy prepares his radioactive phosphorus by the action of neutrons on sulfur in the form of carbon bisulfide. After exposing carbon bisulfide to neutrons from a radon beryllium source for two weeks, he evaporates the carbon bisulfide, burns the residue, and adds the residue to ordinary sodium phosphate. The very minute amount of radioactive phosphorus, amounting to only 10^{-10} grams, which is prepared in this way, can be mixed with considerable amounts of ordinary phosphorus and used in feeding experiments. This phosphorus, which is fed in the form of phosphate, then serves as a tracer for the course of the absorption of phosphorus, its excretion through the kidneys and through the feces, its deposition in bones, teeth, and muscles by the animal. It this way he has been able to make a fairly complete study of the rapidity with which these processes take place. It is not surprising, of course, that the results show that growing animals absorb phosphorus in the bones and teeth much more rapidly than do adult animals. The really interesting and surprising result of their studies is that even adult animals, both man and experimental animals, are able to exchange the phosphorus, which is mostly in the form of phosphate, in both the bones and the teeth in comparatively short periods of time so that neither the bones nor the teeth can be regarded as relatively dead structures so far as their mineral content is concerned. It appears that the phosphate is deposited in the bones, redissolved, and deposited again. In the case of human teeth, about 1 per cent of the phosphorus of the tooth is replaced by phosphorus from the food in 250 days in the case of an adult man. This, of course, does not mean that this is the only exchange of the phosphate that takes place, for, of course, phosphate that is liberated from the bones may be deposited in the teeth, and, since this phosphorus is not radioactive, its deposition in the teeth cannot be detected. It is interesting indeed

that the bones of a rat are replaced on the average within about one month.

Both the stable isotopes and these radioactive isotopes have already found a very important use in studying the enormously complex mixture that makes up living substances. The use of these tracers in the study of the usual chemical reactions and the physical properties of substances, perhaps does not impress one so much as the unraveling of this more complicated biochemical problem. After all, in our chemical and physical laboratories, we work with more simple systems, and, of course, can get very precise and unequivocable answers to many of our problems, but in the study of living organisms we find compounds of great complexity, and an enormous number of varieties, and here any tool that can be brought to bear on the problem is bound to have outstanding results. I myself feel that the use of both the stable and the radioactive isotopes in biochemical problems in the next years is going to be the most important use of any to which these new methods can be applied.

III

RECENT ADVANCES IN THE STUDY OF VIRUSES

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DISEASES now known to be caused by viruses were recognized hundreds of years ago, yet it was not until 1892 that man succeeded in learning something of the nature of these infectious agents. In that year Iwanowski, a Russian botanist, was studying a disease of certain plants which was called tobacco mosaic, because it manifested itself in tobacco plants by causing a light- and dark-green mottling of the leaves resembling that of a mosaic pattern. The disease, which was considered to be bacterial in nature, was known to affect only certain plants and to cause a distortion or puckering of the leaves and a stunting which rendered diseased plants very undesirable from a commercial standpoint. During the course of his work with the disease, Iwanowski pressed out the juice from a diseased plant and applied it to normal plants. These plants promptly came down with the disease, thus indicating that the infectious agent was present in the juice. He then passed the juice through a Chamberland filter, a device that was considered to retain or hold back all known bacteria or living organisms, and applied the filtered juice to normal plants. He expected the Chamberland filter to filter out and remove all bacteria and hence to render the juice noninfectious. He was greatly surprised to find that the plants inoculated with the filtered juice came down with the disease just as promptly as those inoculated with the unfiltered juice. He first thought that his filters were faulty and had al-

lowed bacteria to pass, but careful examination revealed not only that his filters were satisfactory but that no bacteria could be demonstrated in the filtered juice despite its infectious nature. Iwanowski failed to be impressed by these striking results and, despite the fact that he was unable to demonstrate the presence of bacteria, concluded that the disease was bacterial in nature. It remained for Beijerinck, a Dutch botanist, six years later to realize the true significance of these experiments. He repeated and confirmed Iwanowski's filtration experiments and, in addition, by serial passage of filtered juice, demonstrated that the disease could not be due to a bacterial toxin. He established definitely that the disease was caused by an infectious agent that was smaller than ordinary bacteria. He then concluded that the disease was not caused by ordinary bacteria, but by a "contagious living fluid." He desired apparently to convey the idea that the active agent differed in certain respects from known forms of living organisms. Beijerinck was, therefore, the first to recognize that one of the group of infectious disease-producing agents that we now call viruses differed from ordinary bacteria. His discovery was followed by the recognition of the foot-and-mouth disease of cattle as the first virus disease of animals. Later smallpox, louping-ill of sheep, rabies, psittacosis, St. Louis encephalitis, yellow fever, poliomyelitis, fever blisters, fowl pox, dog distemper, hog cholera, horse encephalitis, certain types of tumorous growths in fowls and other animals, various yellows and mosaic diseases of plants, the unusual colors produced in the flowers of plants which in tulips is called tulip break, and the transmissible lysis of bacteria were recognized as diseases caused by viruses.

It is perhaps unnecessary to mention the tremendous suffering and loss of human life that once resulted from smallpox and yellow fever—virus diseases which fortunately are now under control—or that now result from the ravages of poliomyelitis, encephalitis, and influenza—virus diseases which are not yet under adequate control. The animal virus diseases also

present very serious problems today. Swine influenza and horse encephalitis cause the deaths of many animals in the United States every year, and in England and on the continent hundreds of thousands of animals are lost annually because of the foot-and-mouth disease. Plant virus diseases are responsible for an annual crop loss amounting to millions of dollars. There is, therefore, adequate reason for making every effort to learn the true nature of viruses in the hope that eventually we may be able to bring all of these devastating diseases under control.

Although filterability through membranes of small pore size was the earliest recognized property of viruses and has continued to be considered generally as a characteristic of viruses, it should be noted that in recent years filterability has not been found an infallible means of distinguishing between viruses and bacteria. It may be seen from Figure 25, which shows the relative sizes of several selected viruses as compared to those of the red blood cell, *Bacillus prodigiosus*, pleuropneumonia organism, and protein molecules, that the viruses form an unbroken series with respect to size from protein molecules to bacteria, but that at either end there is an overlapping. Certain viruses are smaller than accepted protein molecules and others are larger than accepted bacteria. It is obvious that on the basis of filterability it is impossible to draw lines that sharply divide the viruses from bacteria or from protein molecules. During the course of the last few years other characteristic properties of viruses have been discovered, and it is by means of a combination of properties that it has been possible to recognize and to segregate the viruses, some of which are listed in Figure 25, as a more or less distinct group of disease-producing agents.

The most important property of viruses is that of multiplication or reproduction or growth. The introduction of one unit of a given virus into a susceptible host is followed by the production of millions of units of the same virus. It should be emphasized, however, that multiplication occurs only within the living cells of certain susceptible hosts. Viruses neither multiply nor cause

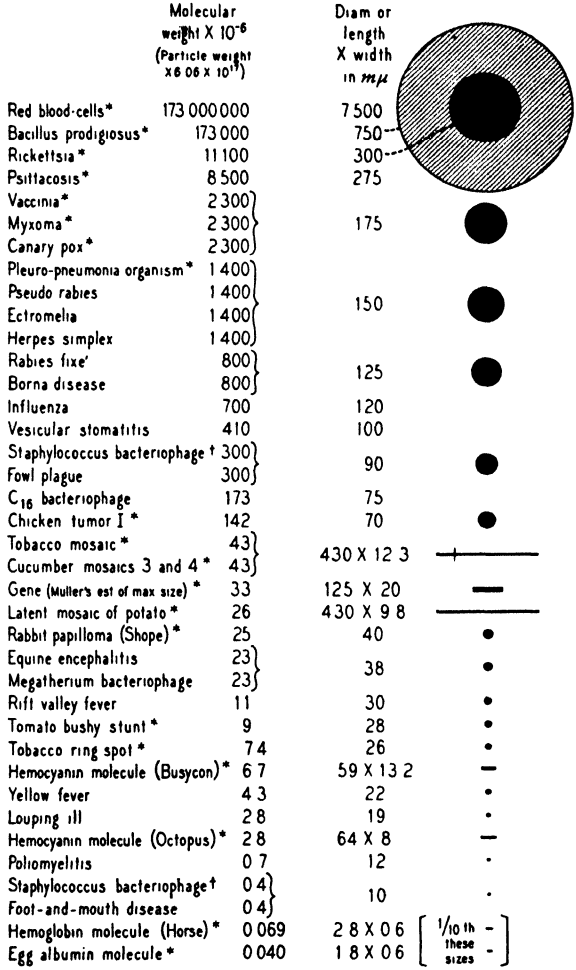


FIG. 25. A chart showing the relative sizes of several selected viruses, including bacteriophages, as compared to those of red blood cells, *Bacillus prodigiosus*, rickettsia, pleuropneumonia organism, and protein molecules. The figures for size have been arbitrarily selected from data available in the literature. Particles known to be asymmetric are so indicated and the estimated length and width and the molecular weight in accordance with the asymmetry are given. In other cases where the particles are known or assumed to be spherical, the diameter and the molecular weight based on a sphere of density 1.3 are given. * = Evidence regarding shape available. † = Large size from filtration and sedimentation of concentrated solutions and small size from diffusion of dilute solutions.

disease when kept in a test tube, or with the living cells of a nonsusceptible host, or in the presence of dead cells or of cell-free extracts of living cells. Viruses have been found to multiply, therefore, only within the living cells of susceptible hosts. Another point that should be emphasized is that, although during the multiplication most of the virus produced is of the same kind as that introduced, there also occurs a remarkable and apparently constant production of a small amount of virus that differs slightly from that originally introduced. It is said that the virus has changed or mutated and given rise to a new kind that is referred to as a new strain. If this new strain is vigorous and able to compete successfully with the parent strain, it will multiply in the host and may be isolated by an appropriate technique and studied apart from the parent strain. One point of considerable practical importance which may be noted here is that some of the strains of virus may be very mild in their effect on the plant, so very mild in fact that they may be present within the cells of a host and cause no readily visible symptoms, yet when present they protect the plant against the invasion of a more virulent strain. It is possible, therefore, to protect a plant from a severe virus disease by deliberately infecting it with a mild strain of the same virus. A similar situation prevails in the case of viruses affecting man and animals, although because of the difference in the circulatory systems the actual mechanics of the protection may be somewhat different. However, it has been found that the immunity produced in mammals by a mild strain of a given virus usually protects against a more severe strain of the same virus. Thus, as you probably know, vaccine virus has been used for years to vaccinate or protect human beings against the more severe strain, smallpox virus. Recently, there was developed a new strain of yellow fever virus which is now used to protect against the more severe strain that has caused such a great loss of life in the tropics.

Two additional points that should be mentioned in a consideration of the characteristic properties of viruses are, first, that

many, but not all, virus-infected cells contain inclusion bodies, masses within the cells which may be recognized readily with the aid of a microscope, and, second, that most, but not all, virus diseases are followed by a lasting immunity in the recovered hosts. Thus, Guarnieri described the inclusion bodies within cells affected with vaccine virus in 1894, and even earlier it was noted that a person recovered from smallpox was then usually immune and could not be reinfected with the virus. It is seen, therefore, that viruses are now recognized, not by the sole means of filterability, but by means of a set of general properties which emphasize not only their small size and the fact that they may change or mutate, but especially the intimate relationship that exists between viruses and their host cells. This relationship is manifested by the fact that viruses grow or multiply only within the cells of certain hosts and have never been found to grow on cell-free media, the fact that many virus-infected cells contain inclusion bodies, and the fact that a host recovered from a virus disease is usually, but not always, immune from a second attack of the same or related virus.

It will immediately be recognized that some of the properties that are used to characterize viruses, such as their reproduction, mutation, and specificity of action, have been regarded for years as being characteristic of living organisms. For this reason and also because the study of viruses has been largely in the hands of bacteriologists, there has been a general tendency to regard viruses as submicroscopic living organisms somewhat similar to the bacteria. No great difficulty was encountered in this conception so long as the larger viruses, such as, say, vaccine virus, were under consideration, for, actually, accepted living organisms were discovered that were smaller than vaccine virus. However, as the methods for measuring the sizes of small particles were improved, it became obvious that certain of the viruses, notably those of poliomyelitis and of the foot-and-mouth disease of cattle, were actually smaller than the hemocyanin protein molecules found in the blood of certain crabs. The gen-

eral situation became somewhat embarrassing for, so far as the general virus properties were concerned, the viruses tended to form a group and there was no biological or pathological property that tended to be a function of size. There was, therefore, no justification for separating on this basis viruses of the size of accepted bacteria from those of the size of protein molecules. However, because it has been considered unlikely that an entity no larger than a protein molecule could support that degree of organization that has been regarded as necessary for life, some of the pathologists, leaders in the virus field, have sought a way out of the difficulty by assuming arbitrarily that the large viruses are living organisms and the small viruses are something else, usually of a nonliving nature. However, such an arbitrary division cannot be executed in reality, for it has not been possible to locate the division line and it is not in accord with the fact that virus properties do not tend to be a function of size. Furthermore, such an assumed division does not aid in the solution of the problem of the fundamental nature of viruses and, as a matter of fact, tends to confuse rather than to simplify a consideration of the fundamental properties of viruses.

It seemed to the writer that the best approach to the general virus problem would be the selection of three or four representative viruses, some large, some intermediate, and some small in size, some plant and some animal viruses, etc., followed by a concentrated, exhaustive study of the properties of these representative viruses. The three viruses that probably had been subjected to the most extensive investigation in the past were vaccine, rabies, and tobacco mosaic viruses. The latter of these was selected for initial investigation because certain of its properties made it an excellent virus for study. It was known to be intermediate in size (Fig. 25) and to be among the most stable of all viruses. The work of Vinson and others had demonstrated that tobacco mosaic could be subjected to extensive chemical treatment without complete loss of virus activity. The virus was known to cause a systemic infection and to reach a

high concentration in Turkish tobacco, a large and very rapidly growing plant, hence it was possible to secure tons of very potent diseased starting material merely by infecting a sufficient number of such plants. Furthermore, as a result of the discovery by Holmes that this virus caused local lesions on the leaves of certain plants about two days after inoculation and that the number of such lesions could be used as an index of the amount of virus applied (Fig. 26), a very accurate method for estimating the concentration of this virus was developed. This

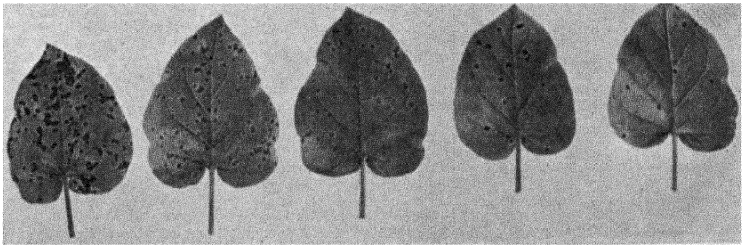


FIG. 26. Local lesions on leaves of plants of *Nicotiana glutinosa* showing effect of diluting juice containing tobacco mosaic virus (1:1, 1:3, 1:10, 1:100, and 1:1000). (From Holmes, 1929.)

method consists in inoculating the left halves of about twenty leaves and the right halves of another set of about twenty leaves with the unknown virus preparation and the remaining left and right halves of the forty leaves with a known or control virus preparation. The number of lesions produced by each preparation is then counted and by means of a statistical analysis of the results, differences in virus concentration greater than about 10 per cent may be recognized. This procedure has made it a simple matter to follow the virus very accurately through a series of manipulations. The great stability of the virus, the ready availability of large amounts of highly infectious starting material, and the fact that the virus could be measured rapidly and accurately caused tobacco mosaic virus to stand out as the ob-

vious selection for an exhaustive study, for no other virus was known to possess such favorable characteristics to a comparable extent.

At the outset of the investigation much of the earlier work on the effects of many different chemical agents, of various enzymes, and of acid and alkali was repeated and extended. The

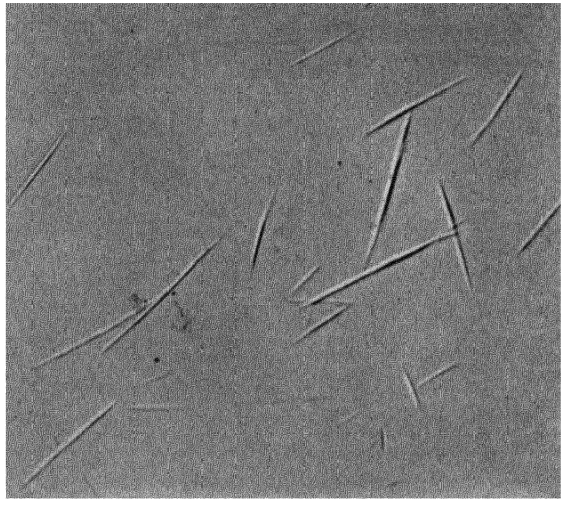


FIG. 27. Crystalline tobacco mosaic virus protein prepared by Dr. H. S. Loring. × 675. (Photograph by J. A. Carlile.)

most important result of this preliminary work was the definite indication that the virus was either protein or closely associated with protein. This lead was followed up and early in 1935 there was isolated for the first time an unusual high-molecular-weight protein possessing the properties of tobacco mosaic virus. This material, crystals of which are shown in Figure 27, was first isolated by means of a chemical procedure which involved the use of one step of Vinson and Petre's lead acetate method of purification, but which was based chiefly on the general

methods of protein chemistry that had been used so successfully by Northrop and associates for the isolation of protein enzymes. Later a simple method involving filtration through celite and precipitation with ammonium sulfate was used, and still later isolation by means of the purely physical method of differential centrifugation was found to be preferable to all other methods. At the present time, the latter method is used at the Institute in Princeton almost to the total exclusion of other methods. Following the announcement of the isolation of tobacco mosaic virus protein by chemical means, there was isolated by the same or similar methods in rapid succession aucuba mosaic virus protein by Stanley; tobacco, aucuba, and enation mosaic virus proteins by Bawden and Pirie; staphylococcus bacteriophage protein by Northrop; tobacco mosaic virus protein by Takahashi and Rawlins, by Beale, and by Best; and the same from tomato plants by Loring and Stanley. This was followed by the isolation, by means of differential centrifugation, of tobacco ring spot, latent mosaic of potato, and severe etch virus proteins by Stanley and Wyckoff and of the Shope papilloma virus protein by Beard and Wyckoff. The proteins of cucumber viruses 3 and 4 were isolated by chemical means by Bawden and Pirie in 1937 and by differential centrifugation by Price and Wyckoff in 1938. Recently, Loring and Wyckoff have studied the isolation of the latent mosaic virus by means of differential centrifugation, and the same virus and related strains and the bushy stunt virus have been obtained by Bawden and Pirie by chemical means. Indications of the presence of high-molecular-weight protein material in highly active preparations of the Rous sarcoma, equine encephalomyelitis, and foot-and-mouth disease viruses have also been obtained. It is seen, therefore, that within three years after the isolation of tobacco mosaic virus protein, over a dozen different specific and highly characteristic proteins possessing the properties of the respective viruses or virus strains have been isolated. Insofar as examined, these materials have been found to give all the usual protein reactions and to

TABLE XI

Analytical Data for Purified Virus Proteins

	C Per Cent	*H Per Cent*	*N Per Cent*	*S Per Cent*	*P Per Cent*	*Lipoid*	*Reference*
Tobacco mosaic*	52.0–53.3	6.9–7.0	16.1–16.4	0.0	>0.1	0.0	Stanley, 1936
Tobacco mosaic†	50.9	7.6	16.7		0.43	0.0	Loring and Stanley, 1937–38
Tobacco mosaic†	50.7	7.6	16.6		0.39	0.0	Loring and Stanley, 1937–38
Tobacco mosaic‡	47.7	7.3	15.9	0.24	0.60		Loring, 1938
Tobacco mosaic†	49.3–50.0	7.2–7.4	14.4–16.6	0.2–0.6	0.45–0.55		Bawden and Pirie, 1937
Aucuba mosaic†	49.1–50.5	6.6–6.9	16.5–16.8	0.24	0.51		Stanley, 1937
Cucumber mosaics 3 and 4†	50.0–51.0	7.1–7.6	15.3–15.8	0.0–0.6	0.55–0.6		Bawden and Pirie, 1937
Latent mosaic of potato‡	47.7–47.8	7.3–7.6	14.5–16.1	1.1	0.51–0.58		Loring, 1938
Latent mosaic of potato†	47.7–49.5	7.1–7.7	15.7–17.0		0.4–0.5		Bawden and Pirie, 1938
Tobacco ring spot‡	50.5	7.3–7.8	14.3–14.9	0.39	3.4		Stanley, 1938
Tomato bushy stunt†	47–50	7.2–8.2	15.8–16.4	0.4–0.8	1.3–1.5		Bawden and Pirie, 1938
Vaccinia†‡			13.1–13.6			6.5–10.1	Hughes, Parker, and Rivers, 1935
Staphylococcus bacteriophage†	40.6–41.8	5.2–5.4	14.1–14.6		4.6–5.0		Northrop, 1938
Chicken tumor I‡			8.6–9.5		0.7	24	Claude, 1938

* An early preparation purified by extensive chemical treatment. Now considered to have been aggregated and fairly inactive and to have contained little or no nucleic acid.

† Prepared by chemical procedures.

‡ Prepared by differential centrifugation.

analyze for protein and nucleic acid (Table XI), or in certain instances for protein, nucleic acid, lipoid, and additional carbohydrate. The viruses appear to consist, therefore, of conjugated proteins. As was expected, none of the viruses isolated was found to be preferable to tobacco mosaic with respect to the desirable properties mentioned earlier. For example, as may be seen from Figure 28, the amounts of the virus proteins of tobacco mosaic and its strains that were isolated were considerably greater than those of other viruses. It was desirable, therefore, to conduct the exhaustive study of fundamental properties on tobacco mosaic virus protein.

It was first necessary to demonstrate beyond a reasonable doubt that the unusual high-molecular-weight protein isolated from mosaic-diseased plants was really tobacco mosaic virus. It was found that the same protein could be isolated from many different batches of diseased Turkish tobacco plants or from other mosaic-diseased plants such as tomato, common nightshade, petunia, spinach, and phlox. The latter finding is of considerable significance, for it has been found that there is no serological relationship between the constituents of normal Turkish tobacco and normal phlox plants, yet on infection there is built up within these two different plants the same virus. The virus protein was found to be highly active as a precipitinogen, for antiserum to the protein gave a specific precipitin reaction with solutions containing only 10^{-7} gm. of virus protein per cc. It is of interest that the protein was found to be only weakly anaphylactogenic when tested *in vivo* and nonanaphylactogenic when tested *in vitro* by means of the Schultz-Dale technique. It was impossible to demonstrate the presence of material other than virus protein in purified preparations even by the sensitive precipitin and anaphylactic reactions. The ultraviolet absorption spectrum of the virus protein was determined and found to agree essentially with the destruction spectrum of virus activity, that is, just those wave lengths of light that were preferentially absorbed by the protein were exactly the ones that caused loss of

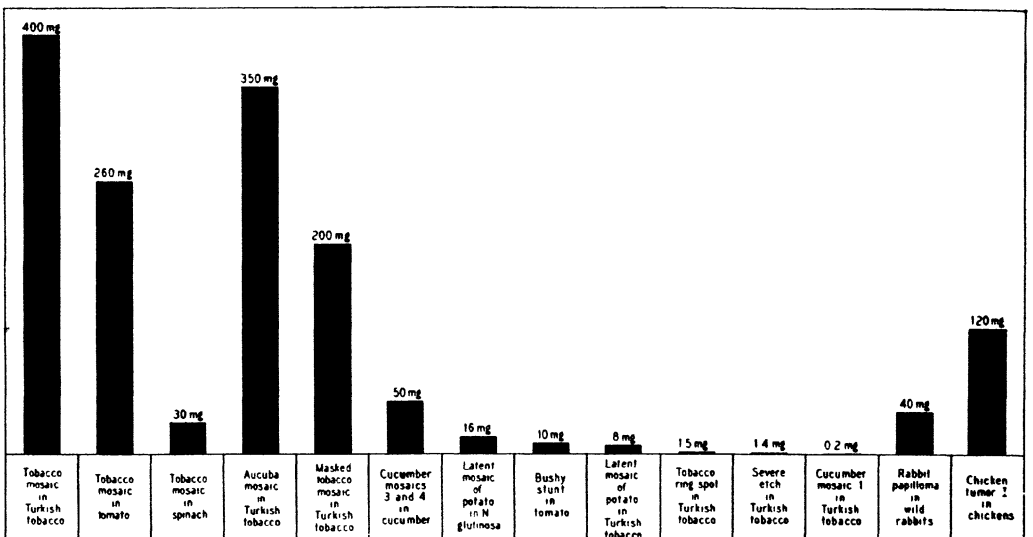


FIG. 28. Approximate amounts of virus proteins isolated from 200-gm. portions of tissues diseased with different viruses.

virus activity. Wave lengths near 2,250 Å, where protein absorbs strongly, were found to be more effective than wave lengths near 2,650 Å, where nucleic acid absorbs strongly. It was found impossible to separate the virus activity from the protein by any one of several different procedures such as by filtration through collodion or other types of filters or by centrifugation of the protein from solution under a variety of conditions. For example, centrifugation of negatively charged, positively charged, or neutral virus protein always resulted in sedimentation of the active agent and protein at exactly the same rate. Sedimentation of the virus from solutions containing various types of low-molecular-weight material always failed to remove activity from the virus protein or to cause the low-molecular-weight material to become active. Gratia and Manil, working with mixtures of virus and phage proteins, were able to effect a partial separation of the two by centrifugation or by crystallization of the virus protein.

It has been found possible to treat the virus protein with ultraviolet light, formaldehyde, nitrous acid, or hydrogen peroxide, and to secure proteins possessing no virus activity yet having physical, chemical, and serological properties either identical or very similar to those of the active protein. For example, antisera to such inactive proteins have been found to give a precipitin reaction with either active or inactive protein and, more important, to have a specific neutralizing action on virus activity. The latter fact is important because it indicates a close relationship between protein and virus activity and especially because it may serve as an example of the immunological potentialities in the control of certain virus diseases. However, it should be noted that in every instance of inactivation it has been possible to detect a change in one or more of the physical or chemical properties of the virus protein. The inactivation by means of formaldehyde has been followed in detail by Dr. Ross, who found that the inactivation followed roughly that of a monomolecular reaction and was accompanied by a decrease in

amino nitrogen as measured colorimetrically or by means of the Van Slyke gasometric method and by a decrease in the color developed by Folin's tyrosine reagent. It was proved that the inactivation was not due to the presence of free formaldehyde or to the formation of an insoluble or toxic compound. Most important are the facts that it was found possible to reactivate the formolized virus to a considerable extent and to demonstrate that the reactivation was accompanied by an increase in amino nitrogen as measured by the color developed with ninhydrin and also by an increase in the color developed with Folin's tyrosine reagent. It should be emphasized that the reactivation of formolized virus differs from all previously reported virus reactivations in that the virus was proved truly inactivated and in that the inactivation was proved to have been accompanied by structural changes in the protein molecule, which were reversed simultaneously with the return of activity. These results indicate that the virus activity is a specific property of the protein and provide some information regarding the structure necessary for activity.

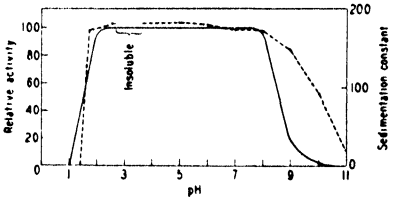


FIG. 29. pH stability range of tobacco mosaic virus protein as measured by virus activity (solid line) and by sedimentation constant (dotted line). (Drawn from data of Best and Samuel, 1936; Stanley, 1935; Eriksson-Quensel and Svedberg, 1936; and Wyckoff, 1937.)

Additional evidence relating virus activity to the protein was obtained by the partial or complete denaturation of preparations by means of heat, acid, alkali, or chemical treatment. It was found that whenever the protein was denatured by such treatments there was a corresponding loss of virus activity. For example, heating solutions of the protein to 75° C. for ten minutes caused denaturation and coagulation of the protein and loss of virus activity. Furthermore, as may be seen from Figure 29, subjecting solutions of the protein to hy-

drogen ion concentrations more acid than pH 2 or more alkaline than pH 8 caused loss of activity and, as indicated by the decrease in sedimentation constant, by the breaking up of the protein into low-molecular-weight material. Over the range between pH 2 and pH 8, the protein remained native and active and, except near the isoelectric point, its size as indicated by the sedimentation constant was unchanged. It should be noted here that similar results have been obtained with other virus proteins and, as may be seen from Figure 30, different virus pro-

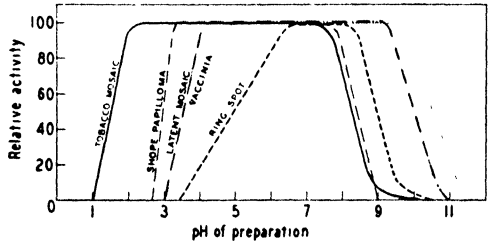


FIG. 30. pH stability range of tobacco mosaic, Shope rabbit papilloma, vaccine, latent mosaic of potato, and tobacco ring spot viruses. (Drawn from data of Best and Samuel, 1936; Stanley, 1935; Beard and Wyckoff, 1938; Beard, Finkelstein, and Wyckoff, 1937; Loring, 1938; and Stanley, 1938.)

teins have different and highly characteristic pH stability ranges.

One of the most interesting developments in the study of tobacco mosaic virus protein is concerned with a series of observations having to do with its size and shape and the effect of salt on these. It was noted early that one of the first samples of protein that had been subjected to extensive treatment in order to render it very pure was in fact quite inhomogeneous when examined in the ultracentrifuge. Eriksson-Quensel and Svedberg found that the inhomogeneity with respect to sedimentation constant became even more pronounced following additional crystallizations that were designed to reduce the inhomogeneity. It seemed very probable, therefore, that the very method of

purification was such as to render the protein inhomogeneous. Surprisingly enough, these preparations, regardless of the inhomogeneity with respect to sedimentation constant, were completely homogeneous in electrochemical sense and gave a uniformly migrating boundary in the electrophoresis cell. One of

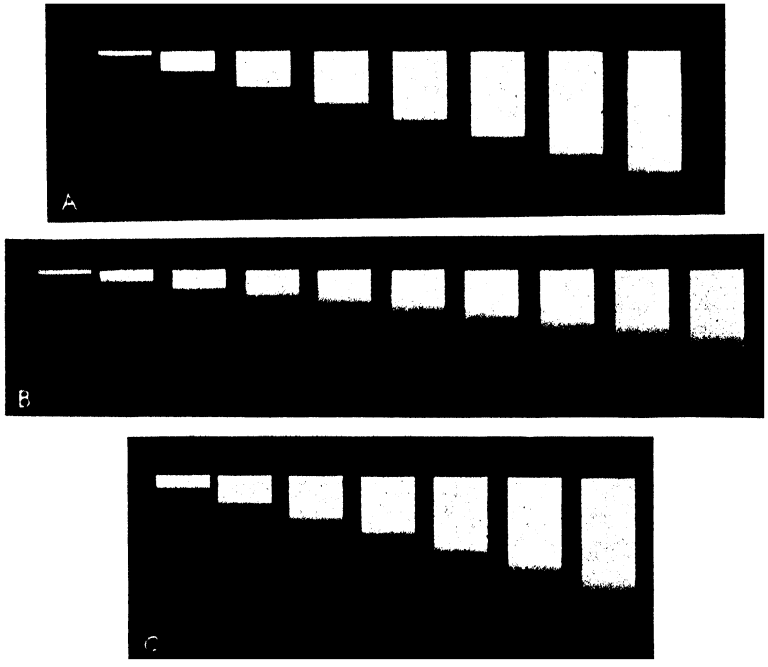


FIG. 31. Sedimentation pictures of solutions of tobacco mosaic virus protein prepared by Dr. Wyckoff. (A) Protein isolated by mild means such as by differential centrifugation, (B) following development of a second component caused by allowing protein to stand in the presence of salt, and (C) following more extensive treatment with salt. (Stanley, 1938.)

the earliest samples that had been extensively treated, and hence was regarded as especially "pure," was analyzed and found not to contain a demonstrable amount of phosphorus (see Table XI, p. 88). Because "less pure" samples were found to

contain variable amounts of phosphorus, it was regarded as an impurity at the time. Later, when the protein was obtained by differential centrifugation, it was found that such protein was completely homogeneous with respect to sedimentation constant (Fig. 31A), contained about 0.6 per cent phosphorus in the form of nucleic acid, and, most important, possessed a virus activity as much as ten times that of some of the early, chemically isolated, supposedly "very pure" preparations. It has also been found that merely allowing this protein to stand in the presence of 0.1 M phosphate buffer for a few days caused it to become inhomogeneous, due to the development of a second component having a sedimentation constant of about 200 instead of the original value of 174 (Fig. 31B). The explanation for the variation in the phosphorus content, the virus activity, and the sedimentation constant appears to reside in the peculiar shape of the protein and in the effect of salt on the protein. The first information regarding the shape of the virus was obtained in 1932 when it was shown by Takahashi and Rawlins that the juice from mosaic-diseased plants exhibited stream double refraction and hence probably contained rod-shaped particles. Following the isolation of the virus protein, these workers as well as Bawden and Pirie and Stanley and Lauffer showed that solutions of the protein also exhibited stream double refraction and that the molecules were probably rod-shaped. Although Bawden contends that it is impossible to assign a molecular weight to the protein from sedimentation data, due to the peculiar shape, it has been shown by Lauffer and by Frampton and Neurath that it is possible to obtain an estimate of the ratio of the length to the width of the protein particle from viscosity data and from this a value for the dissymmetry constant. Lauffer then used this value and the sedimentation constant to obtain an estimate, not only of the molecular weight, but also of the dimensions of the molecule. Neurath has determined the diffusion constant of the protein, which with the sedimentation constant or the viscosity data may be used as inde-

pendent means of estimating the molecular weight. Depending upon the equations used in the calculations, a molecular weight of the order of 40 to 60 millions, a width of about 11 to 12 mμ, and a length of between 430 and 700 mμ are obtained. These values are of the same order as those calculated from stream double refraction, ultrafiltration, and X-ray diffraction studies. Lauffer has shown that if two molecules of molecular weight 42.6×10^6, length 430 mμ, and width 12.3 mμ should join end to end to form a double molecule, the new particle would have a sedimentation constant, not of 174, but of 202, a value in close agreement with that found for the second component formed on treatment with salt. It is known that additional treatment of such double-boundaried preparations with higher concentrations of ammonium sulfate caused it to become less active and quite inhomogeneous and to give a boundary comparable to those obtained with some of the earlier chemically prepared samples (Fig. 31C). Since much of this material may have a sedimentation constant even greater than 202, it is obvious that aggregation greater than that corresponding to double molecules has occurred.

In the light of these new data, it seems probable that the earlier extensively purified preparations of virus protein actually consisted of protein that had lost most of the nucleic acid and that had been greatly aggregated due to the extensive treatment with salt, acid, and alkali, and as a consequence possessed as little as about 1/10 the original activity. It has not been found possible as yet to secure the dissociation of the nucleic acid from the protein by means of moderate concentrations of ammonium sulfate at about pH 7, yet it seems likely that during the chemical purification process conditions were such as to cause a dissociation of the nucleic acid which was then lost from the preparation. Loring has, by means of acid or alkaline hydrolysis, split and isolated the nucleic acid apart from the protein and has proved it to be a nucleic acid by the isolation of adenine, guanine, cytosine, and uridylic acid from the degradation

products. It was not until virus protein prepared in the cold and with a minimum of treatment and especially the ultracentrifugally prepared protein became available that it was possible to be certain that such virus protein possessed its original virus activity. This was due chiefly to the fact that during the earlier work the half-leaf method for estimating virus activity was just being evolved and neither the optimum conditions for carrying out the test nor the accuracy of the method had been determined. It was, of course, recognized that the activity of different samples varied to a certain extent according to the method of preparation and that samples of comparable activity were obtained only when prepared under comparable conditions. It was also known that the chemically prepared samples could be recrystallized repeatedly without measurable change in activity. It is now known that such experiments are possible only when most of the potential aggregation referable to salt has already taken place or when the experiments are carried out rapidly in the cold. Under these conditions, the additional treatment or recrystallizations cause but little additional aggregation and hence but little additional loss of activity. However, with the perfection of the half-leaf method so that differences in activity of 10 per cent or greater were readily detectable, and with the isolation by means of the ultracentrifuge and even by means of careful chemical procedures of protein possessing a uniform and high virus activity, it became possible to evaluate properly the earlier preparations and to conclude there was good reason for believing that at last fully active protein had been isolated.

The tendency for the virus protein to aggregate in the presence of salt is causing considerable difficulty among the English workers, who have adhered to chemical methods and room temperature for the preparation of protein and who, therefore, isolate aggregated, less active preparations. They have noticed that their purified preparations will not pass filters of average pore size, 450 mμ, filters which will readily pass the virus in the untreated infectious juice. Since all of the methods that they have

used have given the same type of preparation, they have concluded that aggregation is unavoidable and that purification of itself causes the protein to assume a different form from that in the plant. Bawden considers that the size of the smallest particle capable of causing infection is unknown, and Bernal has suggested that the virus protein molecule as it exists in the plant juice is a flat prism and that it is not until a sufficient number of these flat prism-shaped molecules become aggregated that rod-shaped aggregates become demonstrable. These conclusions are not at all in accordance with the results obtained in the Institute laboratories at Princeton, where it has been demonstrated that the protein isolated by careful ultracentrifugation is essentially the same as the protein as it exists in the plant juice. The virus activity, sedimentation constant, and stream double refraction of virus protein in juice and following purification were found to be the same. The protein purified by ultracentrifugation readily passed a filter having an average pore size of 450 mμ. If the virus present in the juice were of low molecular weight, it is obvious that it would not be sedimented at the speeds used. Since it is an experimental fact that over 99.9 per cent of the virus activity is sedimented and removed from the supernatant liquid on centrifugation for 1 hour at 60,000 g., it is obvious that, contrary to Bawden's contention, the minimum size of the infective particle is known. Dr. Lauffer has shown that, if the isolated protein of sedimentation constant 174 were composed of aggregated protein, say double molecules, then the hypothetical single molecules would have a sedimentation constant of 149 or less. No evidence has been obtained for the existence of active protein having such a sedimentation constant. It is seen, therefore, that, in view of the evidence relating virus activity and protein and the recent data regarding the size and shape of the protein, there is good reason for believing that the size and shape of the tobacco mosaic virus particle is known with considerable certainty.

Bawden and Pirie have also noted that chemical isolation of

the latent mosaic of potato virus protein yields a preparation of greatly lowered filterability. This virus protein, which was first isolated by Stanley and Wyckoff, has been found by Loring to be greatly affected by salt. Loring showed that the precipitation of ultracentrifugally isolated latent mosaic protein with ammonium sulfate at room temperature caused a loss of filterability and, more important, a great loss of virus activity. It seems likely, therefore, that the salting out used by Bawden and Pirie in their purification procedure had a similar effect on virus activity. It may be concluded, therefore, that the first preparations of tobacco mosaic virus protein prepared by the writer by rather drastic chemical methods, as well as the various purified preparations of the English workers, consisted of aggregated protein having a lowered activity and filterability, and that to date the only preparations of unaggregated protein, that is, of protein in a state and possessing an activity and filterability comparable to that of protein in the untreated juice, are those that have been prepared in the Institute laboratory at Princeton with a minimum of treatment in the cold or by means of careful differential centrifugation. The difference in the filterability of the preparations of Bawden and co-workers as compared with that of the preparations obtained in the Princeton laboratory by differential centrifugation, as indicated by the fact that the former fail to pass membranes of average pore size, 450 mμ, whereas the latter readily pass such filters, and the data of Loring, Lauffer, and Stanley on the effect of salt on activity, filterability, and aggregation emphasize the great difference in the two types of preparations. Thus, although the earlier chemically isolated preparations were comparatively inactive, their isolation and study represented an advance, for it soon led to an understanding of the tendency of certain of the virus proteins to aggregate, and enabled isolation techniques that prevented aggregation and loss of activity to be worked out which subsequently led to the isolation of what may be considered unchanged and fully active virus proteins.

The unusual rod-like shape of the tobacco mosaic virus protein molecule and its great tendency to aggregate have led to some observations of interest to physicists and crystallographers. Bawden, Pirie, Bernal, and Fankuchen first noted that, when a rather concentrated solution of the virus protein was allowed to stand, it separated into two layers having different solids contents and appearances (Fig. 32A). The upper layer was the

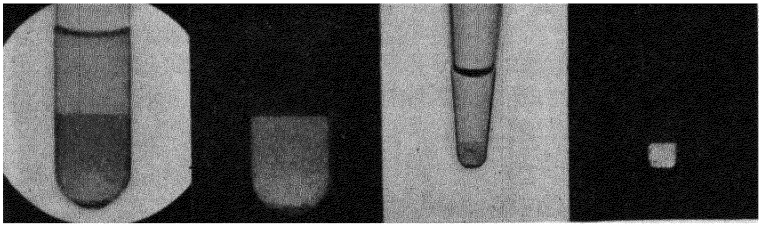


FIG. 32. (A) Liquid crystalline bottom layer of tobacco mosaic virus protein photographed between parallel and crossed Polaroid plates. (B) Liquid crystalline bottom layer of latent mosaic virus protein photographed between parallel and crossed Polaroid plates. (Stanley and Loring, 1938.)

more dilute and showed double refraction only when made to flow. The lower layer was the more concentrated and was spontaneously doubly refracting. Similar results have been obtained with solutions of latent mosaic virus (Fig. 32B). Bawden and Pirie suggested that this layering phenomenon is due to the fact that, when a certain concentration of protein is reached, such as in the lower liquid crystalline layer, the protein molecules become so close together that rotation about the two shorter axes is impossible, although translational motion is unimpeded, and the fluid then consists of a three-dimensional mosaic of regions arranged at random to each other, but in each of which all of the rod-shaped particles lie approximately parallel. The orientation phenomenon is readily reversible, for lower-layer material may be diluted to give upper-layer material, and the latter may be concentrated in the centrifuge to give lower-layer ma-

terial. Bawden considers that this layering phenomenon can be used as an index of the purity of preparations, yet it has been found in the Princeton laboratories that completely inactive virus protein may give liquid crystalline solutions. It is obvious that the ability to form liquid crystalline solutions is only an indication that the particles may be rod-shaped and is no criterion of virus purity. Lauffer studied the variations of viscosity and double refraction of flow of the protein with changes in hydrogen ion concentration, and found that these increase greatly in the region near the isoelectric point of the protein, but that only the viscosity falls sharply to a minimum very near the isoelectric point. He regards this behavior as being due to the end-to-end association of the rod-like molecules, followed by the side-to-side association of the elongated rods as the isoelectric point is approached from either side. Lauffer has also shown that the double refraction observed in the case of flowing solutions of tobacco mosaic virus is due largely to form double refraction rather than to intrinsic double refraction. This was accomplished by studying the double refraction of flow in solvents of different index of refraction. As may be seen from Figure 33, the phenomenon disappeared when solvents having an index of refraction approximately the same as that of the protein were used. It should be emphasized that rod-shaped particles are not a characteristic of all virus proteins, for, as may be seen from Figure 34, while some virus proteins, such as tobacco mosaic

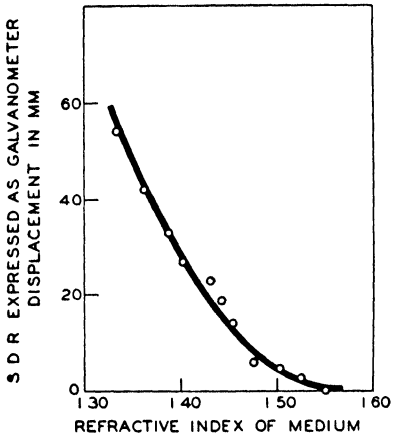


FIG. 33. Dependence of double refraction of flow of tobacco mosaic virus protein on index of refraction of solvent. (Lauffer, 1938.)

and its strains and latent mosaic of potato, exhibit double refraction of flow to a marked degree, others, such as severe etch, exhibit it to a lesser degree and still others, such as rabbit papilloma, tobacco ring spot, and, according to recent data, the bushy stunt of tomato do not show the phenomenon at all.

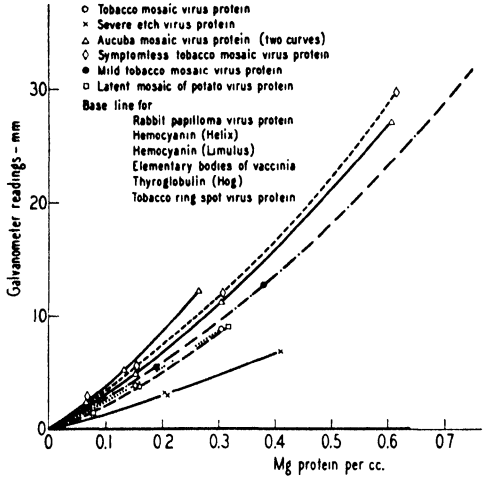


FIG. 34. Double refraction of flow of protein solutions. (Revised from Lauffer and Stanley, 1938.)

