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SCIENCE AND NUTRITION

BY

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WITH A PREFACE BY

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PREFACE

AT no time has Brillat-Savarin's aphorism " La destinée des nations dépend de la manière dont elles se nourrissent " been more apposite than it is to-day. The survival of democracy or its annihilation during the next few years may easily be determined by the measure of attention given in the various countries to what have come to be called the problems of human nutrition.

What are these problems? Thirty years or more of experimental research have revealed that health is quite as seriously menaced by ill-balanced or defective diets as by germs, viruses, and other infective agents. Perhaps in the former case the danger is even greater, because most infections betray their presence by a recognizable group of symptoms calling for treatment, whereas the onset of disabilities due to improper diet is often so insidious that irreparable harm may be caused before the alarm is raised.

The story of the remarkable developments in the field of experimental nutrition during these thirty years or so is simply and accurately told by Mr. Bacharach in the following pages. He speaks with the authority of one who has not only been in close touch with all the important research work of recent years, but who has himself made many valuable experimental studies. His easy and lucid style will at once appeal to the intelligent " man-(and woman)-in-the-street," for whom I am certain this book has been written. I hope they will read

it in their thousands. I hope they will learn from its pages that the great majority of the "problems of nutrition," about which one hears so much nowadays, have been solved by the scientist, and that they are now translated into problems of economics or political science.

Let me give one example. In every part of the world where the very poor have been forced to live on maize as their staple food, there is found a repulsive and often fatal disease known as pellagra. For many years it has been attributed to faulty diet, but the experts could not agree whether it was caused by a poisonous substance in the deteriorated grain, by the poor quality of the maize proteins, by a vitamin deficiency or by one or other of many hypothetical defects. Within the past year a brilliant series of researches in which *mus norvegicus*, to whom—I refuse to say "to which"—the author has so charmingly and appreciatively dedicated his book, played his modest but essential part, has proved that the disease arises from a deficiency of a substance called nicotinic acid, related curiously enough to the highly poisonous ingredient of tobacco. In this compound, which can very easily be produced by synthesis in the chemical laboratory, we now have a certain cure for pellagra.

But important as this discovery is, the main problem does not end there. The foodstuffs which contain most nicotinic acid, and which therefore give protection against the disease, are dairy produce and fresh meat. When these form a reasonable proportion of the diet there is no need to think in terms of nicotinic acid. Not so many years ago pellagra was rife in the Republic of Georgia. By making it possible for the inhabitants to obtain ample supplies of cheese and eggs, the Soviet

Government has practically wiped it out. Conversely we find that the incidence of pellagra in the " corn-belt " of the United States still sharply reflects the rise and fall of poverty and unemployment. It is not a question of nicotinic acid ; it is simply one of £ *s. d.*

The only real problem of nutrition in England to-day is the task of making available to the poorest person the " protective foods," such as milk, butter, fresh salads, and fruit, which are essential for health.

This book should be read not only by the intelligent " man-in-the-street," whom for no very clear reason one assumes to be a layman, but also by the medical profession, until quite recently lamentably behindhand in recognizing the significance of experimental dietary studies, and perhaps even by the politicians who are at the moment very timorously testing the strength of " nutrition " as a party plank.

J. C. DRUMMOND.

June 1st, 1938.

INTRODUCTION

NUTRITIONAL Science, especially on its experimental side, with which this book is mainly concerned, is a borderland subject, where chemistry and physiology meet, often along with physical chemistry, and even physics—to say nothing of contacts with zoology and botany (including bacteriology) and the potent weapon of statistics. It is—let us face the fact—a branch of biology, for it is concerned with what happens when certain living organisms kill, eat, and inwardly digest (or try to) certain other living organisms.

This being so, I fully realize that the predominantly chemical treatment here given to the subject lays me open to criticism from many quarters—more especially from my physiologist friends. To them, therefore, in particular, I put forward both an excuse and (I hope) a justification. The excuse is that I happen, by training and profession, to be a chemist, and that merely prefixing to that designation the syllable "bio" cannot be expected to alter fundamentally the outlook engendered by thirty years' training and practice. The justification is one of both expediency and principle. The interested and educated layman of any class, for whom this book is written, may quite possibly have once learnt some chemistry—at school, at night class, at college, at extension lecture, at university—and may even remember something of what he has learnt. The chances, alas, that he will ever have been instructed even in the

earliest rudiments of animal physiology are still to-day so small as to be negligible. I think, therefore, that for him the chemical approach is likely to be less forbidding than the physiological. I am by no means the first to recognize this, nor have physiologists themselves been blind to it. In the many admirable publications of my friend, Dr. T. R. Parsons (notably in *The Materials of Life* and in the evergreen and ever-tended *Fundamentals of Biochemistry*) though the outlook is essentially physiological—that is, biological—the treatment is very largely chemical.

I think the reason of principle must have operated in him also. Walter Pater said that all art aspires towards the nature of music; is it not also true to say that all biology aspires towards interpretation in terms of atomic physics? It will probably be conceded that chemical interpretations are a little nearer to the physical asymptote than are physiological. If so, the urgent need to guide the layman away from the mists of obscurantism—naturalism, vitalism, and what not—in his application of scientific knowledge to ordinary human and social problems, must surely justify the presentation to him of biological facts in terms as far removed as possible from the dangers of mystification. I honestly believe that there is a better chance of doing this on the chemical than on the physiological plane, fully realizing that this belief may have been largely conditioned by my own chemical training. That, at any rate, is my case.

It is customary for the author of a book in any introductory note to make acknowledgments of various kinds. If my thanks seem exiguous to the point of parsimony, let it be attributed to the fact that I know neither where to begin with them nor where to leave off. I am most grateful to Dr. Maurice Newfield and Dr. R. A.

McCance, who read and helpfully criticized certain parts of the manuscript. Professor Drummond's kind Preface is perhaps the least of his services—to me and to the reader: he has read the whole of the manuscript and saved me from error or obscurity on many occasions. He and those others who have helped me are, however, in no way responsible for my statement of any facts or for my arguments from the facts. They must be credited with much of any virtue in this book; they must be debited with none of its faults. To Professor H. Levy, in a sense the godfather of this book, to my wife, and to the publishers I feel that no adequate acknowledgment can be made of their patience, aware as I am that over this book I have been an unconscionable time writing.

Finally, to the editors of various journals that have thought fit to publish reviews, articles, and notes of mine, I apologize for any unintentional infringement of their copyrights, appreciating that, in re-stating here facts and arguments already stated there, I may well have inadvertently repeated phraseology to an extent not to be covered by "reasonable quotation." My acknowledgments on this score are particularly due to the editors of the following journals: *The Analyst, Chemistry and Industry, Food, Food Manufacture, The Industrial Chemist, The Manufacturing Chemist, Nature,* and *The New Statesman.* Part of the last chapter appeared in substantially the same form in *The Highway* for April, 1938. I am much indebted to the editor of *The Lancet* for permission to reprint the Annotation that appears on pp. 147 and 148.

CONTENTS

SECTION I

THE EXPERIMENTAL BASIS

SECTION II

CLASSICAL NUTRITIONAL SCIENCE

SECTION III

MINERALS

SECTION IV

VITAMINS

SECTION V

DIET AND HUMAN HEALTH

SECTION I—THE EXPERIMENTAL BASIS

CHAPTER I

INTRODUCTORY

§ 1 THE object of this book is to describe, as simply as the size of the book and the nature of the subject ermit, both the results obtained and the methods used ı scientific investigations, especially those carried out nder laboratory conditions, that have influenced our iews of the relationship between human nutrition and ealth. It is customary to jeer gently at publications hich try to show in precise terms how inadequate eeding, qualitative at least as much as quantitative, is actly correlated with general and specific ill-health, on he grounds that this is simply gilding the lily and tating in a complicated way what all open-minded eople know to be the simple truth. I think the jeerers ıre wrong—wrong in fact, for the correlation is by no neans as widely accepted as they would like to believe; vrong in intellectual approach, for they fail to distinguish etween vaguely accepted generalizations and the exact ases of those generalizations, which alone can turn ague acceptance into effective corollary action. So that hey are thirdly wrong as well—wrong tactically, as social eformers, politicians and revolutionaries.

§ 2 In order to carry out our object it will be necessary to try to steer a very careful course between scientific

inexactness on the one side and excessive detail on the other. I therefore apologize in advance to those who will be inclined, doubtless with frequent justification, to criticize me from the safe heights on either side of these narrow straits. To the scientist, and especially to my biochemical colleagues, this book will sometimes appear to be lacking those meticulous qualifications that are essential in accurate technical expositions; the layman, on the other hand, may occasionally find himself asked to take for granted matters that are by no means self-evident.

It is, however, for the layman that this book is mainly intended. I must, therefore, direct his attention to the fact that there are many other books, of not too specialized a nature, from which he will be able to supplement such new information as I may be fortunate enough to give him, should I be successful in arousing in him the necessary interest. In any event, if I occasionally credit him with knowledge that he does not happen to possess, and omit some connecting argument or set of data, I can only hope that he will succumb to the implied flattery and forgive me for any accompanying exasperation.

CHAPTER II

THE USE OF ANIMAL EXPERIMENTS

§ 3 WORKERS in the nutritional laboratory frequently meet with criticism from the non-scientist on two rather fundamental questions. This book could have little justification were either criticism valid, and it is essential to dispose of them at the outset. Each can best be illustrated by an example, and it may be well to take the two examples from the same branch of the subject.

The first criticism, in its extreme form, is a denial that any true progress is made by laboratory experiments on small animals. "What," it is asked, "has been learned about cod-liver oil that was not known decades, even centuries, ago?" Once it was established that cod-liver oil could cure rickets, so runs the argument—and that was discovered by physicians, not by biochemists—the expenditure of thousands of pounds, tens of thousands more likely, on salaries and equipment for laboratory scientists was merely pandering to a purely academic curiosity. It would have been much more practical and of far greater benefit to humanity if the money had been diverted into the channels of preventive and curative medicine, so that cod-liver oil could have been provided for all children suffering from, or likely to suffer from, the disease that it had been shown to prevent and cure.

§ 4 There is first of all a purely practical answer to this question. Go and ask your doctor. Does he take this view? You will find that even the least academically minded medical man will pay at any rate lip-service to laboratory investigations; most physicians, moreover, will assert their sincere belief that the type of work under critical fire has been absolutely essential to the advance of medical knowledge. And it is fairly easy to explain why they hold that view.

It is not true to say that we have known for decades, or centuries, all about the therapeutic value of cod-liver oil against rickets. What we did know was that certain doses of some cod-liver oils had been found efficacious. But there had been no certainty, not even a fifty per cent. probability, that we should strike the right dose of a particular oil for the treatment of any individual case.

True, with all except a very few and unusual samples of cod-liver oil, it would probably be possible to prevent, and even to cure, rickets, provided enough were given. But that would by no means always be practicable, especially with infants and young children. You can't expect them to take an almost unlimited amount of any liquid oil, particularly not of cod-liver oil, which some people reject in even quite small doses.

It was only when the therapeutic properties of cod-liver oil had been fully investigated in the laboratory by the use of experimental animals that any really *quantitative* knowledge became available. As soon as it was established that the therapeutic property was due to the presence of a small amount of an intensely anti-rachitic (rickets-preventing or -curing) substance, subsequently to be known as vitamin D, the hunt was up. Methods were developed for comparing one sample of cod-liver oil with another, and for evaluating the anti-rachitic

potency of any particular sample, and, indeed, for that matter, of any other substance containing vitamin D. At least the physician can now buy " assayed " oils of declared potency. Once he has decided how many " units " of vitamin D—a matter we shall return to later—are required for the treatment of his patient, it is now a matter of simple arithmetic, and not a wild guess, to calculate how much of a given assayed oil he must give to that patient.

§ 5 With the development of the methods of assay, in which the albino rat played, and still plays, an absolutely indispensable part, other sources of vitamin D were investigated. As a consequence, many liver oils have been examined from different fish beside the cod, and Dr. C. E. Bills of Evansville in the United States has actually discovered a liver oil having five hundred times the anti-rachitic power of a good medicinal cod-liver oil. We now know that the vitamin is present to some extent in the fat of human and of cows' milk, and in the yolk of egg. We also know that it is completely absent from vegetable foods like green salads, carrots and turnips, fruits and berries, nuts, and so on; which leads to an interesting query. Why do not the children of vegetarians all die of acute rickets? To this question we shall also return later, as well as to a description of the exciting investigations that led to the production of calciferol, " crystalline vitamin D," a chemical produced in the laboratory from substances having no anti-rachitic power whatever. Though calciferol is now known to be different from the vitamin D of cod-liver oil, for reasons that we shall endeavour to explain, it was the first vitamin to become available to medicine in the pure state; its medical value can be partly appreciated from the fact that it has for human subjects over twenty thousand

times the rickets-preventing activity of good cod-liver oil.

All the investigations leading up to the just-mentioned discoveries hang together inseparably. Had they not taken place, and had the funds so saved been used for the attempted treatment of rickets on the basis of the knowledge available prior to this undertaking, we should still be groping in near-darkness, and the potential, if not the actual, amount of rickets prevention and cure would have been immeasurably less than it in fact is at the present day. Not only have we placed the treatment of rickets on a quantitative basis, but we have also, in so doing, unearthed—or got out of the sea—a whole collection of new anti-rachitic agents, natural and artificial. Besides that, and by no means least, we have turned a flood of light on to the cause—the etiology—and the course—the pathology—of the disease itself, valuable alike for purposes of prevention and of treatment.

§ 6 The second fundamental criticism that must be met before we can conscientiously proceed has a rather greater *prima facie* plausibility. It takes the form of admitting the scientific advances obtained in the laboratory, whether by experiments on animals or by more purely chemical and physical methods, but it asks, "What earthly reason have we for assuming that results obtained on lower animals, or even on monkeys for that matter, can be applied to man?" The answer is, though to many it may seem odd, that we have very little reason, and in any event we didn't make the assumption! The question, in fact, shows understanding neither of the purposes for which experimental animals are used, nor of the general method of applying on a large scale, whether in a factory or a hospital, results obtained in the laboratory.

§ 7 It cannot be too clearly understood that the laboratory animal, especially in nutritional investigations, plays two quite separate, though connected, parts. It serves as a reagent: in just the sense that litmus-paper is a reagent for acids, so a properly prepared rachitic rat is a reagent for vitamin D. It can be used, like a chemical reagent, not only to detect, but under suitable conditions to measure. The fact that acids turn litmus-paper red does not, of course, tell us of their ability to dissolve metals or to burn the human skin. The fact that vitamin D cures a certain experimental condition in the laboratory rat does *not* tell us that it can prevent human rickets.

That fact could only be established by experiment. But there is a world of difference between experiment and purely trial-and-error methods. The latter may not lead to any definite result, even a negative one; the former is based on already ascertained facts, applied with due consideration for the differing circumstances to the problem requiring solution. The knowledge of rat rickets and the effect of vitamin D thereon enabled us to plan intelligently its experimental application to the human subject. The analogy drawn above—between acids and vitamin D—must not, in any event, be pressed too far. There were connections, obvious to the biochemical worker, between rat rickets and human rickets, though the etiologies of the two conditions seemed to differ. But the resemblance was sufficiently close, in pathology as well as in actual clinical symptoms, to make it most probable that vitamin D would be as effective against one as against the other.

§ 8 The first highly concentrated preparations of vitamin D were made from cod-liver oil; the process of concentration was checked at each stage by

means of experiments on rats. When the chemist had got as far as he could, his material was tested on men and other animals by practitioners, medical and veterinary respectively. It worked. But no one could have known in advance *for certain* that it would work. All one knew was that its efficacy was sufficiently probable to make its trial well worth while.

Exactly the same procedure was followed with calciferol and with its less pure predecessors, irradiated oils, irradiated cholesterol, and irradiated ergosterol.

§ 9 It is worth while mentioning here one or two other instances, including one failure, that illustrate the same methodological principles, namely, those necessary for proceeding in a scientifically sensible manner from laboratory to bedside. The fact that pure crystalline ascorbic acid, the chemical name for vitamin C, cured guinea-pig scurvy, only established a high probability that it could cure human scurvy, and therefore also prevent it. Clinical trial was necessary to prove the point, and it did.

Copper is essential to the rat for the adequate formation of hæmoglobin—the red pigment of the blood. That has been proved up to the hilt. But there is even now no complete certainty that it is essential to man. Only very minute traces are required by the rat to supplement the action of the iron in its diet. One cannot produce copper-anæmia in the human being by experimental methods, but iron to which small amounts of copper have been added, used in treating anæmia in men (and especially in women), has occasionally appeared to give better results than unsupplemented iron. It may be taken as highly probable that man needs some copper in his diet, notwithstanding that an excess has undoubted toxic effects.

solid and is being converted to two gases *A* and *B*, and if these are dissipated in one way or another, all of *AB* will be broken down to *A* and *B*.

In the living tissue, naturally, a far more complex state of affairs exists; nevertheless the *biochemical* reactions taking place therein are of a "balanced" type fundamentally similar to the simple examples just outlined. The proteolytic enzymes of the stomach, pancreas and intestine are able to effect a fairly complete breakdown of food proteins to amino-acids so that these, being soluble in the juices of the alimentary tract, get carried away from the site of the enzyme action and pass through the walls of the tract into blood-stream and lacteals; the enzymes thus have no amino-acids available for anabolism—rebuilding to peptones, proteoses, and so on—and proceed to catabolize more protein.

§ 49 If the reader deduces from the last sentence that the *same* enzymes take place in protein catabolism and in protein anabolism, he will have deduced right. There is experimental evidence that they are indeed capable of catalysing both processes. When the amino-acids in the blood-stream reach the tissues in which protein has to be built up—notably the muscles, but indeed also every cell in the living body—enzymes essentially similar to the digestive enzymes almost certainly play the main part in the resynthesis.

Owing to the difficulty, already discussed (see p. 44), of accurately characterizing individual proteins, it is quite impossible to say even approximately how many separate proteins occur in the human body. They are, however, certainly numerous, if one may argue from the proteins of other animals. It can easily be understood that exact knowledge of even the amino-acids present in the proteins of live human tissues is almost impossible of

achievement by direct analysis, and this makes it difficult to know exactly what amino-acids are essential to man. Here again we are dependent largely on animal experiments and argument by analogy. It seems likely that many different enzymes must be involved in protein anabolism; certainly the cellular conditions under which they perform their functions must be extremely diverse.

§ 50 Investigations into the amino-acid requirements of experimental animals, and also of farm animals, have been carried out in dozens of laboratories for several decades. A diet of precisely known amino-acid content, lacking one single amino-acid, but containing all other recognized dietary essentials, can be prepared and its effect on a group of animals compared with that of the same diet to which the missing amino-acid has been added. The increases in size and weight, the general health, the fertility, and other characteristics of the two groups of animals may all furnish evidence by which to decide whether or not the particular animals can dispense with the particular amino-acid. Analysis of the animals' body-proteins will then often make it possible to know if the animal has only appeared to do without the amino-acid, or if it has been able to synthesize it from some of the other food constituents. There is a considerable amount of published information on this subject; some of the earlier work needs rather critical evaluation, because it was carried out before present-day knowledge of vitamins and other "accessory" substances was available. The beneficial effect of a particular amino-acid might have been due to its contamination with some trace of an unknown essential food constituent, absent from all the other dietary ingredients. Nevertheless, despite some difficulties in interpreting these investigations, the general picture is fairly clear.

Most animals need most of the known naturally occurring amino-acids; most animals are unable to synthesize most of these essential amino-acids from other substances. Broadly speaking, the animal kingdom is dependent, directly or indirectly, on the plant world for its supplies of protein, as for its carbohydrates and minerals. It is true that excess protein can be rather extravagantly transformed, *via* its amino-acids, to carbohydrate; but very few wild animals, and still fewer human beings, find it desirable or necessary to adopt so uneconomical a regime.

§ 51 Plant proteins differ from animal proteins both in the number and in the proportions of their constituent amino-acids. It is because of this that the physiologist has come to speak of some proteins as "good" in the biological sense, and of others as "poor." (The antithesis is curious, and should not be indiscriminately applied to the study of social phenomena generally.) A "poor" protein is one that is unable by itself adequately to support animal life; a "good" protein can do so. Few proteins are perfectly "good," but, generally speaking, animal proteins are "good" and vegetable proteins are "poor." Even so, most single animal proteins give inferior results to mixtures, and this is still truer of vegetable proteins. The reason should be fairly clear; we will take a simple numerical example. Supposing that the total body proteins of man, or some other animal, are represented by amino-acids *A*, *B*, and *C* in the proportions

$$6A + 3B + 1C.$$

It is clear that a protein containing only one or only two of these amino-acids will not be able to support that animal's life, assuming that none of the amino-

acids can be synthesized by the animal. If, however, it eats a protein containing equal parts of *A*, *B*, and *C*, then it will have to get rid of three parts of *B* and five parts of *C* for every six parts of *A* that it consumes. The protein in question, though "good," would be extravagant.

If this protein can now be supplemented with a protein, "poor" in itself, that contains five parts of *A* and two parts of *B*, but no *C* at all, then the diet will be adequate if it contains quantities of the two proteins represented by

	$1A + 1B + 1C$	six times
plus	$5A + 2B$	$1A + 1B + 1C$
	$6A + 3B + 1C$ instead of	$6A + 6B + 6C$

Thus there will have been a saving of *five* parts of the "good" protein, for *one* part of it will have been adequately supplemented by, say, two to three parts of the second "poor" protein. This *supplementary action* of proteins is of the greatest importance in nutrition. The foods containing the best proteins are the most expensive foods; they are also not very economical, in the physiological sense, as sources of energy. If, however, they can be taken as sources of the amino-acids absent from the "poor" proteins of many vegetable foods—notably cereals—then the "poor" proteins fulfil an invaluable function in supplying the amino-acids common to "good" and "poor" proteins alike.

§ 52 Consideration of the chemistry and physiology of the proteins in diet leads to the inevitable conclusion that a mixed diet is the best diet. The proteins of meat, milk, and eggs, with those of cereals and legumes, taken together are biologically more useful—and cer-

:ainly cheaper—than either group taken alone or in partial association.

Large sections of the population, especially among the simpler races, would find it a complete impossibility to obtain enough of the essential amino-acids from "good" proteins alone, even if the diet implied by such dietary restriction were not undesirable for other reasons. For those people, by economic, climatic, or geographical circumstances, as for all mankind by physiological necessity, variety is not merely the spice of life, but the soundest road to amino-acid "salvation."

§ 53 There is one other feature of protein metabolism that needs a brief account here. We have already seen that the calorific equivalents of the principal food constituents are accurately known from purely physico-chemical experiments. The amount of heat that should be derived from them in the body can be calculated to a nicety. If a man in a state of rest takes a certain amount of food, in which the proportions of fat, protein, and carbohydrates have been determined by analysis, then his body should be able to produce the corresponding amount of heat, and no more. By what the physiologists call animal calorimetry, the actual heat production during rest—called the basal metabolic rate—can also be measured with considerable accuracy.

It is invariably found that this amount of heat is actually more than can be accounted for by the amount of food consumed, on the assumption that it has all been completely burned up in the body. Further, this excess heat production is almost entirely due to the protein in the food, and indeed to certain, but not all, of the amino-acids therein, and hardly at all to the fat or carbohydrate. This effect of protein on the production of bodily heat (or energy of movement, if the subject is not at rest) is

called the "specific dynamic action" of the protein or amino-acid.

§ 54 The exact mechanism by which amino-acids exert this specific dynamic action is not fully understood. Possibly it is associated with the conversion of the amino-acids to carbohydrate. Be that as it may, one thing is clear. The heat must come from somewhere. It does not come from the food, and must therefore be derived from the combustion of cell constituents already present in the body before that food was consumed. This stimulating effect of food-protein on bodily oxidations has an important consequence. It indicates clearly that a high-protein diet should be efficacious in enabling the body to burn up its existing stores of fat and carbohydrate. A diet of lean meat and lean fish must therefore be a "slimming diet," while hardly at all depriving the body of essential food constituents, for we have seen that protein can be converted into carbohydrate and then into fat, if required. But in being so converted it will cause the combustion of more fat or carbohydrate or protein than it can itself replace. The use of high protein diets, when not otherwise contra-indicated (by impaired kidney action, for example), as a means of reducing excessive weight is, in fact, advocated and does, in fact, succeed.