It may be seen from the results that have been presented that several different virus proteins, each possessing highly characteristic physical, chemical, biological, and serological properties, have been isolated from tissues diseased with different viruses. Tobacco mosaic virus protein has been isolated in comparatively large amounts and has been subjected to rather intensive study. All of the information now available regarding the homogeneity of this and other virus proteins, the correlation between protein and virus activity, the reactivation of formolized virus, the nature of virus in purified as compared to unpurified preparations, and the isolation of different viruses in the form of quite

different and highly characteristic proteins, and of different strains of the same virus in the form of different although closely related proteins indicate that the several virus proteins are in fact the viruses themselves. It becomes necessary, therefore, to consider virus activity in terms of these proteins. One is immediately faced with certain difficulties for, as has been demonstrated, the virus proteins have the properties of molecules, whereas virus activity, involving reproduction, mutation, and specificity of action, has been generally regarded as a property of living organisms. At present there are at least two theories that have been proposed to explain virus activity and hence this apparent discrepancy. According to one, the cell theory of life would be discarded and the virus proteins accepted as elementary living entities, whereas according to the other, the virus proteins would be regarded not as living but as autocatalysts of the type of trypsin and pepsin. These general ideas were expressed at the beginning of this century and, although each has certain advantages, it has at the same time certain disadvantages, so that neither is perfect. There is no question but that a consideration of the virus proteins as autocatalysts makes for a very simple explanation, for by supposing the existence of inactive precursors which are merely activated autocatalytically, the necessity for explaining the origin of viruses, the production of protein molecules and of accepting a protein molecule as living is eliminated. There are, however, certain difficulties. In the first place, there is no definite evidence for the existence of the inactive precursors. It has been impossible to detect them in Turkish tobacco plants by direct isolation, hence, if a precursor exists in Turkish tobacco plants, it must be very unstable or the amount must be infinitesimally small. Furthermore, if a preëxisting inactive protein were converted into virus, you would expect it to retain some of its serological specificity, yet no evidence whatsoever for a serological relationship between virus and normal plant material has been obtained. In addition, it has not been found possible to demonstrate a serological rela-

tionship between the normal constituents of certain different plants such as phlox and Turkish tobacco, both of which are known, on infection, to foster the production of the same virus protein. However, the demonstration of the existence of high-molecular-weight material in normal hosts by Loring and Osborn, by Price, and by Glaser working with Wyckoff may prove of significance in this connection. These materials have been found to be quite unstable and following isolation soon become denatured and insoluble. Similar results on the normal proteins of tobacco and tomato have recently been reported by Bawden and Pirie. The possibility must be considered, therefore, that precursors exist and are stable under the conditions within the cell, but soon disappear following destruction of the cell. Furthermore, there is the claim of Krueger and Baldwin to have produced bacteriophage from cell-free filtrates, which, if substantiated, would of course indicate the existence of a precursor. Although these leads are significant and should be followed, it may be said that at the present time the existence of a virus precursor has not been proved.

Since many host cells are susceptible, not only to many different strains of a given virus, but to different viruses, it becomes necessary, according to the autocatalyst-precursor theory, to postulate the existence of a multiplicity of precursors. Furthermore, the virus reaction differs in certain respects from known autocatalytic reactions. For example, Northrop and coworkers have shown that pig pepsinogen is always converted into pig pepsin, whether it be activated with chicken or pig pepsin. The "strain" of the pepsin is determined by the precursor and not by the activator. The case with viruses appears to be fundamentally different, for the activator alone determines what is produced. The introduction of any one of the several different strains of tobacco mosaic virus results in the production of that particular strain. Since the host cell is ready to foster the production of any one of hundreds of different virus proteins, it becomes necessary to postulate the existence of hun-

dreds of different precursors, each of which is activated only by its own special activating agent and not by even closely related agents. Thus, it is necessary to assume that, upon the introduction of one of the many strains of tobacco mosaic virus into a Turkish tobacco plant, only the precursor of that particular strain is activated, the precursors of closely related strains being entirely unaffected. It is possible, of course, to postulate a type of master molecule that is capable of being converted into any one of a number of different strains or viruses and which would, therefore, make unnecessary the existence of a large number of precursors. However, a further difficulty is that, according to the autocatalyst-precursor hypothesis, it should be possible to produce viruses with cell-free media, yet one of the outstanding characteristics of viruses is their dependence upon the presence of living cells for multiplication. A more serious difficulty and the one that looms as a major obstacle to the acceptance of the straight precursor-autocatalytic hypothesis is one that is inherent with the idea. If viruses are produced by the autocatalytic activation of precursors, then, since a catalyst does not initiate or change the reaction but merely changes the *rate* of the reaction, one would expect the production of viruses *de novo*. The spontaneous activation of some of the hundreds of precursors would most assuredly occur and we would thus have the production of a virus without the addition of virus. However, this does not appear to be the case, for there is no evidence that viruses arise in this manner—in order to produce virus, it is necessary to start with active virus. It is possible that the tendency for activation may be so slight that activation occurs only at rare intervals in the absence of a catalyst, intervals so rare that none has occurred during man's observation, or if it has occurred then under conditions that precluded observation. There are, of course, the coming and going of herpes, the occurrence of spontaneous filterable tumors, especially following chemical or other treatment, etc., yet so far as I know there is no authentic record of the production of a virus from nonvirus-containing materials.

Numerous situations are known where the conditions are most favorable for the production of a given virus, yet the virus does not appear and is not synthesized unless introduced from without. There is, so far as I know, no way of producing viruses at will from nonvirus materials. In this respect, therefore, the viruses appear not as autocatalysts but preëminently as living agents.

It may be seen, therefore, that, although the precursor-autocatalytic hypothesis has certain advantages, it does not fit the experimental facts in that neither the existence of a precursor nor the production of a virus *de novo* has been demonstrated. Although the first difficulty may be resolved by improved experimental techniques and the latter by observation over a longer period of time, I think that a consideration of an alternative hypothesis, that is, of viruses as a type of living agent, is warranted. The general properties of viruses, such as their ability to reproduce and to mutate and the necessity of having active virus in order to obtain more virus; fit in very well with the idea that viruses are living, and there was for many years no serious objection to the acceptance of this idea. However, with Galloway and Elford's demonstration in 1931 that the foot-and-mouth disease virus was only about 10 mμ in diameter and with the recent demonstration that many more of the viruses are no larger than protein molecules and, more important, that at least some of the viruses have the physical and chemical properties of protein molecules, serious difficulties were encountered. It appeared inconceivable that an entity no larger than a protein molecule could contain the organization necessary for life, and there was a tendency to regard the smaller viruses as being different from the larger. However, as I mentioned earlier, the viruses appear to consist of a group and there is no biological or pathological property that appears to be a function of size. I feel that the true solution to the problem does not lie with the arbitrary division of viruses according to size, but rather in the search for a fundamental hypothesis of

virus activity that may be applied to all viruses. There is no indication of a well-differentiated cell wall or of metabolism, and so far as the smaller viruses are concerned it appears very probable that there is not sufficient structure to support the type of organization that we think of in connection with ordinary living organisms. I think that we may as well recognize the fact that, if we wish to regard the viruses as living, we must assume that there is some fundamental difference between them and ordinary living organisms. I wonder if this difference could correspond to the transition from an intermolecular to an intramolecular structure or type of organization. The virus proteins isolated so far have been found to be nucleoproteins or more complex conjugated proteins having unusually high molecular weights. It may be possible that in this chemical combination of nucleic acid and protein of unusually high molecular weight we have sufficient organization within a single molecule to endow it with the lifelike properties that characterize it. Such a molecule might be regarded as the simplest type of living agent, one from which all extraneous material has been removed and which is so highly specialized that it can reproduce only under very special conditions. Green has already pointed out that an intracellular parasite might be expected to undergo a loss of function due to the assumption of such function by the surrounding protoplasm. There is a wealth of evidence that may be used to prove this point, such as the fact that strains of bacteria may be developed whose growth is absolutely dependent upon the presence of certain very definite and in some cases very simple materials, and the fact that protozoa are known which will not grow except in a certain location within a certain host. It is possible, therefore, to consider obligate intracellular parasitism as a characteristic that may be acquired. Degradation, loss of function, or retrograde evolution of a living organism might progress to the point where only a single molecule remains, and this molecule might be expected to possess unusual properties but would be functionally complete only when immersed in a

certain type of protoplasm. Some writers, notably Alexander and Bridges, wish to consider that such an entity might also have arisen by abiogenesis, by the chance coming together of molecules to form a larger molecular complex. It should be noted, however, that, if one accepts this conception for the origin of viruses, it becomes necessary to assume that the conditions for abiogenesis no longer exist, or, if they do exist, then that the actual occurrence is such as to preclude observation, for, as mentioned earlier, viruses have not been found to arise *de novo*. Whether originating by abiogenesis under favorable conditions that existed only at some time in the past or from living organisms by some degradatory process, we would have an entity possessing all the physical and chemical properties of a molecule and at the same time the potential properties of an organism, without it itself being a functionally complete organism. This is the type of entity that we may be dealing with in the case of the virus proteins.

Similar views have been expressed by Laidlaw in his Rede Lecture, and it is obvious that this general conception is uniquely adapted to an explanation of all of the general virus characteristics. Such an entity should remain inert and lifeless under all conditions except when immersed in a certain type of protoplasm where it should spring into action and exhibit the ordinary properties of an organism such as reproduction, mutation, etc. You would not expect the "*de novo*" production of virus, for the appearance of virus would be dependent upon the introduction of virus from without or upon the conversion or mutation of "virus" already present into a detectable form. Viruses would arise only from the action of active virus, and there would be no tendency for their production except in the presence of virus, for only the virus could initiate the reactions that culminate in the formation of still more virus. There would be no need for metabolism on the part of the virus, since the energy for these reactions could come from the metabolism of the cell. A given cell would possess the ability *a priori* to foster the

synthesis of only certain virus proteins depending upon its own special enzyme system and metabolic activity. A change in the genetic constitution of the host cell could, therefore, bring about an altered virus reaction, perhaps even prevent the production of virus and thus render the host nonsusceptible. The intimate relationship between virus and host cell that has been recognized as an outstanding characteristic of viruses for years thus acquires a special significance. Furthermore, the view provides a good explanation for the fact that, although these agents may be grouped together because they possess similar biological and pathological properties, they nevertheless vary greatly in size, for, being functionally incomplete, size becomes but an index of how far evolution or retrograde evolution has proceeded. There would be, according to this view, no sharp line between molecules and organisms, for there would be an imperceptible gradation from one to the other. However, from a theoretical standpoint the line of separation might be considered as the point of transition from an intermolecular to an intramolecular type of structure or organization. Since it is possible to conceive of many molecules uniting to form one molecule having a size greater than that of a smaller number of molecules in the form of an organized, metabolizing, living entity, it is obvious that there might be no sharp line of demarcation between the two types with respect to size. This emphasizes again that the difference between ordinary living organisms and viruses is one of their fundamental properties rather than one of size.

It should be noted here that, although there are difficulties involved in the acceptance of the autocatalytic activation of an inactive precursor as the basis for virus activity, the reactions involved in the synthesis of virus according to the present view may well be catalytic reactions and the net result of the reactions may appear to be that of a single autocatalytic reaction. The present hypothesis is concerned chiefly with an explanation of the characteristic virus properties and since the metabolic activity of the host cell may be considered to be at the disposal

of the virus, no discussion of possible mechanisms of virus synthesis is given. It may be noted, however, that the virus appears to possess a directional influence that may be likened to that of the gene. It is possible that the reactions involved in the synthesis of virus may be similar to those involved in the duplication of genes. Possible mechanisms were discussed by Troland in 1917, by Muller in 1922, and by Stanley in 1938. The properties that have been ascribed to genes are remarkably similar to those of viruses. Both appear to be large nucleoproteins (Fig. 25) that multiply or reproduce only within living cells and that may be altered or changed and then give rise to the new rather than to the old type on multiplication. Neither is produced *de novo*. One point of difference is that, whereas viruses usually continue to multiply, at least for a considerable period of time, within a cell, genes do not. However, the similarity is so striking that some geneticists are turning to a study of viruses as a means of learning about genes. It is possible that a careful study of the production of virus within a cell may aid in the elucidation of the reactions involved in intracellular metabolism. The virus proteins, because of their special activity, may be regarded as marked molecules, similar to the inorganic molecules that are marked by radioactive properties and that are now used in studies of animal metabolism. Great progress in the study of intracellular reactions has been made in recent years. Warburg's work on the respiratory ferment and Keilin's establishment of the nature of cytochrome C represented great advances in the study of intracellular reactions. It does not seem too much to hope that with the aid of the newer knowledge of these materials and of the virus proteins the basic and fundamental reactions involved in the life process may be discovered and understood. At least, it seems probable that with the elucidation of the mechanism of virus formation will come information pertinent to the question of the gene and its mode of action.

The chief difficulty in the acceptance of the general theory that has been presented is, I think, not so much factual as men-

tal. We have always adhered strongly to the cell theory of life and to the idea that a tremendous amount of organization is necessary for life. The idea that a single molecule may possess sufficient organization to endow it with lifelike properties under certain conditions is new and, although I think we should approach it with caution, we should nevertheless approach the idea and give it all due consideration. The viruses have, therefore, caused to be inaugurated a research that started with a study of a plant disease, that continued with problems of interest to the bacteriologist, the pathologist, the chemist, the physicist, the geneticist, and the biologist, and that now appears to be culminating in a problem of interest to all scientists, the problem of life itself.

IV

NEW VIEWS IN VIRUS DISEASE RESEARCH

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THE economic importance of the virus diseases of plants furnishes a continuous stimulus for their study and brings a constant demand for their control. Viruses cause some of the most destructive diseases of such important crop plants as the potato, the sugar beet, the sugar cane, and the peach. They are suspected of causing certain diseases of undetermined etiology, in addition to many well-recognized maladies. Since less is known of viruses than of any of the other agents that cause contagious diseases, they appeal to the imagination of investigators who are not primarily concerned with problems of control, as well as to those interested in the yield of crop plants. It is not surprising, therefore, that there is much activity in this field and that work on viruses is increasing at an accelerating rate. Each new contribution brings new views regarding the things viruses are able to do, and each new view suggests new experiments. The activity will doubtless continue until the nature of viruses is understood and the diseases they cause are controlled. In the meantime, it may be worth-while to give, as I shall attempt to do, a brief account of some recent contributions that seem important at the present time.

TOBACCO MOSAIC

To tobacco mosaic belongs the distinction of being the first disease shown to be due to a filterable virus. To it also belongs

the distinction of having received more attention than any other plant virus disease and more perhaps than any animal virus disease. There are at least two reasons, in addition to early recognition of filterability of the virus, for the popularity of tobacco mosaic and its causative agent as objects of study.

First of all, the entity responsible for this disease is the most stable of the known viruses. It is not inactivated by a 10-minute exposure to temperatures under 90° C., and will endure heating at 70° C. for many days. It is highly resistant to chemicals and persists for years in dried tobacco leaves or in frozen samples of tobacco juice. Therefore, it can be used in many experiments from which most other viruses are excluded because of inactivation.

Second, better and more accurate methods have been devised for quantitative studies on tobacco mosaic virus than are available for any other plant virus. Quantitative methods involving the primary-lesion technique of Holmes and the serological technique of Purdy have made possible much of the recent work on tobacco mosaic. As might be expected, knowledge of this much-studied disease profoundly influences conceptions respecting all other virus diseases. It therefore deserves special consideration in any résumé of new views in virus disease research.

Particulate Nature of Tobacco Mosaic Virus. Juice squeezed from the leaves of tobacco plants having mosaic is highly infectious. If a sample is passed through a porcelain filter with pores of the proper size, all bacteria are removed, but the juice retains its infectivity. This proves that the disease is not due to a bacterium, as was believed by Iwanowski, who first made filtration experiments. If a sample is passed through a collodion membrane with pores of the proper size, the juice loses its infectivity. This proves that the disease is caused by something which is particulate and not by the fluid portion of the sample, as was suggested by Beijerinck. By determining the smallest pore size that will allow virus particles to pass and the relation

of pore size to the filterability of masses of known size, it has been possible to show that tobacco mosaic is caused by particles having diameters of the order of 30 mμ. Since the best microscopes do not possess a resolving power which permits our seeing objects with diameters much less than 200 mμ, it is obvious that this particle is well below the range of microscopic vision. Researches on the nature of the particle are limited by the inability to see what it is like. Its properties and its behavior can be studied only through effects on plants.

Development of Quantitative Methods. Studies on the nature of tobacco mosaic virus require the infection of plants, and the desirability of measuring degree of infectivity of juice samples was recognized many years ago. But previous to the work of Holmes, which showed that tobacco mosaic virus causes conspicuous local lesions at points of inoculation in leaves of *Nicotiana glutinosa* L. and several other species, there was no accurate method for determining degree of infectivity. Such measurements as were attempted involved the inoculation of large numbers of tobacco plants by some uniform method of wounding. Tests by the local-lesion method employing a single *N. glutinosa* plant bring more accurate information regarding infectivity of any sample than tests by the old method with hundreds of plants. The new technique, which consists simply in rubbing *N. glutinosa* leaves with a piece of gauze that has been wet in the juice sample to be tested and subsequently counting the number of necrotic lesions that develop on the rubbed leaves, gave a great stimulus to studies on tobacco mosaic virus. A further stimulus came with the discovery that the virus, or some substance closely associated with it, is antigenic. While serological reactions do not furnish as accurate a measure of infectivity as the local-lesion method, they permit of rapid tests and give an independent means of determining infectivity. It has long been known from dilution experiments that the infectivity of juice samples varies with the concentration of virus in the samples. Undiluted juice is highly infectious, but prepara-

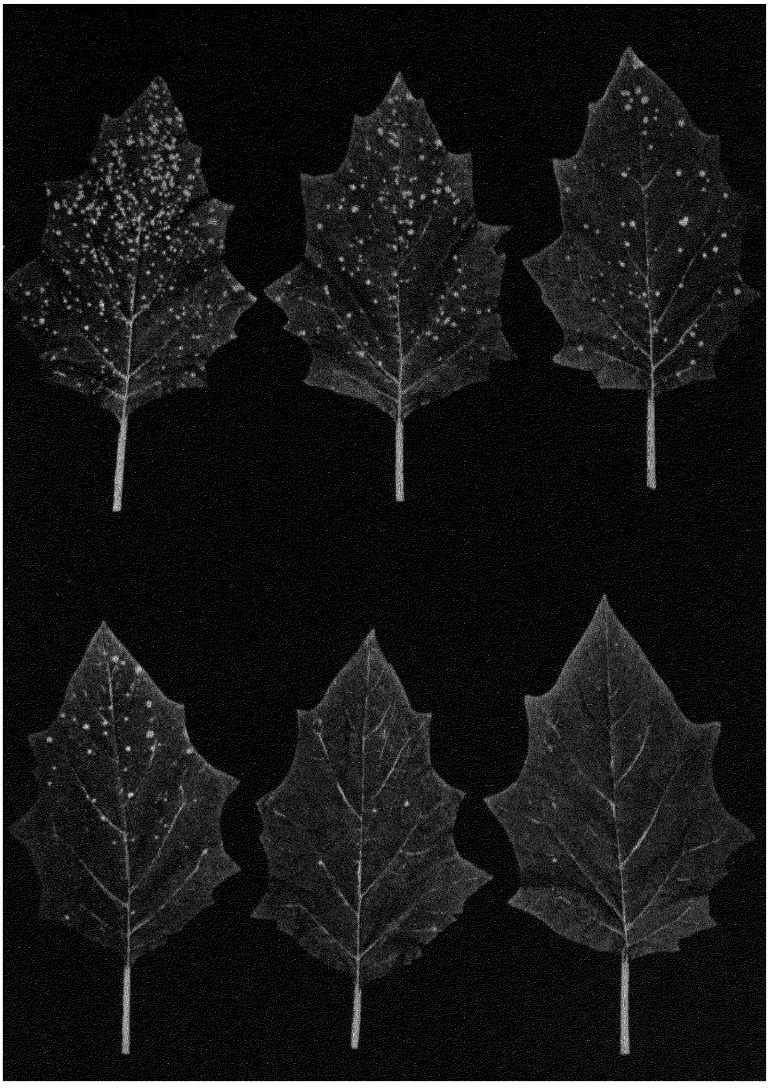


FIG. 35. Six leaves of *Datura stramonium* inoculated with samples of juice from a mosaic-diseased tobacco plant diluted to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}, respectively. The picture shows that as dilution increased the number of necrotic lesions produced decreased. Illustrating the Holmes method of determining virus concentration. (Photographs by J A. Carlile.)

tions containing 1 part of juice in 10 million parts of water are only slightly infectious. If dilution is carried much farther they become innocuous. The infectivity of samples containing more than 1 part of juice to 10 million parts of water is roughly proportional to the concentration of juice in the sample and presumably to the number of virus particles contained therein. Since the development of reasonably accurate methods of measuring virus concentration, work on tobacco mosaic has progressed rapidly.

Isolation of Strains of Tobacco Mosaic. It was soon found that a limited amount of increase of virus takes place in the necrotic lesions produced on leaves of *N. glutinosa* and that the virus cannot move out of the lesions. The disease does not become systemic in this plant. Tissues between the lesions are virus-free. When subinoculations were made from large numbers of individual lesions to tobacco plants, it was observed that some of the plants came down with diseases that differed from typical tobacco mosaic. Evidence was thus obtained that juice from plants affected by ordinary tobacco mosaic usually contains more than one kind of virus. The local lesions furnish a means of isolating these different kinds or strains of tobacco mosaic comparable to that furnished long ago by the Koch plate method for isolating different kinds or strains of bacteria. The discovery that most of the individual necrotic lesions contain only one strain of the disease-producing agent has led to the conclusion that each lesion results from infection by a single unit of virus. Whether the unit consists of one particle or of many particles is not yet known.

Origin of Strains of Tobacco Mosaic Virus. Jensen has isolated well over fifty different strains of tobacco mosaic which seem unlike any that occur in nature. To these, others have added small quotas obtained by well-known experimental methods. The new strains vary as regards the severity of the diseases they produce, from some that cause no symptoms in tobacco plants to a few that cause diseases far more severe than

ordinary tobacco mosaic. Most of Jensen's strains were obtained by subinoculating from bright yellow spots that occasionally occur on mottled tobacco leaves. When viruses capable of causing diseases symptomatically distinct from tobacco mosaic were first isolated from the bright yellow spots on mottled leaves, they were presumed to be contaminants unrelated to tobacco mosaic virus. But when the serological technique was employed in attempting to identify them, Chester was able to show that the different strains isolated by Jensen gave precipitin reactions when tested against rabbit serum sensitized to juice from plants with tobacco mosaic. This proved that the strains are related to tobacco mosaic virus. McKinney was the first to obtain variant strains from the bright yellow spots. He concluded that the strains arise from ordinary tobacco mosaic virus by a process comparable to mutation. The studies of Jensen and others have confirmed this conclusion. All of the variant strains studied up to the present time have produced other variant strains. The process of strain production is apparently a continuous one. The strain that predominates in any host plant is believed to be the one best suited to the conditions in that plant. Tobacco mosaic virus particles seem to be very stable entities. When introduced into susceptible plants, they cause the production of millions of other particles exactly like themselves. Nevertheless, in the course of time, they apparently cause the production of a certain number of particles that are unlike the first. When isolated and introduced into plants, these variants produce diseases differing from



FIG. 36. Yellow spot in mottled tobacco leaf affected by tobacco mosaic. Jensen's strains of tobacco mosaic were isolated from spots of this type. (Photograph by J. A. Carlile.)

that caused by the original particles. The work on strains has shown both the stability and the variability of the agent responsible for tobacco mosaic. It is now recognized that a considerable number of distinct but closely related tobacco mosaic diseases occur in nature and that a new disease of this type may be expected to arise at any time when conditions obtain that are suitable for the isolation and perpetuation of any one of the many variants that are constantly being produced.

Use of Mild Strains in Immunizing against Severe Strains. The variant strains of tobacco mosaic found in nature or obtained experimentally have proved useful in many ways. For example, it has been found that mild strains may be employed in immunizing plants against severe strains including ordinary tobacco mosaic. Tobacco tissues that have been invaded by any strain of tobacco mosaic virus acquire immunity from all other strains of this virus, but they do not become immune from any virus outside of the tobacco mosaic group. The reaction is a specific one and furnishes a convenient and dependable means of identifying new diseases and of showing their relationships.

All that is needed for a demonstration of the immune reaction in plants is a supply of virus strains of appropriate severities. The demonstration is striking only when a very mild strain is used to protect against a very severe strain. If, for example, tobacco mosaic virus of a strain isolated by Holmes and shown by him to cause no visible symptoms in tobacco plants be rubbed over the surface of a mature leaf of *Nicotiana sylvestris* Speg. and Comes, no symptoms are produced in the leaf. However, after three or four days, the inoculated tissues of the leaf become solidly immune from infection by the virus of aucuba mosaic, which always causes necrotic lesions on unprotected leaves. No other method is known of protecting these leaves from infection by aucuba mosaic virus. Immunization in this way is strictly local, because the mild strain of virus does not move readily in old leaves and immunity is confined to tissues that are actually invaded. In order to immunize all the tissues of an

N. sylvestris plant against infection by aucuba mosaic virus, the plant must be inoculated while still young with a mild strain of virus that will move readily through its tissues. Such a strain was isolated several years ago from a field-grown tobacco plant and designated as strain V-9. It causes a mild type of mottling and renders young *N. sylvestris* plants immune from all other strains of tobacco mosaic virus, including a lethal strain isolated by Jensen and designated as J-111. The latter causes a disease which almost always kills young *N. sylvestris* plants. If such plants are inoculated by rubbing the surface of a single leaf with the virus V-9 about two weeks before they are heavily inoculated in the same manner with J-111 virus, the plants do not become infected by the latter. Since they are not seriously injured by the virus V-9, they grow up to maturity and reach almost the same size attained by healthy control plants. Unprotected plants of the same age inoculated in the same manner with J-111 virus quickly die. A still more striking demonstration of immunization is given when young tomato plants infected by the virus V-9 are inoculated with a strain of virus isolated by Jensen and designated as J-14D. The V-9 virus causes a very mild type of mottling in tomato; the virus J-14D causes a severe type of systemic necrosis which quickly kills the plants. However, in this case it is not necessary to inoculate the plants with the protecting strain previous to their inoculation with the lethal strain in order to save them. Plants which have been inoculated for one, two, and even three days with the lethal virus may be saved from death or serious injury by inoculation with the protecting virus. Plants inoculated with the lethal strain for longer than three days cannot be rescued in this manner. Like all other viruses belonging in the tobacco mosaic group, J-14D occasionally gives rise to strains which cause mild types of disease. Almost all healthy young tomato plants infected with J-14D virus die, but if large numbers are inoculated a few will be found that survive. From such plants a mild strain of virus can always be isolated. It is believed that, whenever a mild strain

capable of moving more rapidly than J-14D arises from J-14D within three days after the plants are inoculated, it moves into the tissues that have not already been invaded by the latter and immunizes them. In this way it stops the advance of the lethal



FIG. 37. Five tomato plants of the same age and variety (Bonny Best). The plant on the left is healthy; the other plants are diseased. They were inoculated with the lethal virus J-14D twenty-five days before the picture was taken. They were inoculated with the protecting virus one, two, three, and four days, respectively, after inoculation with the lethal virus. The protection thus given saved all except the last from serious injury. (Photograph by J. A. Carlile.)

virus and saves the life of the plant. The studies on acquired immunity show that the tissues to be protected must always be reached by the immunizing virus before they are invaded by the virus to be protected against. The process seems comparable to that of fighting a dangerous fire with one that is less dangerous.

Mixed infections may be maintained indefinitely in plants inoculated simultaneously with two or more strains of tobacco mosaic virus which move and multiply at about equal rates. Any virus that does not move readily through the tissues of the host

is unsuitable for use as an immunizing strain. Nothing is known of the mechanism by which one strain of tobacco mosaic virus immunizes against related strains. It may be that the strain which first reaches any host cell uses up all of the food or all of the precursor that is needed for the multiplication of viruses of the group to which it belongs. It would in this way stop the invasion and multiplication of a related virus while exerting little or no retarding action on an unrelated virus.

Chemical Work on Tobacco Mosaic Virus. One interesting phase of work which quantitative methods for studying infectivity of tobacco mosaic virus samples have facilitated is concerned with the nature of the virus particle. Many theories dealing with the agent responsible for this mosaic have been postulated since the disease was first recognized. It has at different times been regarded as due to a living fluid, enzymes, gene-like bodies, and ultramicroscopic organisms of various kinds. Recently, the theory that it is a protein of high molecular weight has received much attention.

Vinson and Petre found that, when subjected to chemical treatments, tobacco mosaic virus behaved like a chemical substance and contained a percentage of nitrogen characteristic of some simple proteins. Stanley has recently isolated and crystallized a protein of high molecular weight which seems to possess all the properties associated with virus activity (Chapter III). It has a molecular weight of the proper order to give it the size that filtration experiments have shown it to possess. It is denatured by heat treatments at about the same rate that virus is known to be inactivated by these treatments, and is denatured by acids, alkalis, and other chemicals at about the same concentrations known to destroy virus activity. It has not been possible to separate virus activity from the protein, and Stanley has concluded, tentatively at least, that the protein is the virus. The work on this tobacco mosaic virus protein is of great theoretical as well as practical importance, because it suggests new ways of attacking problems connected with all other virus diseases.

NEW FACTS IN THE INVESTIGATION OF OTHER VIRUS DISEASES

So much for recent changes in our views regarding both the nature and the behavior of tobacco mosaic virus. Many of the facts learned have already proved useful in studies on other viruses which, although less infectious and less stable, are nevertheless similar to tobacco mosaic virus in their effects on plants.

The cucumber mosaic virus has been found to cause local necrotic lesions when rubbed on cowpea leaves. Its concentration in juice samples can be measured by methods exactly like those described above. Variant strains of cucumber mosaic virus have been isolated by use of some of the methods that yielded new strains of tobacco mosaic virus. The mild strains of cucumber mosaic virus have been used to protect plants against severe strains. A high-molecular-weight protein that seems to possess the properties of tobacco ring spot virus has been isolated from juice of tobacco plants affected by tobacco ring spot disease. In the space remaining an attempt will be made to describe some experiments with virus diseases that differ in many ways from tobacco mosaic, cucumber mosaic, and tobacco ring spot.

In respect to symptoms, the virus diseases of plants may be divided into two groups. The first group consists of the spotting diseases in which fusion of chlorotic lesions in leaves frequently results in the production of characteristic mosaic patterns. Tobacco mosaic is typical of the group. The second group consists of diseases in which the tissues of affected plants become uniformly or almost uniformly sick throughout. They are the nonspotting diseases and include such maladies as potato leaf roll, peach yellows, and aster yellows. They never cause typical mottling.

The virus diseases of plants may also be divided into two groups on the basis of infectivity. In the first group are placed the diseases that can be transmitted by means of juice from infected cells, and in the second the diseases that cannot be transmitted mechanically except by tissue transplantation. Both spot-

ting and nonspotting maladies are included in the list of juice-infectious diseases. Tobacco mosaic which causes spotting and curly top of sugar beets which does not cause spotting may be mentioned as examples. The diseases that are mechanically transmitted only by grafting also include both spotting and non-spotting virus maladies. *Abutilon* mosaic and peach yellows are well-known examples.

These groups are arbitrary, but they serve to indicate the range of variability of plant virus diseases with respect to symptoms and infectivity.

Peach Virus Diseases. The diseases requiring tissue transplantation for transmission are probably all spread by insects. Some, such as peach yellows and aster yellows, are known to be spread in this manner, while the means of spread of others, such as *Abutilon* mosaic and *Sida* mosaic, are at present unknown. Work on this group of diseases is more difficult than work on diseases transmitted mechanically by means of juice. Nevertheless, some of them have been used in experiments similar to those employed in the investigation of tobacco mosaic. Since none of the virus diseases of peach can be transmitted mechanically except by grafting, they seem to offer suitable material of the type desired.

Relationship between Yellows and Little Peach. The first objective in work on peach viruses was to determine by plant immunization tests and serological reactions whether or not the named peach virus diseases are all strains of one disease. Experiments were also planned with the object of isolating new strains from one or more of these diseases.

Peach yellows and little peach, which have similar geographical distributions, were suspected of being caused by different strains of the same virus because they were found to be transmitted by the same leafhopper. Attempts were made to obtain evidence of such a relationship by employing the immunization reaction. Experiments were carried out in which trees having little peach, the mildest of the two diseases, were inocu-

lated by inserting buds from yellows trees in their stems and branches. The yellows buds almost always lived and in many instances they produced shoots, but in no case was yellows transmitted to the trees. Healthy trees into which similar yellows buds were implanted always came down with yellows. It was thus shown that trees having little peach are immune from infection by yellows. This result was anticipated, but it was not expected that when a yellows bud inserted in a little peach tree developed into a shoot the shoot would show the symptoms of little peach and none of the symptoms of yellows. This, however, proved to be the case. Other experiments were made in which trees having yellows were inoculated by the insertion of little peach buds in their stems and branches. Similar buds were inserted in healthy trees at the same time. In due course all of the healthy trees so inoculated came down with little peach, but in no case was this disease transmitted to yellows trees. The experiments proved that trees having yellows are immune from infection by little peach virus. This finding, like that yielded by the reciprocal experiments, was anticipated. When little peach buds inserted in yellows trees developed into shoots, the shoots showed the symptoms of yellows instead of the symptoms of little peach. Although in keeping with the results obtained in the reciprocal experiments, this apparent displacement of one strain of virus by another was not anticipated. As the problem seemed to deserve further investigation, many displacement experiments were made. It was thought that, although the shoots developing from yellows buds inserted in little peach trees showed symptoms of little peach, their tissues might nevertheless carry and transmit yellows virus. Subinoculations were therefore made by transplanting buds from such shoots to healthy trees. It was found that the shoot tissues always transmitted little peach although they had grown from yellows buds. Likewise, when subinoculations were made to healthy trees from shoots that had developed out of little peach buds inserted in yellows trees, the yellows disease was transmitted. It was decided to determine

what would happen when healthy trees were inoculated with both viruses simultaneously. In certain experiments, a bud carrying yellows virus was inserted in the stems of trees a few inches above the bud carrying little peach virus, while in other experiments the position of the buds was reversed. It was found that the trees always came down with the disease caused by the virus carried by the bud in the upper position. When shoots developed from buds in the lower position, they showed symptoms of the disease caused by the virus carried by the bud in the upper position and none of the symptoms characteristic of the disease caused by the virus carried by the buds from which they grew. Here again was excellent evidence that either virus is capable of replacing the other in peach tissues.

The demonstration that a virus causing a severe disease like peach yellows can apparently be replaced in certain tissues by a virus causing a mild disease, like little peach, suggests the desirability of making experiments of this type with many other combinations of virus diseases.

A survey of peach orchards in the vicinity of Princeton, New Jersey, showed little peach to be common in this area. Many different strains of the disease could be distinguished by differences in degree of severity. Seven of them were transmitted in series and were found to remain true to type when carried from tree to tree by budding. Trees infected by the mildest of the seven strains were difficult to distinguish from healthy trees by foliage symptoms, but were somewhat stunted. All strains protected against infection by yellows. These immunization experiments fully justify the conclusion that yellows and little peach are caused by closely related viruses. Attempts were made to confirm this conclusion by precipitin tests, but up to the present time specific serological reactions have not been obtained for these viruses or for products associated with them.

Cross-reaction experiments similar to those described above for yellows and little peach were made with yellows and rosette and with little peach and rosette. Neither little peach nor yel-

lows protects trees against rosette, and rosette does not protect against little peach or yellows. It was therefore concluded that the virus causing rosette is not closely related to the viruses of the little peach and yellows group.

Effects of Heat-treating Yellows Trees. As only one strain of peach yellows was found in New Jersey, an attempt was made to isolate new strains experimentally. Since it was not possible to transmit yellows by means of juice, the methods used so successfully by Jensen for isolating strains of tobacco mosaic could not be employed. Exposure of infected trees to high temperatures seemed to be the most promising method available. Heat treatments were therefore undertaken. But instead of yielding mild strains of yellows as had been expected, heat-treated trees always continued to show the typical symptoms of severe yellows or they recovered completely from effects of the disease. It was first thought that trees which recovered might be infected by a mild strain of yellows. However, when it was found that they were as susceptible to infection by yellows as were trees that had not previously been infected, this hypothesis was abandoned. The recovered trees were apparently free of yellows virus. While the heat treatments yielded no new strains, they cured the trees. This seemed as important as the isolation of new strains, and methods of cure were studied further.

Many experiments were made with the purpose of determining the length of exposure necessary for the cure of trees held at different temperatures. It was found that they could be cured by exposure to temperatures as low as about 34.5° C., but at such a low temperature an exposure period of about 24 days was required. As the temperature was raised, the period of exposure could be much shortened. Small trees held at 50° C. were cured by treatments as short as 10 minutes. Dormant 2-year-old trees of the size commonly sold by nurserymen were cured by immersing them in a tank of water at about 50° C. for 10 to 15 minutes. The trees were not seriously injured by the treatment. Diseased bud sticks were readily cured by immersing

them in water at 50° C. for about 4 minutes, and the sticks were not injured by this treatment. They could, in fact, endure a temperature of 50° C. for a period 5 times as long as is necessary for cure. It is presumed that the cure of yellows by heat is due to inactivation of virus in the peach cells. It is hoped that this method of curing trees and bud sticks may prove of some practical value to nurserymen and peach growers.

Heat-treatment experiments were also carried out on the little peach, red suture, rosette, and mosaic diseases of peach. It was found possible to cure trees of all of these diseases except mosaic. The virus of this disease is not inactivated apparently by any treatment peach tissues will endure.

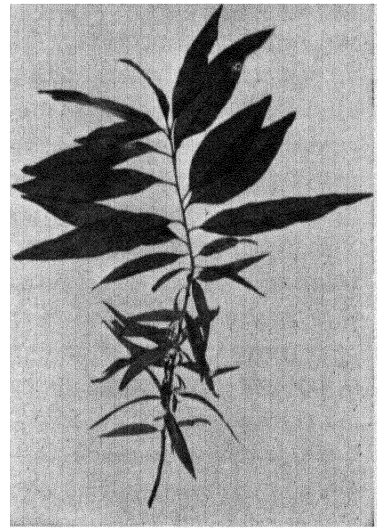


FIG. 38. Twig from a peach tree cured of yellows disease by heat treatment, showing type of growth made before and after treatment. (Photograph by J. A. Carlile.)

Heat Treatments on Aster Leafhoppers. The favorable response of yellows peach trees to high-temperature treatments suggested that other plants affected by virus diseases might be cured by heat. Since aster plants affected by aster yellows bear symptoms similar to those characteristic of peach yellows, they were selected for investigation. Yellows aster plants were placed in the same hot room that had been used in the cure of yellows peach trees. The room was held at about 35° C. Plants exposed for as long as 3 days usually died, but a few lived that were treated for 4 days. During 2 to 3 weeks following treatment these plants showed improvement in health. The young leaves were

a deeper green color than comparable leaves on untreated plants. However, improvement was temporary in all cases. After a few weeks the treated plants were no less severely affected than the untreated. Since aster yellows affects a large number of different species, it was decided to treat the disease in a host more resistant to heat than the China aster. *Nicotiana rustica* L., which is a vigorously growing, heat-loving plant, was selected for testing. Healthy seedlings were exposed to virus-bearing colonies of *Cicadula sexnotata* Fall., the vector of aster yellows. In due course all plants so exposed took the disease. Plants of different ages that had had yellows for different periods of time were placed in the hot room. All that were held in the room for more than 8 days died. A few that were treated 8 days survived. They produced leaves having a deep green color and seemed to be cured of the yellows disease. A number that were treated for less than 8 days also produced healthy-appearing leaves. After periods varying from 2 to 5 weeks, symptoms of yellows reappeared in the tips of all treated plants. *N. rustica* was not cured by any treatment which it could endure. Attempts to cure aster yellows in either aster or *N. rustica* were abandoned, and no effort was made to cure the disease in any other plant. Attention was next turned to the possibility of inactivating aster yellows virus by treating the leafhopper, which was found to be much more resistant to heat than either aster or *N. rustica* plants.

It will be recalled that aster yellows virus does not pass through the egg of the insect or the seed of the plant. Eggs laid by virus-bearing females always produce virus-free nymphs. All viable aster seeds, whether borne on yellows or on healthy plants, give healthy seedlings. Neither the leafhopper nor the aster plant lives for more than one season. Yellows virus passes the winter in perennial plants, such as the daisy and the dandelion. Any aster leafhopper that feeds on an affected perennial plant, even for a few hours, picks up the yellows virus, but no insect is capable of transmitting virus immediately after it first

feeds on a yellows plant. A period of from ten to twenty days must elapse between the time when the leafhopper first feeds on yellows tissues and the time when it is first capable of transmitting the disease. During this interval, which is known as the natural incubation period, the leafhopper carries but is unable to transmit virus. When the period of incubation is over, the leafhopper becomes infective and usually remains infective as long as it lives. Whether the insect functions only as an agent for transferring virus from diseased to healthy plants or is itself a host in which virus multiplies is not known. But, in any event, it should be possible to free infective insects of virus by heat-treating them, providing the thermal inactivation point of the virus is lower than the thermal death point of the insect. On the hypothesis that such might be the case, heat-treatment experiments with insects were undertaken.

Colonies of infective leafhoppers confined on aster or rye plants in lantern globe cages were placed in the hot room used in the curing of yellows peach trees. The thermostat regulating the temperature of the room was set to hold at about 32° C. The insects were transferred from the hot room to a greenhouse after varying periods of treatment. The colonies were then transferred to a succession of healthy aster plants in order to obtain a record of infectivity. All colonies held in the hot room for 1 day or longer lost the ability to transmit yellows. This was rather surprising, since even at 35° C. yellows aster plants were not cured by treatments lasting 4 days or yellows *N. rustica* plants by treatments lasting 8 days. It was soon found, however, that loss of ability to transmit did not mean that the insects were virus-free. Many colonies that were heat-treated for from 1 to 10 days regained the ability to transmit yellows after a certain interval which for convenience has been designated as a heat-induced incubation period. The longer the colonies were heat-treated, the longer it took them to regain ability to transmit, as may be seen from the data recorded in Table XII. The data show the effect of length of treatment on the length of the

heat-induced incubation period. Eight colonies consisting of 50 insects each were used in the test. All were confined on yellows aster plants for 12 days before the experiment was started. Col-

TABLE XII

Rate of Recovery of Infectivity by Insects Determined by Length of Treatment

Transmission Records

Day of Test	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*
Culture 1	+	+	−	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Culture 2	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+	+	+
Culture 3	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−	+	+	+	+	+	+
Culture 4	−	−	−	−	+	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+
Culture 5	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	+	+	+
Culture 6	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
Culture 7	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Culture 8	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

Transmission Records

Day of Test	*23*	*24*	*25*	*26*	*27*	*28*	*29*	*30*	*31*	*32*	*33*	*34*	*35*	*36*	*37*	*38*	*39*	*40*	*41*	*42*	*43*	*44*	*45**
Culture 1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Culture 2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Culture 3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	†		
Culture 4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	−	−	+	−
Culture 5	+	+	+	+	+	+	+	+	−	+	+	+	+	−	−	−	−	+	−	+	+	−	+
Culture 6	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Culture 7	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−	+	−	+	−	+
Culture 8	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

The periods indicated by heavy lines show the length of the treatments. Each culture consisted of 50 insects when experiment was started.

* Cultures 1, 2, 4, 5, 6, 7, and 8 consisted of 6, 8, 8, 8, 4, 5, and 3 insects, respectively, when experiment was ended.

† Culture 3 was lost on the 43d day of the test.

ony 1, which was not heat-treated, transmitted yellows to every plant on which it fed after the 7th day of the test. Colonies 2, 3, 4, 5, and 6, which were heat-treated for 3, 4, 5, 6, and 7 days, respectively, regained ability to transmit after 3, 2, 4, 5, and 6 days, respectively. Except in colonies 2 and 3, the length of the

heat-induced incubation period varied with the length of the treatment. Why colony 3, which was treated for 4 days, recovered ability to transmit on the 3d day following treatment, while colony 2, which was treated for only 3 days, did not recover ability to transmit until the 4th day after treatment, is not known. The virus carried by colonies 7 and 8 had not completed its natural incubation period when the colonies were treated. Virus undergoing natural incubation is more readily inactivated by heat than virus carried by infective insects. The table shows that colony 7 became infective on the 36th day after treatment and that colony 8 did not regain infectivity. It is probable that all the virus carried by colony 8 was inactivated by the treatment. It seems equally probable that almost, but not quite all, of the virus carried by colony 7 was inactivated and that the percentage of virus inactivated in the other colonies varied with the length of time the colonies were treated. The fact that colonies given long treatments require longer periods of time for recovery of infectivity than colonies given short treatments suggests that the virus multiplies in the insect. If this supposition is correct, then both natural incubation periods and heat-induced incubation periods probably represent the time required for virus to reach a concentration sufficient to render insects infective. The fact that colonies frequently transmit for from 8 to 10 hours after being placed in the hot room indicates that they carry virus at a considerably higher concentration than is necessary for infectivity. The longer the insects are subjected to treatment, the less virus they retain after treatment and the longer they require to regain infectivity. The work suggests an explanation of the peculiar relationship of specific insect vectors to the diseases they transmit. Such insects are probably the ones in which the viruses concerned are able to multiply.

An attempt has been made to give in this chapter a brief account of results from recent work on tobacco mosaic and to show how the work on this disease has been facilitated by the development of methods for measuring degree of infectivity of

juice samples. An attempt has also been made to show how new viewpoints obtained in studies on tobacco mosaic and the virus by which it is caused have suggested lines of experimentation that proved fruitful in the investigation of other virus diseases such as peach yellows and aster yellows. The results obtained have not always been those that were anticipated. However, unexpected findings are likely to give other viewpoints which can be profitably employed in further investigations on tobacco mosaic and other virus diseases.

V

VITAMINS AND HORMONES

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MORE than a hundred years ago Berzelius advanced the idea that life phenomena are the result of a play of catalysts, though of different category from the mineral catalysts whose existence had already been demonstrated. Furthermore, he believed that every living organism possessed an infinite number of these catalysts, the nature of which was as unknown and mysterious as was that of life itself. Since the time of Berzelius, the combined forces of biological and chemical sciences have succeeded in removing many of the obscurities surrounding the nature and physiologic role of a multitude of such organic substances which play an important and intimate role in the metabolic activities of living cells.

During recent years we have come to the realization that the once mysterious substances for which the terms "hormones" and "vitamins" were first coined represent merely a group of satellites in a veritable galaxy of chemical substances directing and controlling the processes of development and growth in plant and animal organisms. The term "hormone" originally connoted "any substance normally produced in cells of some part of the body, and carried by the blood stream to distant parts, which it affects for the good of the organism as a whole" (Starling). Current usage now warrants a broader definition to include "any substance which is produced in one part of the organism and is transferred to another part which it affects for the good of the organism as a whole." Vitamins, on the other hand, may be de-

fined as "indispensable organic substances which the organism, lacking the ability to synthesize, must obtain from dietary sources." They are sometimes referred to as "exogenous hormones." Both hormones and vitamins are characterized by their biological effectiveness when present in exceedingly low concentrations.

Yet, as is true of most definitions, sharp lines of demarcation cannot be made. The antirachitic vitamin (D) can be synthesized within the animal body by the action of certain light rays upon sterols in the skin. The antiscorbutic vitamin (C) is apparently synthesized by all animal types except the guinea pig, monkey, and man. Vitamin A can be synthesized in the liver through the action of enzymes upon its precursor, carotene. A dietary source of iodine is a prerequisite to the synthesis of the thyroid hormone.

THE "ACTIVATORS"

As a prelude to the main topic of this discussion, I should like to consider briefly that vast array of organic substances so widely distributed in both plant and animal life which, although possessing certain hormone-like or vitamin-like attributes, are usually set apart in rather poorly defined categories. For convenience in discussion, and to afford a broader concept of the multiplicity and complexity of these substances and the physiologic processes concerned, I shall adopt the concept of Huxley and include under the general term "activator" those chemical substances produced by the organism "which exert specific functions in regard to correlation or differentiation." On the basis of their range of action the various activators may be grouped according to the following classification which, except for the inclusion of growth factors for microörganisms, is essentially that proposed by Huxley, 1935 (Table XIII).

Such a classification is by no means perfect, and probably not entirely acceptable to some morphologists and physiologists, especially to those who do not concur that the mechanisms of

TABLE XIII

Activators

A noncommittal term for those chemical substances produced by the organism, which exert specific functions in regard to correlation or differentiation.

A. Local Activators

1. *Intracellular activators*
   a. Substances produced by genes and causing the cells which produce them to differentiate in a specific manner.
   b. Substances responsible for sex characters, eye color, etc., in certain insects.
2. *Regional activators*
   a. Substances which predetermine specific regions in the embryo; e.g., limb disc, parts of eye rudiments.
   b. Substances responsible for growth gradients.

B. Distance Activators

3. *Diffusion activators*
   a. Direction of diffusion unrestricted—
      e.g., Numerous growth factors necessary for yeasts, molds, protozoa, and bacteria.
      e.g., Molting hormones of insects.
      e.g., Pigmento-motor neurohormones of fishes.
   b. Direction of transport restricted by structural organization—
      e.g., Growth hormones of higher plants.
   c. Action restricted to contiguous tissues—
      e.g., Organizer substances of vertebrate embryo.
      e.g., Lens-inducing substance of optic cup.
   d. Action restricted by specific means—
      e.g., Neurohormones of higher vertebrates.
4. *Circulatory activators*
   a. Hormones, as originally defined.
   b. Vitamins.

Para-Activators

By-products of metabolism, with effects on correlation or differentiation; e.g., carbon dioxide, histamin, etc.

genic action and embryonic segregation are of a humoral nature. The biochemist might also object to the fact that the large group of "enzymes" are not included, or might maintain that many of these so-called "activators" really function as enzymes. The latter point would be well taken, for, as will be emphasized

in the later discussion, certain of the activators that have been grouped under the heading of vitamins (vitamin B_1, riboflavin, nicotinic acid) are readily converted by living tissues into important coenzymes. Regardless of these imperfections, such a classification does afford a broad and helpful perspective of the wide range of biological processes for the successful completion of which the presence of hormone-like or vitamin-like substances has been demonstrated or postulated. A few brief remarks regarding the role of certain of these substances in the lower forms of plant and animal life, and in developmental processes, is now in order.

We are still quite ignorant of the mechanisms whereby the genes and other formed elements of the nucleus, interacting with the other protoplasmic constituents of the cell, control the unfolding of the genetic constitution during the development of the organism.[1] The same may be said of the factors responsible for differentiation of the cells of the early blastula prior to the process of invagination at the posterior lip of the blastopore in amphibia, and for the formation of the primitive streak in higher forms. But we do know, through the work of Spemann and many others, that the cells in the latter regions develop an "organizer" substance which, when reacting with substances present in the overlying ectoderm, is capable of inducing the latter to differentiate into a neural tube and to determine the main axis of the embryo.[2] This "primary organizer" appears to have the chemical properties of a sterol. It seems probable that there also exist innumerable other organizer substances or evocators which, appearing in orderly sequence of time during successive stages of development and interacting with closely related tissues imbued with the necessary reactivity, or "competence," establish the destinies of the various parts of the embryo.[3]

It is now recognized that certain strains of yeasts differ widely

1. Lillie, 1927, 1929; Ströer, 1936. 2. Spemann, 1927; Harrison, 1937.
3. Needham, 1936, 1.

in their requirement of specific substances, necessary for optimum growth, which they themselves are unable to synthesize in adequate amounts. Among these substances, bios I (i-inositol), bios II (biotin), and vitamin B_1 (thiamin) are best established. Bios I is widely distributed throughout the plant and animal world. Bios II is found in the lipides of egg yolk and in malt. Furthermore, while certain types of yeasts and molds can thrive in artificial media lacking vitamin B_1 but supplemented with either the pyrimidine or the thiazole components of the vitamin B_1 molecule, other types or species require both components of the molecule, and still others require the intact molecule for their metabolic needs.

There has grown up in recent years a very extensive and confusing literature dealing with the nutritive requirements of bacteria and protozoa in synthetic media.[4] In addition to their need for indispensable inorganic elements, amino acids, and nonessential growth-stimulating substances, these organisms exhibit very wide variations in their ability to synthesize other substances which, because of their indispensability and their activity in exceedingly low concentrations, possess many of the attributes of vitamins. A few examples may be of interest. *Staphylococcus aureus* requires both the pyrimidine and the thiazole components of the thiamin molecule (vitamin B_1) as well as nicotinic acid (pellagra-preventive vitamin, P-P). The latter is thought to be necessary for the synthesis of cozymase by this organism. Both cozymase and thiamin have been recognized as growth factors for other microörganisms such as the influenza bacillus and propionic acid bacteria. Furthermore, certain bacteria (*Cl. sporogenes, botulinum,* and *welchii*) require an ether-soluble unsaturated hydroxy acid, "sporogenes growth factor," which many other bacteria are able to synthesize.[5] There is reason to believe that continued investigation of the nutritive requirements of bacteria and other simple forms of life may eventually

4. See recent reviews by Burrows, 1936; Knight, 1936.
5. Kögl, and others, 1937.

prove of value in the elucidation of the physiological role of vitamins in higher forms, and even afford more delicate methods for the bio-assay of certain vitamins.

The field of plant physiology has undergone rather revolutionary changes since the idea of growth hormones entered botanical literature about twenty years ago. With the establishment of delicate and reliable methods of assay, the chemical nature of various growth hormones (auxins, phytohormones) has been demonstrated. One of these, known as hetero-auxin or indole-acetic acid, is readily obtainable by chemical synthesis. The formation and transport of these auxins in plant tissues, their role in various aspects of normal and abnormal plant growth, and their practical usefulness to the horticulturalist have been extensively investigated.[6] Furthermore, other compounds only distantly related to the auxins as well as quite unrelated substances (thiamin, biotin, female sex hormone) possess auxin-like activity in sufficient concentration;[7] a condition comparable to the estrogenic action of many substances quite unrelated chemically to the true estrogenic hormones.[8]

Practically nothing is known regarding the role of vitamins in higher plants. The relative abundance of components of the vitamin B–complex and of the fat-soluble vitamins A and E in the embryo of cereal grains, together with the rapid synthesis of vitamin C in the sprouting grain, suggests an indispensability of these substances in certain phases of the plant's development rather than an accidental appearance as by-products of cellular metabolism. In fact, the wide distribution of the water-soluble vitamins throughout the plant world and unicellular organisms suggests that these substances represent some of nature's earliest inventions in the process of evolving life, which have been retained, either in the same or in a more specialized role, in the more complex forms of animal life.

Most of our knowledge of the vitamins is based upon clinical

6. Went and Thimann, 1937.
7. Kögl, and others, 1937.
8. Dodds, 1937.

observation, and upon experimental studies with warm-blooded vertebrates—birds and mammals. Among the lower vertebrates, fish have been studied most extensively from the standpoint of nutritive requirements.[9] Since most of the well-recognized vitamins are present in various organs of the fish, it appears that these substances function here as in higher vertebrates. Experimental studies indicate that fish require not only the vitamin factors common to higher forms but also a thermolabile factor present in fresh meat, to which the designation "factor H" has been applied.

Some progress has also been made in the study of the vitamin and other nutritional requirements of invertebrate forms. A growth factor resembling the "factor H" of fish appears to be necessary for the growth of planaria.[10] The clothes moth requires part, or all, of the components of the vitamin B–complex; but does not require either fat or the fat-soluble vitamins.[11] The vitamin needs of the cockroach appear to be limited to certain water soluble and alcohol-ether soluble factors present in yeast, other than vitamin B_1 and flavin.[12] Isolated studies on other invertebrates also demonstrate exceedingly wide variations in species requirements for vitamins and other dietary components.

In the invertebrates, endocrine factors play a relatively minor role in most physiologic processes, especially those concerned with sex phenomena; the latter being largely controlled by chromosomal mechanisms.[13] During recent years considerable attention has been devoted to the corpora allata of insects. These small glandlike bodies located just behind the brain secrete hormones necessary for the initiation of each moult, for the prevention of metamorphosis until the final moult, for egg development in the adult female, and for development of the accessory glands in the adult male.[14] In certain respects, this

9. McCay, 1937.
10. Greenberg and Schmidt, 1936.
11. Crowell and McCay, 1937.
12. McCay, 1938.
13. Danforth, 1932.
14. Wigglesworth, 1936.

little gland might be compared to the hypophysis of the vertebrates. The adrenaline-like substance present in the salivary secretion of cephalopods by which they paralyze their prey, and in the nerve ganglia of certain leeches and annelid worms endowed with contractile vessels, may be regarded as the phylogenetic antecedents of the neurohormones and adrenal medulla hormone of the vertebrates.

These neurohormones are of two types. First, the pigmentomotor type liberated at the nerve terminals in fishes for control of the melanophores in the phenomenon of color change.[15] Due probably to their lipoid-soluble nature, their diffusion through tissues is relatively slow. In certain fishes, but not in others, a blood-soluble hormone of the neuro-intermediate lobe of the pituitary gland exerts a similar action. The latter alone is responsible for the color changes in amphibia and reptiles. Second, the chemical mediators of the nerve impulse in higher vertebrates, viz., the "sympathins" and "acetyl-choline" liberated at the nerve endings of the sympathetic and parasympathetic fibers, respectively, of the autonomic nervous system. Their range of action is also limited. In fact, the body possesses a specific enzyme, acetyl-choline esterase, which rapidly hydrolyzes acetyl-choline in such manner that its action is restricted to the particular end organ concerned and its escape into the circulation is prevented.[16]

It is thus apparent that the neurohormones of vertebrates represent a primitive type carried over in phylogenetic evolution. The circulatory hormones, on the other hand, represent a specialized group which has evolved in conjunction with the appearance in the vertebrates of highly specialized glands for their production, and a very efficient blood and lymph vascular system for their dissemination. Of these glands, the pituitary, thyroid, adrenal, and sex glands are present in all vertebrates; while the parathyroid and thymus appear first in the amphibia. The corpus luteum and placenta are characteristic only of pla-

15. Parker, 1935. 16. Cannon and Rosenblueth, 1937.

cental mammals. Despite wide variations in the morphologic arrangement and structure of these glands throughout the vertebrates, the chemical nature and physiological activity of any one hormone produced by different species are astonishingly identical.

With this rather limited survey of the nature and role of the "intracellular" and "diffusion" activators in biological phenomena, we may now progress to a discussion of the "circulatory" hormones and vitamins and their relation to avian and mammalian forms. In the course of the discussion to follow, I shall attempt to outline briefly certain progressive stages in the evolution of our knowledge of these interesting substances, to indicate the relation of these epochs of advance to important discoveries in related fields of the biological and chemical sciences, to cite a few of the astonishing developments of recent years, and to indicate some of the close interrelationships between the latter and many other fields of pure and applied science.

EPOCHS OF PROGRESS IN THE STUDY OF THE HORMONES AND VITAMINS

In the case of both the endocrine and the deficiency diseases there may be recognized four major stages of progress dealing, respectively, with (1) *clinical recognition and description,* associated with speculative theories of etiology and treatment, (2) *experimental production* of the disease state, elucidation of the pathologic and physiologic disturbances, and rational therapeutic treatment, (3) *concentration* of active principles, concurrent with the development of reliable methods of bio-assay, and (4) *isolation and synthesis* of active principles, with investigation of their therapeutic applications and their specific physiologic or metabolic roles.

Clinical Recognition and Description. The deficiency diseases are historically older than the endocrine diseases, five of them having been clearly described prior to 1755; at which time diabetes, goiter, and cretinism represented the only endocrine dis-

eases referred to and vaguely described in the medical literature. Since that time no new deficiency diseases have been established as specific clinical entities, despite the ever increasing addition to our alphabetical list of vitamin factors evolving out of experimental nutritional studies on avian and mammalian forms, although a hitherto unsuspected role of vitamins in various clinical states of unsolved etiology has frequently been suggested. The endocrine diseases, exclusive of those just mentioned, have been recognized as disease entities only during the past hundred years. Only about one half were described fully during the last half of the nineteenth century, and several new entities have been added during recent years. The exact type of glandular dysfunction and metabolic disturbance responsible for certain of these diseases is still unsettled.

Prior to the present century, diseases were so generally associated with positive agents (noxious vapors, toxic substances, infections, parasitic agents) that the concept of disease as due to the lack of specific substances either in the diet or as a result of glandular dysfunction was little short of medical heresy. One hundred years ago it was generally believed that there was but one kind of nutriment, termed "aliment," dissolved out of the ingested food by the action of the digestive juices. The term "protein" had not been coined. Concepts of the anatomy of the endocrine glands were exceedingly crude. So little was suspected in regard to their physiology that they were generally regarded as vestigial structures.

One can readily understand the failure of the early seeds of empirical treatment to take root. The time was not right, and the soil quite uncultivated. In fact, only during recent years have we been able fully to appreciate Hippocrates' treatment of hemeralopia (night blindness) with decoctions of liver; Cartier's miraculous cure of scurvy in his exploration party wintering on the St. Lawrence River in 1535 with an infusion from the leaves and bark of the fir tree; the repeated demonstration of the efficacy of certain foods in the prevention and cure of

scurvy and pellagra during the eighteenth and nineteenth centuries; the therapeutic use of seaweed and sea sponges in the treatment of goiter centuries before the discovery of iodine in 1812.

Experimental Production. Beginning about 1891, the gradual development of the experimental method in nutrition centering around the feeding of artificial diets to laboratory animals eventually led to the experimental production of beriberi (1897), scurvy (1907), xerophthalmia (1906), and rickets (1918), and to the establishment of the vitamin hypothesis between the years 1912 and 1915. By 1926, just twelve years ago, the general distribution of vitamins A, B_1, C, D, and E was quite well known, the pathologic changes induced by their absence in the diet demonstrated, and therapeutic treatment of the disease states well established.

During the last half of the nineteenth century and the early part of the present century, attempts to reproduce the endocrine diseases by extirpation of the related organ met with many disappointments and few successes. Obviously, states of glandular hypofunction and hyperfunction could not be reproduced in such manner. Hence, experimental attack upon these diseases has been decidedly retarded through the complexity of the problems involved.

The concept of a gland functioning through the production of a chemical substance was also slow in its development. Not until 1891–92 was the first successful administration of an internal secretion accomplished, when Murray in England courageously employed a crude extract of sheep thyroid for the treatment of a case of myxedema. The survival of this patient to the age of seventy-four years (1919) is an adequate tribute to Dr. Murray's clinical perspicacity. As a result, there was aroused much interest in the preparation of the active principle of the thyroid which culminated in Kendall's preparation of crystalline thyroxin in 1915, followed by the establishment of the chemical constitution and the laboratory synthesis of the

hormone by Harrington in 1927; the second hormone to be so prepared. The hormone of the adrenal medulla (adrenalin), first demonstrated in 1894 and isolated in 1901, was the first hormone to be obtained synthetically, in 1906. However, prior to the discovery of insulin by Banting and Best in 1922, but little advance had been made in the treatment of endocrine diseases, exclusive of thyroid disorders.

Concentration and Bio-Assay. The establishment of sensitive and trustworthy methods for the bio-assay of vitamins has been dependent upon careful analysis of the physiologic and pathologic alterations characteristic of the deficiency disease, followed by careful standardization of methods for measuring the animal's response. As an example I may cite the pathologic analysis of the bone changes in rickets and their indispensability to the establishment of the "line test" method of vitamin D–assay by McCollum and his associates in 1922. Although there exist a number of useful chemical and physico-chemical methods for the detection and measurement of certain vitamins, it seems doubtful that they can entirely replace the biological method of assay. This is well illustrated by the demonstration of the variable response of the chick, rat, and human infant to different antirachitic substances, which has led to the realization that two different forms of the vitamin occur in nature in fish-liver oils and that five or six other forms may be prepared synthetically; a distinction which could not have been made by chemical means.[17]

Progress in the study of the sex hormones was dependent upon a thorough analysis of the structural and functional changes occurring in all portions of the male and female reproductive tracts of rodents, and of other mammals, during different stages of reproductive activity and after castration. This was attained largely through the efforts of American anatomists and biologists. From these advances came the vaginal-smear procedure developed by Allen and Doisy in 1923 for the detec-

17. Bills, 1937.

tion of minute amounts of the female sex hormone; and the uterine-histology test established by Corner and Allen in 1929 for assay of the corpus luteum hormone. Likewise, the discovery and analysis of the regressive changes occurring in the epithelial lining of the accessory sex glands of male rodents

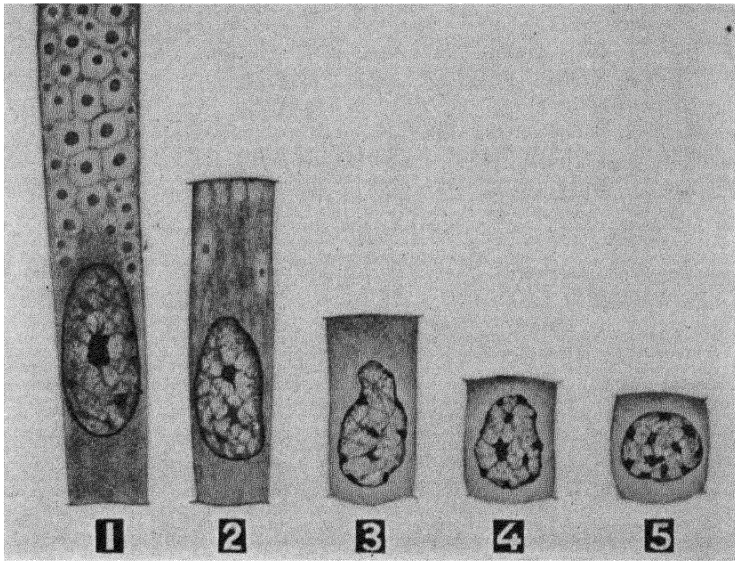


FIG. 39. Changes in epithelial cell of seminal vesicle of rat (1) before castration, (2) twelve hours, (3) two days, (4) twenty days, (5) ninety days, after castration. (From Moore, Hughes, and Gallagher, 1930.)

after castration, which affords a most striking example of the essentiality of a single hormone for the maintenance of functional integrity in a specific cell type (Fig. 39), made possible a reliable method for assay of the male sex hormone, first obtained by McGee from bull testes in 1927. The comb-growth and plumage tests in birds have also proved very delicate and useful criteria for assay purposes. It is of particular interest to note that the chemical isolation of the follicular, corpus luteum,

and testis hormones was accomplished six, five, and four years, respectively, after the establishment of satisfactory methods for their bio-assay.

TABLE XIV

Preparation of Hormones and Vitamins from Natural Sources

Preparation	*Investigator*	*Fresh Material Used*	*Crystals Obtained*
Thyroxin	Kendall (1915)	3 tons fresh thyroid	33 grams
Follicular H.	Doisy (1935)	4 tons hog ovaries	24 milligrams
Testis H.	Butenandt (1931)	25,000 liters urine	15 milligrams
Vitamin B_1	Williams (1934)	per ton rice polishings	5 grams
Riboflavin	Kuhn, etc. (1933)	100 kg. dried egg albumin = 33,000 eggs	100 milligrams

TABLE XV*

Hormone	*Date of Isolation*	*Concentration in Source* *Relative*	*Mg. per Kg.*	*Obtained*
1. Adrenalin	1901	1:1,000	1,000	From beef adrenals
2. Thyroxin	1914	1:2,000	500	From hog thyroids
3. Insulin	1925	1:100,000	10	From beef pancreas
4. Male sex	1931	1:5,000,000	0.2	From human urine
5. Corpus luteum	1934	1:50,000†	20	From corpora lutea
6. Follicular	1935	1:160,000,000	0.006	Concentration in hog ovaries

* From Doisy (1936).

† Its relative concentration in natural sources would have permitted isolation prior to that of the other sex hormones had methods for its detection and bio-assay been established.

Isolation and Synthesis. From the standpoint of effort, patience, and indomitable perseverance, the labors of the biochemists in the isolation of vitamins and hormones from natural sources command our greatest admiration and respect. Some concept of the laborious and costly procedures, and the low concentration of these substances in nature, may be obtained by reference to Tables XIV and XV.

To the chemist, however, isolation is merely the beginning.

With minute amounts of crystalline material in his hands, he can proceed to an analysis of the chemical character and composition, set up and test hypothetical formulae, establish the true chemical structure, and eventually secure an abundant and relatively cheaper supply through laboratory synthesis. Whereupon the physiologist, pharmacologist, morphologist, and clinician reappear on the scene to restudy and verify the action of the synthetic product, and to explore and establish its therapeutic usefulness. I wish now to recount several of the more remarkable achievements of recent years in the chemical identification and synthesis of several of the vitamins and hormones (Table XVI and Table XVII, p. 158).

TABLE XVI

Discovery of the Vitamins

Vitamin	*Existence Established*	*Isolation*	*Synthesis*	*Designation*
A	1912–15	1928	1937	Vitamin A
B	1912–15	(See below.)		
C	1919	1932	1933	Ascorbic acid
D	1922	1927	1927	Calciferol
E	1922	1936	1938	Tocopherol
B B_1	1912–15	1926	1936	Thiamin
B P-P	1925–26	1937	1867	Nicotinic acid amide
B ?	1933	1933	1935	Riboflavin
B B_6	1936	1938	....	....

THE VITAMINS

Vitamin C. The discovery of vitamin C affords a remarkable illustration of the convergence of two rather unrelated fields of investigation. In 1932 appeared the interesting announcement made simultaneously by King and Waugh (Pittsburgh) and Szent-Györgyi (Hungary), that the heretofore elusive vitamin C was actually a relatively simple hexuronic acid. King and Waugh succeeded, where others had failed, in obtaining the

crystalline antiscorbutic substance from lemon juice through persistent and painstaking effort of chemical purification. Szent-Györgyi, not especially interested in the problem of vitamins, came to his conclusions quite indirectly through his investigations of oxidative processes in the adrenal glands and other tissues.

The preparation of an abundant supply of this material by Szent-Györgyi from the Hungarian red pepper, the distribution of these crystals to interested chemists of various nations for verification of its chemical nature and properties, and coöperative arrangements culminating in its synthesis by Swiss and British investigators the following year (1933) afford an unparalleled illustration of the application of modern chemistry to nutritional problems, and the value of international coöperation of research workers of broad vision in the rapid and unselfish solution of a problem of greatest practical and theoretical value.

Vitamin B_1. Equally noteworthy are the fourteen years of persistent and intensive effort of chemists to obtain the antiberiberi (B_1) vitamin in crystalline form, as accomplished by Jansen and Donath in 1926, and the additional ten years of labor before its synthesis was consummated by Williams and Cline in 1936 (Fig. 40). It was the pyrimidine nature of curative extracts obtained from rice polishings which led Funk, in 1912, to coin the term "vitamine" to designate the suspected group of accessory food substances. Interestingly enough the crystalline vitamin (thiamin), unlike other known vitamins, proved to be an amine composed of a 6-amino-pyrimidine and a thiazole ring.[18]

The latter discoveries are superseded only by the astonishing developments of the past few years in the elucidation of the multiple factors of the vitamin B–complex. The term vitamin B originally referred to the growth-promoting and antiberiberi substance found in greatest abundance in yeast, liver, and the

18. Williams, Chapter VI, p. 172.

embryo of cereal grains. Twelve years ago it became apparent that there existed in such sources both a thermolabile antiberiberi vitamin (B_1) and a thermostable antipellagra vitamin (P-P, B_2). It has since been found that there also exist a growth-stimulating substance (riboflavin), a substance prevent-

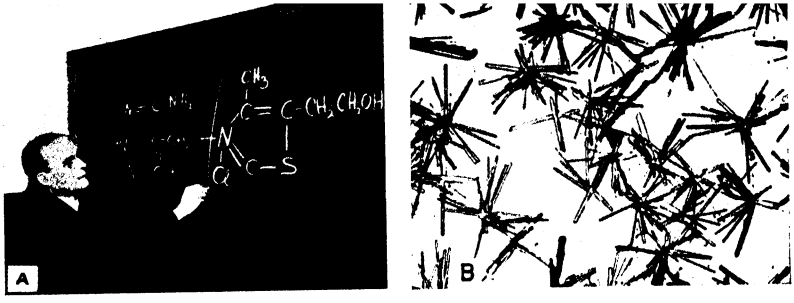


FIG. 40. (*A*) Dr. R. R. Williams demonstrating the structural formula of vitamin B_1. (*B*) Crystals of vitamin B_1 hydrochloride. (From *The Story of Vitamin* B_1. Rahway, N. J., Merck & Co., 1937.)

ing a "pellagra-like" dermatitis in the rat (B_6), and several less well-defined factors apparently required by the pigeon (B_3, B_5) and the rat (B_4, W); all of which, because of their close association with vitamin B_1 in yeast and other natural sources, have been generally referred to as factors of the B-complex.[19]

Riboflavin, first demonstrated as one of the fluorescent pigments of milk as early as 1879, received very little attention until its isolation in crystalline form in 1933 in the course of studies primarily concerned with the chemical nature of the new "yellow oxidation enzyme" obtained by Warburg and Christian in 1932 from aqueous extracts of yeast. By 1935 its synthesis was accomplished. It now appears that riboflavin, which is widely distributed throughout the plant and animal kingdoms, owes its vitamin-like activity to the fact that it is incapable of synthesis by the cells of certain animals which require

19. See reviews by Elvehjem, 1938; Nelson, 1938.

it as a structural unit in the synthesis of Warburg's yellow oxidation enzyme. A phosphoric acid ester of riboflavin, combining with the proper protein, forms this important enzyme which together with the haem-containing pigments (or "cytochromes") and vitamin C (ascorbic acid) constitute the three best-known respiratory catalysts of living cells.

Vitamin P-P (nicotinic acid). Another amazing turn of events came last fall when Elvehjem and his associates (1937) at the University of Wisconsin announced that canine black-tongue, long thought to be the analogue of human pellagra, could be cured by administration of nicotinic acid or nicotinic acid amide. It has since been convincingly demonstrated that pellagra responds in a similar manner to this relatively simple and cheaply produced chemical substance. The rather checkered career of nicotinic acid is interesting. First produced in 1867 by oxidation of nicotine, it was found to be present in the inactive fractions of extracts of rice polishings and yeast in 1912 by Funk, and by a group of Japanese investigators, in their search for vitamin B_1; and later, just one year prior to the discovery of its vitamin-like nature, it was shown to be an essential constituent of synthetic media for the growth of certain microörganisms. Of additional interest is the observation that nicotinic acid amide appears to be an essential constituent of cozymase, and of another closely related coenzyme, which are important in the cellular oxidation system of Warburg and Christian. It has also been suggested that nicotinic acid may be related to the metabolism of porphyrins, the iron-free decomposition products of hematin which are excreted in abnormal amounts in lead poisoning and in pellagra.[20]

Vitamin B_6. And, to add another brief chapter to this interesting story, the preparation of the rat antidermatitis factor, vitamin B_6, in crystalline form has been announced from two different laboratories during the past few months.[21] We do not yet

20. Spies, Cross, and Sasaki, 1938.

21. Lepkovsky, 1938; Keresetesy and Stevens, 1938.

know the exact chemical nature of this vitamin, nor are we certain that it is necessary for the human species, but we can rest assured that its synthesis will soon result and that many new and interesting fields of investigation will be opened.

Pathology versus Physiology. We are now confronted with the problem of correlating our knowledge of the role of vitamins in cellular metabolism with the pathologic evidence that each well-established vitamin appears to exert a fundamental effect upon a rather specific tissue of the body. The latter problem, which has been extensively discussed elsewhere,[22] may be summarized by a simple diagram which I have found quite helpful to those who encounter difficulties in correlating the alphabetical designation of the different vitamins with the corresponding deficiency disease and its associated pathology (Fig. 41). It will be noted that the pathologic lesion due to lack of vitamin A, the two major components of the B-complex, vitamin C, vitamin D, and vitamin E, respectively, are related to tissues which may be represented by those successively encountered in a hypothetical section through the body wall.

It is now recognized that an inadequacy of vitamin A results in impaired secretory function and excessive keratinization in epithelial tissues throughout the body, and that the many manifestations of the deficiency disease (night blindness, xerophthalmia, sterility, increased susceptibility to infection, etc.) are secondary to this primary lesion. Variably specialized epithelia, such as the columnar-ciliated epithelia lining the trachea or the enamel organ of the tooth, revert to a stratified type with excessive cornification of the superficial layers but, interestingly enough, never loose their potentialities for differentiation into their original morphologic type following restitution of the vitamin. In the vaginal epithelium of the A-deficient rat, the metabolic disturbances are such as to prevent this epithelium from responding to hormones which normally induce in it a state of mucification, such as that observed during the latter

22. Wolbach, 1937; Eddy and Dalldorf, 1937.

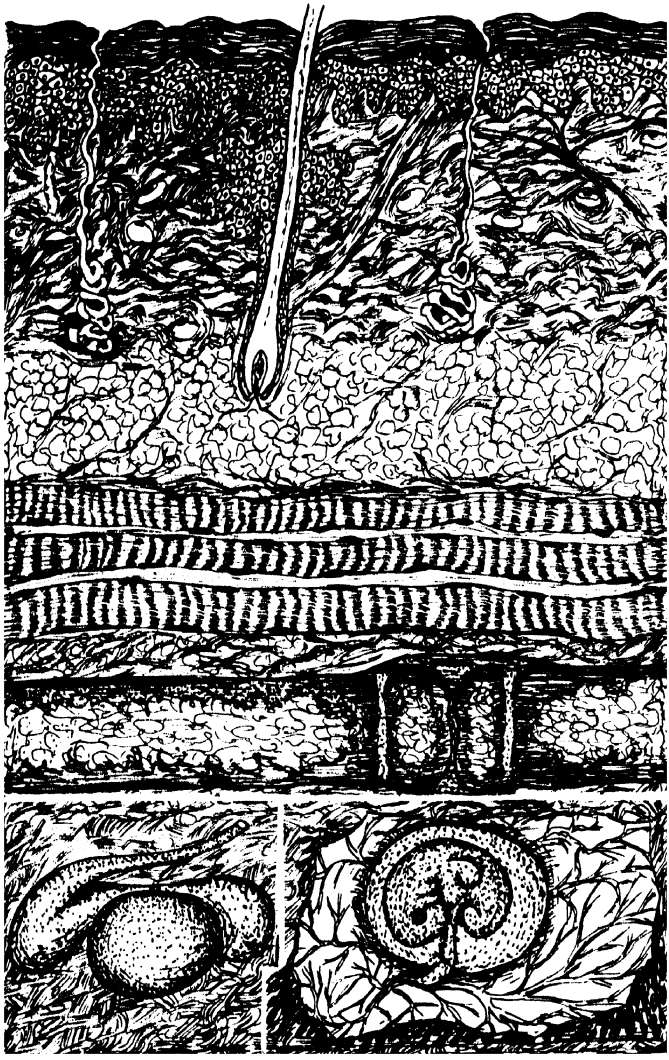


FIG. 41. Schematic section through the body wall to represent types of tissue affected by deprivation of different vitamins.

half of pregnancy. The synthesis of visual purple in the cells of the retinal epithelium is impaired, resulting in the production of hemeralopia (night blindness). Of additional interest is the recent claim that vitamin A is an essential constituent of the cytoplasmic chondriome and of the nucleolus in all living cells.[23] It is to be hoped that the correlation of the existing store of information regarding vitamin A, and the addition of newer knowledge, will soon lead to a better understanding of the utilization of this vitamin in cellular metabolism.

It is now thought that the vitamin B_1 of living cells combines with phosphoric acid to form a coenzyme which has a specific and important function in the oxidative removal of pyruvate formed during the intermediary metabolism of carbohydrates. Both the functional (neuritis) and the structural (demyelinization) changes in the nervous system in B_1-deficiency appear to be referable either to a direct effect of the metabolic disturbance or to an indirect effect related to the diminished energy development in the nerve cells. It is not yet certain to what extent the cardiovascular and other manifestations of B_1-depletion can be attributed to such impairment of carbohydrate metabolism. There is reason to believe that vitamin B_1 has other important physiologic functions not yet revealed, as suggested by its growth-promoting action in lower plants and its auxin-like activity in higher plants, mentioned earlier in this discussion.

The primary pathologic lesion of pellagra and of canine blacktongue consists of edema of the papillae, dilatation of the papillary blood vessels, and deterioration of the superficial collagen layer in the corium of the skin and buccal mucosa. It is not yet possible to correlate the pathology of pellagra with the evidence that nicotinic acid is an essential constituent of cozymase, and that it is concerned in porphyrin metabolism, as mentioned above. However, such observations open up interesting avenues for future exploration.

The lesion of scurvy is characterized by an inability of the

23. Joyet-Lavergne, 1937.

variably specialized connective tissue cells (odontoblasts, osteoblasts, capillary endothelium, etc.) to produce their extracellular products in a normal manner, and a morphologic reversion of such cells to a less highly differentiated type. These phenomena, and their associated clinical manifestations, have been

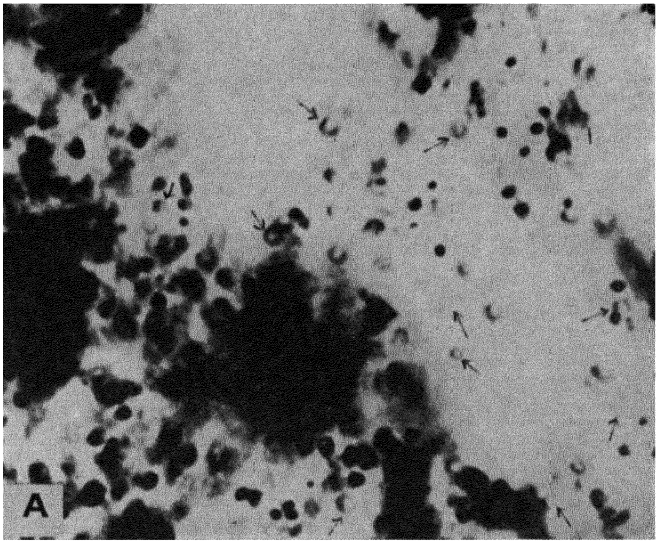


FIG. 42A. Granules of vitamin C in the adrenal gland of a human fetus, investing a core of lipoidal material (indicated by arrows) and completely enveloping the nuclei of cells (large masses of black material).

explained by the assumption that vitamin C has two functions, concerned with (1) regulation of the colloidal condition of intercellular substances produced by connective tissue cells, and (2) cellular respiration, in which it serves as a hydrogen-transport agent between carriers of molecular oxygen.[24] Not only does this vitamin appear to represent a normal cytoplasmic constituent of all living cells, in which its concentration is directly proportional to the general level of metabolic activity, but by

24. King, 1936.

special cytologic methods it can be demonstrated in the form of discrete granules (Fig. 42), probably surrounding lipoid droplets, in all types of animal and plant cells investigated, including the unicellular organisms.[25]

The antirachitic vitamin (D), the first vitamin to be pro-

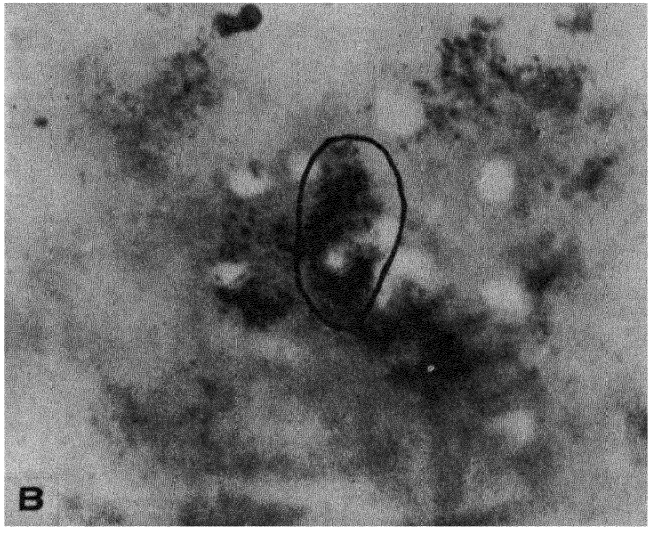


FIG. 42B. Granules of vitamin C in chromophil cells of the anterior pituitary gland. (From Bourne, 1936.)

duced synthetically (1927), appears to function either by increasing the retention of calcium and phosphorus in the body as a whole, or by mobilizing phosphorus from sources within the body tissues. We are still ignorant of the real mechanisms concerned in these processes and in the bone lesions of rickets and osteomalacia, as well as the possible interrelationships between vitamin D and the parathyroid hormone in calcium and phosphorus metabolism.

The antisterility vitamin (E), the existence of which is based

25. Bourne, 1936.

largely upon observations on the rat, mouse, and chick, supplemented by indirect evidence of its necessity for other species of animals, appears to be related to nuclear activities in cells, especially those undergoing unusually rapid proliferation and differentiation (germ cells of the testis, developing embryo). The nuclear chromatolysis and the irreversibility of the lesion in E-deficiency suggest that the vitamin may be concerned in synthesis of certain components of the chromatin molecule. Numerous attempts to relate the sterility of E-deficiency to a direct effect upon the endocrine function of the hypophysis or the sex glands, or both, have ignored many established facts which are quite incompatible with such a hypothesis. With the recent announcement of the structural formula of crystalline vitamin E, and the possibility of an abundant source through chemical synthesis in the not too distant future, a better understanding of the physiologic role of this interesting vitamin can be expected.

Space will not permit a discussion of various other vitamins, or vitamin-like substances, whose existence has been demonstrated or postulated in recent years. A review of the past decade clearly demonstrates that the discovery of each new vitamin has gone hand in hand with increased purification of ingredients in experimental diets, and of vitamin concentrates supplementing these diets. We do not know how extensive the list of vitamin factors may be when the biochemist can express every component of his experimental diet by indisputable structural formulae. By this time, the term "vitamin" will have long since served its purpose and these substances will be labeled with more specific chemical names such as we now apply to the indispensable amino acids and other constituents of diet.

THE HORMONES

Discussion of the hormones will be limited to some of the interesting developments of the past twelve years in the study of the anterior hypophysis, sex glands, and adrenals. The anterior hypophysis commands first attention because of its dominant

anatomic position and its masterly control, through a delicate balance of reciprocal interrelationships, over the major portion of the endocrine system. Despite astonishing advances of the past decade, knowledge of these interrelationships is still quite incomplete. None of the many hormones supposedly produced by this gland has been obtained in a satisfactorily pure state. There is some reason to believe that when more is learned regarding the physiologic and anatomic responses to more highly refined preparations, to unfinished pituitary products, and to secondary products of reciprocal hormone action with other endocrine glands, the number of true anterior pituitary hormones will prove to be decidedly limited.[26] As far as can be determined, these latter hormones are all protein in nature. In this respect they resemble insulin and the parathyroid hormone, whose true chemical nature is likewise unknown, and the less complex hormones of the adrenal medulla and thyroid gland which have yielded their secrets to chemical analysis and synthesis (Table XVII). Further progress in their isolation is primarily dependent upon future developments in the field of protein chemistry which, we hope, will soon materialize and find as effective application as have recent advances in sterol chemistry to the isolation and synthesis of the sex hormones and other physiologically important sterols.

The past fifteen years have witnessed astonishing advances in the field of reproductive physiology. The physiologic and morphologic changes associated with the phenomena of estrus in lower mammals and of menstruation in primates, regarding which much confusion previously existed, are now well understood. In fact, these phenomena can be produced at will in the castrate animal through the injection of chemically pure hormones. Not only has the time of ovulation in man and other mammals been determined, with relation to menstruation or estrus, but verification of this finding has come from the biophysicist as a result of his application of the cathode-ray oscillo-

26. Riddle, 1937.

graph to the detection of striking alterations in electro-potentials at this particular phase of sexual periodicity. However, many problems still exist regarding the interrelationships of hormonal mechanisms involved in ovarian dysfunction, in abnormalities of pregnancy, parturition, and lactation in the female, and in spermatogenic function, testicular descent, and

TABLE XVII

Chemical Investigations of Hormones

Hormones	*Extracts*	*Isolation*	*Synthesis*
Adrenalin	1894	1901	1904
Thyroxin	1896	1914–26	1927
Insulin	1922	1925	. . .
Parathormone	1924	....	..
Ant. hypophysis 8–10 hormones	1921–	....	....
Follicular	1912	1929	....
Corpus luteum	1928	1934	1934
Testicular	1927	1931	1934
Adrenal cortex	1929	1934	....

prostatic pathology in the male. The availability of cheaper and more abundant supplies of the pure hormones, and the extensive use of lower primates in experimental studies, are gradually laying a broad foundation upon which a more rational and effective system of therapeutic treatment will be built.

It is most fortunate that, concurrent with the chemical purification of the sex hormones, rapid advances were being made in the elucidation of the chemical constitution of the sterols which, in turn, came largely as a result of the interest aroused in these substances following the synthetic production of vitamin D by the irradiation of ergosterol in 1927. The sterols are complex hydroaromatic secondary alcohols widely distributed in nature, of which cholesterol constitutes the most familiar ex-

ample. Among the physiologically important sterols are grouped the bile acids, cardiac glucosides such as digitalis, certain venoms, vitamin D, the hormones of the sex glands and adrenal cortex, and a large series of carcinogenic hydrocarbons. There is some evidence that cholesterol may be regarded as the mother substance from which many of these sterols are formed through the metabolic activities of different body cells.

Largely as a result of the contributions from the field of sterol chemistry, the chemical structure of the three sex hormones (ovarian, corpus luteum, and testis hormones) has been determined and the artificial preparation of two of these (corpus luteum and testis) from less expensive sources accomplished, during the past five years. The corpus luteum hormone (progesterone) has been prepared both from pregnandiol, an inactive sterol found in urine of pregnancy, and from the relatively inexpensive stigmasterol obtained from soya beans. The testis hormone (testosterone) has been prepared through chemical degradation of cholesterol itself. Interestingly enough, two other chemically related substances obtained from male urine (androsterone) and from the adrenal cortex (adrenosterone) possess the same biological activity as testosterone, but to a lesser degree.

Similarly, eight estrus-producing substances have been obtained either from the ovary, urine of pregnancy, or the placenta of different animals; of which one (estradiol) is decidedly the most active and is now regarded as the true hormone of the ovarian follicle. Furthermore, a large group of quite unrelated substances, of both natural and synthetic origin, possess estrus-inducing activity if present in sufficient concentration; suggesting that the molecular configuration necessary for this phenomenon, but not necessarily for the other physiologic effects of true estrogens, may be of many types.[27]

It is also known that the biological activity of the hormones of the corpus luteum and adrenal cortex is possessed by several

27. See review by Marrian, 1938.

compounds closely related chemically to the hormone proper. In fact, it is not yet certain which of the chemical substances obtained really represents the true hormone of the adrenal cortex. The close similarity of progesterone and corticosterone in molecular structure is also worthy of note. It seems likely that future advances in sterol chemistry and sterol metabolism may attach greater significance to the fact that the cells of the adrenal cortex, corpus luteum, interstitial cells of the testis, and the interstitial and theca cells of the ovary are (1) strikingly similar in histologic structure, (2) have a common embryonic origin, and (3) produce hormones which, differing but little in chemical structure, represent the only hormones of sterol nature produced by the vertebrate organism.