SECTION III—MINERALS

CHAPTER X

THE MAJOR MINERAL ELEMENTS

§ 1 IN this chapter I am using the word " mineral " in a wide sense; it is taken here to include all the elements, non-metallic as well as metallic, that appear in foods, apart from the four appearing in organic combination as fats, carbohydrate, and protein—that is, excluding carbon, hydrogen, oxygen, and nitrogen, but *including* sulphur and phosphorus, although these occur organically combined in many proteins. The " mineral " elements, in short, are roughly those that the chemist reckons as occurring in the " ash " of a food after ignition.

§ 2 Just as the constituents of food as a whole can be roughly divided into " major " and " minor "—some ninety-five parts by weight out of a hundred being accounted for by fats, carbohydrate, protein, and water—so can the odd 5 per cent. be roughly divided into those elements and compounds that preponderate and those that, however important, are required only in " traces." These trace substances include the vitamins and possibly some other complex organic compounds of less well-defined properties and composition, as well as certain individual elements—both metallic and non-metallic; the preponderating elements on the other hand, are few in number, and the recognition of their importance

belongs to the period of "classical" nutritional theories. Even those who did not in the least suspect the dietary insufficiency of foods containing only fat, carbohydrate, protein, and water, would have been the first to agree that calcium was needed to form teeth and bone, and iron to form blood, and that certain other elements besides carbon, hydrogen, nitrogen, sulphur, and phosphorus were probably not present in the human frame as mere accidental contaminants.

§ 3 On the other hand, it is even to-day by no means certain, however highly probable, that traces of copper, manganese, aluminium, zinc, cobalt, and fluorine have an essential rôle to play in the metabolism of human beings—whatever may be true of experiments with laboratory animals. Intermediate between these and the "major" mineral elements are others, in particular sodium, potassium, manganese, and iodine: present in intermediate amounts, they are certainly ubiquitous and indispensable.

§ 4 The division of these essential chemical elements into two main groups, though it may at first sight appear a little arbitrary, is in fact an historically justifiable as well as a convenient method of presentation. In this chapter, therefore, we shall consider briefly all the *major* items in this group of *minor* food constituents, both those recognized in "classical" nutrition and those of which the essential nature has been more recently established. In the next chapters we shall briefly discuss the "trace" elements and so, we hope by an easy transition, pass to the vitamins, which, though of highly complex organic structure, do their jobs in quantities of the same order as the "trace" elements themselves. To take a single example—the amount of copper required daily by the young growing albino rat is about 8 micrograms per day;

the amount of aneurin chloride (vitamin B_1) required daily by the same animal is about 4 micrograms (a microgram is one thousandth of a milligram, itself one thousandth of a gram, and about one sixtieth of a grain). The discovery of the vitamins, their natures and functions, has in actual fact occurred during the same period as that of the trace metals; both discoveries were undoubtedly to a large extent made possible by contemporaneous improvements in laboratory methods, especially in the technique of animal experimentation.

§ 5 Bone and teeth consist largely of the carbonate and phosphate of lime (calcium oxide). The carbon, oxygen, and phosphorus of these salts could, of course, at any rate in theory, be derived from the major food constituents, since oxygen occurs in all of them, carbon in fats, protein and carbohydrate, and phosphorus in many proteins, as well as in some compounds called phospholipids, closely associated with both animal and vegetable fats, although never present as more than a fractional percentage by weight in food. (A fairly well-known phospho-lipid is lecithin, present in egg-yolk and in soya-bean oil, and in other natural fats and oils.) Even, however, if the phosphorus of protein and phospholipid were adequate for the formation and maintenance of bones and teeth, there would still be other phosphorus requirements of the body to meet and especially, as the perspicacious reader will have foreseen, the requirements for building up the body's phospho-proteins and phospholipids.

§ 6 The phosphorus required for formation of bones and teeth occurs in foods along with calcium, combined as one or more of the various simple or complex phosphates of calcium. Neither element is of any use for skeletal purposes without the other. It is, however,

hardly to be put down to luck that man, like other vertebrates, finds together in foods two elements needed by him for the same bodily purpose. It is more logical to argue that the occurrence together of these two elements in plants and lower animals was itself a determining factor in allowing the evolution of organisms that require for an essential part of their structure both these elements together.

(Whatever the evolutionary truth of this matter, the fact is beyond dispute. Man must have both elements for his bones and teeth: moreover, to direct this exogenous calcium and phosphorus to their structural end in bone and teeth, vitamin D is indispensable. Of this we shall have more to say later. Calcium and phosphorus also both circulate continuously in the blood-stream of adults, as of growing infants and children. The amounts of these two elements present in the blood of healthy individuals oscillate only between rather narrow limits; small departures from these lead immediately to pathological symptoms; large departures may have fatal results. The metabolism of calcium and phosphorus is at least as important to the living vertebrate organism as that of any other element, not even excepting iron, vital for blood formation; fortunately both elements are widespread (though calcium is comparatively abundant only in milk and cheese and a few vegetable products) and are generally absorbed and assimilated with great ease.

§ 7 Magnesium, an element closely related to calcium, seems, on the evidence of animal experiments, to be essential to normal health; its metabolism is closely connected with that of calcium and phosphorus, and it is mainly distributed along with them in foods. It seems particularly concerned with the physiology of the nervous system and the capillary blood vessels.

There seems no reason—at present—to believe that the other elements closely related to calcium—strontium and barium—or the elements related to phosphorus—arsenic and antimony—have any effect other than a toxic one on the human organism, and they may therefore be allowed to pass from the picture being attempted in this book.

§ 8 Iron, though it has other parts to play in the complex chemistry of living processes, is of primary importance to man and other vertebrates because it is an essential element in hæmoglobin, the red pigment of the blood-cells; it is also present combined in those body pigments that control the respiration of individual cells—the burning up of cellular contents to give heat and energy. Hæmoglobin has the fundamental task of carrying oxygen from the lungs to the various parts of the body where the fuel constituents of food are burned. When the iron supply is insufficient, owing to shortage in the food or to defective absorption or inefficient utilization, anæmia develops—perhaps the commonest of all "deficiency diseases" afflicting the human races. This is no place to discuss the different types of anæmia, but it is necessary to mention that those due to iron deficiency have certain well-marked characteristics that distinguish them clearly from, for example, pernicious anæmia, which is due to quite a different cause and is in no way to be cured, or even mitigated, by treatment with iron.

§ 9 It has recently come to be recognized that not all forms of iron are equally well assimilated. Although it is possible to determine the assimilable iron in food by biological tests, they are extremely laborious: fortunately investigators have found a simpler method of distinguishing between the more and the less easily used forms, and are now able by chemical analysis to evaluate quite accurately any food material as a source of iron for

nutritive purposes. It is established beyond any doubt that the simpler salts of iron—the phosphates in foods, the sulphate and chloride in medicinal preparations—are the most effective both in preventing and in curing nutritional anæmia.

Iron in the mammalian body is present as several organic compounds, of which the best known is hæmoglobin; the amount of hæmoglobin in the blood, and of the red cells containing it, can show with considerable accuracy the absence or presence of anæmia, and the extent of its remission under treatment. That is probably why hæmoglobin was formerly recommended as a means of treating iron deficiency and why red meat was regarded as of great value for blood formation. Modern researches have shown this view to be unjustified; the prescription of iron in "natural" association with chlorophyll, the green pigment of plants (itself free from iron), is based upon a like error. Even some of the less complicated iron compounds, such as the "scale salts" official in many pharmacopœias, are inferior to the simple salts already mentioned. For the subject suffering from nutritional anæmia, or even from some of the pathological forms, the simplest iron salts, and foods containing iron that is not in organic combination, have much the greatest value.

§ 10 In order to cure certain pathological anæmias, and also —though to a less extent—simple nutritional anæmia, especially in the severer cases, very large doses may be required even of the best (that is, the simplest) iron compounds. Some investigators have suggested that this may be explained in terms of the amount present of some "trace" element, accompanying the iron in small and variable quantities: they have even named cobalt as a possible activator of iron. We shall return to this question of "trace" elements in the next chapter.

More recently experiments on normal subjects and anæmic patients have pointed to a rather different explanation. Apparently a deficiency in hæmoglobin, even when of nutritional origin, cannot always be made good unless much larger quantities of iron than are required to make the hæmoglobin level normal are consumed and actually absorbed. It is known that by giving extremely large doses of iron, of which a great deal is almost immediately lost in the fæces, a store of iron can be built up in the body, very little passing out by way of the kidneys. It may, therefore, be necessary in some persons to build up, as it were, a considerable "pressure head" of iron in the body in order to promote adequate hæmoglobin formation. It is worth remarking here, by the way, that high doses of iron compounds are, despite popular and even some medical opinion, not in the least constipating, though small doses may be!

§ 11 Whatever the explanation of this mysterious storage of iron at some unknown site or sites in the body, it has one obvious corollary for the dietician. Excess of iron in the diet is harmless; deficiency of iron leads to nutritional anæmia that may require prolonged treatment with large doses of iron to restore a normal blood picture. There are in this country large numbers of anæmic children and adolescents. The advocates of increased milk consumption—essential though this is for public health on other grounds—are doing nothing to remedy this; milk, as we shall have occasion to point out later, is a very poor source of iron. For this we have to depend on green vegetables, some cereals—especially whole-meal flour and oatmeal—legumes, liver, and egg-yolk.

§ 12 It is necessary to point out further that women almost certainly need more iron than men. Hæmoglobin determinations on similar groups of men and

women apparently quite free from outward manifestations of anæmia almost always show a slightly lower figure for the women. Moreover, that figure can be raised to the male level by a moderate iron dosage that has no effect on the hæmoglobin in normal male blood. This gives strong presumption that the women are, indeed, slightly anæmic—as opposed to the suggestion that women's "physiological level" of hæmoglobin is lower than man's. Apart from the requirements of the fœtus in pregnancy (and also the loss of iron in blood at parturition), the monthly loss of blood during menstruation is probably an important factor in determining the higher iron requirements of the adult woman over those of the adult man.

§ 13 The commonest salt known to man is, not unnaturally, common salt. Indeed, its presence in sea-water has determined the whole course of evolution. Those readers who have a taste for both biochemistry and philosophical speculation will find this subject expounded in a most fascinating manner in Dr. Ernest Baldwin's *Comparative Biochemistry* (Cambridge University Press, 1937: 5s.). Common salt—sodium chloride—is as widely distributed inside man as outside: every body-fluid, and especially the blood-stream, contains more sodium than any single other metal. Chlorine is present to a somewhat greater extent, and is the most abundant non-metal of the "minor" constituents in the human frame.

A metal closely related to sodium is potassium, and this is present mainly in the blood corpuscles, as distinct from the serum in which they circulate: incidentally the corpuscles contain no sodium at all.

§ 14 Substances in solution in an enclosed space exercise a pressure on the enclosing walls: this is called "osmotic pressure." If the walls are permeable, and if

there is a watery fluid on either side of these walls, water will pass from one side to the other until the osmotic pressure is the same on both sides. A strong solution inside such a permeable container will become weaker by infiltration of water if there is a weak solution of salt on the outside, while the outside solution will thereby become more concentrated. These osmotic phenomena are to a large extent independent of the chemical nature of the substances in solution: those substances may therefore be regarded as of physical rather than chemical importance. It is broadly speaking true to say that the main function of sodium, combined with chlorine and carbonic acid as chloride and bicarbonate, is of this physical nature, while potassium acts in a more specifically chemical manner. A shortage of sodium in the food of experimental rats permits normal growth for a relatively long period, but potassium deficiency results in early death.