The more we learn about the vitamins and hormones the more we are willing to concede that their biological effect can also be produced, to a rather variable degree, by numerous other chemical compounds obtained from natural sources or prepared by chemical synthesis. This is particularly true of those of sterol nature, many of which possess biological activities characteristic of more than one hormone or vitamin. For example, certain sterols are both antirachitic and estrogenic; others are both estrogenic and carcinogenic; others are both estrogenic and androgenic; while some androgenic substances are also capable of producing progestational activity.[28] Never has there evolved such a happy hunting ground for investigators interested in a study of the relations between chemical constitutions and physiological activity. There is also reason to believe that continued investigations along such lines, particularly as they apply to the problem of normal and abnormal cellular growth, may eventually constitute one of the most strategic points of attack upon the general problem of cancer.

There are many other new and interesting aspects of the vitamins and hormones which have, of necessity, been excluded from our discussion, but I trust that this review has afforded

28. Needham, 1936, 2; Dodds, 1937.

some concept of the general manner in which our knowledge of the vitamins and hormones has developed, of the amazing progress made during recent years, and of the many problems still awaiting more effective experimental analysis. Truly, this is an era in which the chemist, with his keenly sharpened tools of chemical analysis and synthesis, is playing a brilliant part in the elucidation of problems fundamentally essential to further progress in the theoretical and practical application of a wealth of scientific knowledge being made possible by the morphologist and physiologist. Never before in the history of science have seemingly trivial observations of today become discoveries of greatest importance tomorrow. Never before has there been such a challenge to young men embarking upon research careers in the biological, chemical, and allied sciences; for whom there lie unlimited fields of exploration in the search for, and the discovery of, the mechanisms hidden in the functioning organism.

VI

THE GENERAL ROLE OF THIAMIN IN LIVING THINGS

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In a sense it may be said that vitamin B_1 was "discovered" forty years or more ago, for it was during the years 1896–97 that evidence first appeared that the bran coats of grains contain a substance which is essential to the life of animals. Without this substance they develop a paralytic disease called beriberi. The experiments which furnished this evidence were performed by Eijkman in Java with domestic fowls as experimental animals. The experiments were undertaken with the object of demonstrating the supposedly infectious nature of the disease, beriberi, by transmitting it from human sufferers to the animals.

This was in spite of the fact that Takaki had more than a decade earlier eliminated the disease from the Japanese Navy by change of ration alone. That Takaki's evidence was not more generally accepted may be ascribed in part to the fact that the Japanese had not at that time attained a standing as contributors to science and in part to the obsession that possessed the medical profession. It amounted almost to an assumption that all disease is caused by microörganisms. This exaggerated notion of the role of bacterial infection as a causative factor in disease was a natural reaction to the then recent victory of Pasteur over the skepticism of an earlier generation.

The incident also illustrates the fact that the conquest of many diseases has exhibited a series of phases. First comes clinical evidence furnishing a clue but rarely supplying convincing

proof. Later comes the production of the experimental disease in animals which permits sufficiently extensive and well-controlled experiments to convince those who are accustomed to the laboratory method. Still later come further clinical experiments patterned after or guided by the previous animal experiments. Only if these are successful does the idea make an impression upon general practice. The cycle from embryonic idea to common practice is often a decade or two in duration.

Actually Eijkman's discovery of the experimental disease was due to his use of the waste polished rice from the hospital kitchens for feeding his animals. The accidental nature of the discovery should not detract from Eijkman's fame, for it requires an extra portion of discernment to see the significance of facts which are contrary to one's previously conceived hypothesis.

The point which we wish to emphasize here is merely that the discovery of the vitamin took place more than a generation ago. To the chemist it may seem astounding that the isolation and identification of a substance having such tangible physiological properties should have been delayed for such a long period. The explanation lies partly in the fact that the substance in question occurs in the richest food sources such as rice polish only in very small amounts, i.e., approximately fifty parts per million (Fig. 43). It is partly due to the chemical sensitivity of the substance which leads to a destruction of a substantial part of it during attempts at isolation. One should also mention the fact that the world's methods of microanalysis and of physicochemical examination of substances were not sufficiently advanced until about the time of the World War to permit a ready identification had the substance been successfully isolated previously.

By far the greater part of the delay, however, was due to another cause; namely, that there is a multiplicity of factors in rice polish, yeast, or similar products which were used as sources of the vitamin. Each of these factors plays an essential role in the physiological processes of certain animals, and the absence

of each from the diet brings on a distinctive disturbance. Even before the dietary origin of beriberi was generally accepted, there began to develop in Europe evidence that milk and butter fat contained substances which are necessary to the proper growth and development of rats and other experimental animals. Furthermore, in an investigation of ship beriberi which had long taken a heavy toll among Norwegian sailors, Holst and Fröhlich, patterning their experiments after those of Eijkman, encountered not beriberi but scurvy. This was due to the fact that they chose guinea pigs for their experiments, believing that the use of mammals would furnish results of greater significance for human nutrition than the use of birds. Guinea pigs are, however, so sensitive to lack of vitamin C that it was scurvy rather than beriberi which developed first. The world's physiologists were disposed to construe the fact that sometimes one pathological condition developed and sometimes another, as evidence that the whole deficiency idea had a very uncertain basis. They were naturally extremely reluctant to accept all at once the idea that there could be a multiplicity of previously unrecognized factors necessary to fundamental nutrition.

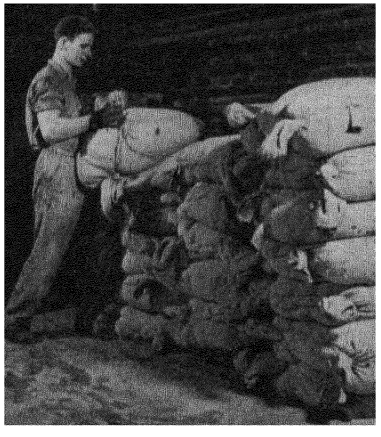


FIG. 43. Tons of rice polish were required to isolate enough natural vitamin for study of chemical constitution.

Precisely this, however, was the thesis of Funk who coined the term "vitamine" in 1911 and in 1914 published his book which ascribed not only beriberi, scurvy, and rickets but also pellagra and other less widely known disorders to nutritional deficiencies. The vision which Funk exhibited in choosing a

name with such broad implications must establish for him a permanent fame.

The proof of his thesis, however, required the labor of hundreds of minds which carried the experimental studies into all the principal countries of Europe, Asia, and America. It was not until 1926 that definite evidence was obtained that the so-called vitamin B consisted of a thermostabile as well as a thermolabile component. The former was at first assumed to be the pellagra vitamin because a deficiency of it led to a dermatitis in experimental animals which more or less resembled that of the human disease. Later the nutritional dermatites of various animals were found to have various causes of a deficiency character. By a long and slow process, it has now developed that aside from the scurvy vitamin and the fat-soluble vitamin there are a dozen or so significant water-soluble substances in the vitamin B group alone. Figure 44 provides in diagrammatic form a notion of the relationships of this group and of the history of their recognition. The first of these to be fully defined was vitamin B_2 or riboflavin, which was isolated in 1933 and produced synthetically in 1935. It has been shown to be a component of an important enzyme system, and is important in human nutrition though no well-characterized human disease is specifically associated with a lack of it.

The next to be characterized was vitamin B_1 or the beriberi vitamin which is the chief topic of our present consideration. Only in September, 1937, a third component of the vitamin B–complex was identified as nicotinic acid or its amide. This was found by Elvehjem at the University of Wisconsin to be curative for blacktongue in dogs, a condition which had long come to be recognized as the fairly precise counterpart of human pellagra. The utility of nicotinic acid in the treatment of human pellagra has also been established beyond question in recent months. It was not until January, 1938, that a fourth component of the B-complex was obtained in crystalline form; namely, vitamin B_6. This is essential to the growth of rats and

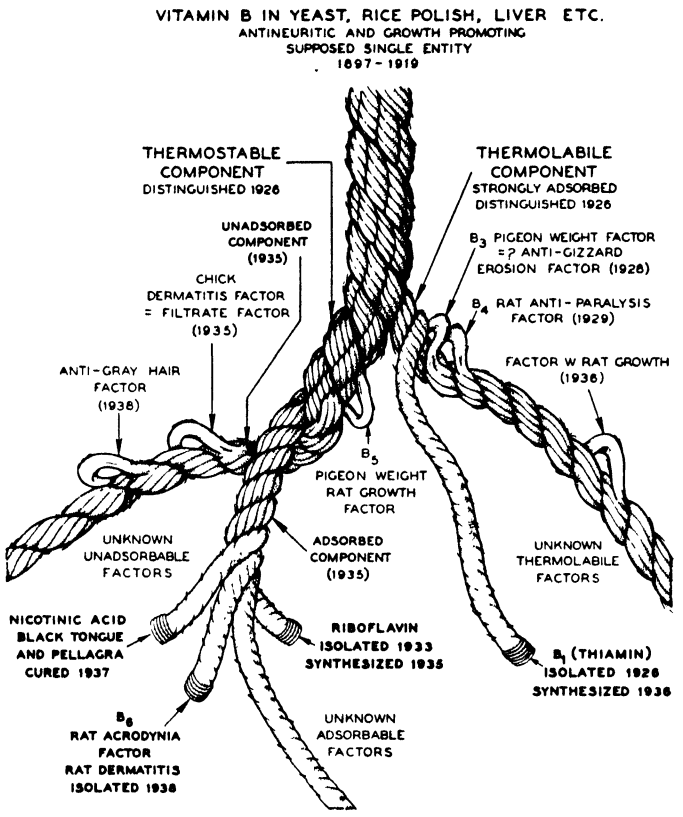


FIG. 44. Differentiation of the components of the vitamin B–complex. The components that have been isolated in the pure state are indicated in heavy lettering; those which have been recognized only by the discrepancies in physiological properties of crude extracts are indicated as loops; the possible still unknown factors are divided according to the principle of adsorbability which has been so useful in experimental differentiation. (From R. R. Williams and T. D. Spies, *Vitamin B_1 (Thiamin) and Its Use in Medicine* [New York, Macmillan Company, 1938].)

for the prevention of a nutritional dermatitis in this species. Its significance for human nutrition is unknown. There is still question concerning the significance or even the existence of many of the remaining B factors, but their study is in progress.

The bearing of all this confusion upon the delay in the isolation of the earliest recognized vitamin will be apparent when one considers that the chemical process of its isolation was of necessity guided by biological tests of successive filtrates, precipitates, etc., upon experimental animals. The various species of experimental animals differ considerably from one another in their requirements; various foodstuffs likewise differ in the proportions of the several physiologically active components. Thus a great deal of confusion arose and many debates resulted as to the significance of the findings. We did not make rapid progress because we did not know well enough the precise physiological properties of the substance which we were seeking.

My own introduction to the subject took place in the Orient as a part of America's venture in colonial enterprise in the Philippines. Beriberi was very prevalent in Manila and the cause of the disease was under active discussion. Fraser and Stanton had just completed their now classical experiment with coolie labor in the Malay States which they reported to the Far Eastern Association of Tropical Medicine in Manila in 1910. Chamberlain and Vedder were then undertaking the eradication of the disease among the Philippine Scouts by substitution of unpolished for polished rice. Other important studies by Andrews and by Strong and Crowell were also in progress in Manila.

It was to be expected that a young chemist who was enlisted in that environment for the study of the significant nutritive components of rice bran should become imbued with a desire to isolate this substance of almost fabulous curative properties. He saw many scores of cases of human beriberi and tried his concentrates upon many of them with sufficient success to strengthen his convictions greatly.

The most conspicuous symptoms of beriberi are in the nature of nervous disturbances. The patient experiences numbness of the extremities, followed progressively by a high degree of anæsthesia or paræsthesia, such as the sensation of insects crawling over the skin. Progressive tenderness of the musculature and

neuritic pains ensue, accompanied sometimes by a noticeable atrophy of the musculature, sometimes by edema of the hands and feet. The former type is known as dry beriberi; the latter as wet. The most useful diagnostic sign is impairment of the patellar reflex, the knee jerks becoming at first exaggerated and later being absent. However, while the nervous symptoms are most painful, the cardiac affection is the most immediately dangerous. Death results from heart failure.

The disease takes an uncertain course, sometimes progressing rapidly to a fatal termination, sometimes enduring for years in a relatively mild form. The latter is especially common among women of child-bearing age, the severity of the condition rising during the puerperium. The breast-fed infants of such deficiently fed mothers present a tragic picture, for the infantile form of the disease often terminates fatally within forty-eight hours after the appearance of the first symptom, which is usually vomiting. The infant mortality of Manila formerly approached or even exceeded 50 per cent, of which the larger part was competently ascribed to beriberi. That this has been reduced is largely due to the free distribution of rice-polish extract through government health clinics. This practice was inaugurated by Dr. E. B. Vedder of the U.S. Army Medical Corps and for several years it was one of the duties of the present writer to prepare the extract. Infants respond to such treatment almost miraculously, but in general a simple rice-polish extract is not very effective for adults. The disease still causes the deaths of many thousands of people annually in each of the principal oriental countries and the number of Asiatics who suffer impairment of health from this cause must be around a million or more. Furnishing an adequate supply of this vitamin by modification of dietary or other measures is one of Asia's major public-health problems.

While the background of my studies in Manila was medical, the objective was chemical, that of isolating the substance for determination of its structure. The work continued from 1910

to 1933. However, the first success in the isolation of vitamin B_1 was achieved by Jansen and Donath in Java in 1926. It cannot be regarded as a coincidence that this success occurred in the same laboratory where Eijkman had discovered the experimental disease twenty years previously. The Dutch in the intervening years had carried on a persistent and thoroughgoing study of the subject. Because of their location in the Orient, their attention was also focused upon the symptoms of beriberi rather than upon the general nutritional state as expressed by weight maintenance or rate of growth of the young. They fortunately continued to use birds as experimental animals, a choice peculiarly adapted to avoid some of the complications which arise from the more variegated requirements of other species, and with singleness of purpose they marched on to a successful isolation. However, Jansen and Donath did not obtain a sufficient amount of the pure vitamin to carry on a very effective chemical study of it. Notably the presence of sulfur in the molecule was overlooked and remained to be revealed by Windaus in Germany in 1932.

Furthermore, the process of isolation as described by Jansen and Donath was so difficult and complicated that it was not possible for others to repeat it satisfactorily until six or seven years later. The experience gained in these attempts enabled us in America to modify the process in such a way as to improve the yields ten- to twenty-fold and thus to obtain a sufficient quantity of the vitamin to undertake a serious chemical study. This quantity did not become available until 1934 and then by means of an elaborate and expensive process. Some of the difficulties of the process will be indicated by the range of the scale of operations in the successive steps. In the first step we used a 1,300-gallon tank for extraction of rice polish (Fig. 45A). In the twentieth and last step the entire resulting product was recrystallized in a test tube by the use of 10 cc. of solvent (Fig. 45C).

Probably no other substance has been isolated from nature at greater cost of time, labor, and money. Contributions to the

successful issue came from all parts of the globe. The chemical researches of the Dutch workers were paralleled and amplified by Fraser and Stanton in the Malay States, by Suzuki and others in Japan, by Funk in Paris and London, by Peters at Oxford, and by Seidell in Washington. Scores of others contrib-

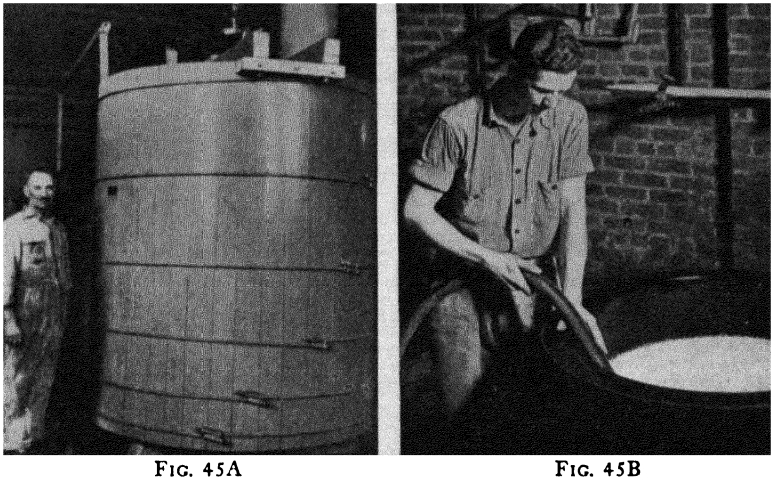


FIG. 45A FIG. 45B

FIG. 45. Descending size of scale operations in experimental isolation procedure. (*A*) Tank used for initial extraction of rice polish. (*B*) Elution of the vitamin from fuller's earth. (*C*) Final crystallization of the batch of vitamin. (Courtesy of *Industrial and Engineering Chemistry.*)

uted in each of these and other countries by developing techniques of testing and by enlarging our knowledge of the physiological role of the vitamin in animal nutrition. The aggregate effort surpassed that associated with any other like objective.

Elucidation of the structure progressed much more rapidly than the development of the isolation process had done. Within a year after obtaining the first gram of the vitamin, we were able to place correctly in a conventional formula about 90 per cent of all the atoms which were shown by analysis to be pres-

ent. This success was largely due to our good fortune in discovering an excellent tool for splitting the vitamin into simpler fragments. For the benefit of nonchemists, it may be well to

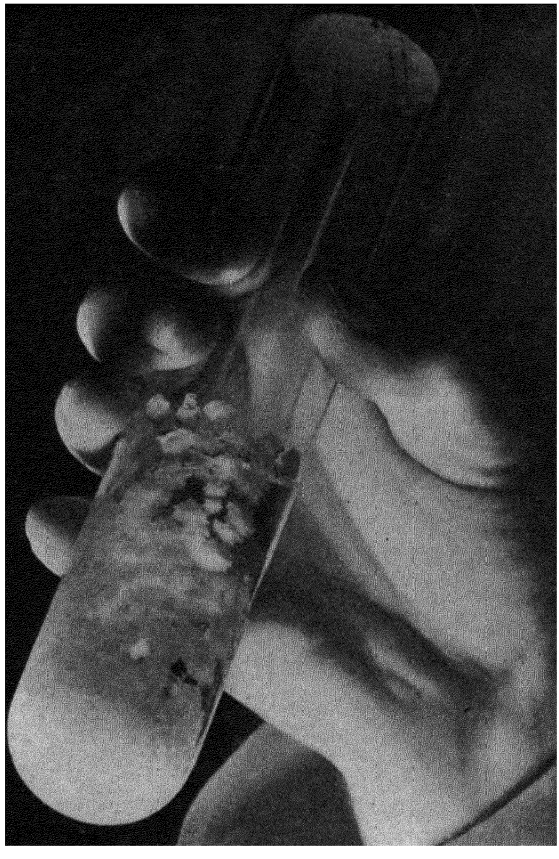
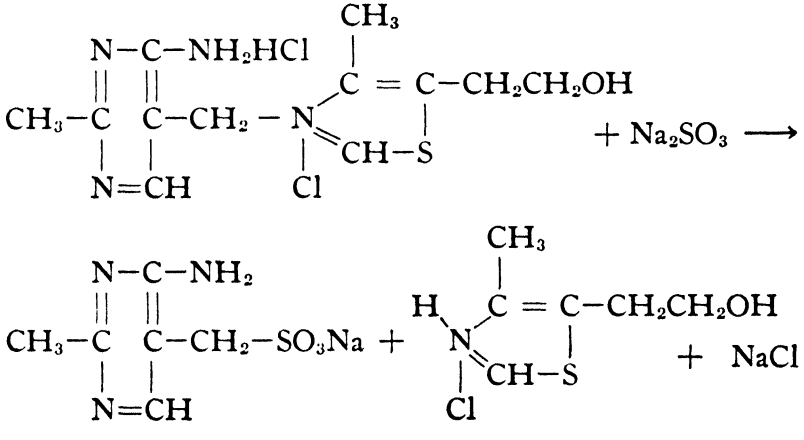


FIG. 45C

explain parenthetically that such a splitting of molecules represents the classical method of approach to their architecture. It is well, if possible, to cleave the molecule in a succession of steps and to analyze and examine the fragments which result

at each stage. Sooner or later such a succession of cleavages is certain to result in compounds sufficiently simple to fall within the category of substances already known. Thereafter the process consists in putting the fragments together again in a reverse series of steps thereby reconstructing the original molecule.

In choosing an agent for splitting, it is obviously desirable to use one so gentle in its action that cleavage will occur only at the weakest point in the molecule. This sort of tool we found in sodium sulfite. Again the important discovery was an accident. It arose from a previous attempt to preserve rice-polish extracts against bacterial decay by the use of sulfites. The bacterial action had been stopped, but the vitamin had been destroyed promptly and completely at room temperature. This was turned to account by applying the action of sodium sulfite to the pure vitamin from which we thus obtained two fragments of nearly equal size, quantitatively in the proportions in which they existed in the original. In the light of our present knowledge, we may write the structural equation involved thus:

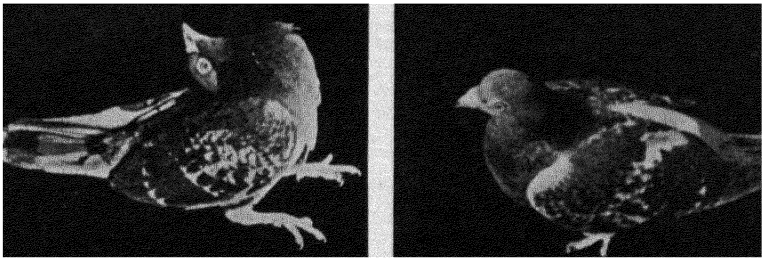


I. 2-methyl-6-amino-pyrimidine-5-methyl sulfonic acid.

II. 4-methyl-5 (β hydroxy) ethyl thiazole hydrochloride.

Of course, the structure of the resulting products was unknown at the time. They had to be converted to still simpler products before recognition was possible.

It is unnecessary in such a presentation as this to describe further the process of elucidation of structure. We need merely say that one of these fragments, I, was eventually identified as a particular 6-amino pyrimidine derivative, the other as a particular thiazole derivative, II. The discovery of a pyrimidine nucleus in a product of such biological importance was no sur-



Paralyzed condition Recovery one day after treatment

FIG. 46. Treatment of beriberic pigeon with synthetic vitamin. (Courtesy, Merck and Company, Inc.)

prise as pyrimidines occur widely in cells. The thiazole nucleus, however, had never been encountered in nature before, although many thiazoles had long been known to chemists as synthetic products. The methods of synthesis which had been devised for pyrimidines and thiazoles were applied with suitable modifications to these particular unique examples and finally the synthetic pyrimidine and thiazole were united to duplicate the natural vitamin in physical, chemical, and physiological properties (Fig. 46). Already hundreds of kilos of the product have been manufactured and sold for medical, commercial, and experimental purposes, although the artificial synthesis was not inaugurated until 1936 (Fig. 47).

What is the basis for such a keen and widespread interest in the use of the substance? We once supposed that beriberi was peculiar to the Orient or to isolated groups of men, such as the crews of sailing ships on long voyages. We also supposed that the vitamin played a peculiar role with respect to the function of nerves. This supposition arose from the fact that chickens and pigeons as well as human sufferers from beriberi exhibit very conspicuous nervous disturbances, such as numbness of the extremities and incoördination of muscular action progressing gradually toward a complete paralysis. However, it has long been recognized, though not strongly emphasized, that in the experimental and human disease most of the vital organs exhibit pathological changes. Notably, it is a failure of the heart which is the immediate cause of death in human beriberi. This heart failure is accompanied by an accumulation of fluid in the pericardium and an extensive hypertrophy of the right side of the heart. The accumulation of such fluids commonly extends to the lungs and often to the tissues of the limbs. In addition, it has long been known that the "beriberi" vitamin is an essential for the growth of young animals; without it appetite fails, food consumption falls off, and there is a general stunting of all parts of the skeleton.

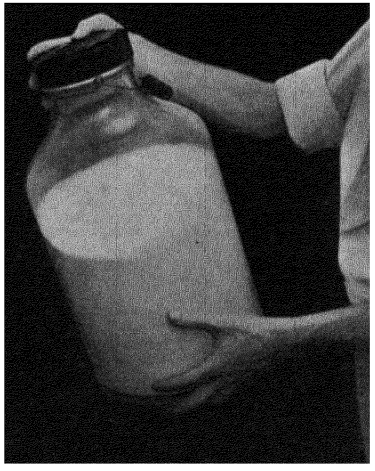


FIG. 47. Synthetic vitamin in quantity. Train loads of rice polish would be needed to produce an equivalent amount.

The conspicuousness of the nervous symptoms is now conceived to be due not so much to the nutritional peculiarities of nerves as to the extremely delicate nature of the physiological

balance which must be preserved in nerve fibers if they are to function as nerves at all. It is now known that the passage of a train of impulses over a nerve fiber results in a chemical alteration of the fiber itself which must constantly be repaired by active metabolic processes within the fiber if it is to continue to respond to repetitions of the stimulus. The curtailment of the oxygen supply to a nerve fiber results in a complete cessation of nerve function. This, however, can be restored after remaining dormant for an hour by a restoration of the oxygen supply. Functioning nerve is, to a peculiar degree, a dynamic system, not a static.

More and more it becomes evident that the vitamin functions as a part of an essential mechanism in all tissues. The nature of the mechanism involved has long been surmised in a general way to be catalytic, for it would not be possible to account reasonably for the very large physiological effects which are produced by minute quantities of the substance except on the assumption that the vitamin is used over and over again. Such a catalytic effect of the vitamin in artificial media was discovered by Peters nearly ten years ago. To put it briefly, he found that there is an excess of pyruvic acid in the brain tissue of polyneuritic pigeons and that this tissue is subnormal with respect to its capacity to consume oxygen from the air. The brain tissue of birds which had been cured of polyneuritis (beriberi) by means of the vitamin was found to possess more nearly normal respiratory capacity. Peters later showed that a restoration of the respiratory capacity can be brought about by adding the pure vitamin to the pyruvic acid medium in which the slices of brain tissue are allowed to respire.

Further evidence with regard to the catalytic function of vitamin B_1 was encountered by Lohmann and Schuster early in 1937. They obtained a substance from yeast which turned out to be the pyrophosphoric ester of vitamin B_1. This has the property in the presence of washed yeast cells of decomposing pyruvic acid to acetaldehyde and carbon dioxide. In fact, the fer-

menting function of yeast as well as its very growth depends upon the presence of vitamin B_1 in the cells. Such a vital function of the vitamin is not peculiar to yeast cells, for Lipmann has found that in the presence of lactic acid bacteria the vitamin pyrophosphate has a catalytic effect which results in the conversion of pyruvic acid to acetic acid and carbon dioxide. In the case of staphylococcus, a common pus-forming organism, it is also true that the vitamin appears to play an essential role for its life and growth, again taking part in its metabolic conversion of pyruvic acid. The significance of these findings is enhanced by the fact that pyruvic acid has been found in excess in the tissues and excretions of human beriberics.

The presence of pyruvic acid has an even broader significance which we must now discuss. It has long been believed that the process of carbohydrate metabolism, whether it occurs in the fermentation of sugar by yeast, or in the germinating seed, in the contracting muscle, or elsewhere in the tissues of the body at large, proceeds by pathways which are largely common to all. Pyruvic acid in all these cases is believed to be one of the intermediate products of the conversion of carbohydrates into vital energy. So much emphasis has been placed upon the special functions which are served by each of the organs of the body that we tend to lose sight of the fact that, specialized as they are with respect to their place in the body economy as a whole, all the cells carry on similar metabolic processes for themselves. In a complex human society, we subsist by the exchange of goods and services with one another, yet each home maintains its own hearth fires and its own kitchen. Similarly, the cells of each organ perform "public services" but also have private duties. More and more it appears that the metabolic processes of all living things involve certain common mechanisms, one of which comprises vitamin B_1.

The majority of these common mechanisms are of the nature of biocatalysts, i.e., enzymes. Hundreds of different enzymes operate in the various complex physiological processes of

the body, each performing its own unique function. In certain of these enzymes, it has been possible to demonstrate the existence of two component parts which can be separated from one another, whereby both lose their catalytic power. The power, however, can be restored simply by remixing the two components. In each case, one of these components gives evidence of being a protein in nature, a giant molecule of distinctive properties; the other is a much simpler crystallizable substance and is called a coenzyme. Vitamin B_2 is known to be the coenzyme of one such enzymic system. Nicotinic acid, now recognized as the pellagra vitamin, enters into the coenzyme component of one enzyme of yeast and another enzyme present in red blood cells. Vitamin B_1 in the form of its pyrophosphate now clearly appears to be the coenzyme of at least one and perhaps several important enzyme systems having to do with the conversion of carbohydrate to energy at some point in the process beyond the pyruvic acid stage.

When one turns his attention to the occurrence of vitamin B_1 in natural tissues, including those which we use as food, he finds many striking confirmations of the idea that this vitamin is a well-nigh universal mechanism for all living things. All natural tissues contain it in small amounts, usually substantially less than one part per million. Only in seeds do we find a marked concentration to the extent of three to five parts per million. The concentration actually is largely in the germ and bran coats in the case of the cereal grains. Commercial products of this type may contain forty to fifty parts per million. Some yeasts are very rich, but these are yeasts which are grown upon whole-grain media from which they extract nearly, if not quite, all the vitamin which they possess. It has become clear that most and probably all living things use this same vitamin for their own physiological processes. This explains its universal presence in their tissues.

The widespread utilization of vitamin B_1 by the plant world has intrigued the plant physiologists who are now engaged in

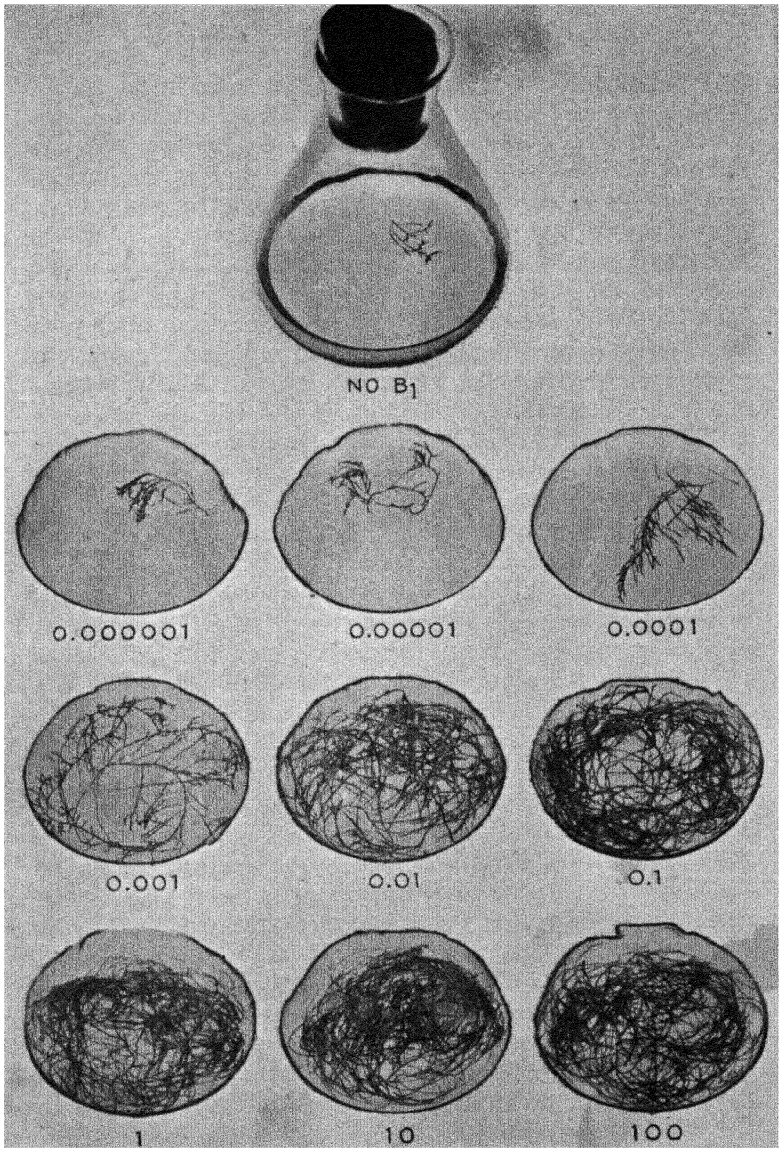


FIG. 48. Increase in growth of excised tomato roots with increasing amounts of vitamin B_1. The figure represents millionths of a gram of vitamin per 40 cc. of medium. Work of Robbins and Schmidt. (From R. R. Williams and T. D. Spies, *Vitamin B_1 (Thiamin) and Its Use in Medicine* [New York, Macmillan Company, 1938].)

very intensive studies of the phenomenon. Their work has been greatly advanced by the discovery that for many plants a mixture of the pyrimidine and thiazole intermediates which we use in the artificial synthesis of the vitamin is as effective as the vitamin itself. By this means, it has been possible to study the synthetic capacity of many plants: (*a*) for the thiazole half, (*b*) for the pyrimidine half, and (*c*) for the union of the two halves. In a general way, the results suggest that the capacity for the complete synthesis of the vitamin is very limited even in the plant world and completely absent in the animal world. The saprophytic plants depend to a large extent upon the decaying remains of higher plants for their supply. Even the roots of the higher plants are parasitic to the tops in several instances which have been studied. Apparently the complete synthesis of the vitamin takes place significantly principally in the tops of higher plants, probably in their leaves. The studies of Robbins, for example, have shown that excised bits of tomato roots immersed in a sugar solution containing minute amounts of suitable salts are dependent for growth upon additions of vitamin B_1 to the medium. The weight of roots produced is proportional over a wide range to the amount of vitamin added (Fig. 48).

It is obvious that in the natural sprouting process the reserve of vitamin B_1 in the seeds serves the purpose of metabolizing the starch which also is stored in the seed to nourish the plant during the period of its early growth. Thereby a root system is established and the first leaves are allowed to form, where in the light of the sun the synthetic process can be renewed. In a dramatic way, these observations point to the folly of mankind in making a practice of consuming the starch in the seed and painstakingly discarding the mechanism provided by nature for its metabolism.

VII

INTERNAL SECRETIONS IN REPRODUCTION

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WHILE a student assistant in biology, the writer collected pig embryos at the nearby packing plant for use in the course in embryology. The first visit provided a never-to-be-forgotten experience. After the blood had been drained from a pregnant sow, after she had been plunged into a scalding bath to remove hair, after she had been laid open and the uterus removed—processes requiring as long as ten minutes—the embryos in her uterus were still living, hearts beating, blood circulating. This illustrates the truly remarkable protection afforded the embryo by the mother's uterus. What an improvement in reproduction over the method still used by lower animals!

After benefiting from this protection and care, the embryo must make its escape and begin an independent existence. This escape at birth is one of the major crises of life.

Another major crisis, which comes before fertilization, is the escape of the egg from the ovary at ovulation. In some respects this is similar to birth. In both birth and ovulation membranes are ruptured, fluid escapes, blood is lost, the individual (egg or baby) is squeezed through a small opening, and the event marks the beginning of a more independent existence. Both deliveries, ovulation and birth, are managed by the same methods. Both involve *growth by cell division* and the slow separation from surrounding cells by "biologic hydraulic mining methods" through the *accumulation of secretion.*

Ovulation and birth, in common with other sexual and reproductive functions, are dominated and controlled by internal secretions or hormones which use the blood stream as a common carrier. Certain hormones profoundly affect specific organs and have little or no effect on others. They seem to be like radio broadcasts over specific wave lengths, to be picked up only by organs "tuned in" to these lengths. Sometimes they work independently; at other times it may require several hormones working together or in succession to complete a reaction. Some of the internal secretions work with that other great correlator, the central nervous system, to produce a reaction, but in most cases the reproductive hormones can accomplish their functions after all nervous connections have been cut.

The Cyclic Nature of Sex Function in the Female. In the male, hormone secretion by the sex glands is rather steady and continuous. The female, however, is subject to flood and ebb tides of hormonal influence upon her reproductive organs. These cycles of sexual activity are apparently necessary to produce eggs and provide uterine conditions for reproduction. Reduced to their simplest terms they are spurts of growth and function of the genital organs, followed in each case by periods of retrogression. The latter are necessary; otherwise the female genital organs would grow out of all proportion to the body. The growth involves the eggs and follicles in the ovaries, the vagina, the uterus and oviducts, and the mammary glands. This growth is stimulated and controlled by the ovarian hormones.

Study of a series of ovaries at several stages in the estrous cycle shows several interesting points about growth and elimination of eggs. Figure 49 shows ovaries from two pigs obtained at least ten days before mating time. The clear blisters are follicles containing eggs. There are many when the follicles are small. The ovary on the left is from an immature animal which had never produced an egg. That on the right is from an adult animal and shows, besides the follicles, large opaque bodies, the corpora lutea. These structures remain after the eggs have been

ovulated. They are a little larger than follicles containing ripe eggs. Ovaries from adults taken at the time of estrus, that restricted period, a day once in three weeks, in which the sow mates, contain follicles about ready to rupture to permit the escape of eggs. A process of selective elimination causes a radi-

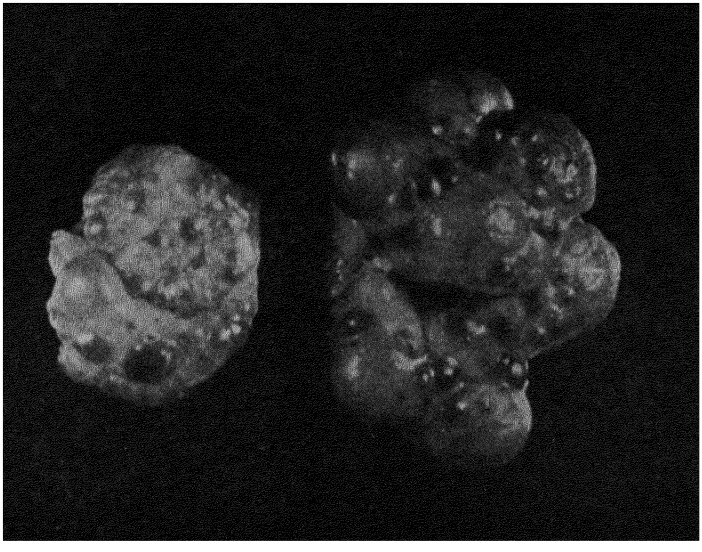


FIG. 49. Photograph of normal pig ovaries showing clear follicles containing eggs, and large, opaque, spherical corpora lutea (right) from which a previous generation of eggs had been extruded (Allen, Kountz, and Francis).

cal reduction in the number of follicles even in the last ten days of their growth.[1] For each egg that successfully attains ovulation, there are many that die, just as in the general population people die at all ages.

The microscopic study of prepared sections through ovaries shows the eggs in different stages of growth and development. The small eggs begin to grow from the outside of the ovary and sink down toward the center. As they do this they enlarge,

1. Allen, Kountz, and Francis.

and groups of cells grow around them to form follicles. As the follicle grows larger, small bodies of fluid develop among the follicle cells and leave the egg attached to the wall at the top of a small hill of cells. As this blister enlarges by accumulation of fluid, the hill of cells is dispersed as though washed away by hydraulic mining. This sets the egg free in the follicle, and it floats in this fluid (liquor folliculi) which contains the primary sex hormone in high concentration. Then the wall of the follicle bursts, releasing the egg into the uterine tube. After ovulation, the wall of the follicle thickens, and becomes a solid sphere of cells, the corpus luteum, which changes its type of secretion to a second hormone, progesterone.

Figure 50 is a drawing of a human ovary at operation. The uterus or womb at the lower right and the uterine tube, with its end an open funnel (lower left) which picks up the egg and carries it into the uterus, are shown. The surgeon is aspirating through a hypodermic needle the fluid from follicles in the ovary, which contains the principal female sex hormone.[2]

Adolescence: the Beginning of Menstruation and the First Ovulation. The time of adolescence is another crisis in reproductive life. Adolescence in children extends over several years. Its outstanding feature is growth of the genital organs, slow at first but increasing in momentum, and culminating in sexual maturity. Increasing amounts of hormones from the sex glands can be recovered from the urine as this occurs. Sexual maturity can be produced in experimental animals at early ages by injections of sex hormones.

The main events of this maturing process in females are extremely interesting. It will perhaps be easier to describe them as they occur in the chimpanzee and monkey, since these primates are subject to no restrictions of morals or customs. A young male and young female chimpanzee, caged together and watched for the development of adolescence, began sexual relations gradually as a sort of play, just as other behavioral re-

2. Allen, Pratt, and Doisy.

actions develop.[3] The first sign of approaching adolescence in the female was a swelling around the genital organs. The adult female chimpanzee has a marked genital swelling. This region "ballooned up" for two weeks or longer. Mating occurred at

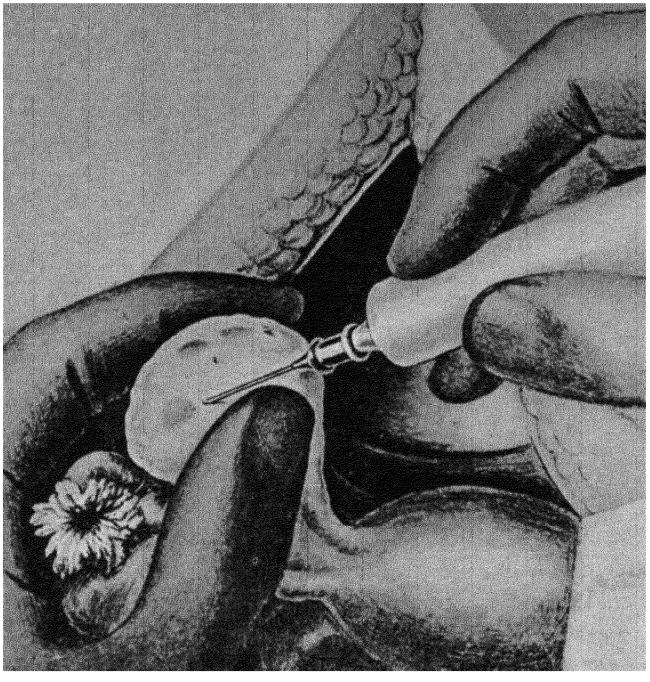


FIG. 50. Drawing of the human ovary, tube, and uterus seen at an operation. The fluid being withdrawn from the ovarian follicles contains the estrogenic hormone (Allen, Pratt, and Doisy).

this time. Then the swelling decreased and disappeared for eight or ten days and sexual activity also declined. Four or five successive enlargements and shrinkages occurred at approximately monthly intervals before anything else happened. Then, in between two of these periods of swelling, the first menstrual

3. Yerkes, Tinklepaugh, and Elder.

hemorrhage appeared. This particular female had four menstrual cycles before she became pregnant. Soon after she became pregnant, the genital swelling decreased and disappeared and menstruation ceased. She gave birth to a normal baby and nursed and cared for it during its infancy.

Conditions in the monkey are similar. First the secondary sex

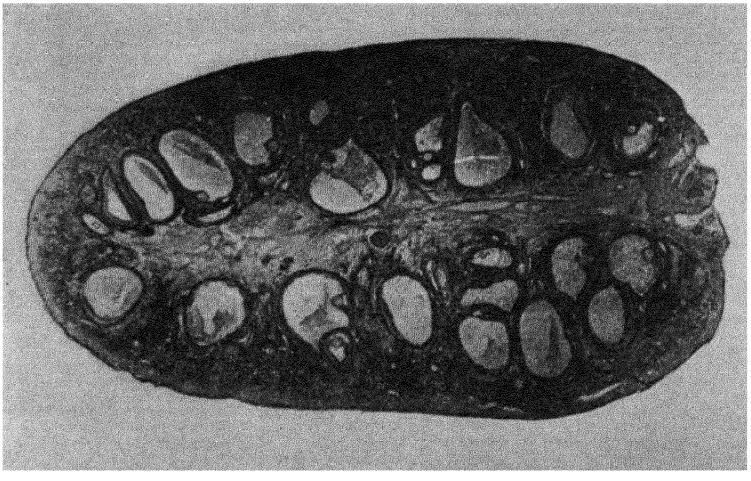


FIG. 51. Section through the center of an ovary of a monkey after eight menstrual periods had been recorded during the previous year. Many follicles, no corpora lutea; hence no ovulations had occurred (Allen).

characters develop gradually—the early growth of the mammary glands and the reddening and swelling of the "sexual skin." Then menstruation begins. Usually the first egg is produced and ovulated only after several menstrual cycles. The monkey from which the ovary shown in Figure 51 was removed was thought to be mature. Eight menstrual periods had been recorded for her before the ovaries were removed. Microscopic study of the ovaries showed that she had never ovulated. This emphasizes the fact that sexual maturity does not happen suddenly. Although we might think a girl is sexually mature at the beginning of menstruation, she really does not become ma-

ture until the first egg is produced. This evidence involving the occurrence of menstrual cycles without ovulation in adult monkeys in summer shows conclusively that menstruation may occur without ovulation.[4] Sections of ovaries from adult monkeys during fall, winter, and spring may contain recently rup-

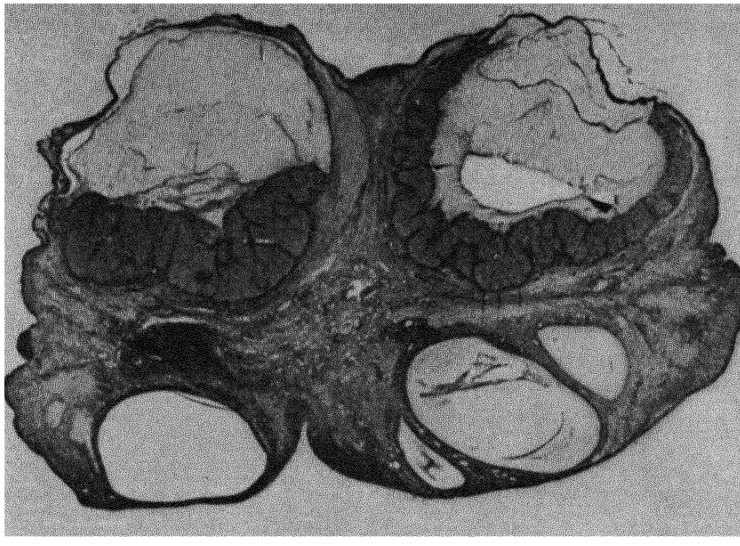


FIG. 52. Section of a human ovary containing two corpora lutea (at top) at the same stage of development; therefore, after twin ovulations (Halpert).

tured follicles. Several corpora lutea may still persist as evidence of previous ovulations. These become scars which may be recognized for six or eight months before they finally shrink and disappear, but they show how many eggs have been produced.