§ 15 Sodium, potassium, and chlorine are not merely abundantly present in food, but they are also very widely distributed. Almost any diet is probably an adequate source of all three; the biological origin of taking salt with meals is rather difficult to explain. The requirements for these elements are, moreover, not great in normal circumstances, but it should be noted that perspiration is very rich in sodium chloride. Consequently continuous very violent exercise or hard manual labour may result in a temporary shortage of salt in the body-fluids, and the condition known as miner's cramp is due entirely to this. The miner's remedy—to take extra salt, and even to add salt to his drinks, or to any water he may consume—is the result of traditional knowledge that is physiologically unimpeachable. There is, however, some evidence that gradual acclimatization to

work at high temperatures may make an increase of salt intake unnecessary; the body, apparently, learns to sweat more water and less salt, and so not to disturb the balance of osmotic forces between the fluids inside and outside the cells in the body.

§ 16 Chlorine plays one other important rôle, besides keeping sodium and potassium, as chlorides, dissolved in body-fluids and tissues. It is needed to form the hydrochloric acid—a compound of hydrogen and chlorine—secreted by the stomach, where it takes part in some of the early digestive processes—particularly in assisting the breakdown of proteins, as we have already described. The actual *amount* of chlorine required for this purpose is, however, relatively small. The quantity of hydrochloric acid in gastric juice seldom reaches more than one quarter per cent. In other words, a pint of gastric juice contains an amount of hydrochloric acid that could be derived from $2\frac{1}{2}$ grams of common salt—under one-tenth of an ounce. Actually, of course, the volume of gastric juice in the stomach is much less than one pint: the salt equivalent of the hydrochloric acid in the normal stomach, even when it is at its maximum after a meal, does not exceed half a gram. As the equivalent of 10 grams of sodium chloride may be lost daily in the urine, apart from any passing out in the fæces, it is clear that the amount of chlorine needed for the formation of gastric hydrochloric acid, essential though it is, is small compared with other bodily requirements. The blood alone in a man weighing 10 stone contains chlorine equivalent to about 60 grams (2 ounces) of sodium chloride or 80 grams of potassium chloride.

CHAPTER XI

THE HORMONES—IODINE

§ 17 A BRIEF consideration of the endocrine or ductless glands—also called the glands of internal secretion—is necessary at this point. The elements from which the active principles of these glands, known as hormones, are built up must originally have come from food. There is no evidence whatever to suggest that man ingests any of his hormones as such, and it may quite safely be taken that he normally can synthesize all of them.

The glands of the animal body are divided broadly into two classes: those with special channels, called ducts, in which their secretions are carried into the appropriate organs, and those that discharge their secretions directly into the blood-stream. There is no reason why one gland should not fall into both categories; the pancreas, in fact, does.

§ 18 During recent years much progress has been made in identifying, isolating, and even synthesizing the individual hormones. One of them, adrenalin, has been available as a synthetic "fine chemical" for more than twenty years. Adrenalin is the hormone from two small glands situated quite close to the kidneys; they are sometimes called the suprarenal glands, and the hormone has been called epinephrin and suprarenin. The terms adrenal and adrenalin, however, are most generally accepted. Its property of raising the blood-pressure is

due to its ability to constrict the smaller arteries or arterioles—a fact made use of by the dentist, who frequently injects local anæsthetic and adrenalin simultaneously.

§ 19 More recently thyroxin, the essential substance from the thyroid gland, has also been synthesized. It is distinguished from all other known hormones by containing iodine, and it is for the production of thyroxin that iodine is required as an essential nutritional element.

The thyroid gland plays a fundamental part in the control of the body's oxidative processes. The amount of energy required by a resting organism—for maintenance of body temperature, for the action of the muscles that continue to work even during sleep, such as the heart muscles—is called the basal metabolism. Lowered thyroid activity reduces the basal metabolic rate and has many other serious effects. In extreme cases it may lead to a condition known as myxœdema, or to cretinism, or to one form of goitre. Investigations in many parts of the world have shown a close correlation between the incidence of endemic goitre and the amount of iodine in the soil, food, and drinking-water. Exophthalmic goitre, however, is due to excess of thyroid activity—hyperthyroidism; it is not due to iodine shortage or cured by its administration.

Goitre is never endemic near the sea or in other places where iodine is relatively abundant; where, however, endemic goitre exists, iodine supplies are always found to be abnormally low. It is possible practically to exterminate endemic goitre by enforcing legally the iodinization of table-salt and cooking-salt. The amount needed is relatively very small; the requirement of the average adult is about one quarter of a grain *per year*! Quantities of iodine far in excess of this amount are

quite harmless—it is doubtful if they would seriously affect the few rare individuals suffering from hyperthyroidism—that is, excessive production of thyroxin; the cost of adding to all salt for human consumption an amount of sodium iodide sufficient to ensure that everyone obtained, say, an additional grain of iodine spread over twelve months would be almost negligible. (Children appear to require about three times as much iodine as adults.) There is, therefore, a good deal to be said for compulsory iodinization of salt in all countries, even where there is no question of endemic goitre.

However that may be, there remains the indubitable fact that certain forms of hypothyroidism—an *endocrine* disturbance—are undoubtedly caused by *wrong food* and can be cured by making good the dietary deficiency. This is the only clear and proven instance of such a connection.

§ 20 It must, of course, not be forgotten that, as has already been pointed out, *all* our hormones are ultimately of dietary origin. But the fats, carbohydrates, and proteins of our food contain all the elements necessary for building up those other hormones of which the elementary constitution is known. Some contain only carbon, hydrogen, and oxygen, like the fats and carbohydrates. Others certain nitrogen as well, and must presumably derive this from dietary or bodily proteins (see Table I, p. 72). At least one hormone—insulin—contains sulphur, and this may be derived either from certain proteins (or their appropriate amino-acids) or, less likely, from mineral sulphates.

It may be objected that the new-born infant's body contains sufficient of all these elements to cope quite easily with all its hormone requirements to the end of its days, and that therefore no exogenous origin of the

hormonic elements need be postulated. To this the answer is simple: the life of the organism does not begin at birth, but at conception: the fertilized ovum—at any rate in mammals—is clearly not sufficiently endowed for its subsequent endocrine demands. If the new-born infant were so endowed, this could only be the result of inter-uterine nutrition. In other words, if the elements required for our hormone production are not derived from our food after our birth, they *must* be derived from our mother's food before our birth, for the embryo certainly does not carry with it into the world a full adult complement of hormone. Actually, of course, there is no need to assume so remote a connection between food intake and hormone production: the simpler hypothesis is most likely the right one. Our hormones, like all other bodily secretions, are built up from elements ingested in our food.

§ 21 When we remember the relationship between iodine intake and thyroid secretion, it is not at all fantastic to suggest that there may be other disorders of a similar nutritional origin caused by a lack of some element or group of elements necessary for the production of a hormone—either by actually entering into its composition, or by taking part in the chemical reactions necessary to produce it.

Of the hormones besides adrenalin, insulin, and thyroxin, little can be said here, since nothing but a general connection can be traced between them and nutrition. One hormone, only recently isolated, is that of the adrenal cortex (the rind of the gland); it is called corticosterone, and has been successfully used in the treatment of Addison's disease, hitherto always fatal in a very short time. The disease is characterized by bronzing of the skin, nervous symptoms, gastro-intestinal

disturbance, and sexual decline. A clue to its cause was given by the fact that it showed some marked similarities to the condition of rats deprived of their adrenal glands.

Unfortunately the pure hormone—whether it be of natural or of synthetic origin—is extremely expensive, and the necessary dosage is prohibitive to all but the very wealthy. It is therefore interesting to note—and germane to a discussion on connections between nutrition and endocrine physiology—that the dosage of corticosterone required for treatment of Addison's disease can be considerably reduced by increasing the amount of sodium chloride (common salt) in the patient's diet. The adrenal cortex through its hormone is apparently concerned in some very fundamental way with those osmotic phenomena mentioned in § 14 of the last chapter; for Addison's disease is accompanied by increased excretion of water and of sodium chloride and a reduction of mineral matter in the blood. The successful combination of dietary with hormone treatment of this disease again calls our attention to the importance of research in the borderland between nutrition and endocrinology.

NOTE.—A table showing the hormones and the chemical elements present in them will be found overleaf.

TABLE I

Elements and Hormones

GLAND	ACTIVE PRINCIPLES	ELEMENTS PRESENT
PITUITARY ANTERIOR LOBE	Possibly several	Probably Carbon, Hydrogen, Nitrogen, Oxygen.
POSTERIOR LOBE	α- and β-hypophamine	Possibly Sulphur, Phosphorus, and others.
PANCREAS	Insulin	Carbon, Hydrogen, Nitrogen, Oxygen, Sulphur.
GONADS TESTIS	Testosterone Androsterone	Carbon, Hydrogen, Oxygen.
OVARY	Oestrin Progesterone	Carbon, Hydrogen, Oxygen.
ADRENAL MEDULLA	Adrenalin	Carbon, Hydrogen, Nitrogen, Oxygen.
CORTEX	Corticosterone	Carbon, Hydrogen, Oxygen.
THYROID	Thyroxin	Carbon, Hydrogen, Oxygen, Nitrogen, Iodine.
PARATHYROID	Not isolated	Probably Carbon, Hydrogen, Nitrogen, Oxygen. Possibly others.
THYMUS, PINEAL BODY, SPLEEN	Endocrine activity uncertain	

CHAPTER XII

TRACES

§ 22 THAT many elements, previously thought to be present in food as accidental and inert contaminants, are almost certainly essential to the well-being of those that consume the food, has come to be recognized only during this century. Two main causes have contributed to the change of view. First of all, our knowledge of the fundamental changes brought about by the presence or absence of minute quantities of certain substances—the hormones, the vitamins, the enzymes, in particular—is of only recent origin. Secondly, the detection of many of the trace elements had to await the necessary refinements of analytical chemistry. Some trace elements, indeed, are not yet to be accurately estimated by chemical means, and the spectroscopist has had to be called in to establish their presence or absence. Spectroscopy, too, has sharpened its weapons, and is to-day capable of a delicacy of detection and an accuracy of measurement not dreamt of fifty years ago.

§ 23 One word of warning must, in honesty, be given here. Conclusive though the evidence is for the essential part played by several trace elements in the metabolism of animals, including often domesticated farm animals as well as those in the laboratory, direct information as to *man's* requirements is difficult, if not impossible, to obtain. The conclusion that man also needs many, if not all, of these trace elements is almost

entirely a matter of inference. The wide distribution of these elements, their detection in the human body when careful chemical analysis has been part of any post-mortem examinations, the general principle that substances essential to the life of one species of mammal are likely to be essential to others, the occasional, though rare, occurrence in human patients of conditions similar to those produced in experimental animals deprived of some trace element, and similarly amenable to treatment by its addition to the diet—all these facts, and others, make a strong *prima facie* case for holding that man does indeed need these traces.

§ 24 Though it is not possible here to describe, even in outline, the many researches that support these views, it is proposed to give some details of one of them, perhaps the most important, because of the satisfying completeness of the investigations and the general acceptance commanded by the results. It is the story of how copper came to be recognized as indispensable for the formation of hæmoglobin, a story of which the main scene is the laboratory of Professor E. B. Hart and his colleagues at the University of Wisconsin in Madison.

If rats are fed on a diet of milk only, or milk and some highly purified cereal product, like starch, they ultimately die. Supplementing this diet with any or all of the known vitamins in pure or highly concentrated form does not ward off this end. The Wisconsin workers soon found that the immediate cause of the rats' decline is anæmia, a shortage of hæmoglobin and the red-blood cells that carry this pigment. They proposed to call the condition, which is brought about by simple dietary restriction, milk anæmia or, more generally, experimental nutritional anæmia.

The classical, and to us obvious, remedy for anæmia—

that is, the administration of iron to the patient—gave very variable results when applied to these anæmic rats. Sometimes they got better, sometimes they did not. The treatment was particularly ineffective with very pure iron, given as one of its salts, the chloride or sulphate. On the other hand, quite small doses of liver were dramatically effective. Liver was known to be a relatively rich source of iron, but this could hardly be the cause for a cure not to be brought about by iron itself, especially when purified.