A section of an ovary from a woman who had already borne twins is shown in Figure 52. Two early ruptured follicles (corpora lutea) of approximately the same state of development are

4. Corner, Allen, Hartman.

present. Therefore, just before this ovary was removed this woman produced two eggs from the same ovary. If fertilized they would have produced fraternal twins. Several degenerating follicles in the process of elimination appear along the lower border.

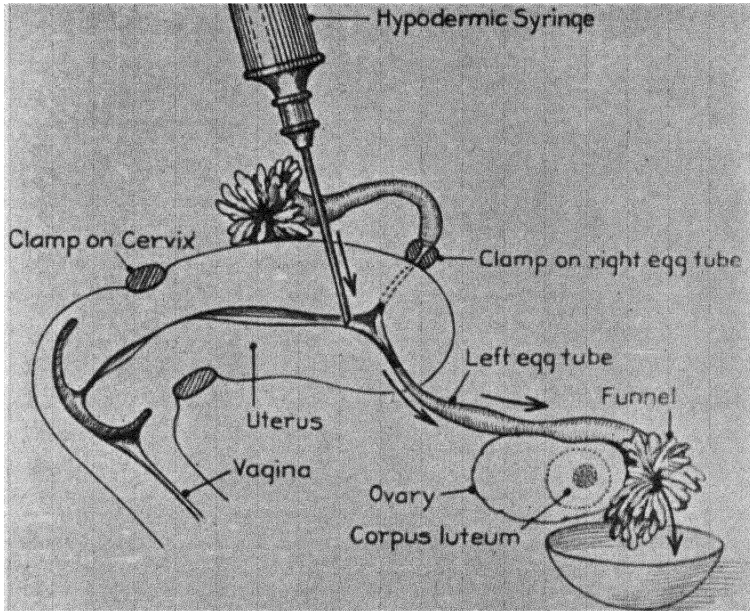
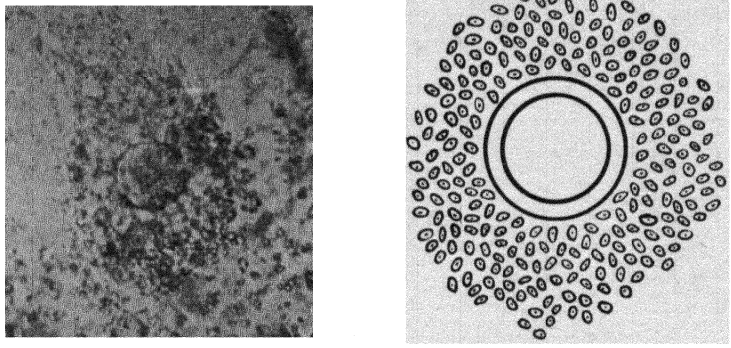


FIG. 53. Method of washing ripe human egg from the egg tubes at operation (Allen, Pratt, Newell, and Bland).

Recovery of Ripe Human Eggs and the Time of Ovulation in the Menstrual Cycle. The appearance of the human egg while it is in the ovary has been known for some time, but the ripe human egg had not been seen until 1928. One of the reasons was the difficulty in knowing just when ovulation occurs in relation to the menstrual cycle. It was thought at first that an egg was produced just before menstruation. There had been many attempts to find ripe human eggs and many failures, per-

haps because they were searched for in abnormal or diseased tubes.

The method used in obtaining eggs was by securing permission to search in the normal oviducts of women undergoing operations for other reasons. Figure 53 shows how a hypodermic needle was inserted into the cavity of the uterus, after clamping the cervix and one tube, and fluid injected to wash the egg from



A B

FIG. 54. (*A*) Living human egg (center) recovered from the egg tube and photographed in saline solution. Ovulation must have been recent, for the ovum is still surrounded by cells from the follicle (Allen, Pratt, Newell, and Bland). (*B*) Camera lucida drawing of the living egg and surrounding follicle cells shown in *A* as it appeared when seen through the microscope. The two circles represent the egg capsule (zona pellucida) surrounding the ovum. The diameter, including the capsule, is 132 microns.

the other tube. It was then recovered by searching under the microscope. By lucky circumstance, among the five eggs recovered, two were twins; one from each tube of a thirty-eight-year-old woman. The appearance of one of these while living in warm salt solution is shown in Figure 54. The twin eggs were not naked when ovulated, but were still surrounded by many of the cells of the follicles. These ripe human eggs were 130 microns in diameter—about the same size as eggs of rabbits and monkeys. When the eggs are sectioned (Fig. 55), the chromosomes

which carry the hereditary characteristics of the mother can be seen and also the first polar body in which half of the chromosomes are discarded to compensate for those which the sperm will introduce at fertilization. The human egg is therefore similar to those of most mammals in accomplishing this stage of maturation before ovulation. The dog's egg is apparently an exception, for it is ovulated with nucleus intact.

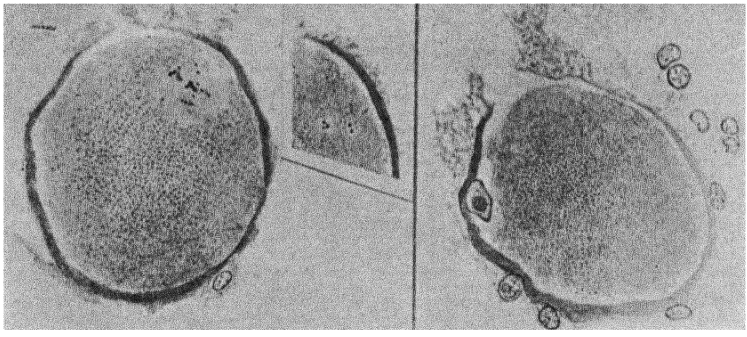


FIG. 55. Sections of a human tubal ovum showing first polar body (left) and chromosomes of second maturation spindle (right and center).

Much evidence from the monkey[5] and the recovery of human eggs from the tubes definitely places the time of ovulation in a majority of cases at about the twelfth to fifteenth day after onset of the previous menses in regular cycles of twenty-eight-day length, or midway between the onset of two successive menstrual periods. This time varies in irregular cycles and there are undoubtedly exceptions, but a majority of ovulations probably come at this time.

The Ovarian Follicular Hormone. While this preparation for the escape of the egg by growth and secretion of fluid in the follicle is going on gradually, the follicle is also secreting its hormone ("estrogen," "theelin," or "folliculin") into the blood

5. Corner, Allen, Hartman; Allen, Pratt, Newell, and Bland.

stream. This hormone starts growth in the uterus, vagina, and mammary glands and toward the end of the period of growth promotes the sex urge. In some animals the female spontaneously feels energetic: the female rat at this time may run more than a thousand rounds in a rotary cage. This is the natural time for mating. In some animals, this mating period is brief

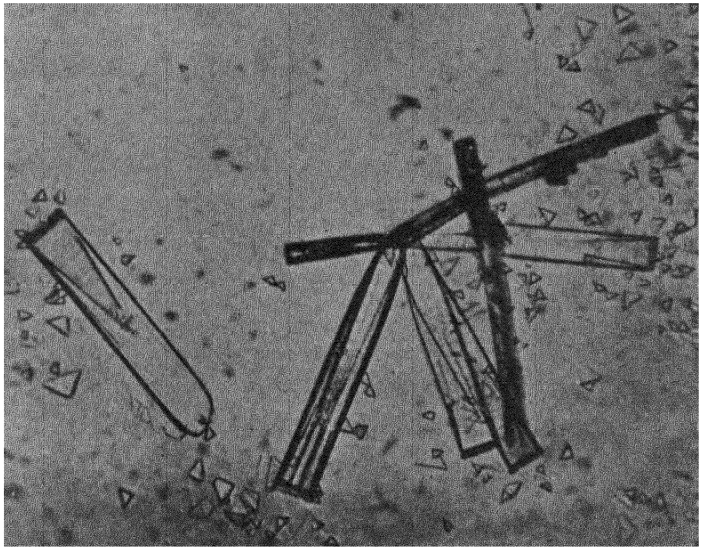


FIG. 56. Crystals of follicular hormone from pig ovaries. Two forms, needles and platelets (McCorquodale, Thayer, and Doisy).

and they will not mate at other times. In some primates, mating may occur throughout a greater part of the cycle. By injections of estrogen, it is possible to induce mating behavior followed by coitus in animals from which the ovaries have been removed.

This hormone has been extracted from animal tissues, purified, crystallized, and recently synthesized by Doisy and many others. It contains only carbon, hydrogen, and oxygen, arranged in phenanthrene-ring structure. Figure 56 shows two types of crystals of the hormone extracted from follicular fluid of pig

ovaries.[6] The male hormone is made of the same elements but contains more hydrogen. It, too, has been synthesized from inorganic compounds.

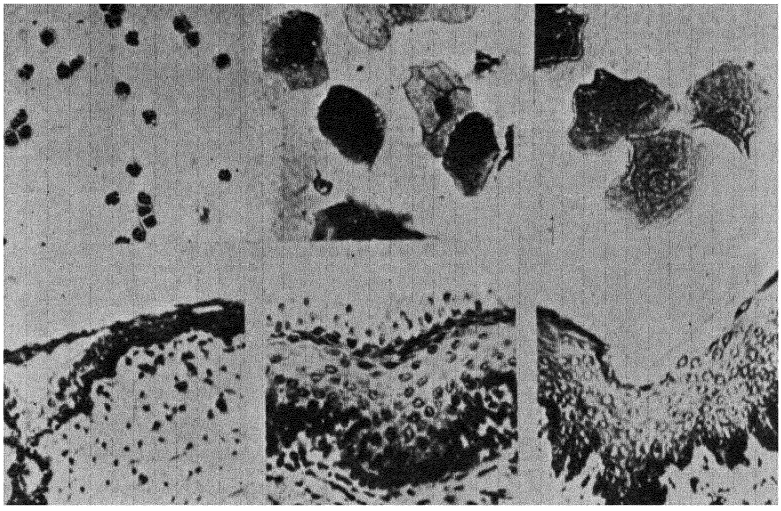


FIG. 57. Effect of estrogens on the vaginal epithelium of ovariectomized rats or mice. Above, left to right, the three types of cells indicative of growth changes in the epithelium which are used in the rat test for standardization of the hormone. Below, the vaginal wall of the control (left) and two stages of growth (center and right) following injections of estrogen (Allen, Doisy, and others).

One of the outstanding reactions to this hormone from the ovarian follicles is growth of the uterus, vagina, and mammary glands. There is a spurt of growth in these organs in women for two or three weeks and then a shrinkage or retrogression. In the wall of the vagina of the mouse and rat, there is such rapid growth that in two days a complete new wall is built (Fig. 57). This involves a change from a thickness of two or three layers in the animal from which the ovaries have been removed to a thickness of ten to fifteen new layers of cells and develop-

6. McCorquodale, Thayer, Doisy; Gallagher, Koch; Butenandt.

ment of a cornified layer such as occurs in calluses. This reaction constitutes a test for standardization of the follicular hormone and other estrogenic substances.

As these cells in the wall of the vagina are dividing rapidly, in response to the hormone from the ovarian follicles, it is possible to hold them in division for a time by the use of a drug, colchicine. A little of this drug injected subcutaneously arrests



FIG. 58. Sections of mouse vagina from immature control (left) and hormone stimulated animal (right). The drug, colchicine, has arrested cells in division along the basal layer of the epithelium (Allen, Smith, and Gardner).

all cells in division. When the animal is killed and the tissues sectioned, the cells which have divided in the last 10 or 15 hours before death are held in an easily recognizable condition for counting. In this way, a good idea can be obtained of just how many cells are dividing. Figure 58 shows at the left the control tissue which is growing very slowly or not at all, and at the right the total growth in 38 hours in response to hormone stimulation. The cells which have been caught dividing in the last 14 hours of growth appear as dots along the basal border

of the epithelium. In one particular section of vagina, after 36 hours of growth following injection of follicular hormone, there were 1,585 cells counted in division in the last 9½ hours. If the drug had not been used, 15 or 20 dividing cells might have been found, for division is accomplished quickly and then the cell goes into a resting stage.

Estrone is necessary for growth of the mammary glands. If the ovaries are removed from a monkey, the mammary glands shrink, lose their foliage (secreting alveoli), just as leaves drop from trees in the fall, and become very small. Figure 59 shows

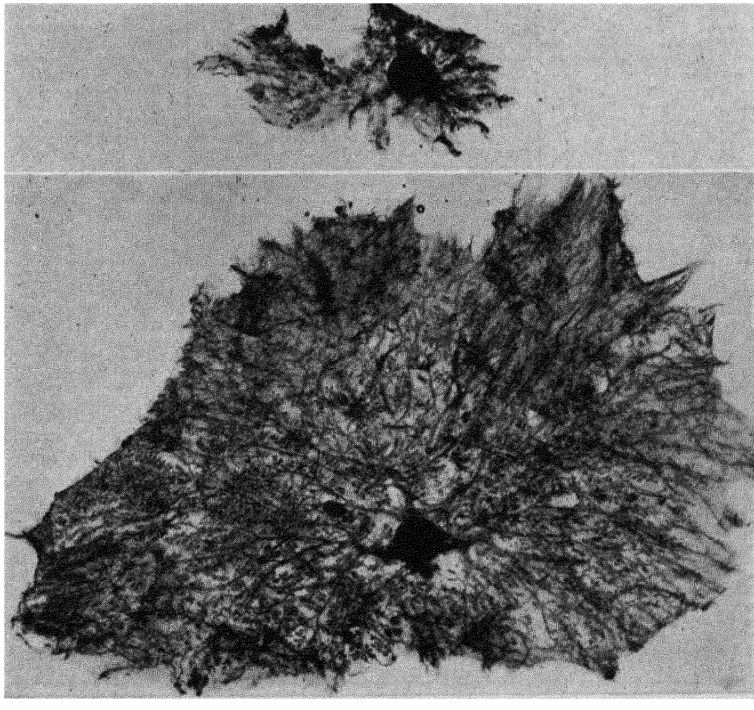


FIG. 59. Mammary glands from a monkey after removal of the ovaries. The glands atrophy after several months in the absence of ovarian hormonal stimulation. Above, atrophic left gland. Below, growing right gland from same animal after three weeks of estrogenic stimulation (Allen).

mammary glands of a monkey from which the ovaries had been removed several months previously. Above is the atrophic control gland. Below is the other mammary gland from the same monkey after three weeks of injections of follicular hormone.

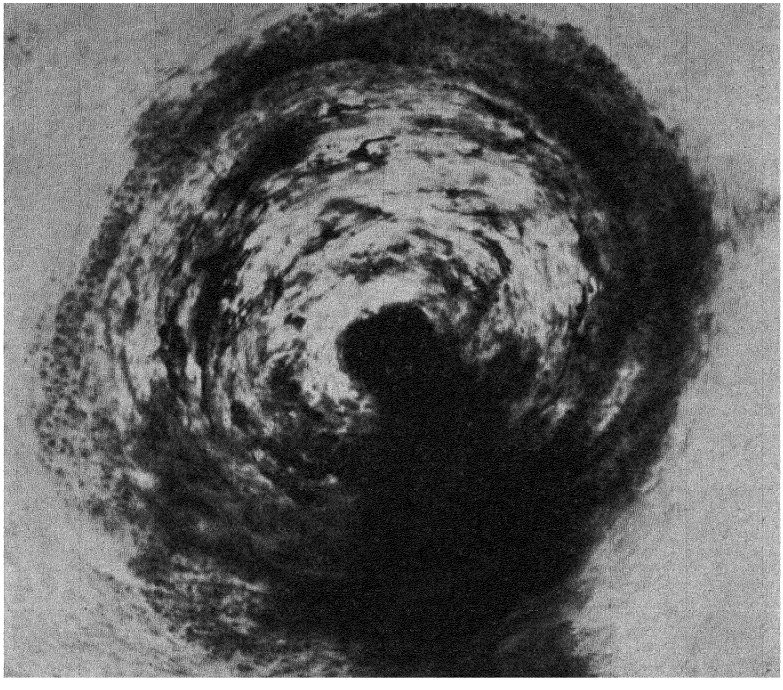


FIG. 60. Photograph of cleared whole mount of a nipple from a mouse. The black shadow (below) is the opaque duct. Rapid growth is indicated by a "collar" of several rows of cells arrested in division by colchicine (Allen, Smith, and Gardner).

There has been a considerable growth of the branches and twigs of the mammary tree and a partial development of foliage.[7]

By using the drug colchicine, it is possible to get clear evidence of growth of the nipple in response to estrone. A surface

7. Allen, Turner, Gardner, and others.

view of a whole mount of the nipple of a mouse is pictured in Figure 60. The black shadow is the opaque main duct. All around the base of the nipple is a zone 3 to 6 cells wide of dividing cells, held in division by the action of the drug. This was taken 36 hours after the injection of estrogen in an ovariectomized mouse and these cells all began division during the last 9½ hours of that time. It is almost as though one were flying over a hill in an airplane and saw below him several furrows plowed around the base of the hill. This "collar" of growing cells results in the eversion of the nipple. That this same hormone is responsible for the development of the "sexual skin" in the monkey, and other secondary sexual characteristics, has been proven adequately by injecting estrone into animals and experimentally producing these results.

One of the most striking changes in secondary sex characters is the change of the feather to the "henny" type. A striped feather can be produced, one stripe female, the next male, by alternating injections of the sex hormones. The width of the stripes can be regulated, depending upon the time the hormone acts during the growth of the feather.

Students of physiology have been interested in the uterus from earliest times because of its two extremely important functions: first, primate menstruation; second, that of harboring the embryo during pregnancy. Within the last decade much experimental evidence has accumulated to explain these functions. The follicular hormone produces growth in the uterus. Not only is the organ increased enormously in size, but the tissues are induced to secrete, and in rodents an accumulation of secretion greatly distends the uterus. The early secretion is watery, a fluid medium for transport of sperm to the uterine tubes where the eggs are fertilized. Later, glycogen forms in the secreting cells, supposedly for nutrition of the embryo when it arrives in the uterus. A certain amount of growth is followed by motility. Peristaltic contractions somewhat similar to those in

the intestine run down the uterine cornua.[8] The luteal hormone quiets this motility.

The Experimental Production of Menstruation. The normal menstrual cycle is dated from the onset of bleeding. The first two weeks is a period of growth of follicles in the ovaries, several starting growth but usually only one attaining complete development to produce and ovulate its egg. Ovulation may occur at about the fourteenth day, in which case a corpus luteum develops in the ruptured follicle; or there may be no ovulation, all eggs of that group dying and their follicles being resorbed. The cycle without ovulation cannot be distinguished externally from the ovulatory cycle.

It has been known for a long time that if the ovaries are removed, menstrual function ceases—the individual has a premature menopause. If the ovaries are removed at 10 to 14 days after the beginning of menstruation there will follow 4 or 5 days in which nothing detectable externally happens, and then menstruation begins a week or more before it would have appeared normally. If a monkey from which the ovaries have been removed is now given a series of injections of follicular hormone for 10 days or more, growth is again started in the uterus (Fig. 61, left). After injections are stopped, there follows a latent period of 4 or 5 days, and then a menstrual period begins. This experimental period cannot be distinguished from normal menstruation either in duration or amount of flow.[9] It is possible in this way to produce 10 or 12 successive menstrual cycles and carry a monkey without ovaries through a year of menstrual function. Short or long menstrual cycles can be produced as desired depending upon the amount of hormone injected and the time over which it acts. Menstruation has also been produced experimentally in women after removal of both ovaries.[10] Apparently only this one hormone is necessary. Follicular hor-

8. Brouha; Reynolds and W. Allen.
9. Allen, Smith, Engle, Hartman, Hisaw, Corner, Zuckerman.
10. Werner and Collier, and others.

mone starts growth in the uterus and then this is followed by menstruation after the growth stops. With large doses of estrone, the uterus can be kept growing for months without menstruation.[11]

The Hormones Regulating Pregnancy. Additional changes occur in the uterus if an egg is ovulated and a corpus luteum de-

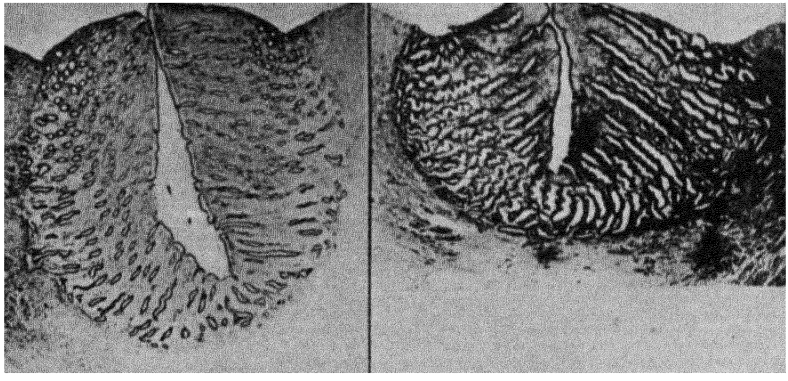


FIG. 61. The uterus of the monkey stimulated by ovarian hormones. Left, section of a lateral half showing the intermenstrual condition produced experimentally by the follicular (estrogenic) hormone (Allen). Right, a further development (premenstrual) produced by successive action of estrogenic and luteal hormones (Hisaw). Withdrawal of either estrogenic or luteal stimulation is followed by menstruation.

velops in the ovary. Figure 61 shows sections of the wall of the monkey's uterus in the region where the embryo implants. At the left is the intermenstrual condition produced by injections of follicular hormone alone. This type of structure will not allow implantation and support the life of an embryo, but it will menstruate when the hormone is withdrawn. At the right is shown the postovulatory-premenstrual transformation produced by the successive action of follicular and luteal hormones. What has actually happened is that the tubular glands have

11. Hisaw, Zuckerman.

grown so fast that they have coiled up into springlike structures. This is the type of a wall of the uterus which is present at the time the embryo reaches the uterus. Various combinations of estrone and progesterone have been used in producing this effect, and the amounts needed in both monkeys and women are now known.[12] By the proper balance of these two hormones, plus stimulation at just the right time, maternal placentas (implantation sites) can be formed in the wall of the monkey's uterus. Various workers have shown that the experimental menstrual periods which follow discontinuance of injections of estrone can be postponed by injections of progesterone.

The ovaries of the rabbit have been removed after the eggs had been produced; then by injections of progesterone the wall of the uterus has been modified to allow implantation of the eggs and carriage of embryos through full gestation.[13] This constituted the experimental demonstration of this function by the hormone of the corpus luteum (progesterone). The two ovarian hormones work together; first, the follicular hormone produces growth in the wall of the uterus, and then the luteal hormone, building on the foundation thus prepared, transforms it into the structure which will sustain pregnancy.

Early in the study of the female sex hormones it was found that large amounts of estrone could be extracted from the placentas of several animals. The question arose as to whether this hormone was actually produced in the placenta or whether it was collected there and stored. This has finally been answered in women by several cases in which the ovaries have been removed during the first few months of gestation without interrupting the course of pregnancy or the excretion of the hormones in the urine of the pregnant mother. Therefore, the placenta may substitute for the endocrine function of the ovaries and secrete substances similar to the ovarian hormones.

Photographs of living rabbit eggs are shown in Figure 62.

12. Smith and Engle, Hisaw, Zuckerman, Kaufmann, and others.
13. Corner.

They are taken from the "Warren Lewis' motion picture of segmenting rabbit eggs." These rabbit embryos were taken from the tube of the mother and raised in tissue culture. At the time the developing embryo arrives in the mother's uterus, it is still inclosed in a shell-like envelope, the zona pellucida. This envelope restricts growth just as does the hard outside skeleton of the crab or lobster. In rabbit eggs raised in tissue culture, it was

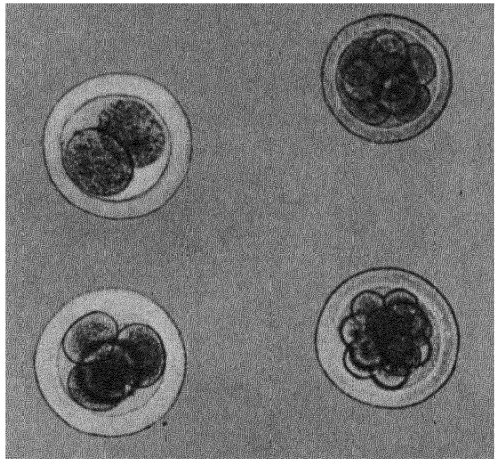


FIG. 62. Living, dividing eggs (two, four, eight, and sixteen cells) of rabbits enclosed in the egg capsule (zona pellucida) from which the embryo must escape before implantation in the uterus (Lewis and Gregory).

necessary for the embryo when it began growth to break this envelope. Recently it has been shown by Hall that this envelope can be dissolved by a slight increase in acidity. It is very probable that the acidity of the uterine secretion is regulated by ovarian hormones, thus providing another escape for the growing embryo.

There are several important modifications of maternal structures during pregnancy. Growth of the uterus and mammary glands is outstanding. In some animals, like the pocket gopher,

it has been found that the canal through the pelvis is not big enough to allow birth. In these animals a hormonal mechanism has been perfected which resorbs the bone of the anterior joint of the pelvis and enlarges the birth canal to make possible the birth of large embryos. In the pocket gopher this occurs at puberty; in the mouse a similar change occurs, but it is a progressive transformation completed only toward the end of pregnancy. Similar loosening of pelvic joints occasionally appears in women with successive pregnancies.[14]

Another interesting experiment involving the hormone from the corpus luteum has been the lengthening of the term of pregnancy in the rabbit. If new corpora lutea are induced to develop in the ovary toward the end of pregnancy, before function of earlier ones ends, a pregnant rabbit, instead of producing her young in thirty-two days, may carry them for several days longer.[15] During this time the young may grow much larger than the usual rabbit at birth (Fig. 63). This shows that the hormone of corpus luteum plays an important part in the birth mechanism.

The Male Sex Hormone. The male may also be dominated by his sex hormones. The testis is similar in some ways to a tubular boiler. Inside the tubes the sperm cells are produced. Outside the tubes, like packing between them, lies the interstitial tissue which probably secretes male sex hormone. Like the two hormones from the female, this hormone has been isolated from testicular tissue and urine: purified, crystallized, and synthesized.[16] In the male, it appears that the chief function of sex hormone is to maintain the seminal vesicles, prostate, and ducts of the testis in functional condition so that they elaborate the secretory products necessary for keeping the sperm alive and those required for mating.[17]

Male sex hormone is necessary for the growth of the comb

14. Hisaw, Gardner, Thoms.
15. Snyder and Wislocki.
16. Gallagher, Koch, Butenandt, and others.
17. Moore, Nelson, and others.

in the cock. When the testes are removed from a cock, the comb wilts and becomes pale. If enough of the male hormone is injected, the comb begins to grow markedly and becomes bright red. This constitutes the bird test used for standardization of the male hormone.[18]

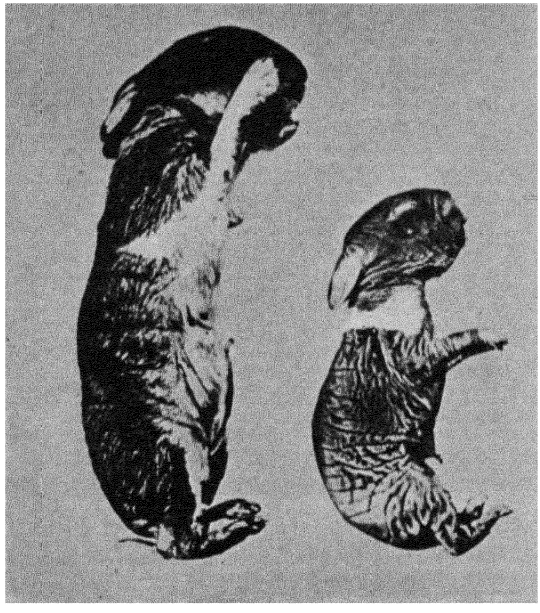


FIG. 63. Postmature rabbit fetus (left) carried thirty-eight days *in utero* by extension of action of luteal hormone which delays birth, compared with the normal fetus (right, thirty-two days) at birth (Snyder and Wislocki).

The Importance of Pituitary Secretions in Reproduction. After the female hormones were fairly well known, the discovery was made that the sex glands could not function normally unless they had the secretion of the pituitary gland. If this gland is damaged by disease, or removed, both the ovaries and the testes stop producing germ cells. If, then, the hormones

18. Gallagher and Koch.

of the pituitary are injected, the sex glands are renovated and made to produce eggs and sperm again and to secrete their hormones.[19] Normal young females injected with one of the pituitary hormones produce many more follicles than usual. The almost quantitative nature of this influence of the pituitary on

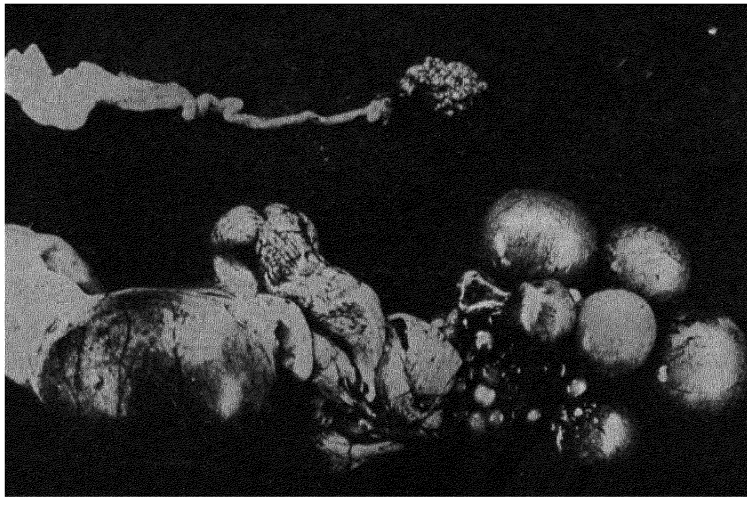


FIG. 64. Added artificial light, to lengthen the day in winter, stimulates the pituitary gland, which secretes its hormone and activates resting ovaries to produce eggs. The ovaries in turn secrete estrogen, which induces growth in the oviduct. Above, quiescent duck ovary (right) and duct (left) during winter. Below, experimentally stimulated duck ovary and duct containing egg (Benoit).

ovarian follicles is shown by Engle in the production of multiple ovulations, as many as sixty eggs being produced at one time by the treated mouse or rat, the usual number being seven to ten. Ovulation of five eggs at a time in the monkey has also been reported by Hisaw.

Now that multiple ovulations have been produced experimentally, there is every reason to believe that unusually large

19. Smith and Engle, Zondek and Ascheim, and many others.

amounts of secretion of the anterior pituitary may account for fraternal twins or other multiple births. It is also interesting to speculate on how evolutionary forces operating upon our ancient maternal ancestors, who probably bore litters of young, might have reduced the number of offspring by reduction of this pituitary secretion.

The Effect of Light on Reproduction. The amount of light, namely, the increased length of day in spring, is an important factor in the onset of reproductive activity in certain birds, such as the starling, sparrow, and duck.[20] Their reproductive activities, usually quiescent in the winter, begin in early spring. By subjecting these birds to increased amounts of illumination during the winter, it has been possible to start their reproductive cycles prematurely. Apparently the light affects the eyes and through them the pituitary gland. The pituitary gland then secretes its hormone, prematurely activating the sex glands in both males and females. Figure 64 shows the ovaries and oviducts of the duck before and after illumination of the birds. The sizes of the testes are shown in Figure 65. They may be thirty times as heavy as the controls. These results can be obtained by three weeks' illumination for several hours a days. The ferret, a seasonally breeding mammal, has been shown to react similarly by Bissonnette. The optic nerve can be cut and the retina removed and if the light is still brought in contact with the pituitary gland, the reaction occurs. Light on other parts of the body is not effective according to the results of Benoit.

Experimental Production of Descent of the Testes. In the monkey at birth, the testes are in the inguinal canal, or already down in a fairly well-developed scrotum. During the month or six weeks after birth, they ascend again into the body cavity and the scrotum shrinks and disappears. This is not true in boys where the testes, though small, normally remain in the scrotum. It has been possible experimentally to cause the descent of the

20. Rowan, Bissonnette, Benoit.

testes in young monkeys by injections of the anterior pituitary hormone or by male sex hormone.[21]

Change of Sex of the Embryo by Hormone Action. If a cow produces two eggs, one from each ovary, and they are fertilized and one starts to develop as a male and the other as a female, the embryonic membranes may extend down into the junction

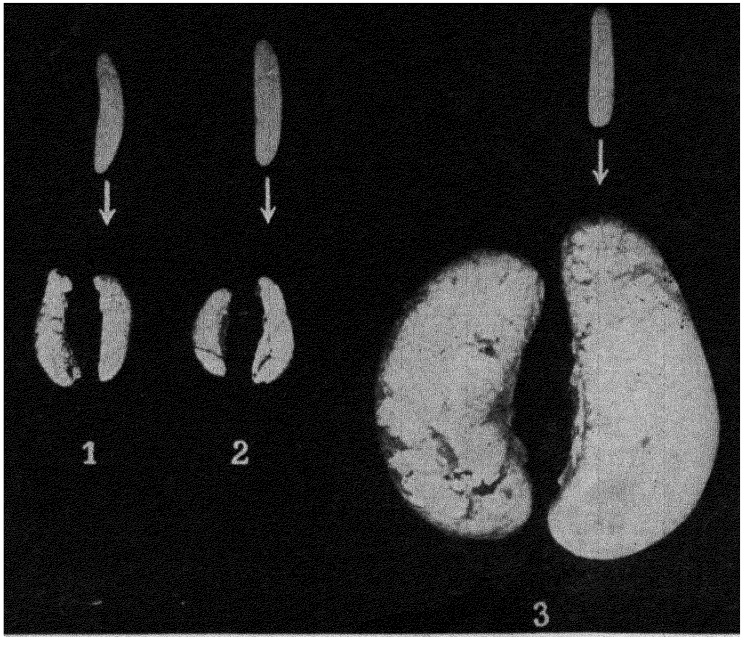


FIG. 65. The "light-pituitary" stimulation of duck testes: *1* and *2*, controls; *3*, experimental (Benoit).

of the horns of the uterus and fuse at the crotch of the "Y." The fetal vessels may then fuse so that the two embryos of opposite sex have a common circulation. It has long been known that the testes begin to develop before the ovaries. Consequently, the male hormone appears first in the common circu-

21. Wislocki, Engle, Hamilton.

lation. This apparently sterilizes the female. The male may be fertile, but the female becomes a sterile sex intergrade known as a "freemartin." It has been found that a similar condition can be produced experimentally in chick embryos. If fertile embryos are incubated, and then injected with a hormone from the opposite sex, the genetic sex of the embryo may be reversed. Willier has found that it is necessary to do this before the twelfth day of incubation.

Castration Changes in the Pituitary and Their Prevention with Sex Hormones. The dependence of ovaries and testes upon the pituitary has already been described. Sex hormones also react upon the pituitary. If either the ovaries or the testes are removed, certain changes occur in the cells of the anterior pituitary gland. They become vacuolated and stain differently. The injection of either male or female sex hormone will prevent these cytological changes in the pituitary. This emphasizes the endocrine interaction between sex glands and pituitary.

The Excretion of Sex Hormones in the Urine; Tests for Pregnancy. Much work has been done recently on the excretion of sex hormones in the urine. Small amounts of estrogen can be recovered at puberty and at certain times in the menstrual cycle. Recently the excretion of a metabolic product of progesterone has been recovered by Venning and Browne from the urine during the last half of the ovulatory menstrual cycle. One of the earliest signs of pregnancy after the failure of a menstrual period to appear is the recovery of anterior pituitary hormone in the urine of women. There is a marked increase if a woman becomes pregnant, and this can be discovered very early after the time of supposed impregnation. Later in pregnancy large amounts of estrogenic substance are also excreted. These tests are therefore used to diagnose pregnancy.

The Lactogenic Hormone of the Pituitary. It has been found recently that an active extract can be made from the pituitary gland which will start secretion by the mammary glands.[22] It

22. Stricker and Greuter, Riddle.

is necessary first that the gland be grown under the action of the ovarian hormones.[23] Then the hormone, prolactin, from the pituitary starts the secretion of milk. There is apparently a balance between the follicular hormone of the ovary and this lactogenic hormone of the pituitary, as shown by Nelson, for milk secretion can be prevented by injecting large amounts of follicular hormone.

The lactogenic hormone also causes a secretion of crop milk in the pigeon. Two areas of epithelial lining of the crop grow very rapidly under the action of lactogenic hormone and produce the crop milk, a cheeselike, cellular material which the pigeon regurgitates to feed the young. This occurs in both the male and the female. Injection of prolactin not only causes production of pigeon's milk but it induces broodiness and other parental behavior. It also affects the sex glands, restraining their reproductive activity.[24]

It was thought that some phases of maternal behavior in mammals might be due to this material. It has been reported that maternal instincts such as nest building and retrieving the young by rats and mice can be experimentally induced by injecting prolactin. Recently, however, it has been demonstrated that these animals experience parental instincts even after the anterior pituitary glands have been removed.[25]

Sex Hormones as Old Age Approaches. Coincident with aging there is a decline in sex function. This may be abrupt in the female, but is more gradual in the male. It is apparently due primarily to a failure of the sex glands to continue production of their specific hormones. At the menopause there is usually an increase in excretion of the hormone of the pituitary. Many have been led to the hope that by the use of sex hormones a rejuvenation could be brought about. At present, however, results in this direction are extremely transient. There may be a temporary rejuvenation, but rapid retrogression follows cessation of treatment.

23. Turner, Gardner. 24. Riddle. 25. Leblond and Nelson.

Coöperation of Internal Secretions and Nervous System in Controlling Ovulation. A most interesting relation exists between mating and ovulation in the rabbit, cat, and ferret. The act of mating apparently releases a trigger mechanism to cause ovulation. In the rabbit, ovulation occurs ten hours after mating; in the ferret and the cat, a longer time elapses. Some stimulus from mating evidently is carried through the nervous system or the blood stream to act either directly upon the pituitary or upon a center in the brain in a way similar to the effect of concentration of gases in the blood upon the respiratory center of the medulla. The pituitary then secretes its hormone which stimulates the follicles and induces ovulation.

If a rabbit is mated and then the pituitary gland removed before thirty minutes have elapsed, the operation prevents ovulation. If the pituitary is removed ninety minutes after mating, ovulation occurs anyway.[26] Therefore, something happens within ninety minutes after mating, some pituitary effect on the follicles which is necessary for ovulation. Recently, Friedgood and Dawson have found that, after mating, the carmine-staining cells in the anterior pituitary rapidly increase in number. This indicates a transient function of these cells for three or four hours, probably indicative of some special secretion which affects the ovarian follicles.

It has also recently been shown by Haterius that electrical stimulation of a small nucleus in the floor of the thalamus in front of the pituitary gland will induce ovulation. It may be that this is one of the relay stations in the nervous circuit from a sexual center in the brain. It seems to be essential, for cutting the stalk of the pituitary prevents ovulation.[27]

Before ovulation the egg must free itself from its attachment to the wall of the follicle. This is done by the formation of small pools of fluid which disperse the surrounding cells (Fig. 66). The egg then floats free in the follicular fluid. While this is happening, interesting things occur inside the egg. The nu-

26. Parkes. 27. Brooks.

cleus, usually central in position, migrates to the periphery, the nuclear membrane breaks down, the chromosomes line up in a plate which divides in half. Then half of the maternal chromosomes are discarded in the first polar body and the other half moves into a second maturation spindle. The chromosomes stay in this condition during ovulation. If the egg is fertilized, they

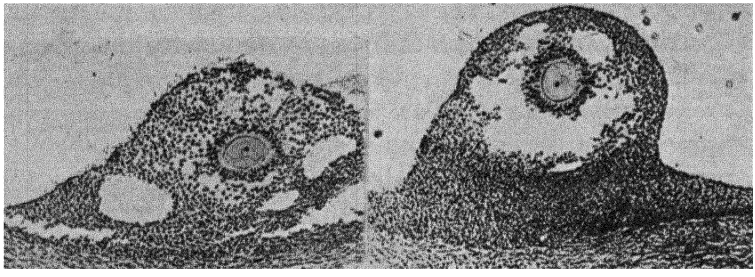


FIG. 66. Human eggs being released from their attachment to the walls of large follicles in preparation for ovulation. Secretion of fluid disperses the surrounding cells (Allen, Pratt, Newell, and Bland).

then combine with the chromosomes introduced by the sperm as previously indicated (p. 189).

The Observation and Photography of Ovulation and Changes in Electrical Potential. No one had seen the escape of the mammalian egg from the follicle until Walton and Hammond observed it in 1928. The follicle slowly bulges from the surface of the ovary due to accumulation of fluid within. As the pressure increases, a small white spot appears, probably due to pressure closing the small blood vessels. Then at this point a little pimple-like protrusion is "blown out" and becomes the rupture point through which the follicular fluid and egg are extruded. These authors describe ovulation as a slow process comparable to the rupture of a boil.

Since it is difficult to get the rabbit to produce eggs when desired we decided to take a moving picture of ovulation in this animal. From the study of this film it seemed clear to us that

the process was quite explosive in nature. This is the only point of difference from the description of Walton and Hammond. It led, however, to a search for some outward sign of ovulation, for such an explosive rupture should "reverberate" through the animal. Using a new potentiometer perfected by Burr, Lane, and Nims, and attaching the electrodes to the genital regions of the anæsthetized rabbit, a marked change in electrical potential at the time of ovulation was discovered. As the time for ovulation approaches, the potential rises from the base line of 500 to 2,500 microvolts to as high as 7,000 to 30,000 microvolts and then drops again to the base line, the change taking several minutes. In the first animal, three changes were noted in a half hour. When the rabbit was opened, three rupture points were found, two in one ovary and one in the other. Further observations showed that as each follicle is distended, the potential rises. After rupture at ovulation, it falls again. This result has been confirmed by Reboul, Friedgood, and Davis. It provides a method of telling the exact instant of ovulation in the unoperated animal. In selected cases it is proving useful in giving additional evidence for the time of ovulation in the menstrual cycle in women.[28]

28. Burr, Musselman, Rock.

VIII

RECENT DEVELOPMENTS IN OUR KNOWLEDGE OF CHROMOSOME STRUCTURE AND THEIR APPLICATION TO GENETICS

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WE are going to consider the recent developments along that frontier of science where cytologists and geneticists meet in a united effort to gain a more thorough understanding of the nature of the hereditary units, the genes, and the roles they play in nature and in the life of the individual. Here, as so often happens in science, the great advances in our knowledge during the past few years and the present widespread activity rest upon a simple but very timely discovery of a new type of material and the development of a technique which have allowed us to attack some of the problems brought into the foreground by the intensive study of the fruit fly by Morgan and his school, and by Muller's discovery that the irradiation of germ cells may cause the genes to mutate and the material of the chromosomes to undergo rearrangements. Among these problems are certain ones of outstanding interest to the general scientific public as well as to theoretical biologists: How are new species formed in nature? Where are the genes? What are genes, and how do they function?

No biologist, and few well-informed people, doubt that there has been a gradual evolution of animals and plants from simpler forms of life. No longer do we question that in a struggle for existence the fittest tend to survive and the unadapted

to die out. But natural selection itself is not a creative force. Rather it is a sieve which sorts out and tends to perpetuate the best adapted; it does not produce the variants from which it selects. Today, as for decades past, the gist of the problem of evolution lies in two questions: How do variant forms arise in nature? Is natural selection the only force which operates in the formation of new species? Each generation of biologists has attempted to answer these questions. A decade ago most of us were convinced that it was only sudden changes or mutations in the physical or chemical nature of the genes that gave rise to new forms. At the present moment, one might well hesitate to say that this is the only source of heritable changes which have played a part in evolution, for it is now apparent that the position which a gene occupies with regard to its fellows may profoundly modify the effect of the gene without the unit itself being changed in any way, so far as we can tell.

The particulate way in which characters are inherited in organisms has forced geneticists to assume the existence of discrete and independent units or genes which control these characters. For more than fifty years now, a large group of biologists have dedicated all their researches to the general problem of determining where the genes are located within the cell. In a moment we shall very briefly consider some of the evidence which led to the conclusion that they are carried by the chromosomes. But this is only the first step in a search whose ultimate goal is an understanding of the physical and chemical make-up of the genes and how they function during the ontogeny of the individual. There are many who will insist that the final answer to these questions must be given by the physical sciences, but at present all will agree that the next step is for the cytologist to determine the fundamental structure of the chromosomes and then for the cytogeneticist to tell us where, within the chromosome, the genes are to be looked for. These last are the questions with which we shall deal at some length because it is here that the study of giant chromosomes has proved most useful.