§ 25 Hart and his associates therefore next turned their attention to liver. They burnt liver in crucibles, and tested the effect of the resulting ash on the anæmic rats. If the ash was fed as such, there was very little result; but if the ash was dissolved in a little acid and fed in a dilute aqueous solution to the rats, they recovered. This, at any rate, established one conclusion of fundamental importance; whatever it was in liver that cured the anæmia, it was certainly not any of its *organic* constituents—fat or protein or carbohydrate, for example—for all of these are entirely destroyed during the process of incineration. The next question to tackle was, which of the inorganic constituents in liver-ash, soluble in acids, was the curative agent.

§ 26 To settle this, they did something so simple that many other workers probably want to kick themselves for not having thought of it. When the analytical chemist is confronted with a solution of metallic salts—that is, of metals or their oxides dissolved in acid—and wants to find out which metals are present, he follows a very old-established procedure for dividing up the metals present into groups by the use of suitable reagents. He makes his solution very acid with hydrochloric acid, and the relatively insoluble chlorides of silver and lead

are thrown down as precipitates, which he can separate from what remains in solution by simple filtration. He then passes hydrogen sulphide gas through this solution, and all the metals present with sulphides insoluble in hydrochloric acid are thrown down as those sulphides. Other reagents are successively employed, and then by similar procedures each group is itself subdivided until every individual metal is recognized by the use of some reaction distinguishing it from those that have accompanied it.

This is exactly what the Madison investigators did with their solution of liver ash. But instead of wasting time—and animals and material—by separating *all* the metals present, some of them in minute quantities, they carried out preliminary tests on the precipitates in each group. The insoluble chlorides, brought back suitably into solution, were inactive; so were iron, aluminium, zinc, manganese, cobalt, and nickel, which are thrown down together in "Group III" as hydroxides. The alkali metal group, in which calcium, strontium, and barium occur, also proved useless. But the "Group II" precipitate, in which are to be found the sulphides of mercury, copper, arsenic, and cadmium, was remarkably effective. A solution of this precipitate in acid, diluted with water, could cure, and would prevent completely, the development of milk anæmia in Hart's experimental rats.

§ 27 From this point on, developments were rapid. None of the metals in this group, whether separated as sulphides from liver ash, or taken from the laboratory reagent bottle as soluble salts, was of the slightest benefit to the anæmic rats—except copper. That element worked every time. It mattered not whether the chloride or the sulphate or the nitrate was used; extremely small

quantities of any of them were completely effective in counteracting the evil effects of the milk diet. With these traces of copper, the rats grew nearly normally, and their blood carried its full complement of red cells and of hæmoglobin. Even better results were obtained with copper *and* iron, though pure iron by itself, as we have already seen, was useless.

§ 28 Milk, especially cow's milk, never allowed to come into contact with iron or steel vessels, is a poor source of iron, and is also practically devoid of copper; both these elements are essential for the formation of normal blood. The reason why the young rat could manage fairly well without any iron at all to supplement its milk ration, but was doomed without copper, was subsequently found to be easily explained; it is born into the world with sufficient reserves of iron in its liver to carry it over the early stages of its life. But this iron cannot be mobilized in the absence of copper, which is present neither in the infant rat's own body nor in the milk diet. This phenomenon of an "iron reserve" in the liver of the new-born has been found to be quite general: even the infants of ill-nourished mothers seldom show signs of anæmia before the age of six months. Their reserves, however, are not only soon exhausted, but are built up at the expense of the mother's own supply. That is the reason why modern nutritionists can hardly emphasize sufficiently the need to secure a high iron intake for expectant and nursing mothers.

§ 29 For the reasons already mentioned, we cannot be absolutely certain that man requires copper as the rat does. Fortunately, however, most foods that are sources of iron are also sources of copper, and medicinal iron salts, too, are nearly always accompanied by traces of the other metal. The Wisconsin workers found that

the optimum ratio of copper to iron was only about 1 to 50. There is some evidence that certain cases of nutritional anæmia in the human subject respond better to treatment with copper plus iron than with iron alone, but certainty as to the essential rôle of copper in human nutrition has not been reached. It is practically impossible to arrange for human beings, even if they agree to become experimental animals, to receive a copper-free diet, and without establishing the effect of such a diet we cannot get a final answer to the question. That man does need copper remains a conclusion based upon indirect evidence, but the weight of that evidence is impressive to the point of conviction.

§ 30 Our knowledge of man's need for other trace elements is less firmly grounded. But there are several of which it can be asserted with confidence that the experimental rat cannot live normally without them. Manganese, for example, can only be completely withdrawn from their diet at the expense of their reproductive system. There is also evidence that manganese can play a part similar to copper in the formation of red blood-cells, but this has been disputed by some investigators. It has, however, been pointed out that the wide distribution of manganese, and especially its regular occurrence in the reproductive organs of animals and plants, is a further reason for regarding it as an element essential to normal health. Attempts have been made to detect bodily changes due to diets containing quantities of certain other elements well below what is normally found in foods: no evidence that laboratory animals need zinc, nickel, silicon or fluorine has yet been obtained experimentally.

§ 31 One metal, however, is of unusual interest in this connection. Some years ago it was found that

adding cobalt to the diet of young rats produces a large increase in the number of blood-cells, a condition known as polycythemia. It has not, however, been possible to show clearly that deprivation of cobalt, normally present in diets only in minute quantities, has any adverse effect, whether on blood composition or otherwise. Nevertheless it has been proved beyond the slightest doubt that one species of animal—the sheep—is incapable of living without it.

In some parts of Australia and New Zealand an apparently incurable disease in sheep was found to be associated with soils that contained only a negligible quantity of cobalt. This correlation was established only after much painstaking research, and after many false clues had been arduously followed. The disease, called "pine" or "coastal sickness" and by other names, was attributed to iron shortage, to infestation by parasitic worms, and to various causes now known not to be the real source of the trouble. The administration of cobalt to these sick animals has an almost incredibly dramatic result. As little as one milligram a day will restore to health a sheep that is literally a bag of bones, doomed to almost immediate death from this costly disease. In a week or so the sheep is "as good as new"; a similar result can be secured indirectly by dressing the soil with appropriate preparations of cobalt and transferring the sheep to grass growing on the treated soil, or even to pasture on which other sheep thrive and where cobalt is presumably present in adequate amount. There is reason to believe that the lack of cobalt in some districts of England—for example, on parts of Dartmoor—is responsible for outbreaks of illness among the sheep there.

The fact that cobalt is indispensable to the sheep but has not been found essential for the rat is further warning

against arguing too readily from one species of animal to another, and leaves its necessity to man still an open question. Nevertheless, when we remember the relative sizes of sheep and rat, and the minute dose required by the former, we can well imagine that the latter's demand for cobalt may be so small that analytical methods have failed to detect the necessary traces in its food.

§ 32 This difficulty has immensely complicated all research in the field of "trace" elements. Even the now-accepted need for manganese took a lot of proving, and was indeed not proved until sufficiently refined chemical and physical technique made it possible to prepare diets almost entirely free of manganese. There is never any possibility of saying that *any* element is absent from a given satisfactory diet: all that the analyst can do is to say that it contains less than a certain quantity—the smallest quantity, namely, that his methods are able to detect. Experimental animals are almost always *small* animals, and their requirements may be undetectable by available methods. The most we can say of man's requirements for a trace element is either that there is evidence that he needs it, or that there is no evidence: the lack of evidence may be qualified by considering the distribution of the element in ordinary foods and in the earth's crust, and other relevant facts.

Progress in this field has been satisfactorily rapid during the last two decades, and no one will be surprised if other elements have to be added to those classified in this chapter as essential to one species or another—namely, copper, manganese and cobalt: sooner or later we may have to add to them zinc, aluminium, fluorine, silicon, and even other less abundant elements.

SECTION IV—VITAMINS

CHAPTER XIII

VITAMINS IN GENERAL

§ 1 To talk, as is often done, of the discovery of the vitamins—whether the credit thereof be given to Hopkins, Funk, McCollum, Babcock, Ragnar Berg, Eijkman, Grijns, or any other of the distinguished scientists who first made laboratory experiments with "accessory food factors"—is to sacrifice historical accuracy to subconscious hero-worship. The pioneers in the field would probably be the first to admit that their early investigations originated in no sudden genial flash of inspiration. An increasing interest in the scientific bases of nutrition accompanied a growing awareness that diet and health were closely connected, and this was itself perhaps in part a reaction against the predominatingly bacteriological view of disease. People were beginning to wonder, moreover, not only at the prevalence of disease despite great advances in social hygiene and the prevalence of poverty despite the economic triumphs of the Victorian age, but also at the patent correlation between disease and poverty.

§ 2 Those who were well versed in what we have called the "classical" views of nutrition knew that many of its exponents, years before, had hinted at the possible existence of essentials other than fat, carbohydrates, protein

and minerals. There is to-day hardly a book on vitamins —and there are several excellent ones—that does not open with some quotation from an eighteenth-century author, or perhaps even an earlier one, suggesting lines of thought that might well be considered the beginnings of modern vitamin science.

It must, however, always be remembered that speculation may be far removed from knowledge, especially in those sciences that are experimental rather than observational. For advances in these sciences are at least as much dependent on the state of experimental technique at any time as on the brilliance of their practitioners. And the state of experimental technique in a given science is in turn related to that of the contiguous sciences.

The development of vitamin science in the last three decades has been made possible only by the application of modern methods in chemistry and physics to problems that had themselves been recognized for many generations. Scurvy, beri-beri, rickets and pellagra figure in the chronicles of travellers and discoverers since the beginning of world navigation. But micro-analysis, that enables a chemist to assign a formula to a compound of which he has only a few milligrams at his disposal, and spectroscopy, that can detect a fraction of a part of one metal in the presence of a million parts of another, are products mainly of the twentieth century. Why these technical weapons became so sharpened at this particular time is a matter that the student of scientific history will find well worth while pursuing.

§ 3 The simple question "What are vitamins?" is often asked of those who speak or write on the subject. As will be seen by the readers of the following pages, it is one to which no simple answer can be given.

The even simpler question "What is a vitamin?" is almost as difficult to answer with a clear-cut definition. Moreover, to-day a definition in general terms is hardly called for; most readers of the daily and weekly press have an idea, however vague, of the word's broad meaning.

As we shall see, every known vitamin—by which is meant every vitamin that has been isolated in the pure or almost pure state—is a single, clearly-defined chemical compound; this statement is in no way invalidated by the fact that we have instances of more than one compound having the same vitamin action. The fundamental fact is that all of them are entities in exactly the same way that a fatty acid or glucose or an amino-acid or an iron salt or a hormone, is a single chemical entity. They are not "states of matter" or "forms of energy" (save in the ultimate analysis of atomic physics, like all other substances). They are individual chemical compounds.

If we have stated the fact rather more often than the reader thinks tactful, we would offer him two reasons. First, that there is still so much loose writing in the lay press (and indeed not always only in the *lay* press) about these food constituents as to make it necessary to do everything possible to try to destroy the obscurantism that still largely surrounds them—an obscurantism that breeds charlatanism and dishonest commercialism with terrifying fecundity. Secondly, that we have written of the vitamins as *individual* chemical compounds, and we have done this with intent. By virtue of their individual nature, the vitamins have individual properties. They can in no circumstances whatever replace one another. The man who takes his morning orange-juice because he thereby gets his daily requirements of "vitamin" has failed to grasp this essential point.

Apart from the fact that fruit-juices are of nutritional value for reasons other than their vitamin content, orange-juice is a valuable source of one vitamin, and of only one vitamin. Crates of oranges are useless in the treatment of rickets, for they contain no vitamin D whatever: only crates of oranges could mitigate night-blindness or beri-beri, for they are very poor sources of carotene and aneurin. But a few oranges a day will effect the complete cure of all but the most advanced cases of scurvy. And an orange a day will most certainly keep scurvy away from all normal subjects.

§ 4 There are two loose statements made about the vitamins that call for brief comment here. It is often said that a complete diet will give any individual *all* the vitamins that he needs, and further that man is entirely dependent on his food for his supply of vitamins. The first statement is true only if we *define* a good diet for the dweller in a modern industrial city as one containing fish-liver or fish-liver oil: the second statement is equally questionable, for normal man in natural surroundings of fresh air and *sunshine*, where this is not obscured by the urban smoke-pall, is as fully able to synthesize his own vitamin D as his own thyroxin or insulin.