The search for the hereditary material took a concrete form with the enunciation of the cell theory in 1838, for if ultimately every animal or plant can be traced back to a single cell, it follows that some part of the original cell must carry the genes. But it was many years before we came to know much about the finer structural elements of cells because very little can be seen in living protoplasm, even with our modern instruments; and cellular morphology had to await the development of adequate methods of coagulating or fixing the living substance so that it is rendered insoluble and can be stained and preserved. This took time but, nevertheless, by the early eighties of the past century the principal parts of the cell were known, and the first hint was had of where the hereditary substance was to be found. It was observed that within the nucleus of each cell, just before division occurs, the deeply staining material, called chromatin, unites into discrete bodies (the chromosomes) and later these chromosomes divide so that each daughter cell receives exactly the same number of chromosomes and exactly the same amount of chromatin. It was the exactness of this division process which led Wilhelm Roux and August Weismann and others to think that the chromosomes were the carriers of the hereditary substance, and the theoretical writings of the two men just mentioned did much to stimulate an intense interest in these bodies. For the next two decades a vast amount of work was done on chromosomes, centering about two general themes: either it had to do with establishing the fact that these units have an individuality and persist as discrete structures from one cell generation to the next, or it was concerned with the behavior of chromosomes during the formation of mature germ cells. Both aspects are extremely important. It must be realized that chromosomes can be seen as such for only a short period in the life of a cell, and the rest of the time the chromatin appears to be scattered irregularly about the nucleus in a granular form. And the behavior of chromosomes in maturation laid the foundation for an understanding of the mechanics of modern genetics.

By the late nineties the individuality of the chromosome was generally accepted. In addition it was known that each body cell carries ordinarily two of each kind of chromosome, that the number of chromosomes in a species is constant, and that when mature sex cells are formed the chromosome number is reduced to half by the simple method of having a germ cell receive only one of each kind of chromosome. Subsequently, by the union of an egg and sperm to form a new individual, the full species number is restored and there are two of each kind of chromosome within each body cell.

In addition to these basic facts regarding chromosomes and their behavior, there was a good deal of other indirect evidence that these bodies are qualitatively different. The first direct evidence of this was McClung's discovery that, in grasshoppers, the presence or absence of a definite chromosome is associated with sex determination.

Such was the state of our knowledge about chromosomes when Mendel's Laws of Heredity were rediscovered in 1900. We usually say that modern genetics had its birth on this date and in a sense that is true, for Mendel gave us our modern concept of discrete and independent hereditary units. At the same time, the interest which attended the rediscovery of these laws was greatly stimulated by the fact, first pointed out by Sutton in 1902, that if we assume that Mendel's unit characters (genes) are carried by the chromosomes, we have a simple mechanical setup in the maturation of germ cells which would account for the segregation of like unit characters into separate gametes. It was the timeliness of the rediscovery of the Mendelian Laws, quite as much as the facts, which led to the rapid development of genetics during the next three decades, for previous work had brought a thorough understanding of chromosomes and their behavior and most of the investigators who entered this new field, especially here in America, were "chromosome-minded."

By 1925 the modern concepts of genetics had been well

worked out, largely through the leadership of Morgan and his associates Bridges, Muller, and Sturtevant, and many of these concepts were based on the work done with the so-called fruit fly or vinegar gnat *Drosophila melanogaster.*

Morgan was able to prove that the genes are carried by the chromosomes; and then with his co-workers, especially Sturtevant, to establish the linear order of the genes. They made genetic or crossover maps of all the chromosomes of the fruit fly, showing the order of the known genes along the chromosomes and their spacial arrangement with each other in terms of crossover units. During this period little was done with the chromosomes themselves. It was known, of course, that there are four pairs of chromosomes in *D. melanogaster,* and which of these elements carried the various genes, but under the microscope there was little hint of the great complexity of organization which the crossover maps indicated must be present. All that was visible, with the best optical equipment, was tiny V-shaped, rod- or dot-like bodies which stained a uniform black with the dyes commonly used.

The key to the more exact localization of the genes was given when Muller found that if mature germ cells are exposed to X rays, the chromosomes are often broken and the material rearranged in a new order. From his genetic data, Muller was able to determine what genes were separated by a given break, and a study of these broken chromosomes by the writer, in collaboration with Dr. Muller, enabled us to construct the first chromosome map of the X-chromosome. About the same time, using similar methods, Dobzhansky made maps of the second and third chromosomes. The methods used in this work were very simple. A chromosome broken between genes A and B was examined under a microscope. The point at which it was broken being determined, it was possible to draw a line across a schematic outline of the normal chromosome and say that the gene A lay to one side of the line and gene B to the other. With a series of breaks, all in the same chromosome, it was soon pos-

sible to demonstrate directly, for the first time, the linear order of the genes which geneticists had assumed from crossover data (Fig. 67). However, these maps were based on quantitative data; we could say a chromosome was broken in the middle, or near one end, but no region differed visibly from any other sec-

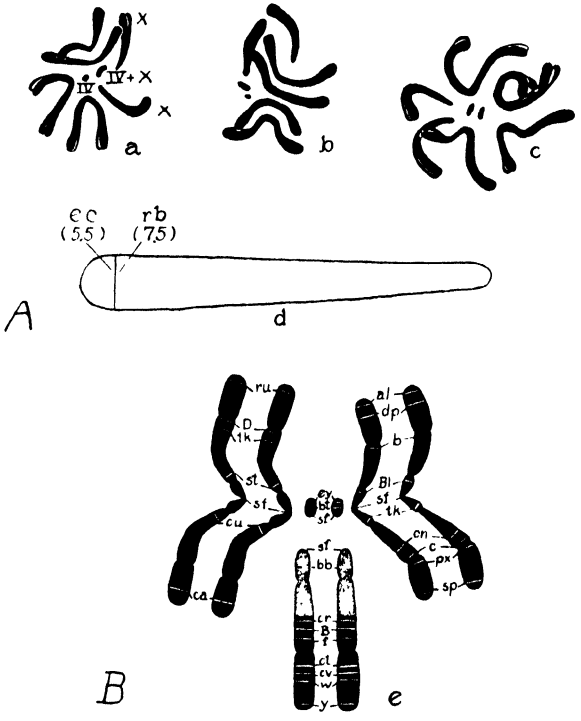


FIG. 67 shows how the positions of genes are determined from metaphase chromosomes. (*A*) Following irradiation, a mutual translocation was obtained between the rodlike X and the dotlike chromosome IV. The X is broken between the genes echinus and ruby. *a* and *b* show the chromosomes of a female heterozygous for the translocation, and the size of the X fragment can be judged by comparing the normal IV with its mate attached to the piece of the X. *c* was made from a female homozygous for the translocation. *d* is a diagram of the X-chromosome showing the estimated point of breakage. Echinus lies to the left of the break and ruby to the right. (*A* after Muller and Painter.)

B shows the position of a number of gene loci in *Drosophila melanogaster* determined by the method outlined above. (*B* after Dobzhansky.)

tion of the same chromosome; and the chromosomes were so small in the cells currently used for such studies that differences in size less than a fourth were difficult to distinguish because of possible foreshortening. It was obvious that if we were to make further progress in our search for the genes we would have to find a new and more favorable type of material for a study of chromosomes. These considerations led to the study of salivary gland nuclei in *Drosophila* larvae and to the discovery of the giant chromosomes which have played such an important role in the recent development of genetics.

We need not take the time here to consider why the salivary gland tissue was selected for study or why earlier investigators had failed to realize the value of these cells for cytogenetic studies. In science, as elsewhere in human affairs, our interests and our interpretations are colored by the standpoint from which we view the facts. Time and again, we note that a discovery in order to be immediately useful must be made at the right time and in the right place. The salivary gland work is a case in point. Some months after the writer had begun to study salivary chromosomes, two German cytologists, Heitz and Bauer, published a paper dealing with these chromosomes as they appear in *Bibio*, another genus of flies. They presented evidence showing that the wormlike structures within the nuclei of the salivary gland cells were chromosomes, and they noted differences in these elements and saw that the homologous chromosomes were closely associated together. But the interest of these able workers was primarily morphological, and we find no hint in their paper of any genetic implications in their findings. On the other hand, it was the good fortune of the writer to approach this type of tissue from a genetic standpoint, and to work in a laboratory where large numbers of chromosome rearrangements were available. As a result, it was possible not only to identify individual chromosomes but to locate individual genes along these, within very narrow limits.

If one were to place a few earthworms in a small glass flask

and view them from the bottom side, he would have a good mental picture of what the salivary gland nuclei look like under the microscope, when they are fixed and stained. By proper manipulation the nuclear wall of the salivary cells may be broken and then the wormlike chromosomes tend to spread out so that often a whole chromosome lies free and fairly straight (Fig. 68). In general proportions these chromosomes resemble earthworms, there is a nonstaining cylinder of material, and across this there are a large number of deeply staining chromatic bands. The latter show a great diversity of form; some are broad and obviously compound, others are thin and sharply outlined, others are dash- or dot-like. The pattern these make along the chromosome, taken together with swellings and constrictions, makes a very striking picture. The most important point about this banding is its constancy; long study of the same region of a given chromosome has shown that this constancy of pattern extends to the finest visible detail.

A second very important feature of the salivary chromosomes is the fact that, for some unknown reason, the two like or homologous chromosomes undergo an intimate union, which I have called "somatic synapsis." During this process similar bands on the pairing chromosomes become continuous, and the two fused chromosomes appear as one structure. The use which we have been able to make of these giant chromosomes in genetic studies rests largely on this union of homologous parts, as will be seen presently.

A third feature of the giant chromosomes is that only the relatively active genetic areas of the chromosomes show the banded form. The so-called "inert material" in the fruit fly appears ordinarily as an amorphous, deeply staining mass, a structure to which Heitz has given the name "chromocenter." Many interesting and important questions arise in connection with the so-called heterochromatin of the chromocenter, but we lack space to consider them here.

The salivary chromosomes are about 125 times as large as

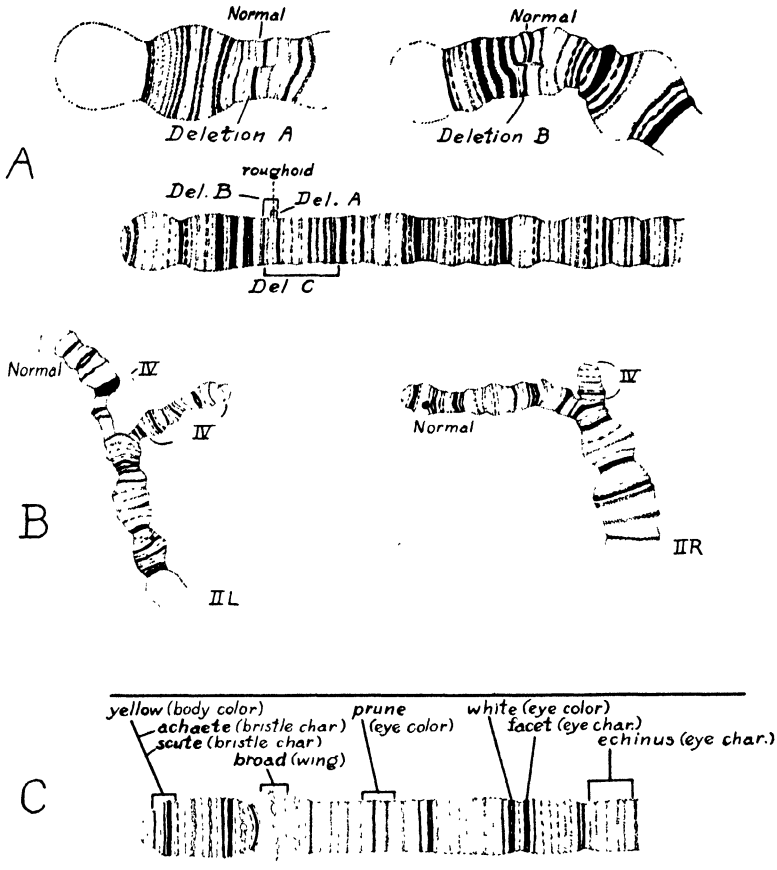


FIG. 68 illustrates the methods by which gene loci are located on salivary gland chromosomes. *A* shows three deletions used to determine the position of roughoid, an eye character in the left arm of the third chromosome. The drawing showing Deletion A was made from a larva carrying a normal and a deleted chromosome. One band is absent from the latter and genetically the normal gene which suppresses roughoid is absent. In Deletion B, three bands are absent and genetically the normal allelomorph of roughoid is out. The lower of the figures shows the normal pattern of the left arm and the limits of deletions A, B, and C. From the evidence it is concluded that roughoid is located on the very fine line indicated in the figure.

(*B*). Two translocations are illustrated, one involving an exchange between the fourth and the left arm of the second chromosome and the other the right arm and the fourth. Each of these breaks separated genes which are normally linked together, a fact which must be determined genetically, and gives us physical limits for the loci involved.

(*C*). A normal map of a short section of the X-chromosome showing the positions of various genes.

the chromosomes of the fruit fly which had formed the basis of previous study. In addition, each element has its own characteristic pattern expressed in the sequence of the various types of bands, as well as other features which make it possible to identify not only the separate chromosomes but even small sections of a single chromosome. Most useful of all, there is a strong tendency for similar regions to unite in somatic synapsis so that it is a relatively simple matter to trace out the rearrangements produced in normal cells after treatment by the agency of X rays.

HOW GENES ARE LOCATED

As has been already explained, the Drosophila workers had been able to show, by their crossover method, that the genes are arranged in a linear order along the chromosomes much like beads along a string. As soon as the true significance of the salivary gland structures was understood, the first problem attacked was the more exact localization of the gene loci with regard to the band pattern. To study this, it is essential to have breaks in the chromosomes accompanied by visible rearrangements of the parts and to know what genes are affected by the changes. Such breaks and rearrangements occur spontaneously in nature, but they are rare and hard to detect. On the other hand, Muller's first irradiation experiments showed that rearrangements were commonly induced in the chromosomes of mature sperm when these were exposed to heavy doses of X rays, and we had in the laboratory a large number of breaks of known genetic constitution which had been accumulated as a result of genetic experiments carried out, for the most part, by my colleagues, J. T. Patterson and Wilson Stone.

In general, chromosome rearrangements are of three types, translocations, deletions, and inversions. Translocations occur when a part of one chromosome breaks and becomes reattached to some other chromosome. There is usually a mutual exchange of parts. In deletions, sections of chromosomes are removed and

lost so there is a deficiency for the area involved. Often these deletions are very short, removing from one to half a dozen bands. Inversions result when a section of a chromosome becomes turned around so that the normal *a-b-c-d* order becomes, for example, *a-c-b-d*. All of these rearrangements are studied in larvae which are heterozygous, that is, which carry one normal and one rearranged chromosome. If we are dealing with a translocation, the normal and broken chromosomes show a perfect fusion or synapse up to the point of breakage where the nonhomologous bands diverge, if the exchange has been mutual. By studying the matched bands, it is a simple matter to determine just where the chromosome is broken. Knowing what genes are separated by the break, we are able to place these on either side of the fracture. One translocation would not tell very much, but ten or twenty breaks between a number of genes in one chromosome, or one arm, make it possible to localize a given gene, often within the space of two or three bands. In deletions, the normal and deficient chromosomes synapse band for band, except at the point at which there has been a loss. Here the normal element buckles and one can readily note the bands which have been lost. Knowing what gene is lacking from the deficient chromosome, it is possible to say that it lies on or near one of the missing bands. When only one band is deleted, as sometimes occurs, the gene's locus can be restricted to the immediate neighborhood of the absent band. Inverted chromosomes are used like translocations. Knowing just where the inverted area begins and ends, as well as the genes affected, we can restrict a given gene to a definite region. The accompanying Figure 69 illustrates the points just described and gives a chromosome map of the X-chromosome of *D. melanogaster.*

The introduction of the salivary gland chromosome method to the study of genetic problems has initiated a new area in this field of biology. Chromosome rearrangements induced by X rays can be analyzed quickly by a cytological study, and many

of these have been utilized for the study of a host of different problems. I wish here to call attention to three salients where the method is proving most fruitful.

The discovery that individual genes may be associated with definite chromatic bands at once raises the question, how are the

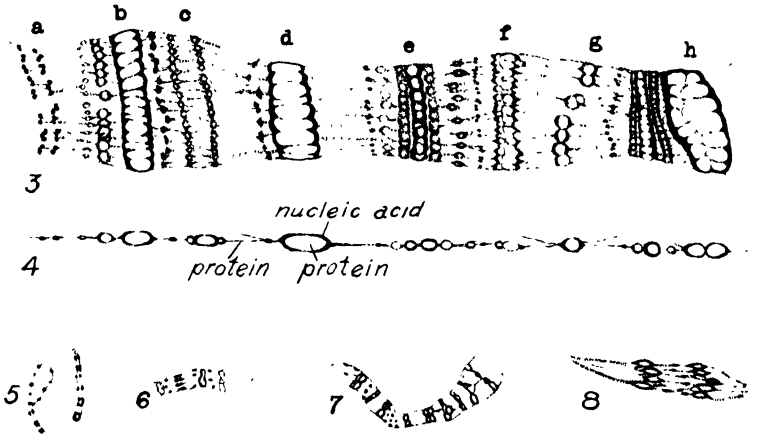


FIG. 69. Section 3 is a camera lucida drawing showing the details which appear on the upper surface of a fully developed salivary gland chromosome taken from *Simulium virgatum*; 4 is a semidiagrammatic sketch of the types of chromomeres which make up the bands in section 3; 5 to 8 are steps in the growth of the salivary chromosome of *S. virgatum*, beginning with a simple chromomeric thread (sec. 5). Note that the increase in size is due mainly to the growth or hypertrophy of the chromomeres and not to numerous divisions of them.

genes related to the bands? Are the bands genes, or the houses in which the genes live, or are they landmarks which incidentally give the key to the gene's location without being in any way concerned with the gene's structure? These questions have focussed attention on the nature of the bands and the structure of salivary chromosomes in general, which must be thoroughly understood before further analyses of the genetic units can be hoped for.

One of the most puzzling features about salivary gland chromosomes is their relatively enormous size as compared to

ordinary metaphase chromosomes. To account for this, Bridges and Koltzoff suggested, independently, that the giant elements represent ordinary prophase chromosomes which have undergone a number of divisions without the nucleus itself having divided. On this basis, the bands represent rows (discs in reality) of chromomeres, the number in each row depending upon how many times the original (paired) chromosomes have split; and the fine lines traversing the areas between bands are the longitudinal threads which connect the chromomeres of a single strand or chromonema. In effect, the giant chromosome is a twisted cable of many chromonemata. Each separate chromonema is thought of as having the structure of the ordinary prophase chromosome; that is, we have a series of individual chromomeres connected linearly by a fine thread. This interpretation gives a simple explanation of the observed facts and has been widely accepted by cytologists and geneticists, and a good deal of work has appeared supporting it, notably Bauer's studies on *Chironomus* larvae. On the other hand, Metz, who has devoted a great deal of time to the study of *Sciara* chromosomes, questions the validity of the chromonema interpretation. He thinks that, in reality, salivary chromosomes have a honeycomb-like structure; that is, are made up of droplets of material. When the chromosome is stretched (the details of structure are best seen when the elements are drawn out), the droplets are elongated and their walls are the "fibers" seen by other workers. Chromomeres, as separate units, do not exist; the chromatin is a sort of granular chinking between the abutting ends of droplets.

Here it might be explained that the order of size of the structures with which we are dealing is far, far too large for them to be direct expressions of the fundamental structure—fibrillar or alveolar—of the living protoplasm. It has been estimated that the largest known protein molecule, having a weight of about 17,000,000, is three or four times too small to be visible in the best microscope with light rays, and many of the chro-

momeres or alveoli are several microns in diameter, while the chromatic granules compare in size with small bacteria.

The chromonema and alveolar interpretations were formulated, initially, from a study of fully differentiated chromosomes of different species of *Diptera*, and it must be admitted that either gives a plausible explanation for the images seen. On the other hand, one theory postulates that the large size is due to a repeated duplication of the original prophase chromosome (or pair), while the other assumes a growth which does not involve the visible reduplication of the parts of the chromosome. Now all stages of growth in nuclear size and, as we shall see, of chromosome differentiation, may be found in the salivary glands of larvae of different sizes, and it is obvious that the most critical evidence for the origin of these chromosomes should be sought in the developmental stages. For more than a year now the writer, together with Mr. Allen Griffen, has been studying this problem from the ontogenetic point of view, and I wish to summarize our findings here because presently I shall discuss in terms of visible structure the question, Where are the genes?

For our study we used the larvae of the black fly, *Simulium*, which is extremely favorable material, not only because the chromosomes are very large, but also because somatic synapsis is not so intimate, thus making it easier to observe the different parts of the elements. In addition it has proved possible to follow through the development of the chromosomes from the earliest prophase stages and to present critical evidence as to just how the giant chromosomes are formed. Before taking up the development, let us examine a fully formed giant chromosome of *Simulium*.

Figure 69 (3) shows the details which appear at the surface of a short section of a *Simulium* chromosome of the "large vesicle" type which was killed and stained with aceto-carmine and then stretched somewhat when the cover glass was placed over the tissue. The essential features shown can be seen in an un-

altered living chromosome, so there can be no question of the validity of the fixation image. In the figure the crossbands appear to be made up of rows of similar chromomeres and between the bands are fine lines or threads running parallel with the general axis of the chromosome. It must be remembered that the chromosomes have a cylindrical form, and that the chromomeres extend as transverse plates or discs through the element. Looked at from the side, rows of units appear, but, viewed from the end, the disc looks much like the head of a pepper box if the chromomeres are small, or like a dish of bubbles if the chromomeres are large.

In most of the bands the individual chromomeres appear as discrete units and are vesiculate, that is, there is an outer rind or hull of chromatin surrounding an inner mass of nonstaining material. But in some rows, as at *a*, these units appear as solid masses of chromatin. Where the chromomeres are very large, as at *b* or *d*, the chromatin is forced to the free ends by the crowding of the vesicles, and forms, as a result, two deeply staining cross lines separated by a layer of thin-walled vesicles. In this way a "double band" is formed, a type which is especially prominent in *Sciara*, and one can readily understand why Metz thinks that the chromatin extends as a transverse partition across the chromosome. In *Simulium*, however, bands like *b* and *d* are in a minority; more usually the chromomeres appear as separate discrete units. Furthermore, in larvae with smaller vesicles, these same bands *b* and *d*, appear exactly like the units of ordinary rows; the chromatin more or less surrounds each vesicular space. The collection of the chromatin at the free ends then either must be a later stage in normal differentiation, or is associated with a hypertrophy of the vesicular material.

"Double bands" may be formed in other ways than by the hypertrophy of vesicles. At *c*, for example, in ordinary preparations, we would appear to have double "fuzzy edged" lines, while in reality there are three separate rows of chromomeres

associated end to end. Many of the "bands" in *Simulium* are complex in this sense.

It is almost impossible to count the exact number of chromomeres present in the discs of fully developed chromosomes. We estimate the average number as about sixty-four, but it is apparent that not all discs have the same number of chromomeres. In the bands *b* or *c*, one can count from fourteen to sixteen units at the surface of the upper side, while at *d* and a little farther to the right eight or nine chromomeres show. The fine lines which connect the bands *b* or *c* run parallel, while in the area between *c* and *d*, we note that at several points two threads arising from separate chromomeres in *c*, converge and unite in pairs before they connect with the less numerous units of row *d*. This suggests that the chromomeres at *d* have really double the valence of the units of *b* or *c*. Likewise the chromomeres of band *f* are about twice as numerous as in the other bands to the right or left and here again the separation and convergence of pairs of threads are noted.

It is difficult to show in a pen-and-ink sketch the character of the fine lines which connect the chromomeres in linear order. As one studies them, he gets the impression that they are tiny bundles of more minute threads. In any event, they give a very different image from that made by the closely appressed vesicular walls seen in bands *b* or *d*.

The whole picture of chromosome structure, which one gets from a study of such areas as that represented in Figure 69 (3), is that we are dealing with a cable-like bundle of chromonemata; the individual chromonema is made up of a series of chromomeres connected in linear order by threads, but the separation of the parts may not be complete; two or more threads may be connected to a single compound chromomere which either is slow to divide or has division planes not visible with the technique employed.

In very early stages before the onset of somatic synapsis, each

separate homologue appears as a typical split prophase chromosome with the two halves twisted about each other. The individual chromatids consist of a linear series of apparently solid chromomeres connected by a fine thread (Fig. 69 [5]). At the four-strand stage (Fig. 69 [6 and 7]), that is, when there are as many as four chromomeres in the incipient bands of a single homologue, the chromosome is much broader than would be expected from a simple reduplication of the original two strands. The cause of this hypertrophy is the growth without a visible subdivision of the individual chromomeres. These not only increase several times in diameter but many of them become vesiculate. The eight-strand stage shows a further growth of the chromomeres and in the thirty-two-strand stage, such as is shown in Figure 69 (3) (the two homologues have about sixty-four strands), there is still more increase in size. Since there are initially four chromonemata (each homologue is split) and, in the fully developed salivary chromosomes, some thirty-two or sixty-four strands, it is clear that in *Simulium* each original strand has divided only three or four times. This number of divisions is not sufficient to account for the increase in size of the giant chromosomes, nor for the observed increase in nuclear volume; the latter indicates that there should be some 512 strands per chromosome. The explanation for this discrepancy in strand number and nuclear volume appears to be that the great increase in the size of the individual chromomeres is due to a growth and reduplication of the chromomeric substances many times without an accompanying visible subdivision of these units with the technique employed. Thus the original explanation advanced by Bridges and by Koltzoff to account for the size of the giant chromosomes is essentially valid.

These studies on salivary chromosomes bring once more into the foreground the old string of beads concept of chromosome structure and indicate that fundamentally a chromonema is to be regarded as made up of chromomeres connected linearly by a fine thread. But our newer ideas involve extensions of the

older concept and much that is new. Instead of a few hundred chromomeres, we think in terms of thousands of them; in *D. melanogaster* the number of chromomeres is variously estimated at between five and ten thousand. And the chromomeres, instead of being tiny solid chromatic knots all much alike except for small variations in size, show a surprising diversity of form. Most significant of all, perhaps, is the realization that in each chromomere the chromatin seems to form simply a hull about a center of nonchromatic material. So the old definition that chromatin is the stuff out of which the chromosomes are made is no longer adequate. Indeed, the present trend of opinion is to attribute to this nonchromatic part of the chromomere the specific properties of the genes and to regard the chromatin as accessory material.

The ordinary cytological methods tell very little about the chemical nature of the chromomeres beyond the fact that the parts show different affinities for dyes. But recently a Swedish chemist, Caspersson, has studied the giant chromosomes of *Chironomus* and by the ingenious application of ultraviolet absorption spectra and a special proteolytic enzyme he has shown that the chromatin is in the form of nucleic acid, as has long been supposed, and that the thread and nonchromatic part of the chromomeres are proteins.

If the chromomeric concept of chromosome structure is accepted, it is hard to avoid the conclusion that the genes should be looked for in the chromomeres. For the genes lie in a linear order along the chromosome, and judging from their diverse effects we should expect a wide range in chemical constitution. The chromomeres, in turn, are arranged in a linear order and they, and not the connecting thread, show morphological differentiations such as might be anticipated if they were qualitatively different. The parallelism is striking and very convincing; and I think that most cytogeneticists are convinced that the genes and chromomeres are somehow associated. But in tracing the gene's lair to the chromomere, we find that the latter shows

a complex structure and we are confronted with the question as to which part of the chromomere is the more likely site of the gene's locus. At the present moment I think the answer would be the protein center of the chromomere. Several lines of thought point in this direction. First of all, as Gulick has recently pointed out in discussing this question, analyses of the sperm of many animals have shown the nucleic acid component to be remarkably similar in different groups. On the other hand, the proteins show an extraordinary degree of specificity and *a priori* one would expect the genes to be made up of this type of material. Again Wrinch, who has approached the question of chromosome structure from the standpoint of molecular physics, thinks of a whole chromosome as a chain of amino acid units linked in a linear order, and the chromatin or nucleic acid simply indicates which units are basic in this long molecule.

Closely associated with the problem of the gene's locus is the question, How many genes are there in a given organism? Since our *Simulium* studies throw some light on this problem, brief reference may be made to it here. Belling was the first to attempt to count the number of genes in an organism. Assuming that the ultimate chromomeres represent genes in some way, Belling counted these in one of the lilies and concluded that there were about twenty-five hundred genes present. Bridges made a count of the number of bands on the salivary gland chromosome of *D. melanogaster* and concluded, at first, that there were about twenty-six hundred of them and hence at least that many genes. More recently, on the basis of very detailed studies of the chromosome pattern, he concludes that the number of genes must be five or six thousand, and Muller and Prokofieva, basing their estimate also on salivary chromosomes, think that there are from five to ten thousand genes in the fruit fly.

The reason that we are finding more and more bands in the fruit fly is because most of those seen in ordinary preparations are really compound, and it is only when a given region is

stretched a great deal that the component parts become visible. In the very large salivary chromosomes of *Simulium*, the individual chromomeres stand out more clearly and the complex nature of most of the bands is easily detected (Fig. 69 [3]).

THE POSITION EFFECT

One of the fundamental tenets of Mendelism, and of modern genetics, is the constancy of genes. It matters not whether the gene is thought of as a single molecule or a molecular aggregate; it is an extraordinary fact that living in an environment of seething chemical reactions the genes function, grow, divide, and pass through millions of cell generations without undergoing any permanent physical or chemical change. Of all our biological concepts, the integrity of the gene seems to be among the best founded. But it may be asked, Do not genes mutate? The answer is Yes, on very rare occasions; and it is the relative infrequency of gene mutations in nature which has caused some to question whether or not, after all, gene mutations, at the present-day rate, can account for evolution.

Now, no one has ever seen a gene to know it, nor has the chemist seen the molecule with which he works, nor the physicist the electron; in each instance each is known by its action. But we are finding cases in which the action of the gene is influenced by its neighbors without the gene itself having undergone any detectable physical change. This so-called "position effect" is, at the moment, one of the most important fields confronting the cytogeneticist, for, if it proves to be of widespread occurrence, the tempo of evolutionary changes may be more rapid than has been thought. While it is true that the suggestion that there was a position effect was made by Sturtevant before the advent of the salivary chromosome technique, the latter has given the crucial evidence and proved the fact.

In the fruit fly, the dominant gene, "Bar eye," reduces the normal eye facet number of about 850 to 68 when a female is homozygous for this condition. Sturtevant found that occasion-

ally, due to unequal crossing over, one chromosome may carry two Bar genes. Now a female carrying two Bar genes in two chromosomes has more facets (68) than a female with one double Bar and one normal chromosome (45). In both instances, two Bar genes are functioning, but it appears that when these genes lie side by side in the same chromosome, their total effect is greater than when they lie separately in different chromosomes.

A still more striking case of a position effect has been recently brought to our attention by Panshin. There is in *Drosophila* a recessive gene, "curled," which, when it is homozygous, affects the wing form; but when there are a normal and a curled gene present together, the wing is normal. Panshin produced a translocation by irradiation, between the right arm of the third chromosome and the fourth which broke the former at a point considerably removed from the place where the normal allelomorph for "curled" lay, as a study of the salivary chromosomes showed. In effect, he separated the normal gene, which suppresses "curled," from many other genes with which it is ordinarily connected and found that it was no longer able to produce normal wings. In his analysis of this case, Panshin was able to exclude the possibility of modifying genes and proved quite conclusively that a normal allelomorph, in the truncated arm, no longer acted as a suppressor of "curled."

I am sure that those familiar with chemical chain reactions will find no difficulty in understanding how a gene's position might affect its action. Genes probably act, somehow, as little chemical factories, each manufacturing products which react with those of other genes and with the cytoplasm of the cell to give, in the end, a red eye, a normal wing, or a straight bristle. A change in the position of a factory might remove it far enough so that its products would not easily combine with the products of other genes necessary for its normal expression. If a break at one point in the chromosome affects the action of genes at other loci, it is clear that one must begin to think, not only in terms of local gene action, but in terms of chromosome action as a

whole, as Goldschmidt has recently insisted. At the same time I must emphasize the fact that we are here right at the edge of a new frontier. There are several indubitable cases of a position effect, but many alleged cases of this may be easily explained in other ways, and at present there are no good grounds for assigning to the gene's locus a far-reaching influence in the evolution of animals and plants.

THE SPECIATION PROBLEM

For almost two decades animal and plant cytologists have been comparing the metaphase chromosomes of closely related species with the hope that such studies would throw light upon the speciation problem. It was clearly recognized, by all, that the methods of comparison were crude, but at the time most of the work was begun there was no other way of attacking this very important aspect of the general problem. These metaphase chromosome studies, sometimes embracing as many as fifty to one hundred or more species, have shown that, in general, closely related species usually show the same chromosome number and chromosome configurations. Aside from polyploidy, a few variants are encountered in any extensive comparison, but such cases constitute a small minority. So far as metaphase chromosomes are concerned, there is little evidence of marked changes in these structures between species.

With the development of the salivary gland technique, the author realized and pointed out that it provided a very exact way of comparing the chromosomes of related forms, and this has proven to be the case. It is often possible to cross species and obtain viable offspring, but the fact that such progeny are sterile has been a bar to further analysis. But for the salivary chromosome comparisons, all that is needed is a hybrid larva; the forces of somatic synapsis will show the homologies of parts.

Of the many aspects to the speciation problem, the one that interests us here is the question of the role which chromosome rearrangements have played in the evolution of new species. It is well known that translocations, deletions, and inversions act

as physiological barriers in the sense that there must be the proper genetic setup to insure a normal fertility, and under such conditions a physiological race could develop which might be the initial stage of an incipient species. As more has been learned about the genetic behavior of these arrangements, it has appeared probable that the most important type, from a theoretical standpoint, is the inversion. Are these theoretical expectations realized in nature?

Comparison of the salivary chromosomes in hybrid larvae have been made for three different species crosses, and these all agree in showing that numerous small rearrangements have occurred since the two species concerned arose from a common ancestor. Dobzhansky and Tan report around fifty new gene associations in *D. pseudoöbscura* and *D. miranda* and one of my students, comparing the chromosomes of *D. melanogaster* and *D. simulans*, has found evidence for some twenty-four changes, all but one involving just a few bands. Obviously, if shifting the position of a gene affects its action, or the action of the chromosome as a whole, we have in these numerous rearrangements an adequate basis for the differences seen between the compared species without invoking any mutations in the genes themselves.

It is too early as yet to appraise the value of these and like discoveries for the speciation problem. We are here again on the frontier of a most promising land, where we may be destined to travel far in our search for the factors which operate in organic evolution.

We have now reviewed three frontiers of cytogenetics which have been opened by the salivary chromosome technique. I realize fully that in each of these fields questions have been considered to which no answers have been given, or can be given until we have penetrated farther into the unknown. At the same time, I hope that the nature of the problems which are being attacked has been made clear and that the reader, in some measure, may share with the specialists in this field a keen interest and anticipation of what future researches will reveal.

IX

ELECTRICAL POTENTIALS OF THE HUMAN BRAIN

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With every heartbeat an electrical potential arises which can be detected by placing metal electrodes on the body and leading the electric current to a suitable recording device. The pattern of this well-known heart potential is complicated, but much can be learned about the working of the heart from a study of the form of the curve as a function of time. The electrocardiographic apparatus is an accepted diagnostic tool.

In a similar manner, electrodes placed on the scalp of a person can detect electrical changes arising in the brain which are correlated with cerebral activity. They may also indicate pathological conditions, although the diagnosis of the abnormal by this method has just begun.

The brain potentials, recorded from the scalp, are so small that very high amplification is necessary to detect them and special precautions must be taken to prevent disturbing electrical fields or movements of the individual introducing an artifact into the record. Therefore, the subject lies quietly or sleeps on a bed in a soundproof room, completely lined with wire netting to shield from external electrical influences. The screened room may also contain the amplifiers, while the recording apparatus is some distance away in another room to prevent disturbing the sleeper.

The amplifier is the important part of any brain-potential outfit. Its characteristics limit the size of potentials which can be detected because of a peculiarity of any type of amplifier,

potentials arising in the set itself. This is seen when a high-gain amplifier is perfectly shielded and not connected with any source of current. Fluctuations of potential of the order of one microvolt appear, due to irregular emission of electrons from the filament of the first tube and thermal agitation of electrons in the resistances. Connected with a loud-speaker, these potential fluctuations give rise to "background noise" and effectively limit the useful amplification, just as the wave length of light effectively limits the magnification possible with the lenses of a microscope.

When the amplifier is connected with small discs of metal making good contact with the scalp of a person, very regular rhythms of potential occur, ten to one hundred times larger and quite distinct from the random fluctuations of the amplifier. They are commonly referred to as "brain waves," but have no relation to radio or other electromagnetic waves. They have merely the form of a wave when voltage is plotted against time.

That these potentials really come from the brain is certain. They are not found when electrodes are placed on the skin over other parts of the body. They change as a result of stimuli to the subject or during various emotional states or the concentration of visual attention. They differ markedly during lack of oxygen or sleep or anæsthesia. They differ in a child as compared with an adult and the pattern varies from person to person. Finally, electrodes placed on the brain itself detect the same potential patterns, affected by the same change in mental states, only the potentials are one thousand times greater, measured in millivolts rather than microvolts. In animals, Dusser de Barenne and McCulloch have shown by successive thermocoagulation of the various cell layers of the cerebral cortex that the potentials arise chiefly in the outer three layers.

Thus, the possibility that these potentials are due to scalp muscle, eye movements, or muscle tremors of any kind can be eliminated and their cerebral origin definitely established. Early skepticism as to their origin has been completely re-

moved. Similar potential rhythms and patterns also appear in cerebellum, thalamus, and other regions of the brain, but are not accessible from the surface of the scalp.

Brain-potential recording thus gives us an objective method of measuring some of the things which occur in the cerebrum and of correlating these with mental states and neural activity. The presence of the meninges, skull, and scalp merely reduces the size of the potentials and makes their localization less precise without greatly affecting the form or fundamental frequency of the rhythms. The method is crude because of the complexity of the processes being studied and because of our distance from them (thickness of scalp and skull). We are in the position of an observer outside a telephone exchange, trying to find out what is happening by recording the magnetic fields that accompany the ingoing and outgoing currents and the working of relays and cross connections inside. A new field of investigation has begun, that of electroencephalography, and it is fair to say that the start has been made possible by the recent great improvement in methods of amplification. We may predict that its importance will equal if not exceed that of electrocardiography in the study of the heart.

It is not surprising to find electric potentials arising in the cerebral cortex. Action potentials accompany the transmission of the nerve impulse and have been known since the time of Du Bois Reymond. From a nerve, the potential "spikes" in individual fibers appear in the record as a random jumble of fluctuations which are almost impossible to interpret, whereas from the brain the fluctuations are frequently so regular and sinusoidal that they appear to be drawn by a tuning fork.

Earlier workers had observed potentials from the exposed brains of animals, but the possibility of recording them from the head of man was not considered until Berger's investigation, begun earlier but published in 1929 and extending over succeeding years, in which the fundamental facts were thoroughly outlined. Partly through difficulties in technique and partly

through skepticism, little attention was paid to Berger's work until Adrian and Matthews (1934) in England and Jasper, Loomis, Gibbs, Davis, and others in the United States repeated much of it and called attention to the importance of this fascinating field of investigation on the borderland of physiology and psychology.

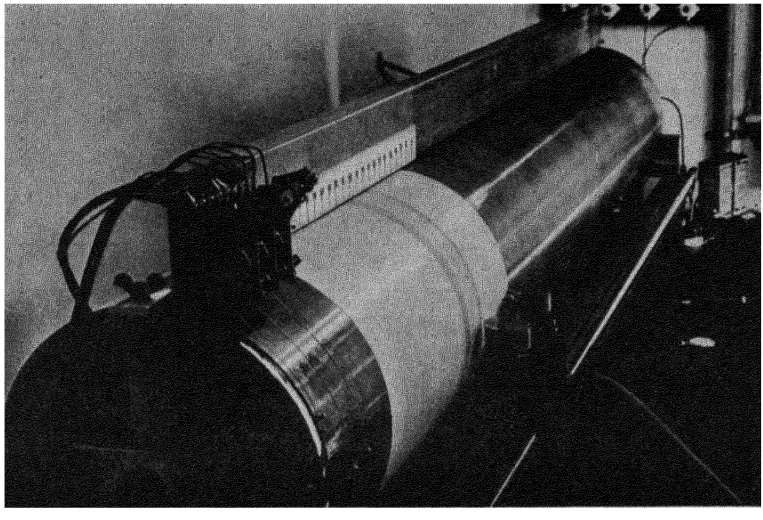


FIG. 70. Photograph of eight-foot drum, partly covered with paper, and pen carriage (lower right) which moves slowly to right describing three lines of brain potentials as the drum revolves. (*Jour. Exp. Psychol.*)

At the present time, many groups of investigators are continuing the studies so ably started by Berger. It is not possible to cover adequately the findings of all groups of workers in the space allotted. An excellent review has been published by Jasper in the *Psychological Bulletin* for 1937. I propose to point out the principal findings in the brain-potential field with special attention to work carried out in collaboration with Alfred L. Loomis and Garret A. Hobart III at the Loomis Laboratory, Tuxedo Park, New York. In this laboratory, ingenious equipment has been especially designed and constructed for record-

ing brain potentials on paper over long periods of time, as during a night's sleep. The electrodes are merely small discs of metal, one eighth inch in diameter, covered with salt paste and attached to the scalp by collodion. Fine wires lead from these to the amplifiers.

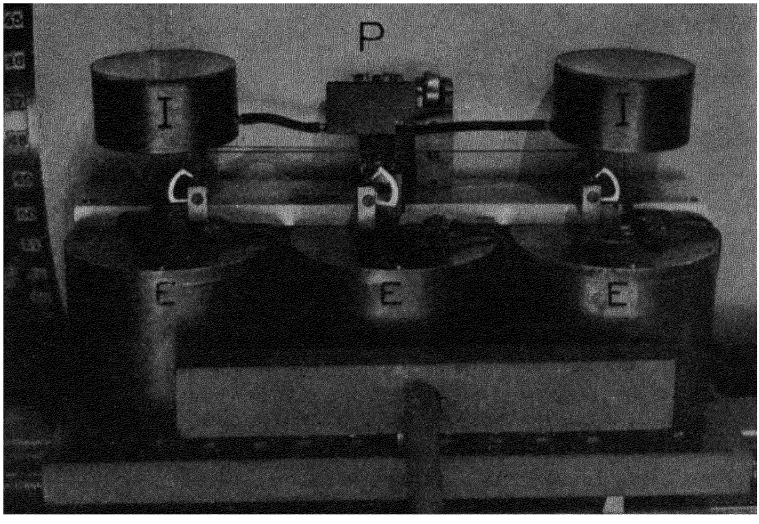


FIG. 71. Photograph of pen carriage (Fig. 70) with three pens (*P*) in position to write on drum. The pens are activated by levers from three coils of wire (through which the amplified brain currents pass) in the center of three electromagnets (*E*). Reserve ink reservoirs (*I*) supply ink to the pens. Drum in the background. (*Jour. Exp. Psychol.*)

In earlier work, three quite independent amplifying systems allow comparison of potentials at three different parts of the head simultaneously. The fluctuation in potential is recorded by three pens, actuated electromagnetically, which describe a curve on paper wrapped on a revolving drum or kymograph. This drum, shown in Figure 70, is a steel cylinder eight feet long and forty-four inches in circumference, revolving once a minute. The pen carriage, shown in Figure 71, moves slowly sideways as the drum revolves, the pens thus describing a close

spiral of three inked lines which may run for many hours. Part of such a record is shown in Figure 78.

Microphones and loud-speakers in sleeping room and in recording room allow two-way or one-way communication between the two rooms as desired. Signals may also be automati-

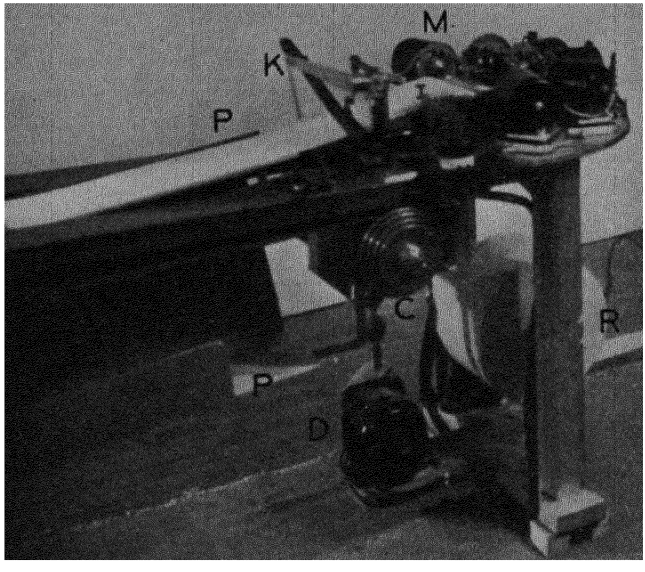


FIG. 72. Paper-cutting brain-potential recorder showing roll of paper (*R*) which passes under pens activated by six magnets (*M*) and is cut by a knife (*K*) each thirty seconds, turned over, and laid in a pile (*P*). The machine is driven by motor, *D*. The contact wheels *C* are so arranged that stimuli can be sent to the subject at predetermined times. (*Jour. Neurophysiol.*)

cally sent to the subject at a predetermined time and recorded on the drum, or the subject may signal and thus correlate subjective phenomena with objectively recorded changes in the brain.

A more recent development with six amplifiers allows potentials from six different regions of the head to be simultaneously recorded in detail on paper from a roll seven inches wide.