What constituents of his food are necessary to enable him to effect this synthesis we can hardly even guess, but we know that some of the lowliest vegetable organisms can synthesize sterols, with which vitamin D is closely related, from simple food constituents like glucose. There is also experimental evidence that other animals, such as rats, can synthesize sterols: a laying hen puts out more cholesterol in her eggs and excreta than she eats in her food. Even if man has, in course of evolution, lost this particular synthetic talent, for practical purposes

the two statements just criticized remain demonstrably false. Rural man can make his vitamin D for himself, and is therefore not solely dependent on his food for his supply of vitamins as such; urban man, and especially the urban child, is likely to suffer from vitamin-D deficiency, and to be prone to rickets even on the best "all-round" diet, unless he receives some medicinal source thereof.

§ 5 This damnable iteration of the facts of vitamin specificity seems to be sadly needed. The quotation below appeared in a daily paper little over three years ago. If the doctor has been misrepresented, the sub-editor must be charged with ignorance otherwise to be laid at the medical officer's door. Anyhow, this is the report:—

> "Dr. ——, County Medical Officer, said he was afraid that the explanation for the large number of school pupils who were receiving after care massage treatment for rickets was due to the diet they were given.
>
> "They were not receiving sufficient protein or meat diet. The parents could only buy meat once a week, and at school the pupils got soup, bread, and pudding, but no meat in it."

If that is what he really did say, it shows that his heart may have been in the right place, but that his information was a little lacking. *No* amount of protein in the diet can have the slightest effect on rickets, and the undoubted ill effects of an inadequate protein supply are entirely distinct from rickets, nor will vitamin D or calcium or phosphorus additions make good the deficiency.

§ 6 The number of vitamins—or groups of vitamins—to-day recognized as occurring in foods cannot be

stated exactly. A few of them have been isolated, analysed and synthesized in the laboratory—and even on a large scale in the chemical factory. Another has had its exact chemical constitution determined but no synthesis of it has yet been achieved. Then there are some vitamins for the existence of which, and their necessity for mankind, there is strong, sometimes uncontrovertible evidence, although they have not been obtained in a state of purity—or even approaching purity. For this reason it cannot be known whether any particular one of them is indeed only one; it has happened several times in the history of vitamin research that a condition first thought to be due to a single deficiency has later been found to be of multiple origin—and it is only by the effects of their absence that the vitamins have come to be characterized.

§ 7 There are two further complications that make it impossible to answer in one word the question "How many vitamins are there?" We know of at least two substances that are not strictly speaking vitamins, but are turned by the body into vitamins. They are frequently spoken of as pro-vitamins. Carotene is turned into vitamin A, possibly by the liver, and it is certainly not the same as vitamin A, for the chemical constitution of both are known, and they are different. Again, the skin contains pro-vitamin D, a substance without any known direct physiological activity: under the action of certain ultra-violet radiations, natural or artificial, it is converted by the body into vitamin D.

Further, we know of at least one instance where two substances, different though closely related in chemical constitution, both occur in plant or animal products and both have the same vitamin action. Also, a number of artificial substances have been made in the laboratory

having the same action as naturally-occurring vitamins, if to a less extent, but differing slightly in constitution from the latter.

These facts will be discussed in a little more detail in the following chapters, but they afford another warning against the glib generalizations in which the subject is all too rich. In no circumstances is it justifiable to make artificially simple a position that is naturally complex, if this can be done only at the expense of accuracy. A refusal on scientific grounds to answer a particular question is not necessarily an admission of weakness; as often as not it means that the wrong question has been asked.

§ 8 There are, however, a number of questions that may legitimately be asked, because they can be scientifically answered—how the existence of a particular vitamin has been recognized, how its quantities in different sources have been estimated, how it has been purified, what constitution has been assigned to it, whether this constitution has been confirmed by synthesis, what are known to be the consequences of its absence from, or deficiency in, a human dietary, and whether it has any uses besides making good those deficiencies when they are liable to occur or have occurred.

It is impossible in this book to answer each of these questions for all of those vitamins about which the relevant information is available. We must therefore have recourse to a compromise. We shall try to give the answer in some detail to each one of these questions for at least one particular vitamin, and content ourselves, if not the reader, by summarizing the answers for the others—where the answers are known or suspected. In the next chapter, therefore, we shall discuss the question of recognizing a vitamin's existence, with special reference

to vitamin A. In the following chapter we shall consider the comparative and absolute measurement of vitamin C (ascorbic acid). Then we shall describe some of the chemical work on vitamin D (calciferol and certain allied substances). After that we shall consider the various deficiency diseases that are now held to be solely or primarily due to shortage of one or other vitamin. In the last section of this book certain aspects of human nutrition will then be briefly discussed.

CHAPTER XIV

DETECTING VITAMINS

§ 9 To treat this subject historically would involve considering many irrelevancies and following side-tracks that have led away from the main road of advance. They are inevitable in an experimental science, especially one that is concerned with the complex processes of living organisms. There are so many unknown factors that cannot be controlled by the investigator just because they *are* unknown. We shall, therefore, occasionally find it necessary to illustrate the arguments in this and subsequent chapters by describing experiments that may not have been carried out in the order or manner set forth. Nevertheless, they will all be, as far as it is humanly possible to make them so, accounts of what would have happened if the experiments had indeed been made and of what would happen if they were made to-day.

§ 10 Let us suppose we have available some young rats of the kind preferred by workers in nutritional laboratories. We want to see if it is possible to bring them up on a simplified diet of purified substances—satisfying the requirements of "classical" nutrition, and as complete as those requirements involve. It would be composed roughly as follows: first there would be some "good" protein—say the casein that is precipitated by acid from skim-milk. The casein would have been submitted to special treatment for removal of

any butter-fat that may have escaped separation from the skim-milk, for we want to control each major dietary constituent separately, and we intend to look after the fat-content next. This we shall do by including 10 per cent. or so of a good vegetable oil—say arachis (pea-nut) oil. We shall add a carbohydrate, cane-sugar or starch, and a mixture of mineral salts, not forgetting the trace elements, iron, copper and the rest, which we discussed in the last section of this book.

§ 11 This diet can conveniently be made into a paste with water and may be offered to the young animals *ad libitum*, to make sure that their partial starvation does not complicate our experiment. On such a diet it will be found that the animals, though taking enough food for calorific requirements and perhaps for some growth, would actually cease growing in a week or so, or even in a few days, and would all be dead in three or four weeks. "Classical" nutrition is not enough, evidently; the rats require something besides protein, fat, carbohydrates, minerals and water. If we add to the diet 10, or even 5, per cent. of dried yeast, the difference will be phenomenal. The rats will grow rapidly, remaining lively and healthy-looking for several weeks. The yeast has clearly supplied something besides its content of fat and protein (both of which occur in the yeast plant), for these were already present abundantly in our "basal" diet. But even the yeast will not suffice for continued health and life. After a few weeks—eight or ten or twelve, depending partly on circumstances connected with the nutrition of the young rats' mothers—growth will again cease; the animals begin to look sick, and a suspicious redness appears round their eyes. This is followed by a discharge of pus and blood from the eyelids, and bacteriological examination of this discharge

will reveal that the outer surface of the eye is heavily infected with micro-organisms. Some of the animals will develop pneumonia, to which the tame albino rat is rather prone; these will undergo a catastrophic fall in weight, ending fatally in a day or so. Others may merely lose weight slowly, but all will decline, and not a rat will be left at the end of two or three months.

§ 12 If, however, we take some of these animals before they are too far gone in their decline, and give them a single drop of medicinal cod-liver oil each day, a change will occur very nearly as dramatic as that brought about by yeast. The eye condition will clear up rapidly, and will become quite normal, unless the infection (known as xerophthalmia) has proceeded too far, in which event the animal may be left blind in one or both eyes, though the infecting organisms will have disappeared. For the most part the animals will begin growing again, and may live to a good old age on the original "basal" diet with its additions of yeast and cod-liver oil. In two respects, however, they will differ from normal rats and resemble their less fortunate brethren whom we may not have tried, or may have failed, to cure with cod-liver oil. They will be more or less permanently under-weight for their age, and post-mortem examination of their various organs will reveal the presence of many abscesses, now perhaps healed, in mouth and alimentary canal, as well as other lesions in kidneys, liver and so on. These abscesses and lesions would be found much more prominently, however, in the animals that died before any cod-liver oil was added to their diet.

This effect of cod-liver oil could also have been demonstrated prophylactically. That is to say, had we added the cod-liver oil to the basal diet at the beginning of the experiment (along with the yeast, of course), then there

would have been no xerophthalmia, no slackening or cessation of growth, no deaths from pneumonia, no internal abscesses or lesions—nothing, in short, to distinguish the rats from those brought up on a complete diet of natural foods. (This statement is not quite literally true; it is extremely difficult, even to-day, to devise a semi-synthetic diet that gives quite as good growth as, say, one made up of kitchen scrap, or one containing milk and meat and green leaves. Investigations in my own laboratory, however, suggest that the difference may be almost entirely one of palatability, and that the experimental diet and the diet of natural foods would be equally effective if the rats would eat as much of the former as of the latter. Broadly speaking, however, the supplemented basal diet can be considered as complete for the rat as present-day knowledge allows us to make it.)

§ 13 It is quite clear that the cod-liver oil contains something essential to the rats' health, and something that it needs in relatively small quantities, for we know that the one drop of cod-liver oil, about 20 milligrams or $\frac{1}{3}$ grain, consists very largely of fat similar in nutritive properties to the arachis oil abundantly present in the basal diet.

Now the use of cod-liver oil to cure rickets in the human being has been recognized in Western Europe and the United States for several decades, and on the coast of Norway, where it might almost be counted as a common food, for centuries. This led many investigators to the idea that the same constituent in cod-liver oil might be responsible for curing (and preventing) rickets in the human infant as for the dramatic effect on the deficient rat. Two sets of experimental facts, however, demonstrated that this could not be true.

§ 14 By choice of a suitable basal diet, rather different from the one described in § 10, it is possible to give to newly-weaned rats very severe rickets in the course of three or four weeks. This is cured or prevented by quite small quantities of cod-liver oil: in this and many other respects the condition is remarkably similar to the clinical rickets of the human young. It is called "experimental rickets"; the ability to produce experimental rickets in rats was discovered independently by two different schools of American scientists some fifteen years ago, and was one of the supreme technical advances in vitamin research.

Cod-liver oil, then, besides curing human rickets, will also cure experimental rickets in rats as well as the condition of deficiency associated with xerophthalmia. It looks more than ever as if the same factor were at work in all three cases. This appearance was soon dispelled. It was found that other substances, notably green vegetables and butter-fat, entirely or almost entirely ineffective in the treatment of human *or* experimental rickets, were as well able as cod-liver oil to cope with the deficiency cured by cod-liver oil. It was also found that cod-liver oil, after it has been heated to a moderate temperature and had air blown through it the while, though it remained a highly effective anti-rachitic agent, was quite incapable of adequately supplementing our original basal diet plus yeast.

There was only one conclusion to be drawn from these observations. There must be in cod-liver oil *two* dietary substances, one of which (or something very like it) is also present in butter-fat and green leaves and deals specifically with the deficiency condition that includes xerophthalmia; the anti-rachitic substance is clearly something different, is absent from butter-fat and green

leaves, and, incidentally, is much more resistant to the action of heat and aeration. The first of these two substances is now known as vitamin A—the growth-promoting, anti-xerophthalmic vitamin, at one time also called the "anti-infective" vitamin, but not very happily, for increased liability to infection is associated with shortage of other vitamins also. The second substance is now called vitamin D—the anti-rachitic vitamin.

§ 15 In similar ways, using basal diets so constituted as to bring about specific deficiencies in suitable animals, the existence of other distinct vitamins can be demonstrated. Cod-liver oil, as we have already mentioned, can be shown to contain a second vitamin different from vitamin A and quite unable to deputize for it. Vitamin D, as it was called when it had been clearly differentiated from vitamin A, is the substance—or group of substances—capable of preventing or curing "experimental rickets" in rats, as well as an almost equally clear-cut abnormality in young growing chicks; the condition is generally referred to, rather colloquially, simply as "leg-weakness."