Thirteen pens write on the paper, six from untuned amplifiers, six from amplifiers tuned to any desired frequency, one a signal pen. When ninety centimeters (30 sec.) of recording has occurred, the paper is automatically cut by a moving knife, turned over, and placed in position over the previous sheets ready to

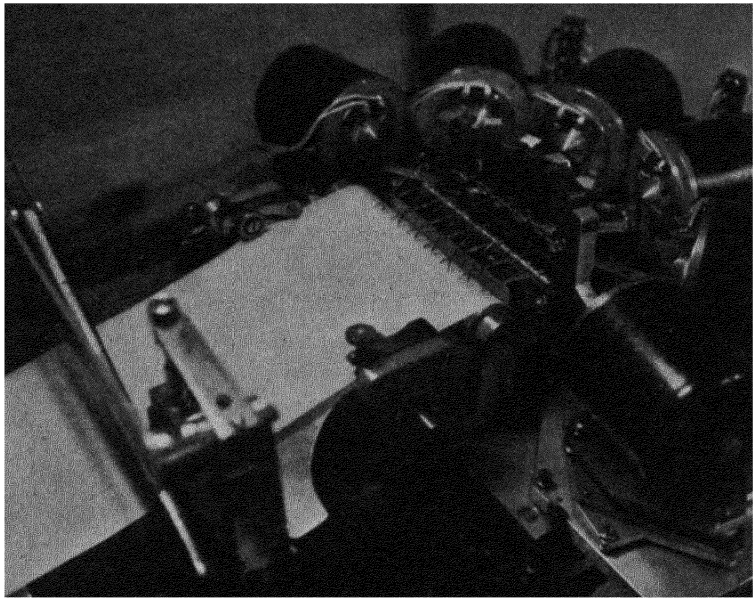


FIG. 73. Enlarged view of electromagnets and thirteen pens. The flying shears are clearly shown. (*Jour. Neurophysiol.*)

be bound together for permanent keeping. The new recording machine is shown in Figure 72 and the electromagnetic ink writers in Figure 73.

It is apparent that there are many different approaches to the study of brain potentials, which may conveniently be considered as follows: (1) the standard electroencephalogram—types of potential; (2) variability of pattern—effect of age, sex, mental and emotional states, attention, stimuli, etc.; (3)

distribution of potentials over cerebral cortex; (4) sleep, hypnosis, anæsthesia; (5) effect of drugs; (6) pathological conditions.

The work at Tuxedo Park has been concerned almost entirely with normal individuals and particularly with sleep. Over 200 records have been taken from 80 individuals, many for an 8-hour period, and representing some 820 hours of recording.

If a normal person rests quietly with eyes closed, rhythmic potential differences can be obtained when any part of the scalp is compared with any other part. If some neutral point like the ear or mastoid process (which is not undergoing changes in potential) and the back (occiput) of the head are used, a surprising amount of regular electrical activity is continually recorded. Figure 74 shows this activity in two different persons. The best-known pattern is the so-called alpha or Berger rhythm, a ten per second variation in potential of ten to one hundred microvolts amplitude. Because of its striking character and because it disappears when the subject opens his eyes to view an object or is startled or emotionally upset or asleep, it has been most studied. The form is that of a sine wave and in some individuals it is continually present, in others it is almost completely absent. Thus, we can immediately group persons into the alpha type and the nonalpha type with all possible gradations between the two extremes. These can be designated by an alpha index giving the per cent of the time the alpha rhythm is present under certain specified conditions. In four hundred normal adults, Davis and Davis find the mode for alpha index distribution at 75 per cent. Rubin has studied its distribution over the head.

The amount of alpha rhythm is extraordinarily constant in any one individual from day to day and apparently must represent some fundamental peculiarity in the make-up of an individual. This idea is supported by a study of identical twins in which the records are found to be almost identical in general pattern. The similarity is most striking in some cases where

both individuals of a pair of twins present a unique peculiarity of pattern not found in the record of any other person. It would seem that the alpha and nonalpha characteristic is possibly an inheritable quality.

Studies so far reveal no obvious relation to high or low intelligence, to sex, to superficial likes or dislikes or other clearly

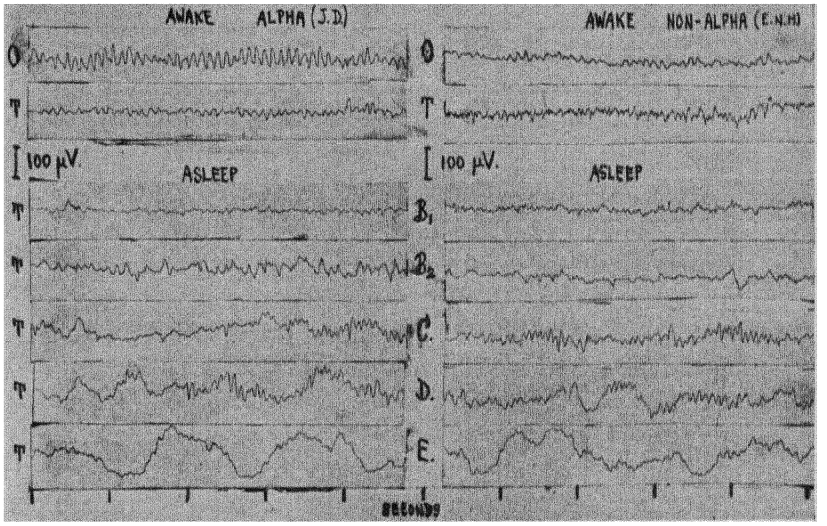


FIG. 74. Brain-potential activity in an alpha type of person (left) and a low alpha type, awake and asleep. O = occipital region, T = top of head. B_1, B_2, C, D, and E refer to states of sleep. Note that when asleep the pattern is much more alike than when awake.

apparent characteristics of the person. According to Saul, Davis, and Davis, a group of persons undergoing psychoanalysis showed the same distribution of alpha index as is to be found in the general population. However, there appears to be a relationship to certain

fundamental psychological trends, usually more clearly revealed in dreams and their associations than in the superficial behavior of everyday life. Strong and plentiful alpha waves are found in passive, dependent, patient, receptive individuals, while alpha waves are weak and few

in men and women with a consistent internal drive, particularly a competitive drive for prestige and power.

The alpha rhythm persists if the mind is made as blank as possible and also if a line of thought is followed as when the person is read to. If the attention is directed to listening for a just-audible sound, the alpha rhythm is even more pronounced, but if the eyes are opened and the attention is directed to anything visible, even a faint point of light, the alpha potentials in most persons disappear immediately, to return when the light disappears. On the other hand, mere light stimulation itself without visual attention, without an attempt to see, will not affect the alpha rhythm.

In some persons opening the eyes in a completely dark room will abolish the rhythm, while in others this does not occur unless they expect to see something, or it is suggested that they will see something. The effect is especially marked in a person hypnotized. In the dark, one can suggest that an object is seen and the alpha rhythm disappears to return again when the suggestion is made that it is dark. The converse is also true for the hypnotized (but not for normals), that in the light the alpha waves appear if it is suggested the subject sees nothing.

A sudden sound to which the attention is markedly directed, or apprehension, or a situation in which there is the element of puzzlement or the desire to act suppresses the alpha rhythm, which is then replaced by more irregular potentials of higher frequency and lower amplitude.

An interesting situation, which is analogous to conditioning, is observed when a subject lies quietly in the dark with eyes open. A low tone stimulus lasting a few seconds will not abolish the alpha rhythm, but a light stimulus lasting the same time will. If the low tone and the light are both presented simultaneously several times in succession at half-minute intervals, the alpha waves will of course stop, due to the attempt to see induced by the light, but if now the tone alone is sounded, the alpha rhythm also stops although no light appears. However,

the effect of the tone alone will not last more than one or two times, as the conditioning is not permanent. Man, unlike the lower animals, is easily conditioned and easily unconditioned.

Persons with little alpha rhythm, nevertheless show potentials of eighteen to thirty per second which can be roughly grouped together as beta rhythm or, if still higher frequencies (thirty to fifty) are found, they have been called gamma rhythms, but are almost impossible to separate from muscle potentials. It must be pointed out, however, that these rapid rhythms are not as regular as the alpha and the word "frequency" can only be applied to them if we use it in the sense of an average frequency.

There are also to be clearly distinguished the characteristic high-amplitude spikes of epileptic and petit mal seizures and the slow or delta waves as well as the random potentials, the K waves, and the fourteen per second "spindles" of sleep, to be considered later.

The normal awake pattern of potentials varies with age. Two points are of note: first, the lack of potential in young babies a few days old and the lack of an alpha rhythm in babies a few months old, whose record looks much the same, awake or asleep; second, the increase in frequency of the alpha rhythm from four or five a second at four months until, as the child grows older, the normal nine to eleven per second is established at the age of nine or ten years (Lindsley). This rate may then be found in persons eighty years old, a perfectly regular rhythm apparently extending through life.

The distribution of potential pattern over the surface of the brain offers some interesting problems. It has long been recognized that in most persons, but by no means in all, the alpha rhythm is most marked from the occipital region at the back of the head. Fortunately, large areas of the head exhibit the same potential pattern simultaneously, so that if six electrodes are attached to the scalp and potential changes in far front, top, and back of head on both right and left sides recorded, a very

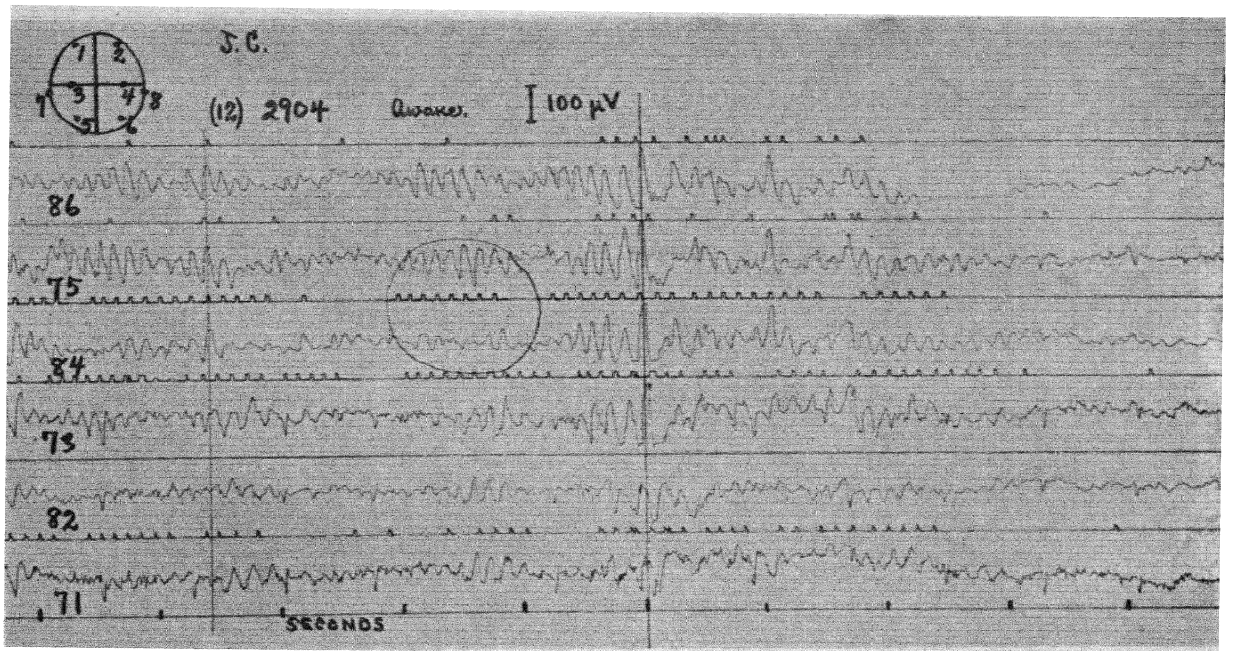


FIG. 75. Brain-potential activity in a person awake but drowsy, recorded from six different regions simultaneously. Figures at left indicate placement of electrodes looking down on head from above as in diagram (upper left corner); 7 and 8 are the left and right mastoid bones, places where brain-potential change does not occur. Note that the whole top of head changes in potential simultaneously, except occasionally, as in circle, where alpha rhythm occurs on 5 but not on 4. Disappearance of alpha rhythm at right indicates drowsing. (*Jour. Neurophysiol.*)

good idea of the distribution of potentials can be obtained. It is found that a rather large potential from one head region will not appear in the records of other regions. Consequently, the electrodes tell what is going on under them and do not pick up much electrical spread of potential from near-by areas. Front top and back, generally, have their characteristic patterns, although large areas of the head in the top and back regions may change in potential simultaneously. Corresponding symmetrical positions on right and left sides usually give identical or similar potential patterns, but a disturbance may occasionally appear on one side and not on the other. It is impossible adequately to describe the detail of the ever changing patterns, for at times any region may exhibit a potential pattern relative to any other. When a spontaneous disturbance occurs that alters the pattern or a change in pattern as a result of external stimuli appears, large areas of the brain change simultaneously. This is well seen in Figures 75 and 76. We are led to the conclusion that the cerebral cortex generally acts as a whole, but with secondary differences in different regions.

Potential distribution over the human brain can best be visualized by comparison with the disturbances in a slowly boiling pool of liquid in which there are eddies and bubbles of gas continually rising to break in ripples at the surface. Large bubbles give rise to large slow waves, while small ones give rapid rhythms. Sometimes the disturbance occupies a large area, sometimes a small. Bubbles appear in a nearly symmetrical pattern on right and left, although a short disturbance may occasionally occur on one side only. On the other hand, front and back show marked differences. In general, many small bubbles arise at front (beta rhythms) and medium-sized, very regular bubbles at back (alpha rhythms). The distribution is continually changing, but any large disturbance affects the whole. The pattern alters completely as sleep begins, passing through a definite sequence of changes until finally the whole liquid is pulsating to large regular bubbles, about one a second.

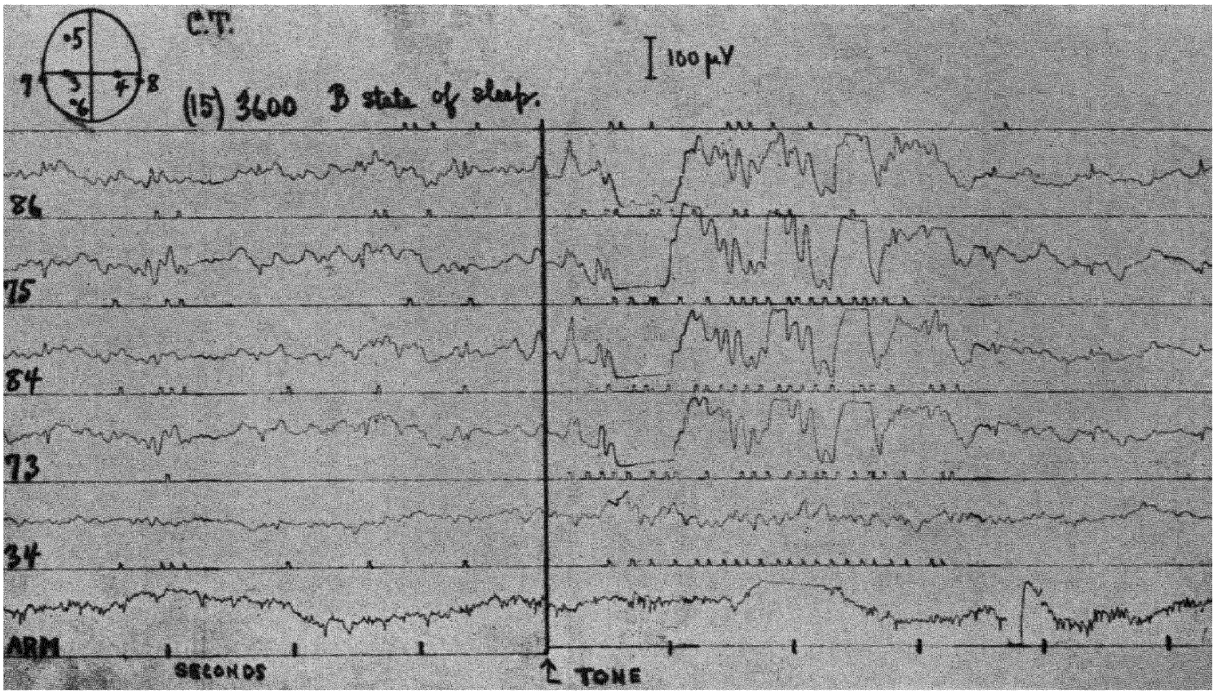


FIG. 76. Brain-potential activity in a person asleep recorded from four different regions simultaneously. Figures at left indicate placement of electrodes as in Figure 75. Note that the disturbances (K waves) as a result of a tone signal occur simultaneously at 3, 4, 5, and 6. (*Jour. Neurophysiol.*)

We are thus led to look upon the cortex, not as an inactive network of neurons, but as rhythmically functioning groups of cells which are affected by incoming impulses from the exterior or from other regions of the brain. Whether the rhythms of the cortex are normally initiated by some pacemaker in the lower centers of the brain or not, there can be no doubt of spontaneous electrical activity from regions completely cut off from incoming sensory impulses. Gerard and Young have obtained them from the isolated olfactory lobes of the frog's brain, and Bremer from the cortex of the cat, severed from all incoming pathways.

Rhythmical activity is common in the nervous system. Sense cells and motor nerve cells discharge at rates from ten to one hundred per second, depending on the intensity of stimulation. Each discharge is an action current, a spike. In the brain, spikes can also be observed, but the alpha rhythm is a slow sinusoidal potential change which can best be referred to as a group of neurons beating in unison. We may then regard the disappearance of the alpha rhythm on light or other stimulation as due either to (1) a true inhibition of the beat or to (2) a break-up of the rhythm, in which the cells discharge at random rather than synchronously. We may compare the situation to that of a company of marching soldiers. They may receive the command to halt or to break step. In either case, the orderly rhythm of the march is broken. Applied to cortical potentials, it is almost impossible to differentiate between these two views.

Some of the most striking changes in brain-potential patterns occur when a person goes to sleep. At least five different, distinct states of sleep may be recognized that occur in a definite sequence and that correlate with depth of sleep. In a person in whom alpha rhythm is prominent these are:

A. Interrupted Alpha. The normal, regularly occurring alpha rhythm becomes diminished in amplitude, slightly slowed in

frequency, and interrupted for increasing periods of time. It can be shown that these interruptions are correlated with a depression of sensory perception and a feeling of "floating." They may appear from one part of the brain and not another, as if the brain "went to sleep" in one region before another. Such a situation makes any attempt to define the "moment" of sleep rather meaningless.

B. Low Voltage. Complete disappearance of the alpha rhythm with some random and delta (low-frequency) waves.

C. Spindles. Moderate delta waves with the appearance of fourteen-per-second rhythm lasting one to two seconds (spindles).

D. Spindles plus Random. The spindles continue together with large delta waves, 0.5 to 3 per second and an amplitude as high as 300 microvolts.

E. Random. The spindles become inconspicuous, but the large delta waves persist.

These states of sleep are illustrated in Figure 74.

While the alpha rhythm is generally of greatest amplitude from the occipital region but may be greater on top or even from the front of the head, the spindles are most marked from the top and the random comes from all parts of the head, but predominantly top and front.

The nonalpha type of individual passes into the same states of sleep as the alpha type, but it is much more difficult to distinguish states *A* and *B*. State *B* is characterized by less high frequency potentials and the appearance of short delta waves. In general, the nonalpha type of record looks more alike, awake or in light sleep, until the later stages of spindles or random waves appear.

One very clear fact has appeared from all sleep records, the continual shift of a person from one state of sleep to another. The changes may be very sudden or they may be gradual, espe-

cially from *C* to *D* and *D* to *E*. In Figure 77 these shifts are plotted for two persons of the alpha and two of the nonalpha type. The *major* movements of the individuals are also marked by crosses. Such a record may be called a hypnogram. The actual potentials in a similar record are shown in Figure 78.

It will be noted from Figure 77 that during a night's sleep there is not always a slow continuous change from one state or

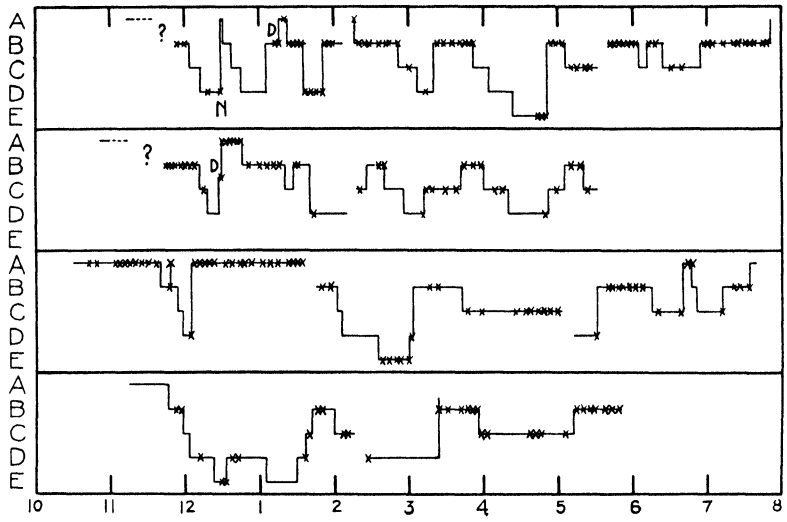


FIG. 77. Night sleep records (hypnograms) of four individuals, the first two nonalpha type, the last two alpha type, showing changes in states of sleep. *A*, *B*, *C*, *D*, *E* (vertical) during the hours of the night (horizontal). The crosses represent major movements. *N*, noise, *D*, a dream. (*Jour. Exp. Psychol.*)

type of sleep to another, no continuous curve of sleep, but there is a continuous shift of states, sometimes down, sometimes up. Frequently these changes are associated with movement, but not always so. Frequently stimuli—noises, light, or mechanical disturbances of one kind or another—will shift the sleeper to a higher level, sometimes to the state immediately above and sometimes to two or three states above. The record gives us an objective method of studying the effect of stimuli on a sleeping

person. Blake and Gerard have correlated the potential pattern with depth of sleep.

Dreams are not represented by any peculiar type of potential, but probably occur in a state of sleep. Subjects have been observed to report dreams immediately after the *B* and *C* states.

It is interesting to note that during hypnosis, where the typical condition of catalepsy was apparent and the subject was amenable to suggestion of the hypnotist, the alpha waves were prominent, as in the awake state, so that the term "hypnotic sleep" is a misnomer.

The effect of suggestion on the alpha rhythm in relation to vision in a hypnotized person has already been described. Another interesting experiment related to startle was carried out during hypnosis. After suggestion that the subject would feel nothing, a pin was pushed into his arm. No outcry or movement was made and no muscle potential appeared in the record, but the alpha waves stopped for some seconds and then returned. When the pin was removed, no change in the alpha rhythm occurred. Evidently the brain responded to a situation which was completely overlooked by the individual.

One feature of sleep is loss of consciousness. Davis, Gibbs, and collaborators find that if this same mental state is brought about by breathing mixtures low in oxygen the alpha rhythm disappears, to be replaced by large slow waves similar to those of sleep. The same is true of the barbiturate anæsthetics and carbon-monoxide poisoning. Dusser de Barenne and McCulloch have shown that the convulsant drugs, like strychnine, applied to the cortex of animals, give large spikelike potentials which spread to the boundaries of an area and often to other regions, but these have not been systematically studied in man, although Lennox, Gibbs, and Gibbs have produced artificial epilepsy waves by injection of camphor.

It is obvious that brain-potential investigation has special interest for the psychologist and the psychiatrist. Travis, Knott

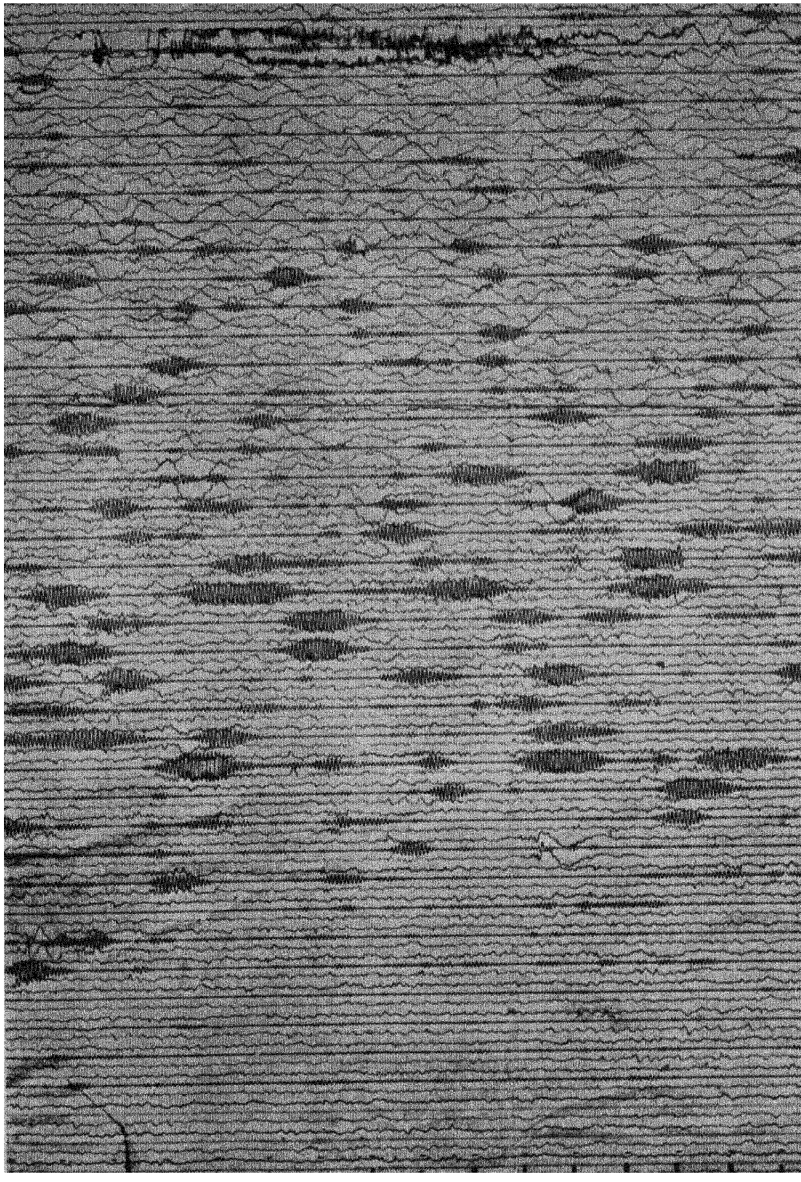


FIG. 78. Section of an eight-hour record during a night's sleep taken on the eight-foot drum. Three pens record potentials from different parts of the head, one pen from an amplifier tuned to fourteen per second rhythm and showing the "spindles" very clearly. From bottom to top the states of sleep are: *B*, *C*, *D*, and *C*. The black high-frequency potentials at the top are due to movement of the sleeper. (*Scientific Monthly*.)

and his collaborators, and Durup and Fessard have applied the method in psychological studies. Berger's original purpose was to use brain-potential records for studying mental disorders. A large amount of work has already been carried out, and in certain disorders definite potentials can be associated with the disease. In other cases the situation is far from clear.

The most apparent relation between the form of brain potentials and a disease is in epilepsy, both the grand mal and the petit mal type, studied by Berger, Jasper and Hawke, Golla and collaborators, and particularly by the Gibbses and Lennox. In the grand mal attack (Fig. 79), there is usually decreased amplitude of the normal rhythm for several seconds before any outward sign of an attack appears, increased frequency in the tonic phase, followed by an explosion in the clonic phase of large potential bursts, 500–1,000 microvolts in amplitude. The form is difficult to make out because of movement artifact and muscle potential, but they are fundamentally sharp spikes. These bursts slow down and drop out and are followed by a period of little potential change or of delta waves, corresponding to the period of postparoxysmal stupor.

In petit mal, the seizure patterns, usually preceded by a few slow, irregular waves, also appear several seconds before any clinical signs of disturbance. They consist of spikes, each followed by a slow wave, repeated some three times a second, which gradually become fewer and disappear.

The origin of these specific types of wave, and similar ones which have been described as psychomotor attacks, can be traced by localization experiments and their spread over the cortex followed. In grand mal the whole cerebrum is involved. At other times the above wave patterns may appear for a short time with no clinical evidence of a seizure. Environmental conditions may convert these incipient patterns into a definite attack. Epilepsy is thus described by Gibbs, Gibbs, and Lennox as a "paroxysmal cerebral dysrhythmia," a disturbance of the normally regular brain-potential rhythms. In grand mal these become

abnormally fast, in a psychomotor attack abnormally slow, and in petit mal they alternate between fast and slow.

In superficial brain tumors, as shown by Berger, by Walter,

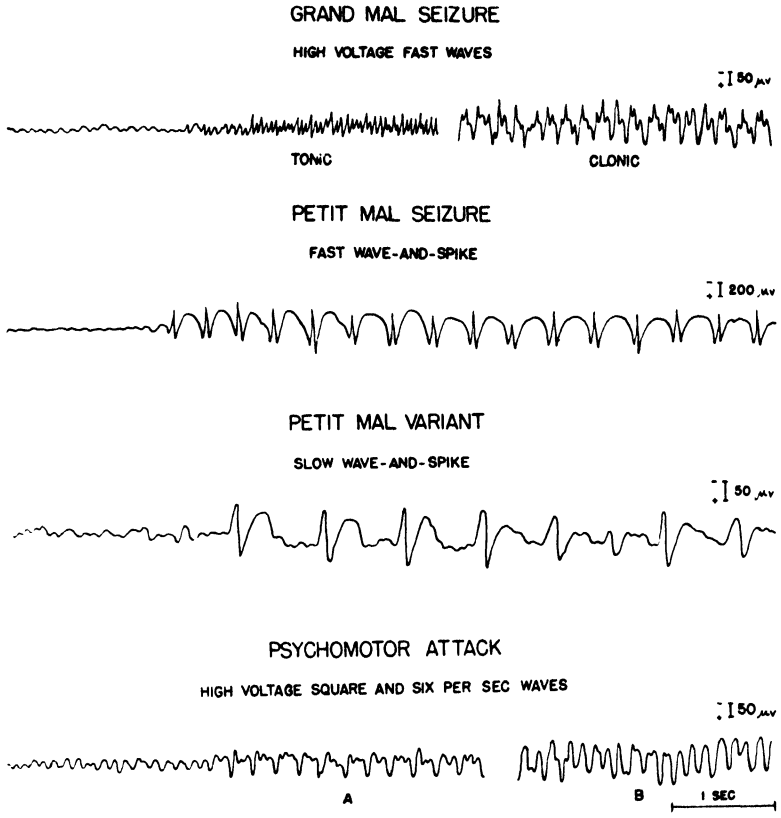


FIG. 79. Epilepsy and psychomotor potentials recorded by and reproduced through the kind permission of Dr. F. A. Gibbs of the Harvard Medical School.

and by Case and Bucy, a change in the electroencephalogram can be noted, namely the appearance of slow waves. These come not from the tumor itself but from regions around it. Similar slow waves are also characteristic of increased intracranial pres-

sure in general. If the pressure is local, as around tumors, the position of the tumor can be determined by localization methods.

On the other hand, there are many mental disturbances, studied by Berger, Lemere, Davis, Hoagland, and collaborators, in which no marked change in brain-potential pattern can be detected. This is true in some types of manic depression and in schizophrenia, although in others there have been described large slow waves or large irregular waves measured quantitatively by a delta index. The situation is not yet entirely clear in schizophrenia, but "episodes" of abnormal pattern do occur in many patients.

In congenital feeble-mindedness it is not always possible to say the record is abnormal, although again there is a tendency to slow waves, especially in the very deficient cases. Slow waves are characteristic of children and the most apparent generalization that Kreezer could make from a study of idiocy is that the record is a reflection of the mental age and not of the chronological age of the afflicted. Slow waves and irregular waves are also characteristic of sleep. Perhaps we have here more than a mere coincidence. Perhaps parts of the brain of the feeble-minded and the mentally ill do go into a condition similar to that of sleep, similar to that of early childhood. A study of brain potentials during sleep may throw much light on the changes in certain mental disorders.

X

ANIMAL METABOLISM: FROM MOUSE TO ELEPHANT

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DOMINATED by the basic thesis that the study of animal physiology is primarily of value in accordance with the extent to which it amplifies our knowledge of human physiology, the Nutrition Laboratory at Boston of the Carnegie Institution of Washington has for three decades been making surveys of the energy metabolism of many different animal species.

Comparative anatomy shows precisely the relative lengths, for example, of the femurs of mice, men, and mastodons; the ultimate size which the different species may attain; and the rate of growth and development. All these measurements are important and particularly helpful in unfolding the close relations, as shown in paleontological research, between the animals of the past geological ages and those now present, but measurements of the relative lengths of the femurs of mouse, man, and elephant have a restricted value otherwise, for there is no real common denominator.

Comparative physiology, which deals with the *living* rather than the *dead* animal, introduces many measurable factors that are based on a common denominator, namely, vital activity. Those signs of life represented in body movement, though vastly different in, for example, the canary and the chimpanzee, nevertheless are not only visible but also measurable with respect to their rate, intensity, and extent. It is, however, difficult, if not impossible, to appraise quantitatively comparable visible

activities in themselves. To record each visible movement, gross or minor, and attempt to measure life activity by the number of times the limbs of an animal are moved is impracticable. Even were such a quantitative record of body movements in any sense approximated, there still remain numerous other important activities which are not visible. In the quiet, resting animal, for example, heat production may be entirely due to invisible activity. Hence, though body movements, in general, have been classified in several groups, their summation really offers no approximation to a common quantitative measure in an attempt to evaluate these various visible activities.

Since, however, every movement, visible or invisible, is inevitably accompanied by the production and liberation of heat, and the amount of this is directly proportional to the intensity of movement and the length of time spent in movement, the physiologist has a real common denominator for the measurement of animal activity or metabolism in the unit of heat measurement, the calorie. No matter what the size of the animal, its vital activity is expressed in calories, and these calories can, under the proper conditions, be exactly measured. The calorie is a measure of vital activity, a yardstick, applicable to every living animal. A mouse produces heat and an elephant produces heat, and both of them have this function in common with the most interesting of all animals—Man. It is with the use of this caloric yardstick that we are chiefly concerned in the following pages.

No argument is necessary to show that a 4-ton elephant produces more heat than a 21-gram mouse, but this very comparison raises the important question as to whether there is a basic relationship between these two enormously differing heat productions. Less disparity exists in heat production between a mouse and an 80-kilogram man. To consider extremes for a moment, there is a breed of mouse weighing but 8 grams, the so-called "dwarf mouse." In comparing the heat production of the 8-gram mouse with that of the 80-kilogram man, it must be

borne in mind that the human body is the equivalent in weight of 10,000 8-gram mice. Is the heat production of the man 10,000 times that of the mouse? This is an important question and, fortunately, it can now be answered. The 8-gram mouse, when quiet and not digesting food, may have a metabolic rate as low as 1 calorie in 24 hours; that is, in 24 hours, *if it remained quiet,* it would produce but 1 calorie. This is the lowest level of heat production that has ever been noted with any strain of mice. On this basis the 10,000 mice will produce 10,000 calo-

TABLE XVIII

Basal Metabolism of the Dwarf Mouse at Thermic Neutrality (34° to 35° C.)

Mouse No.	*Weight*	*Heat Production per 24 Hours*		
		Total	*Per Kg.*	*Per 9 $w^{2/3}$*
	Gm.	*Cal.*	*Cal.*	*Cal.*
29	7.98	.918	115	256
30	8.09	.979	121	270
31	9.09	1.118	123	283
46 & 32	8.14*	.985	121	271
Avg.	8.30		120	270

* Average weight per animal.

ries per day, whereas the 80-kilogram man, equaling in weight the 10,000 mice, will produce under the same conditions of minimum activity hardly 1,600 calories per day, or about one sixth of the daily heat production of the mice.

There is little here in the data just given to suggest any correlation between the two animals in their heat production, and this fact troubled the earlier physiologists a good deal. About 1880 an ingenious concept was brought forth to the effect that animals cannot be compared directly on the basis of size. The argument was somewhat as follows: Animals produce heat to keep themselves warm. They lose heat from the body surface and, hence, their heat production must be closely related to the

surface area of the body. The efforts made to measure the surface area of the animals studied by mechanical, photographic, and other means furnish a history in themselves. Suffice it to say that from the experimental data it was established that if the two-thirds power of the weight in grams is multiplied by the constant factor 10, the surface area of the body in square centimeters is fairly closely approximated. With some animals the constant is not 10, but may range from 9 for the mouse to 12 or more for the pig. For purposes of comparison the value 10 may reasonably be used for all animals and, given the weight, the surface area is readily calculated.

To compare animals with respect to their metabolism, the complete index of all vital activities, the several species must be measured under comparable standardized conditions. Owing to their pronounced effects upon metabolism, muscular and digestive activity must obviously be absent, but even with these two disturbing factors ruled out it must be certain that the animal is not producing heat simply to combat the cold. Hence it must be measured at thermic neutrality, usually in warm-blooded animals, at an environmental temperature of not far from 28° C. Animals differ enormously in their reaction to cold. It is easy to understand why the hairless mouse or dog shivers in a temperature in which the polar bear is quite unconcerned, but it is not so easy to understand, for example, why a sheep withstands 0° C. without an increase in heat production, while the densely feathered goose, with a covering seemingly as protective as that of the sheep, reacts as soon as the temperature goes below 15° C. Finally, it is now well established that the metabolism is specifically high during the growth period, and hence measurements for comparative purposes must be made only on adults. The necessity of clearly recognizing all these important factors influencing metabolism has been slow to be realized, and but now for perhaps the first time the physiologist is in the position to make a survey of a rather extensive field in which the comparisons will not be vitiated by muscular or di-

gestive activity, by the production of heat to combat cold, or by the error of comparing the youth with the adult. In all the comparisons given below, these four factors are as rigidly ruled out as is possible with temperamental, noncoöperating animals. At least it can be said that never for a moment have these factors been forgotten.

It is quite unnecessary to go into the details of the techniques employed for all of the many animal species studied, but because of the peculiar importance of the *mouse* at one end of the weight range and the *elephant* at the other and because the present study was basically made for the express purpose of throwing light, if possible, upon the physiology of man, we shall look briefly into the techniques employed for the measurement of metabolism in mice, men, and elephants.

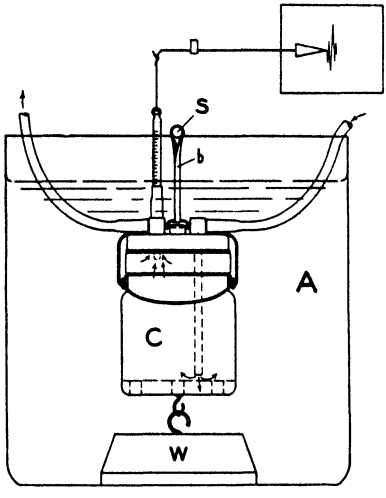


FIG. 80. Schematic outline of respiration chamber used for metabolism experiments with mice.

For the mouse, a very small chamber is needed, a half-pint fruit jar, ventilated with about 20 cc. of air per minute. All the air passing through this chamber is collected in a spirometer, and a sample of this air is taken directly from the spirometer and analyzed to determine the carbon dioxide increment and the oxygen deficit, as compared with outdoor air of known and constant composition.[1] The basic processes employed—ventilating a respiration chamber of suitable size with pure outdoor air, measuring the air used for ventilation, drawing an aliquot sample and analyzing it on the

1. This can be done satisfactorily at present only with the gas-analysis apparatus of Dr. T. M. Carpenter.

gas-analysis apparatus—can be used for all animals, the elephant as well as the mouse.

The record of activity is of the utmost importance with all animals. As can be seen in Figure 80, the respiration chamber of the mouse is literally suspended in water and so delicately balanced that the least change in the center of gravity of the mouse in any direction is registered by the sensitive recording device. If the mouse remains quiet, the kymograph line should theoretically be straight, with only occasional breaks as a result of minor movements. Unfortunately, however, a mouse is almost never still; a quiet mouse is a rarity. Therefore, hours have to be spent making innumerable collections of air samples, a great many of which are later rejected as unfit. Of those analyzed, only a small proportion may be accepted as obtained under conditions approximating basal, that is, with the mouse exhibiting a minimum of activity.

Since the mouse is the lightest animal used in these studies and thus must be taken as the origin of any general curve, obviously every attention must be paid to the establishment of its true basal metabolism. It so happens that within this species there is a very wide weight range. The average white or albino mouse weighs about 21 grams, but there are two other races which show great differences in weight. The so-called "fat mouse" may weigh 70 grams and the so-called "dwarf mouse" only about 7 or 8 grams. Thus these two races show a tenfold difference in weight. The dwarf mouse has an incredibly low basal metabolism when measured under standard conditions. The first reported basal value for the common mouse was 1,188 calories per square meter of surface area per 24 hours. But each successive research on the mouse has indicated a lower basal value. The three races differ in their metabolism, for, as can be seen in Table XIX, the minimum heat production ranges from 110 to 135 calories per kilogram of body weight and from 250 to 475 calories per square meter of surface area per 24 hours. Here in one species, then, is a variation in basal heat produc-

tion, referred to surface area, of from 250 to 475 calories, or a difference of nearly 100 per cent. The 415 calories per square meter produced by the albino mouse is approximately one third

TABLE XIX

Minimum Heat Production of Three Races of Mice

Mouse	*Body Weight*	*Heat Production per 24 Hours*		
		Total	*Per Kg.*	*Per 9 $w^{2/3}$*
	Gm.	*Cal.*	*Cal.*	*Cal.*
Albino	21	2.85	135	415
Fat	59	6.44	110	475
Dwarf	8	.90	110	250

of the 1,188 calories originally established for this animal. The present value has been determined under conditions with minimum activity of the experimental animals.

In no way can the great metabolic differences present in small animals be better realized than by the examination of a curve made only four years ago, representing the values *then* avail-

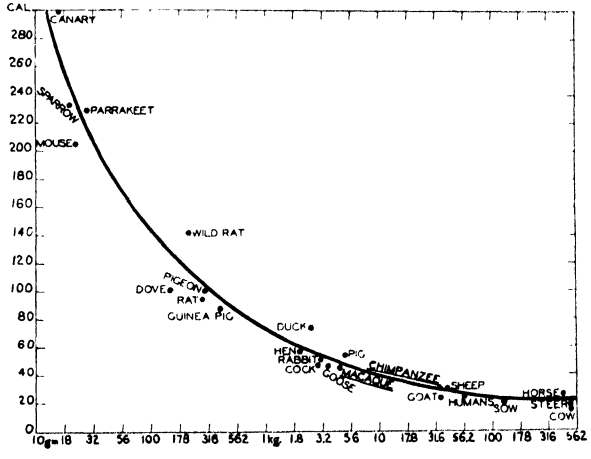


FIG. 81. Heat production per kilogram of body weight per twenty-four hours of different species of animals.

able for all animals. At that time the heat production of the mouse was recorded as about 210 calories per kilogram, whereas at the present day, as can be seen in Table XIX, the value is but 135 calories per kilogram. This curve (Fig. 81) also gives the first general picture of the trend of metabolism per kilogram of body weight for all the various animals studied, beginning with the very small birds and mice and extending, in this particular case, to the horse. At that time the elephant had not

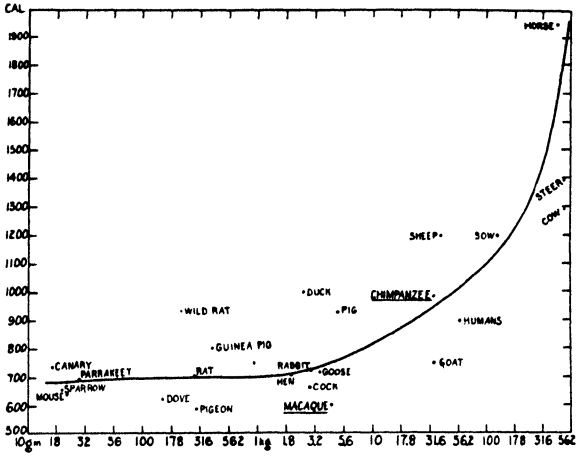


FIG. 82. Heat production per square meter of surface area per twenty-four hours of different species of animals.

been studied, and in order to record all the measurements in one chart, it was necessary to plot the weights on the logarithmic basis. There is no question but that, as has long been recognized, there is a general tendency for the heat production per kilogram to decrease as the weight increases.

If the data presented are examined on the basis of the heat production per square meter of surface area (Figure 82), it will be found that though formerly it was believed, as stated above, that the metabolism per square meter was uniform for all animals, the situation is anything but this. In fact, the heat produc-

tion per square meter is lowest in the small birds and, generally speaking, increases with the weight of the animals. It is important to remember that in these curves the mouse has not been assigned the very low values known to exist at the present day.

The position of the human organism in these charts is well worth noticing, and in the heat production per square meter we begin to see wide differences in animals of the same weight. For example, the metabolism of the macaque is much lower than that of the duck or the pig, and the metabolism of the sheep is much higher than that of the goat. These are striking species differences which demand further consideration and study. The curve in Figure 82 shows at once that the concept of the uniformity of heat production per square meter of surface area must be immediately given up.

From this survey it was clear that, if possible, more accurate determinations must be made upon the mouse in order to find the precise point at which to start the curve. Likewise, that studies must be made on as large animals as possible, one of these animals obviously being the elephant. In the chart shown in Figure 82, the mouse is assigned a value of about 650 calories. At the time this graph was made (1934) the dwarf mouse had not been studied, but as already shown the albino mouse, in accordance with the present values, produces only about 400 calories, and the metabolic rate of the dwarf mouse is much lower than this, or 250 calories (Table XIX).

The modern techniques of measuring metabolism in man are so well known in nearly every laboratory that it is quite unnecessary to go into the description of them here. Suffice it to say that with the present techniques the basal metabolism can be determined in periods as short as ten or fifteen minutes or even less, and hundreds, if not thousands, of measurements are being made practically every day. For many years the Nutrition Laboratory has been accumulating its values on perfectly normal individuals. We distinctly differ with those who assume that hospital patients can be considered normal. Observations

on hospital normals may have their value for comparison with those on hospital patients, but they do not have a physiological value. Our own subjects were all normal individuals, many of them laboratory assistants, students—intelligent, coöperative subjects—and in the course of time many data have been accumulated. Those for men are shown in Figure 83, in which the

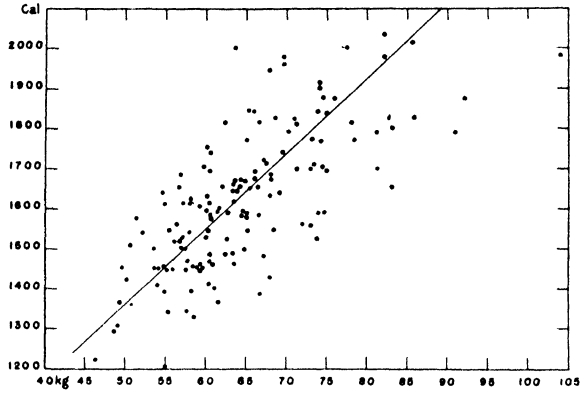


FIG. 83. Total twenty-four-hour basal heat production referred to body weight —Caucasian men. (Nutrition Laboratory Series I and II.) The ages represented are from twenty to sixty years.

total heat production per twenty-four hours is referred to the body weight. There is, as can be seen, a wide scatter of the plotted data, but there is little question as to the general *trend* of the metabolism with increasing weight, as shown by the slope of the line.

For women, similar studies have been made, and here again the data are rather widely distributed, but there is a fairly clear indication of the general trend of the metabolism as the weight increases. Since the scales for plotting are the same in both cases, one can instantly see that in the second group, the women, the curve indicating the average trend of the metabolism (Fig. 84) is at a lower level than that for the men. This instantly emphasizes that there is a sex difference in metabolism, but this will

be discussed further when the general comparisons are considered. With this brief review of the observations on humans, we can dismiss mice and men and turn to the elephant.

There are two methods of introducing the subject of the elephant. One is to recount that international tale of a group discussion on the characteristics of the elephant, and the other is to

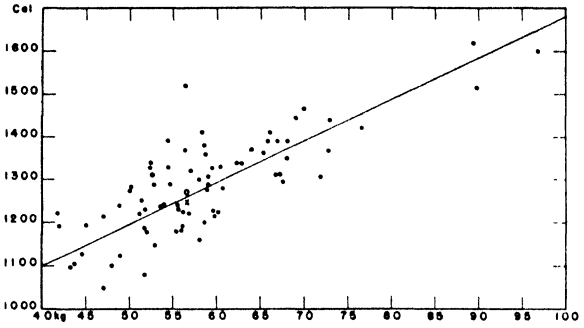


FIG. 84. Total twenty-four-hour basal heat production referred to body weight —Caucasian women. The solid dots represent the Nutrition Laboratory data (Series II) and the data of Stark and Tilt. The cross represents the average value of Coons and the hollow circle the average value of Hetler.

talk about Jumbo. Since both these approaches have distinct physiological slants, I shall use both.

The international elephant story is very old, no one knows exactly how old, but it received its greatest publicity when told by Paderewski at the height of his political activity. As he told it, a group of men of several nationalities gathered in a Paris restaurant got into a controversy about the elephant. After a long discussion they came to the conclusion that they knew altogether too little about this animal, so they agreed to disperse and spend the next year, each in his own way, in gathering information. They made a solemn agreement to return in one year to compare notes. As the story goes, the Englishman went to London, purchased an elephant gun, pith helmet, tinned

biscuits, and other hunting equipment, spent part of the year in India, and returned to write a book on *The Elephant: How To Kill Him.* The Russian gathered a number of his friends in Paris, imbibed a large amount of vodka, and emerged with a fiery pamphlet entitled, *The Elephant: Does He Exist?* The Frenchman talked with several of the keepers at the Jardin des Plantes and wrote a sonnet, "L'éléphant et ses amours." The German consulted the libraries in Berlin and the natural history museums and made a voyage to the Cameroons, and appeared finally with a four-volume treatise entitled, *An Introduction to the Study of the Elephant.* The Pole, like the Russian, remained in Paris, and with some of his friends concocted a pamphlet on *The Elephant and the Polish Question.* Some rather bumptious American has since tried to paint the lily, so to speak, by adding a fellow countryman to the original group, who on withdrawing sent a cable to a friend in America, followed by a letter, and ultimately appeared with a large advertising sheet, "How To Obtain Better, Bigger, and Busier Elephants."

This story, with its apt international slant, suggests both the wide variety of interests manifested in the elephant, and the various methods of study that have thus far been attempted.

The second method of approaching the elephant discussion, by saying something about Jumbo, Barnum's famous elephant, brings us much nearer home, for the word "Jumbo" has become synonymous with everything large, indeed, immeasurably large. There is no question but that Jumbo was a *very* large elephant, and there is equally no question but that no elephant was more frequently or more successfully lied about than Jumbo. A great deal has been written about his prodigious size and weight, but there is good evidence that the large African elephant, Khartoum, formerly of the New York Zoölogical Park, was as tall as, if not, indeed, taller than, Jumbo.

Jumbo serves to draw our attention to the striking differences between the African and the Indian elephant. Jumbo was an African, characterized by enormous ears, a curving forehead,

and, to the expert, quite different anatomical make-up from that of the Indian elephant. The ear of the African elephant is so much larger than that of the Indian that even the African pygmy's ear may actually be larger than that of a full-grown, adult Indian elephant. The Indian elephant, which is the one most commonly seen, has a blocklike and rectangular head, and legend has it that this was designed by God so that the mahout could sit on the elephant's head and direct his training. The fact that it would be almost impossible for a trainer to perch on the sloping head of the African elephant has been cited as one reason why this animal cannot be trained. Needless to say, this, like so many other elephant stories, is not true, for African elephants are trained and trained very successfully. However, the African elephant is a rarity, almost never seen on the road with circuses, and in only a few parks. Perhaps the two best specimens at present in America are Jumbino, a rather large, not too well-behaved, female at Washington, and Josephine at the Philadelphia Park. The discussion here will deal almost exclusively with Indian elephants, since our experience with Africans has been limited to two pygmy elephants with one of the large circuses.

In spite of the interest that mankind in general has always taken in the elephant as the largest living terrestrial mammal, almost no productive scientific studies have been made of it. To be sure, in the wild its habits have been observed in a general way; it has been hunted for sport and for ivory, and in India it has been subjugated and put to work. But all this has produced very little information of scientific value. We know far more about the frog than about the elephant. Anatomists were the first to show any signs of scientific interest. Without doubt Galen participated in an autopsy of an elephant. Subsequently there have been a few reasonably careful dissections, but really no physiological studies as such. There is an entire lack of knowledge of the constitution and physiological activities of the animal.

Few, if any, historical incidents involving the elephant have attracted more attention than the celebrated campaign of Hannibal, who started to cross the Alps with thirty-seven of them. This campaign, the subject of innumerable treatises, has, in the light of present-day knowledge, a number of important physiological angles, such as the matter of the food supply for the animals, the effect of altitude, and particularly the protection from, or resistance to, extreme cold. Because of its imposing size and massiveness, the elephant was in early times much used in warfare, but its inherent nervousness and excitability usually rendered it fully as destructive to its own troops as to the enemy. With the introduction of firearms, its use in war ceased entirely, save perhaps as a baggage carrier, for its emotional nature could not stand the noise of firearms.

In the somewhat extensive, though fragmentary and scattered literature, are many observations on the size and height of elephants, but they are very uncertain. No means existed, until comparatively recently, for weighing animals of four or five tons. The mere technique of measuring their height is by no means simple.

The most extensive survey made in the present studies was of the Ringling herd at Sarasota, Florida, where I spent practically a week with the animals, night and day. In this large herd, one of the first concerns was with the measurements of weight and height. As would be expected, the animals varied greatly in weight. The average circus elephant weighs around three tons, while a "large" circus elephant would weigh more than seven tons. The maximum weight that the elephant can attain has always been subject to exaggeration on the part of various showmen. The autopsy of Bolivar, at the Philadelphia Zoölogical Park a number of years ago, gave an accredited weight of 12,000 pounds. Khartoum, likewise, was weighed at the New York Zoölogical Park by Dr. Noback and found to be 10,390 pounds. The heaviest elephant whose actual weight has been ascertained was a gigantic African male shot recently by

Dr. George Crile of Cleveland. This animal was cut up in parts and weighed in sections, on carefully tested scales. Making a modest allowance of 10 per cent for loss of blood and body fluids by seepage and by drying out during the process of dissection, this animal is credited with a weight of 14,641 pounds, which is probably a slight under- rather than over-estimate. The fact that these animals can reach seven and a quarter tons is noteworthy. The heaviest subjects we encountered were Modoc of the Ringling herd and Babe of the Al G. Barnes herd, each of which weighed about 9,000 pounds.

Measuring the height of the elephant would seem to be a relatively simple problem, but it is not. It partakes of the nature of an engineering feat. Elephants are measured at the shoulder, much as are horses, and a great deal of care is required to get the true height. We used a special leveling rod and plumb bob, and made a large number of determinations. The average height of the circus elephant is seven feet four inches, but any elephant over eight feet at the shoulder is a very tall animal. Khartoum, and doubtless other animals, have actually measured, however, somewhat over ten feet, which is probably not far from the true height of Jumbo. Because of the difficulty of making accurate measurements, a number of rule-of-thumb procedures have gained considerable credence among elephant men. The circumference of the right forefoot, for example, is said to be exactly one half of the height. Or a tape may be thrown over the shoulder to measure from the outside of the left front foot to the outside of the right front foot. This obviously marks an arch, rather than the true vertical height, and can be dismissed as inaccurate. The former method has such current use that it seemed advisable to check it up, and we got some very interesting results.

Of course the circumference of the foot varies with the weight the elephant places upon it. The foot may mushroom out somewhat as the weight shifts. However, measured with all possible care, twice around the right forefoot of our subject

was 7 feet 8 inches. Measured by instruments the animal was 8 feet 1 inch. The tape thrown over the shoulder from foot to foot indicated a height of 9 feet 1 inch. It is interesting that this common method of measuring the foot actually underestimates the height of the animal, and thus the showman has not made full use of his possibilities.

Physiology has a great deal to learn from a careful survey of an animal presenting the wholly unique features of the elephant, namely, immense size and absence of external heat-insulating material, such as fur. It is astonishing how little is known with regard to elephants other than their size. For example, there is almost nothing on record regarding their quantitative eating habits, the digestibility of their foods, the chemical nature of the excreta, both feces and urine, and the respiration rate. Outside of two unconnected records, nothing was known previous to this study with regard to the heart rate. There were no data on the water balance or even the water intake, on the volume of urine and the water of the feces or on the gaseous metabolism and, particularly, the total energy transformations or heat output associated with metabolism.

On the other hand, practically all of these functions have been, in the course of time, thoroughly examined in other species of warm-blooded animals. In studying the laws governing vital activity, specifically metabolism and heat production, one may ask, Do the same laws apply to the small, well-furred, 20-gram mouse as to the 4,000-kilogram hairless elephant? The whole course of animal metabolism has been investigated between the limits of 20 grams and 700 kilograms (Figs. 81 and 82), but, to extrapolate, for example, a curve from 700 kilograms to 4,000 kilograms is dangerous, to say the least. And there remains the whale, with its possible 125,000 kilograms. To attempt to pass from data at a 700-kilogram level to data at a 125,000-kilogram level is wholly outside of reason. Hence it can be seen how important it would be to obtain values on an animal of, say, 4,000 kilograms. If the zoölogical picture of

vital activity was to be in any sense complete, data on a large elephant were imperatively needed.

The fantastic notions about the elephant are legion. There is a classic story of a circus elephant which, with that insatiable curiosity all elephants have, thrust its trunk into the open window of a tailor shop it was passing. The tailor sitting there pricked the sensitive tip with a needle. A year later, when the circus returned to the town, the elephant stored up some muddy water at a puddle several blocks away from the tailor shop and squirted it all over the tailor and his goods. Again, Pliny states that the python suffocates the elephant, by coiling around the trunk or by closing the orifices, and drinks its cool (?) blood (thus he assumes the elephant to be a cold-blooded animal). Pliny's report and the legend of the elephant and the tailor both have very important physiological sidelights. Pliny suggests that the elephant breathes only through the trunk, and therefore the python could suffocate it by constricting the trunk or by plugging its air holes. The tailor story suggests that the elephant can readily carry water in his trunk a long distance and therefore *must* breathe through the mouth. What is the truth?

Since one problem, perhaps the most important one, was to study the total energy output or metabolism of the elephant, and this involved a study of the gaseous metabolism, it can be readily seen that the whole question as to how the elephant breathes was a fundamental one. If he breathes exclusively through the trunk, it would be a simple procedure to place a mask or tube over the end of the trunk and measure the gases passing into and out of the lungs.

There is an old English recipe for rabbit pie which begins with the trite remark, "First catch your rabbit." This applies to elephants as well as to rabbits. There are any quantity of animals available for scientific study. There are plenty of mice, plenty of rats, any number of guinea pigs, dogs, sheep, even cows and horses; but to secure elephants for study is another story. There may be a hundred million guinea pigs, but there

are hardly one hundred and fifty elephants in the whole United States. When the physiologist asks himself, Which of these can serve my purpose? he thinks of a host of financial and diplomatic, to say nothing of physiological, problems involved in making a selection of a suitable animal from this very small number. In the first place, to have experiments of any comparable value one could not take just "any old animal." The subject must fulfil certain basic conditions, which were as follows.

First, it must be a large elephant, the largest possible. Why? This is not a pure Americanism, an attempt to secure "bigger, better, and busier" elephants. The dominant purpose in studying the elephant was to contribute to knowledge of the vital activity of the largest mammal. Hence a small elephant would only partly serve the purpose.

Second, the elephant must be docile. It must be used to handling, and it must be handled regularly. This ruled out all zoölogical park animals, but the circus animal is moved from town to town every night, put on the cars and taken off again, and receives plenty of handling.

Third, the animal must be normal; that is, it must be in good health, well nourished, and not too old; in other words, it should be in the full vigor of life.

Fourth—and here is a special requirement—it should not be restless. To anybody who has watched a line of elephants, this would appear an impossible condition. Close study of a good many elephants, however, shows that although they are seemingly incessantly active, the activity is for the most part confined to rather continuous movements of small parts, that is, parts that weigh very little compared with the weight of the entire animal, such as the tail, the end of the trunk, and the ears. But there is one form of activity engaged in by most elephants that would be detrimental in any study of the comparative metabolism, and that is their so-called "weaving." This is a process of shifting the weight from one front leg to the other and swaying from side to side, which goes on by the hour.

Weaving of itself involves a tremendous amount of activity which would increase the heat production and hence would give nothing approximating basal metabolism. This narrowed down drastically the selection from one hundred and fifty animals. The zoölogical park animals were ruled out; there are less than a hundred in circuses, and of these the large majority are "weavers."

Another requirement was that the animal be capable of being used alone, and here another difficulty appeared. Elephants are very gregarious and generally insist upon having a playmate, usually an elephant, but it may be a horse or a dog. It would be impracticable to study the metabolism of two elephants simultaneously and be sure that both fulfilled all the other conditions outlined, and we were not interested in studying the metabolism of an elephant plus a horse. This, then, narrowed the search to a very small number, for the "solo" elephants can probably be counted on two hands.

These are but a few of the imperative qualifications for the elephant, but above everything else it was necessary to have a nontemperamental elephant, and, as it transpired also after we began our search, a nontemperamental keeper, a combination by no means easily found.

The hunt for such an animal went on for practically twenty-five years. Zoölogical park animals were, almost without exception, immediately ruled out. They were too old, too decrepit, or too unruly, not infrequently having been discarded by a circus because of the hazard of exhibiting them. Or else they had lived such a quiet, well-regulated life in the park that they could not adapt to a new situation.

Circus elephants, on the contrary, met this condition of adaptability perfectly. They are put into cars every night and hauled a hundred miles or so, and then unloaded the next day. They are marched in street parades to the accompaniment of steam calliopes, to say nothing of fire apparatus, and are used to all sorts of excitement. They are continually subjected to new en-

vironmental situations, and never know uniformity or quiet save in winter quarters. But like the zoölogical park elephants, perhaps even more so, the circus elephants are in "groups," and they will not play alone.

Single animals that are not zoölogical park animals are for the most part confined to the smaller circuses which cannot afford more than one elephant. Frequently they have been discarded from the larger shows because of an unruly disposition or some physical defect, and cannot be considered prime animals.[2]

The great discovery was made one afternoon in Stuyvesant Square, New York, when I saw what even at a distance appeared to be the ideal elephant. She was wearing an advertising banner on her side and surrounded by two or three hundred children. She was moving extremely cautiously, apparently thoroughly used to city traffic and its sudden noises. I talked with the keeper and realized instantly that she met many of our qualifications: she was a large, well-nourished animal (weighing, I estimated, nearly four tons), quiet, calm, alone, and, apparently, judging from her reaction to the children and the surrounding noises and from what her keeper told me, as near nontemperamental as one could expect. It turned out that she belonged to Mr. Donald Bish, who generously agreed to our making the observations we desired.[3]

2. The Hagenbeck Brothers and their extraordinarily capable scientific director, Dr. Zukowsky, were most helpful in the search, as were Mr. John Benson of Nashua, New Hampshire, Dr. Blair, Dr. Ditmars, and Dr. Noback of the New York Zoölogical Park, and Dr. William M. Mann of the Washington Zoölogical Park.

3. Professor Samuel Brody, of Missouri, had made experiments on a circus elephant a year or two before this, and there seemed to be some possibility that this was the same animal. Identifying the elephant was an interesting problem. It would seem rather simple to distinguish and identify each of the one hundred and fifty elephants in America. Needless to say, every keeper recognizes his animals instantly, and the elephants know not only their own names but also every keeper. To identify the animal used by Professor Brody, we resorted to what might be termed "elephant fingerprinting," which in this case meant comparing

To find this object of a life's search housed in a small cellar stable on West Forty-second Street in New York City was indeed surprising. At this time, in February, the cellar was unheated and very cold, and Jap, as she was called, wore a blanket. This protection from cold plays a very important role in

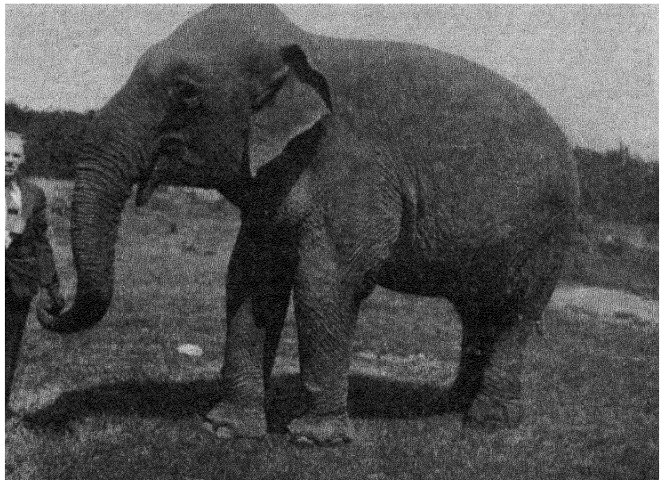


FIG. 85. "Jap." Elephant used by Dr. Benedict in a study of the physiology of the elephant.

the subsequent study of the animal. Normally, she was housed in a barn at Campgaw, New Jersey, about thirty miles from New York City, and here we were allowed to construct a respiration chamber.

The physiological studies of the elephant were concentrated upon this one animal first, for the principal task was to secure measurements that could not be obtained by superficial observation of animals in a circus tent or a zoölogical park. The big

the configuration and pigmentation of the ear. The configuration and spots on the ear turned out to be identical with Jap's. Subsequently, we located the keeper who had charge of the elephant during Professor Brody's experiments, and he confirmed the evidence of the "fingerprinting."

problem was measuring the respiratory interchange, that is, the amount of carbonic acid gas exhaled and especially the oxygen absorbed. This would involve either collecting the expired air from the trunk or, better still, placing the animal inside a respiration chamber and thus securing the entire gaseous emanations. These measurements would be the main objective of the study, and other factors incidentally observed could be subsequently pursued in group studies with various circus herds.

The construction of a respiration chamber and of respiration apparatus adequate for the occasion was our first and most important problem. It was possible to attach a simple respiration apparatus to Jap's trunk—a tube that fitted over the trunk, with inspiratory and expiratory valves, and a large gas tank for collection of the expired air. A number of experiments were made with this apparatus. The apparent total ventilation of the lungs was measured on several occasions, and the respiration rate was determined from the rise and fall of the spirometer bell. The air expired from the trunk was found to contain a significant amount of methane, a product of intestinal fermentation. With the exception of the above statements, little can be said concerning these experiments. When the elephant was placed inside a large respiration chamber and the entire gaseous exchange was measured, the story was very different; the trunk-breathing experiments had to be dismissed as of no practical value.

Putting a large animal, like an elephant, inside a respiration chamber was more a problem of engineering than of physiology. The chamber, when constructed, was between one half and one third the size of an ordinary freight box car (Fig. 86.) It was ventilated adequately by sucking air through by means of three vacuum-cleaner motors and blowers. The air that passed through the chamber discharged through a 150-light gas meter. Samples were collected in rubber bags suspended from the main air pipe and were subsequently analyzed. The usual control test of the apparatus was made by admitting

a known amount of liquefied carbon dioxide from a weighed cylinder and attempting to recover it by the experimental technique, including the respiration chamber, the ventilating sys-

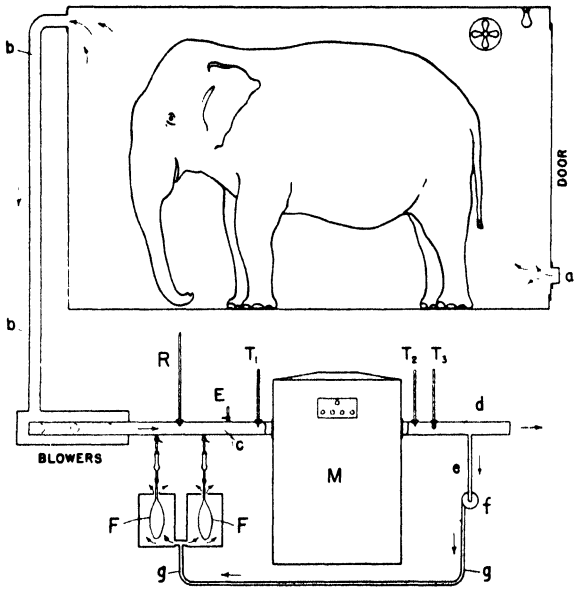


FIG. 86. Diagram of ventilating, aliquoting, and metering systems of respiration chamber used for elephant: *a*, pipe for introduction of outdoor air; *b*, *b*, pipe for outcoming chamber air; *c*, pipe connecting blower box with meter *M*; *R*, rotamesser indicating rate of ventilation; *F*, *F*, rubber bags for collection of samples of outcoming chamber air; T_1 and T_2, dry bulb thermometers; T_3, wet bulb thermometer; *d*, pipe for discharge of air from meter through wall of barn outdoors; *e*, pipe connecting with small blower, *f*, which forced portion of air discharged from meter through the pipe *g*, *g*, into boxes enclosing sampling bags; *E*, petcock for attachment of sampling pump.

tem, the sampling device, and the gas-analysis apparatus. The recovery tests were satisfactory.

Just before the experiments started there had to be a change in keepers. The new keeper claimed to have had charge of Jap several years before and did everything in his power to coöper-

ate, but he obviously did not know the animal's cues, and this hampered the work considerably.

During the construction of the chamber, the elephant was led in on the floor every day to accustom her to entering and leaving it. The interior of the chamber was lined with galvanized iron. The sides were galvanized iron (which was airtight), fastened to 2 × 4 uprights. In order to prevent the animal's coming in contact with the sides of the chamber, two heavy eyebolts were placed in the floor, to which she was chained. Although she was extraordinarily docile and quiet, there was always a possibility of her leaning against the side or hitting it with her trunk or kicking it, and thus demolishing the chamber. Hence it was finally decided that the keeper must remain in the chamber with the elephant. We knew the weight of the keeper and could estimate his metabolism with considerable exactness.

The routine was to place the animal in the chamber head first, and seat the keeper at the rear of the chamber under an electric light, with a fan going to circulate the air in the room and a speaking tube in the wall near his head. But Jap, although accustomed to all the noise and confusion of the New York streets, was like every other elephant in not caring to have anything going on behind her that she could not see. We made our first mistake in putting her in head first. When we attempted to put on the large door in back of her she became very much upset. A good part of one morning was consumed in training her to allow the door to be put on. Finally, at the keeper's suggestion, the door, instead of being brought up from the rear, was slid on from the side, much as the door of a boxcar would be slid in place. This seemed to satisfy the animal, although the door was never closed without a certain amount of anxiety on her part, and, indeed, on the part of the keeper—to say nothing of the investigators.

When the door was put in place and the ventilation started, the measurements were begun. The periods were a half hour in length, and usually eight periods were run each day. At the

very start an extraordinary uniformity was observable in the carbon dioxide production and the oxygen consumption of the animal. Experiments were conducted over a number of days, with remarkably consistent results. From the results of these experiments, it was possible to calculate the total heat production, the most important measure of vital processes (Table XX).

TABLE XX

Respiration Chamber Experiments—23° C. (Ventilation—1,500 Liters per Minute. Methane—661 Liters per 24 Hours)

			Per Minute	
CO_2 *Increment Per Cent*	O_2 *Deficit Per Cent*	*Apparent R.Q.*	CO_2 *Eliminated Liters*	O_2 *Consumed Liters*
.763	.805	.95	9.31	9.83
.690	...	...	9.75	...
.634	.635	1.00	9.36	9.37
.650	.628	1.04	9.62	9.30

In taking metabolism measurements of animals, the two most important factors affecting metabolism, that is, muscular work and digestive activity, must be ruled out: the first by using only periods of complete muscular repose, and the second by insuring that the animal is measured only in the so-called "post-absorptive condition," in which a sufficient length of time elapsed, after the last food was removed, to insure absence of digestive activity. So far as repose went, Jap did not weave, but it remained to observe the extent of her extraneous muscular activity. An exhaustive series of what might be called "motion studies," patterned somewhat after the studies of Gilbreth and Taylor, was made, from which it was concluded that her activity was more apparent than real. Although the ears flopped frequently, the tail switched, and the tip of the trunk in particular was in continuous motion, these were insignificant in proportion

to the weight of the animal. The head movements were relatively slight and body movements comparatively rare, so that, in spite of apparent activity, the animal was really as quiet as a cow or horse in a stall.

Ruling out digestive activity was more difficult. Most animals withstand fasting infinitely longer than one would predict, but it was plain almost immediately that, with this particular animal, withholding food for twenty-four hours was unthinkable. A steer can be made to fast three or four days. For the elephant, a fast of twelve hours was too long, and, indeed, Jap herself insisted that she be given food every few hours. I have emphasized Jap's docility, but one thing that she refused to put up with was an empty larder; if there was no hay in sight there was trouble. She would trumpet and become restless and almost pull her stakes, and on one occasion did. At night when the keeper was asleep on a cot near by and Jap was hungry, she would throw dirt and refuse from the floor to the bed. If this did not wake him, she would search for a stone. One morning we found several stones as large as hen's eggs on his cot, where she had thrown them. There was no peace until he got up, went over to the hay barn, and came back with a forkful of hay. To attempt to force a fast on Jap would have defeated our purpose. We might have lessened her digestive activity, but there would have been a tremendous increase in muscular activity and restlessness. Therefore the attempt to study the elephant in a post-absorptive condition had to be abandoned.

From the respiratory interchange it was easy to calculate the total metabolism of the animal under the conditions measured. The first comparison made was with the metabolism as calculated from the trunk-breathing experiments, and it was found that the metabolism as measured inside the chamber was approximately 40 per cent greater than that measured by the trunk. This was conclusive proof that the collection of expired gases from the trunk was very imperfect and that there must have been considerable mouth breathing.

Several weeks after the respiration experiments were completed an extensive study of the excreta was made. Urination did not occur during the experimental periods inside the respiration chamber. Mr. George Lee and Mr. Carl Hatch spent ten days at Campgaw observing the elephant night and day, in an effort to collect the total feces and the total urine. They succeeded in gathering the total feces for the ten days, and thus obtained the data for computing the digestibility of hay. It proved impossible, however, to collect a twenty-four-hour specimen of urine.

The animal, although usually good-natured, seemed to resent the collection of urine and frequently kicked over the container and spilled the contents, even endangering the assistants. In the course of ten days, however, by faithful attendance night and day, urine was collected in one period of sixteen hours, two periods of over twelve hours, and a number of others of eight hours, which provided sufficient data for calculating with reasonable accuracy the probable twenty-four-hour output of the animal, particularly the nitrogen output. From these data and the digestion experiments, it was possible to calculate the energy balances. Incidentally, many observations on sleep, the respiration rate during sleep, the weight of water drunk, etc., were made during this period of study.

To determine whether the findings applied to elephants in general, the study of Jap was supplemented by work with several circus herds. It was obviously impossible to continue the respiratory measurements with other elephants, but supplementary observations were made on feeding, activity, sleep, and the chemical composition of the urine and feces. Thanks to the cooperation of many circus managers, we had access to some seventy elephants; notably, the thirty-six animals of the Ringling herd, eighteen of the Barnes herd, nine of the Downie herd, and scattered animals with smaller circuses and several zoölogical parks. Owing to the tractability of the circus elephants and the unusual rapport existing between the trainers and their ani-

mals, the surveys of the circus herds were most complete. The Ringling herd was observed at Bangor, Maine, on two different occasions, and again at its winter quarters at Sarasota, Florida. The Downie herd was studied at Machias, Maine, and later at Newtonville, Massachusetts; and the Barnes herd was studied at St. Stephen, New Brunswick. The findings are based almost entirely upon the average results obtained from these larger numbers of animals, though the individual animals showed no marked deviations from the general findings.

In measuring the metabolism and physiological functions of man and other animals, a fairly close correlation has been noted in general between the heart rate and the intensity of metabolism within a given species. Hence it was desirable to get records of the heart rate of the elephant. A stethoscope could not detect it, and so a portable device much like an electrocardiograph was employed. The heart rate was counted by noting the maximum amplitude of the galvanometer with each heart impulse. The animals were brought forward and made to stand with their front feet on two large electrodes. When these were connected with a Boas cardiotachometer it was easy to read the major impulses. At Sarasota almost the entire herd was examined in this way. Later, when the Ringling herd came to Boston, it was possible to supplement the counts of the heart rate by electrocardiograms. The heart rates of some of the animals were taken when they were lying down as well as standing. The electrodes were held against the soles of the feet of the prone animal or attached to the feet with tape.

Contrary to every other animal studied, the elephant was found to have a higher heart rate when it was lying down than when it was standing; the average standing rate was twenty-eight beats per minute, and lying, thirty-five beats per minute. No satisfactory explanation for this phenomenon is as yet apparent.

The elephants were given a sort of life-insurance examination or physical survey which included, among other measure-

ments, the determination of the temperature. This is difficult. In India the mahouts insert thermometers in the rectum and hold them there, but this is impracticable under circus conditions. However, if the temperature of the large quantities of excreta could be determined immediately after voiding, it should be very close to the true temperature of the animal. The temperature of the feces was read by thrusting a previously warmed clinical thermometer into the fecal mass. The urine was collected in a large, wide-mouthed thermos jar which had previously been filled with water at 38° C., that is, approximately the temperature of the human body. The moment the voiding began the water was turned out and the jar filled with urine. Sometimes this in turn was thrown out and a second collection made. A thermometer was instantly inserted in the liquid and the temperature read. It soon became apparent that the temperature of the feces was actually above that of the urine, due to the fact that there was considerable fermentation going on in the feces. The average body temperature, as determined by the urine temperature of all the elephants examined, was 35.9° C. or 96.6° F. The average temperature of the feces was 0.7° C. or 1.3° F. higher than that of the urine.

The food of elephants in captivity is for the most part hay, supplemented by bran and oats. An indication of the amount that elephants will eat is the fact that Jap, a relatively large elephant, consumed 150 pounds of hay per day. The feces collected for measuring body temperature were found to consist exclusively of large pieces of hay. A ball of feces about the size of a derby hat, when softened in a pail of water and thrown on a cheesecloth screen, broke up into pieces of hay from two to six inches long. This challenged attention immediately, for the tooth equipment of the elephant is, perhaps, one of the most highly developed structures for grinding material known in the animal world, and yet here was clear evidence that the teeth are very little used for grinding purposes. The mere fact that a large bolus of feces can fall from a height of six feet, strike the

ground, and retain its original form shows that it is composed of very coarse interwoven material. Hunters report that in the native wilds, likewise, the elephant feces retain their round shape, showing that even there food is very poorly comminuted. At each defecation, five or six large boluses are egested. These may weigh as high as two kilograms or about 4½ pounds each. There are about fourteen to eighteen defecations per day, with an average total daily weight of some 250 pounds.

The singular absence of any sign of swallowing or chewing, in spite of the complicated tooth structure, led to a series of studies to determine how rapidly the elephant ate. Each animal was given a loaf of bread, including the wax paper covering, and the loaf was taken whole. Frequently the bread had disappeared entirely in twenty seconds, and rarely did it take one minute. By placing inside the bread pieces of rubber cut from an inner tube of an automobile tire, it was possible to study the rate of the passage of food through the intestinal tract. This was found to be very rapid for such a large animal, requiring only about twenty-four hours.

A full-grown elephant drinks 50 gallons or one barrel of water per day. The water is drawn up into the trunk, about 5 liters or 1½ gallons being taken each time. It is then forcibly ejected, from the inner end of the trunk, deep into the throat. There is no sign of swallowing. With this enormous water consumption, one naturally expects large volumes of urine. A single discharge of urine proved to average 5 liters or 1½ gallons, while the maximum noted was 11 liters. The average per day was 50 liters, or 15 gallons. The urine was usually straw-colored, with no pronounced odor, turbid, generally acid in reaction, and characterized by a very heavy deposit of calcium oxalate crystals. The average specific gravity was essentially that of man, namely, 1.019.

The study of Jap included a consideration of heat production. Jap, weighing about 8,000 pounds, produced, when standing up and feeding inside the chamber, a total of 65,000 calo-

ries per day. This is equivalent to the heat production of about 30 men. Making allowance for the calories consumed in standing and in digestion, the animal produced in the minimum or basal metabolism 49,000 calories. If this is referred to the weight, we have the equivalent of 13 calories per kilogram of body weight per 24 hours, or 2,060 calories per square meter

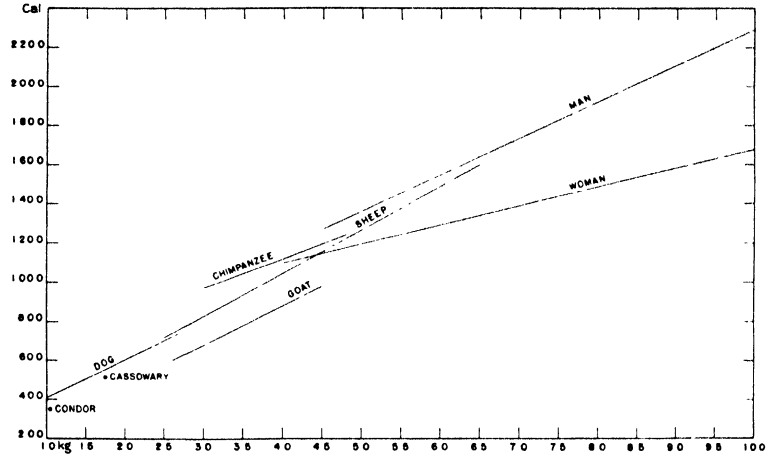


FIG. 87. Trend of total heat production with increasing weight—animal species in weight range from 10 to 100 kg.

of surface area. These values are of particular importance in comparing with data obtained on other animals and in extending the curve for heat production per kilogram and per square meter, which was discussed earlier (Figures 89, 90).

With the metabolism of the elephant fairly definitely established, it is now possible to examine a series of animals and see where the elephant metabolism lies on the curve. No one group of animals is of greater interest than that in which the body weights approximate those of humans, and of the large number of comparisons possible, we shall consider, first, the curves for animals ranging in weight from 10 to 100 kg. Figure 87

shows the total basal heat production per 24 hours referred to increasing weights. Few of the animals reach the weight of man; for although the sheep may weigh as much as 60 kg. or more, the goat 45 kg., and the chimpanzee nearly 50 kg., a large proportion of men and women are available with weights much higher than these. In the survey it was soon apparent that the most important relationships, by far, were to be found in the vertical comparison, that is, in the comparison of animals with the same weight but having different metabolisms.

Three relationships are especially to be noted on the curve in Figure 87. In the first place, the metabolism of the chimpanzee corresponds fairly closely to that of man and of woman. It should be emphasized that the curves for the human male and female are based upon a large number of rather widely divergent results, but the general trend of the heat production with increasing weight is clearly shown by the data. In this respect the chimpanzee certainly falls well in line with man. Second, there is a striking difference between the metabolism of the goat and that of the sheep. These two animals are somewhat similar, and yet the goat has a metabolism perceptibly lower, weight for weight, than the sheep. Finally, there is a wide divergence in metabolism between men and women, especially at the higher weights. Increasing weight in woman is not accompanied by as great an increase in heat production as is increasing weight in man. A similar analysis could be made of the metabolic relationships in other weight groups, notably those from 10 grams to a kilogram and from 100 kg. to 4,000 kg., but the one curve given will serve as an illustration.

In the weight range from 10 kg. to 100 kg., the differences in metabolism of individuals of the same weight are even more strikingly brought out when the curves are plotted on the basis of the heat production per kilogram of body weight, as in Figure 88. The general rule for all animals and all species is that as the body weight increases the metabolism per kilogram decreases. Here the difference between the sheep and goat is even

more evident. The difference between man and woman is also clear, as well as the general tendency for the metabolism of the chimpanzee, a primate, to follow that of man and woman. Unfortunately, dogs were not studied as extensively as they should have been in this series. It is evident that, at a given weight, for

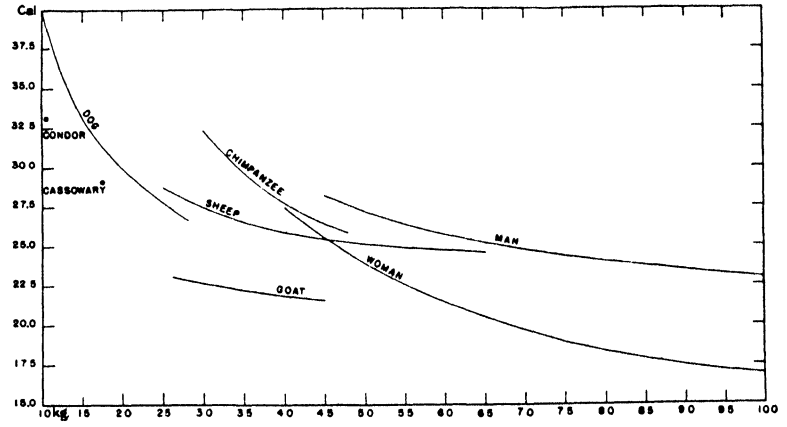


FIG. 88. Trend of heat production per kilogram of body weight with increasing weight—animal species in the weight range from 10 to 100 kg.

example, at 45 kg., the heat production of the goat is much lower than that of the sheep. On the other hand, a 45-kg. sheep and a 45-kg. woman have about the same heat production. The chimpanzee at this weight has a little higher metabolism, and man higher still. In this comparison any role that might be played by the surface area would be negligible, since these wide differences in metabolism are noted in animals of precisely the same weight and hence the same surface area.

With the revision of the values obtained for mice, and a large number of observations on other animals, consideration can now be given to our latest general survey of all the warm-blooded animals shown in Figure 89. In the curve here shown, the heat production per kilogram of body weight is referred to the aver-

age weight of the particular animal species. On the basis of the heat production per kilogram of body weight, it will be noted that the small birds (canary and sparrow) have the highest metabolism, and the elephant the lowest, though not significantly lower than that of the cow and the steer. Here, again, the vertical comparison as has been noted above is of special interest.

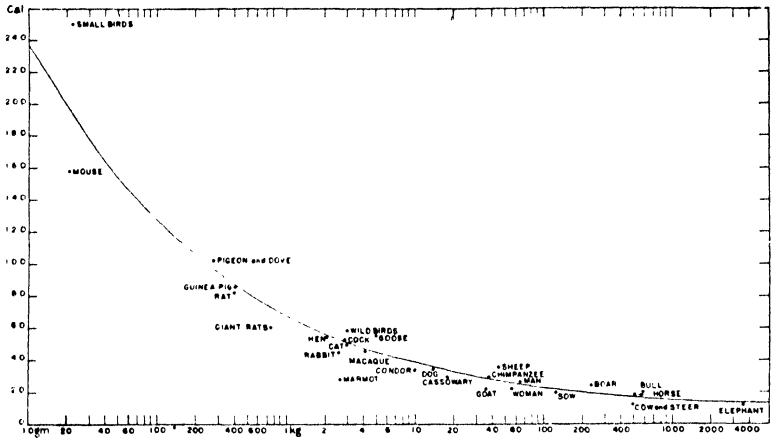


FIG. 89. Semilogarithmic chart showing the trend of the average heat production per kilogram of body weight of each animal species referred to the average body weight—weight range from 10 g. to 4,000 kg.

At about 3 kg., for example, the differences between the marmot and the macaque and between the cat and the wild bird are striking. The difference between the albino mouse and the small birds, both weighing about 20 gm., is also marked. And as shown previously (Fig. 81), the metabolism of the dwarf mouse is 110 calories per kilogram, which is far below even that of the albino.

Finally, since it has been commonly believed heretofore that the heat production per square meter of surface area is constant, it will be of value to observe the latest figures based on surface area. For this purpose the surface area has been calculated by

multiplying the two-thirds power of the body weight in grams by 10, and the total heat production has been divided by this result (p. 258). The values have been plotted in the chart shown in Figure 90. Here again, in order to record the data for all the animals, it was necessary to use the logarithmic basis for the ab-

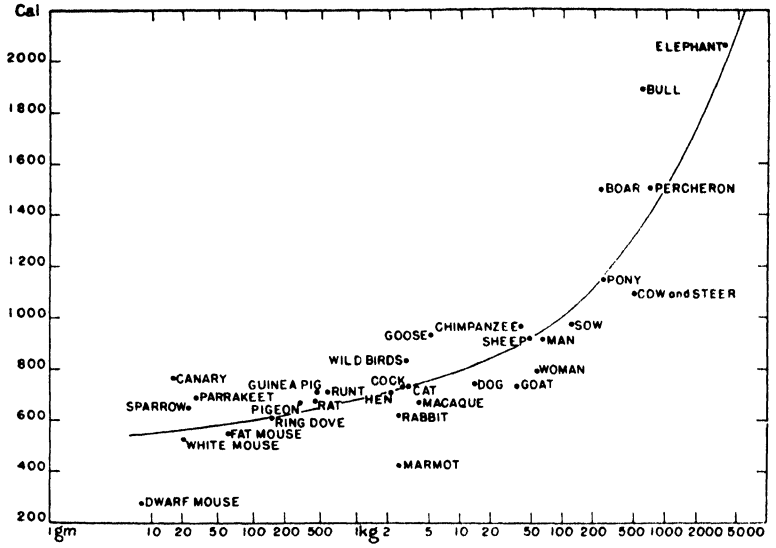


FIG. 90. Semilogarithmic chart showing average heat production per square meter of surface area of each animal species *vs.* average body weight—weight range from 10 g. to 4,000 kg.

scissae, that is, the body weights. On this chart the three varieties of mouse are represented—the dwarf, white, and fat mouse—and the elephant likewise appears. Here the differences in the vertical scale are more strikingly shown than in any of the other charts. At the start of the curve is the dwarf mouse, which produces about 250 calories per square meter of surface area. This is the lowest measured metabolism per unit of surface area ever recorded. The surface area was calculated from the two-thirds power of the weight multiplied by the factor 9. If the

common constant of 10 had been used, the heat value for the dwarf mouse would be still lower.

The value for the marmot is very low. In the vertical scale the marmot, the rabbit, the macaque, the cat, the cock, the wild bird, and the goose show a continually increasing heat production per unit of surface area, although these animals have essentially the same body weight. Finally, the elephant has the highest heat production per unit of surface area of all the animals, namely, about 2,000 calories. When the metabolism of the dwarf mouse, a little over 200 calories, is compared with that of the elephant, about 2,000 calories, it appears that there is practically a tenfold increase in heat production per square meter of surface area, a striking contradiction of the long-maintained view that the heat production per unit of surface area is constant for all warm-blooded animals. No clearer illustration of the absence of connection between surface area and heat production could be given than this curve. It is thus plain that we must forego any theory of heat production being related to surface area, and must think in terms of heat production and not heat loss.

Striking differences in the heat production of animals having precisely the same weight and the same surface area are too often revealed in these charts to be ignored. It is evident that there are great differences in the heat-producing powers of these animals. The metabolic survey outlined above, which has occupied nearly three decades, has disposed of the surface-area concept, for although the simplicity of the concept leaves nothing to be desired, it is quite clear that the heat production varies with various animals. The laws governing their vital activity are not based upon a simple physical law of the loss of heat from one temperature to another. In thus disposing of the so-called "surface-area law," however, the survey has uncovered a great many important problems, and inquiry should now be directed toward discovering why metabolism varies so greatly in animals of the same weight and surface area. To what extent,

if at all, these differences may be based upon purely physical loss of heat, and to what extent they are based, as is probable, upon differences in chemical composition of the body and the blood, the oxygen-carrying power, minute circulation, and, above all, distribution of the blood, remain still to be solved. All these questions are fruitful fields for research.

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CHAPTER X

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