§ 16 Diets can be devised that, though ample in their contents of vitamins A and D, are still incapable of supporting growth in young rats. Yeast contains at least two further vitamins, called vitamin B, before it was established that more than one vitamin must be present to account for its effect on rats receiving all the major constituents of diet, the essential minerals and vitamins A and D. One of the yeast vitamins, vitamin B_1, now called aneurin, is also necessary for pigeons; these birds—and other species on whom similar experiments have been conducted—develop a complete nervous breakdown if fed on a diet consisting entirely of grain deprived of its "bran" or germ. The B_1-deficient pigeon goes into a curious condition of spasm, with its neck bent back, known

as opisthotonos, and is in such a state of unco-ordination that it will actually turn "cartwheels" in its cage. A few grains of yeast, or a few drops of a yeast extract suitably prepared, will effect an astounding change, if forced down the crop of such a polyneuritic pigeon. In less than an hour the bird looks and behaves like a normal animal.

Yeast also contains a second vitamin that was at first called vitamin B_2, but is now known to consist of at least *three* different substances. One of these, called lactoflavin (because it was actually first clearly demonstrated in milk or whey), appears to be primarily a growth-promoting vitamin; two more—called provisionally the "filtrate factor" and the "eluate factor," having reference to their method of preparation from yeast—seem to be necessary partly for growth and partly to prevent certain skin lesions that develop in their absence.

§ 17 Green vegetables and fruit juices—particularly those of the lemon and orange—contain a vitamin that is not needed in the rat's food, for this animal seems to be able to make its own. But the guinea-pig dies without an adequate supply of vitamin C, or ascorbic acid, as it is now called. This is the anti-scorbutic vitamin, for the condition developed in its absence by the guinea-pig is "experimental scurvy."

§ 18 Finally, among the established vitamins must be mentioned vitamin E, the anti-sterility factor for rats. Without this the male rat soon loses permanently all power of producing living spermatozoa, and therefore of effective mating; the female rat receiving a vitamin E-deficient diet, on the other hand, is capable of mating, but not of giving birth to a live litter. Instead, the embryonic animals undergo disintegration and are reabsorbed into the maternal tissues—a process called

gestation-resorption. Restoration of vitamin E to the diet of the female rat enables her once more to produce full-term living young; vitamin-E deficiency in the female rat has been held to differ fundamentally from the male in its reversibility. There are, however, some reasons for thinking that the effects on the female organism may be more lasting than has hitherto been believed; it will be recalled that the lesions of a rat that has been deprived of vitamin A are still present in animals apparently cured by restoration of the vitamin to their diet.

§ 19 For the moment, this list of the vitamins must suffice; we shall later have to consider their relationship with human health, and particularly with certain clearly defined "deficiency diseases." It may, in passing, be mentioned that it is often the practice to divide the vitamins into two main groups—the fat-soluble (vitamins A, D, and E) and the water-soluble (the members of the "vitamin B complex" and vitamin C). This division is, however, of little interest except to the chemist, who, in attempting to isolate or manufacture vitamins, will want to know as much as possible of their properties, and to the pharmacist, who, in dispensing medicinal preparations of them, will have to consider suitable media for their presentation or "exhibition," as he sometimes calls it, preserving a rather quaint term from medical phraseology of a bygone age.

Other vitamins have been suspected of a separate existence and given fancy names or distinguishing letters or both; in this alphabet we find F, H, K, P and T being discussed at present. It can safely be said that some of them have a very shadowy existence, and at least one is a substance of known composition, requiring no individual place in the alphabet, since it has a name of its own. But all of them, and others to which mercifully

no letters have yet been assigned, are of no direct concern to us in this book, because there is no evidence one way or another as to any part they may play in the maintenance of human health.

§ 20 The recognition in cod-liver oil of some specific dietary constituent, present in quantities that had eluded ordinary chemical analysis, raised immediately a number of questions. What kind of a substance was it? Could it be separated from the known constituents of cod-liver oil—the fatty acids and glycerine for example—which were certainly incapable of curing or preventing xerophthalmia? Could tests for the vitamin be found that would make investigators independent of laborious and costly animal experiments? And what other animal or vegetable sources contained the vitamin?

It was very soon found that the vitamin was associated with the unsaponifiable portion of the oil, to which we have already made reference earlier in this book. Since this portion of a normal cod-liver oil is only about 1 per cent. of the weight of an oil, it follows that it must be some hundred times as active, weight for weight, as the oil itself. In other words, an effect produced by way of prevention or cure with, say, 20 milligrams of oil could be produced with only $\frac{1}{5}$ milligram of unsaponifiable matter, assuming that this can be separated from the rest of the oil without any destruction of the vitamin.

By removing from the unsaponifiable matter the whole of the sterols, which show no vitamin-A activity whatever, the activity of the concentrate could be once more increased; sterols make up approximately half the unsaponifiable matter, so that their removal about doubles the activity.

§ 21 Research along these lines was facilitated by two discoveries. First of all, cod-liver oil was found

to give, under appropriate conditions, an intense, though rather evanescent, blue colour with antimony trichloride, and the depth of the colour goes roughly parallel with the vitamin-A activity. This reaction enables the chemist to follow up the fractionation of the vitamin, and to avoid wasting time by biological tests on any portions that do not give a positive antimony trichloride reaction.

Next it was discovered that the vitamin, or something very closely associated with it, absorbs a certain wave-length in the ultra-violet part of the spectrum, making possible its detection by spectroscopic analysis. This was the first introduction of spectroscopic methods into vitamin research; it has played a part also in elucidating the distribution of properties and structure of vitamin B_1 (aneurin), lactoflavin, vitamin C (ascorbic acid), vitamin D and vitamin E—in fact, of all the vitamins about which we now possess detailed chemical knowledge.

§ 22 With the "blue test" and the spectroscope at their disposal, biochemists soon discovered that the livers of other fish than the cod contained oils much more suitable for investigations, for they were far richer sources of the vitamin than cod-liver oil. Whereas a relatively rich cod-liver oil seldom contains, as we now know, more than one part of the vitamin in two thousand of oil (one part in ten or twenty of the unsaponifiable matter), some halibut-liver oils, though by no means all, were found to contain as much as 2 per cent.; the unsaponifiable portions of these oils, however, are not proportionately richer, as they constitute nearer 5 than 1 per cent. of halibut-liver oil. Even so, chemists were able to prepare sterol-free concentrates of halibut-liver oil containing as much as 60 or 70 per cent. of the vitamin. These concentrates, submitted to further careful fractionation, finally resulted in the isolation of a

pale yellow, viscous oil that defied further subdivision, and was some three or four thousand times as active as ordinary cod-liver oil. This product was, until recently, accepted as the "pure" vitamin, but some further investigations have suggested that an even more active fraction can be separated. The technical difficulties for those working in this field mostly derive from the facts, first, that the highly purified vitamin is a rather unstable substance, and secondly, that to establish the purity of a non-crystalline compound is one of the hardest of tasks for the chemist. Recent work in the United States has led to claims for the production of pure crystalline vitamin A, though the crystals melt to a viscous oil at well below ordinary atmospheric temperatures.

§ 23 Before we leave the subject of vitamin A, we must record one other fact of very great importance. This vitamin is an exclusively animal product, occurring most abundantly in liver oil or fat, whether of fish, bird or mammal, but also present in small quantities in other parts of the body. In particular it is secreted in the fatty portion of milk; summer butter is an important dietary source. Plants, on the other hand, contain, in relatively small quantities by weight, a bright red pigment—carotene—often masked by the simultaneous presence of green and yellow pigments. Carotene is chemically distinct from vitamin A, but closely related to it. The animal body can convert carotene to vitamin A, probably in the liver, where the resultant vitamin is anyhow stored. Carotene can completely replace vitamin A, not only for the experimental rat, but also for the chicken, the pig, the cow, and almost certainly for other vertebrates, including man. That is why fruitarians do not die of vitamin-A deficiency. It is, however, stated that carnivores have no power to convert carotene into

vitamin A—quite a subtle economy of metabolic effort! Milk-fat (butter) contains both carotene *and* vitamin A, in proportions that vary with the breed of cattle and with the cows' diet. The carotene is all derived from the cow's food, chiefly from pasture; that is why summer milk contains more vitamin-A activity (that is, the summed activity of carotene and vitamin A proper) than winter milk. A yellow butter—to which no artificial colour has been added—is certain to contain carotene in useful amounts, but a pale butter from a different breed of cow may be an equally good source, or even a better one, if its deficiency in carotene is counter-balanced by a larger amount of vitamin A, which does not affect the appearance of the butter at all. At best, however, summer butter seldom has more than one twentieth of the total vitamin-A activity of an ordinary cod-liver oil.

CHAPTER XV

MEASURING VITAMINS

§ 24 It is not possible to draw anything but a quite arbitrary line between detecting and measuring vitamins. When we detect a substance by its action on a living organism, we are in fact making a measurement. We may give the organism more than it needs for the production of a given effect, and in that sense our measurement is indeterminate in one direction. But we have certainly established the fact that this particular effect does not need for its evocation *more* than the particular amount of the substance used. And that is a measurement.

In a series of experiments, however, we could gradually cut down the amount of the substance, until we had also found out what is the smallest amount that can produce the given effect. We should thereby have conferred a further degree of exactitude on our measurement. As soon, however, as we begin to make this kind of serial experiment, we are met with a real difficulty. Suppose we have thereby established that, say, 1 millilitre (about $\frac{1}{30}$ oz. or a third of a teaspoonful) of lemon-juice is insufficient to prevent one particular guinea-pig from getting "experimental scurvy" on a diet devoid of green-stuff, while 2 millilitres is sufficient to protect another guinea-pig, are we justified in concluding that, say, about $1\frac{1}{2}$ millilitres will be just enough to protect all guinea-pigs whatsoever?

§ 25 The answer to this question, as to so many others, is only to be found by further experiments. In this

instance we must follow the same fundamental principles as have already been discussed in describing the methods for detecting vitamins. First, we must get our basal diet right. We will add to the oats and bran, on which we know *all* guinea-pigs develop scurvy, some baked milk-powder; the vitamin A present is necessary to replace the carotene in the green leaves, on which a guinea-pig depends for its vitamin C as well. The baking is to destroy the small amount of vitamin C that is now known to be present in raw milk and in certain kinds of dried milk. Thus our experiment will not be complicated by the superposition of a vitamin-A deficiency on the experimental scurvy. On this diet, then, of oats, bran and baked milk-powder the guinea-pig stops growing in three or four weeks, and soon develops the weak hind-legs, the sore gums, the hæmorrhages under the skin and the softened bone-junctions that characterize experimental scurvy and resemble very closely the symptoms of human scurvy.

Moreover, if we give to other guinea-pigs the same basal diet with supplements of cabbage or lettuce leaves or of orange or lemon juice, they will remain normal and healthy. We have, in fact, shown the diet to be satisfactory for our purposes by carrying out "negative control" and "positive control" experiments with it. We can now proceed to investigate the problem of the *minimum* curative dose.

§ 26 A dozen or so of young growing guinea-pigs are allowed to eat freely of the basal diet (and water, normally derived from the now missing green leaves). When they have all ceased to grow and begin to show clear signs of scurvy, we will give every one of them 1½ millilitres of the lemon-juice that we are trying to test for its *antiscorbutic activity*—that is, for its content of ascorbic

acid (vitamin C). We shall then find that some of the animals—two or three, perhaps—recover completely, another half-dozen or so cease to lose weight, but do not grow normally, and are still far from well, while the rest die, though probably not as soon as if they had received no lemon-juice. What, then, is the answer to the question, "Is $1\frac{1}{2}$ millilitres of the lemon juice a minimal curative dose for experimental scurvy in guinea-pigs?"

The answer is that there is no answer to the question; this is just one of those instances where the wrong question has been asked! The dose of lemon-juice used is sufficient, or more than sufficient, to cure some guinea-pigs, and quite inadequate to cure others. Clearly, to express the vitamin-C content of the lemon-juice in quantitative terms, we must find some way of getting over the ever-present difficulty of individual variations in the test animals.

§ 27 Indeed, there are two problems to solve here, rather than one. First we have to find a method of measuring the response of each individual animal to a dose, and then we have to consider how best to express the response to any one dose of a group of animals, taken as a whole. In the kind of experiment described above, we shall find that a suitable dose of lemon-juice makes some animals increase in weight more than others. If the dose is too low, no animals will gain at all, and the effect of the dose on the group will be nil; if too large a dose is chosen, all the animals will show the maximum growth possible. Such a dose may clearly have *any* value above a certain amount, and is too large for any kind of comparative measurement. But some intermediate dose will permit of sub-maximal growth; doses of that kind must always be used for measuring physiological activity by tests on animals.

§ 28 Roughly speaking, the most we can make these guinea-pigs gain in weight is about 20 grams a week. The *smallest* dose of lemon-juice able to bring about this result will vary from animal to animal, but there will be a certain dose that produces this effect on all guinea-pigs, as obviously will all larger doses. If we give to a group of guinea-pigs, all receiving the scurvy-producing diet, a dose of lemon-juice about half that able just to produce maximum growth in all guinea-pigs, we shall get the kind of results described in § 26 above, Suppose there are twelve animals in the group. We shall assume that the limiting optimal dose (as discussed above) is 1 drachm (3·5 millilitres) of fresh lemon-juice, so that every animal in the group will be given half a drachm of juice. Then the weekly weight increases of the animals will range themselves something like this.

1 animal gains 20 grams
3 animals gain 15 grams
4 animals gain 10 grams
2 animals gain 5 grams
1 animal weight stationary
I animal lost weight and died.

The average increase in weight for the whole group of twelve animals is $\frac{20+45+40+10}{12} = 9.6$ grams. We can now say that for a particular group of guinea-pigs, twelve in number, half a drachm of the lemon-juice produces an average weekly increase in weight amounting to just under 10 grams. There is one way, and only one way, of judging how accurately this weight increase measures the amount of vitamin C in the lemon-juice. We must repeat the experiment with yet more groups of

guinea-pigs, and again determine the average weekly gain in weight of each group. We shall, in fact, find that this is fairly constant; for some groups it may be as low as 8 grams, for others as high as 12, but most groups will give values round about 9, 10 and 11 grams. In fact, the average weight increases of groups will show fluctuations similar to those shown by individual animals, but much less marked, and the *larger* the groups the *smaller* the fluctuations.

§ 29 For most practical purposes the kind of fluctuations we have attributed to groups of twelve animals will not matter seriously: with groups of, say, twenty animals we should get reasonable results, if we used the same sample of lemon-juice. For this technique we can, therefore, say that the *same* amounts of vitamin C will produce in groups of twenty guinea-pigs approximately the *same* average weekly increases in growth, provided the amount of vitamin C is round about half that just necessary to produce the maximum weekly growth increase possible.

From this it is fairly obvious how to compare the amount of vitamin C in the given sample of lemon-juice with, for instance, the amount in a sample of potatoes or cabbage-leaves or black-currants. We must find the amount of this other source that will also give an average weekly weight increase of about 10 grams, when it is fed to a group of guinea-pigs receiving a scurvy-producing diet. The larger the group receiving the potatoes or cabbage or currants—that is, the *test* group—and the larger the group receiving the lemon-juice—that is, the *control* group—the more accurate will be our comparison. By statistical methods, moreover, it is possible to analyse our results and actually to estimate the degree of their accuracy.

§ 30 Such analyses have led to important conclusions as to the best conditions under which to carry out these biological comparisons in order to obtain the maximum amount of accuracy for a given number of animals. The experiments in both groups should be carried out contemporaneously and the animals in the two groups should be closely "matched"; every animal in the test group should be controlled by an animal as nearly like it as possible. Such pairs of animals should be of the same age, weight and sex; they should come from a uniform (that is, a highly inbred) stock whose diet before the experimental period, including the diet of their mothers during pregnancy and lactation, should be as constant as it can be made. Wherever possible, each pair of animals should come from the same litter.

If these conditions are observed, then the number of animals required to give a certain accuracy of comparison may be very considerably less than, perhaps as little as half, the number required if no attention is paid to the "matching" of pairs in the two groups.

§ 31 Now, it is clear that such conditions can be fully realized only inside one particular laboratory. Dr. A may find that the growth given by 1 drachm of lemon-juice is identical with that given by 10 grams of potatoes —that is, these potatoes contained about one-third of the vitamin C present in the lemon-juice. But how do these compare with a sample of cabbage-leaves being tested for vitamin-C content in the laboratory of Professor Z? It would obviously be quite wrong to assume that Professor Z's animals respond in the same way to vitamin C as those in Dr. A's laboratory. This would have first to be proved experimentally, if any sort of comparison is to be made of substances tested independently in the two laboratories. If, however, Professor Z could test the

same lemon-juice as Dr. A, he could then compare his cabbage-leaves with it, and thus make possible by indirect means a comparison of his cabbages with Dr. A's potatoes.

Lemon-juice does not keep very well: moreover, even though it may continue to look and taste the same, it loses its vitamin C slowly when exposed to air, especially if the temperature is somewhat high. Unless, therefore, the two laboratories are in close proximity, and suited in other ways for carrying out contemporaneous tests with a common sample of some agreed "standard" preparation, they will have no common measure at all for comparing their two sets of results.

It was to meet this difficulty that the Health Organization of the League of Nations decided a few years ago to set up "Standard Preparations" of certain vitamins, to define international units of these vitamins in terms of the Standard Preparations, and to make those preparations available for distribution anywhere in the world where it was required to carry out biological estimations of the vitamins in question.

Four International Standard Preparations exist at the present moment, for the testing of vitamins A, B_1, C, and D. As we have confined ourselves in this chapter to the measurement of vitamin C, we will describe the standard preparation and unit of that vitamin in a little detail.

§ 32 Fortunately ascorbic acid itself is available as a pure, crystalline, stable, white solid, and the International Standard Preparation of vitamin C is a particular sample of very pure ascorbic acid, of which a considerable stock is held, under conditions ensuring its stability, at the National Institute for Medical Research in London. Any worker who wants to carry out a vitamin-C assay on guinea-pigs can obtain from the

Institute (or from his appropriate national distributing centre) a sample of this Standard Preparation, without any charge whatever. The International Unit of vitamin C is *defined* as the anti-scorbutic activity contained in one-twentieth of a milligram of the sample of ascorbic acid that constitutes the International Standard Preparation.

§ 33 Let us, then, consider briefly the procedures of Dr. A and Professor Z, to both of whom the International Standard Preparation of vitamin C is available. Each worker will prepare two carefully matched groups of guinea-pigs—or whatever number of pairs of groups is necessary to get finally an equal growth response in test and control group; each will obtain a supply of the International Standard Preparation, and each will find by experiment how much of that preparation is necessary to give about half the maximum possible growth response, for reasons that we have already discussed.

Dr. A finds that $\frac{1}{10}$ milligram of the Standard produces an average weekly gain in weight of 11 grams in a group of fifteen guinea-pigs receiving a scurvy-producing diet and that an average gain of 12 grams is produced in another group of fourteen guinea-pigs by $\frac{3}{5}$ of a gram of potatoes. Hence, in anti-scorbutic activity $\frac{1}{10}$ milligram of Standard is very slightly less effective than $\frac{3}{5}$ gram of potatoes. But $\frac{1}{10}$ milligram is two international units, by definition. Therefore, $\frac{3}{5}$ gram of potatoes contains a little more than 2 units, and 1 gram of potatoes contains a little more than $3\frac{1}{3}$ units, say, $3\frac{1}{2}$ units of vitamin C.

Professor Z, on the other hand, finds that $\frac{1}{15}$ milligram of the Standard is needed to give an average weekly weight-increase of 10 grams in fourteen animals, and his cabbage-leaves produce an average weekly gain of 9 grams when each animal of fifteen receives $\frac{1}{12}$ of a gram daily. Hence, in anti-scorbutic activity $\frac{1}{15}$ milligram of Standard

is very slightly more effective than $\frac{1}{12}$ gram of cabbage. Therefore, 1 milligram of Standard (20 international units) is slightly more effective than $\frac{1}{12}$,—that is, $1\frac{1}{4}$ grams of cabbage. Thus $1\frac{1}{4}$ grams of cabbage contains slightly less than 20 units, so that 1 gram of cabbage contains about 15 International Units of vitamin C.

§ 34 It is very important to note the result of both workers comparing their test material with the Standard Preparation. Suppose they had not done so, then the only possibility for a comparison would have been to have compared the average weight increases of Dr. A's guinea-pigs receiving $\frac{3}{5}$ grams of potatoes, and gaining 12 grams, with Professor Z's guinea-pigs receiving $\frac{1}{12}$ gram of cabbage, and gaining 9 grams. We should then have argued that, as $\frac{3}{5}$ gram of potatoes is distinctly richer in vitamin C than $\frac{1}{12}$ gram of cabbage, 1 gram of potatoes is therefore superior to $\frac{5}{36}$ gram of cabbage, and equal to, say, $\frac{1}{6}$ gram cabbage, or less. So that the cabbage is six times as rich as the potatoes, or even still more potent. Yet the evaluation in International Units actually showed a ratio of 15 : $3\frac{1}{2}$—that is, a little over four to one. This is the more correct relation, for the use of the Standard Preparation has allowed the two investigators to correct for the *different* average responses of their animals to the *same* dose of ascorbic acid.

§ 35 We have dwelt at considerable length on this matter, because it is one to which even to-day quite inadequate attention is given. The most careful workers in vitamin assay are certainly those in this country, though many American investigators are equally punctilious. There are also in other countries honourable exceptions, but it is broadly true to say that there is still a mass of material being published all over the world purporting to state the amounts of various vitamins in all sorts of foodstuffs, but based on experimental methods

that almost completely ignore the essentials of biological assay described above.

It must be pointed out, however, that the criterion adopted of response to vitamin treatment for purposes of measurement need by no means always be growth—that is, gain in weight. For vitamin-D assay, the biochemist uses the extent of bone calcification; for vitamin-B_1 assay he may use the increased heart-beat of rats on a vitamin B_1-free diet when B_1 is added to it, or even the effect on the rate of increase of fermenting yeast-cells; in vitamin-C assay itself he may use the effect on tooth structure. As long as he has some kind of scale by means of which he may attribute a numerical value to the response of each individual animal in a group, then he can average the responses of all the animals in the group, and express numerically the average response given by a measured dose of the Standard Preparation. *Unless* he can do this, he cannot carry out a vitamin assay; if he has not done this, he *has* not carried out a vitamin assay.

That is why we have at present no means of expressing, for example, the amount of vitamin E in a natural product or a medicinal preparation. There is no Standard Preparation, international or otherwise, and there can, therefore, be no unit and no assay. The best that can be done is, by experiments on carefully matched groups of animals, to express in terms of each other the vitamin-E contents of two contemporaneously tested sources. But that is *not* an assay, and affords no basis for comparison with another substance tested on other animals in different places or at other times. The absence of the yard-stick—the Standard Preparation—makes it impossible in any way to allow for variations in response between the animals used in comparing the first two substances and those used for testing the third.

CHAPTER XVI

IDENTIFYING VITAMINS

§ 36 NEAR the beginning of this book reference has already been made to the use of cod-liver oil for curing rickets; as we have there pointed out, it would be more accurate to speak of the ability of some samples of cod-liver oil to produce some cure in some cases of rickets! In the light of the knowledge to be discussed in this chapter and later, we can say quite confidently that many of the samples of cod-liver oil used in past centuries must have been completely devoid of any anti-rachitic activity. Even to-day samples of cod-liver oil produced under careful and up-to-date conditions may contain under 50 units per gram, while a very active cod-liver oil may contain as much as 300 units. Moreover, there are other fish-liver oils many times as rich, and values as high as 50,000 units per gram have been recorded.

Nevertheless, the association of anti-rachitic properties with cod-liver oil became firmly established, especially in the Scandinavian countries, and the variable responses of different cases to treatments apparently identical were just put on one side as unexplained mysteries. They are, of course, still partly unexplained, but to-day we do at least know what are some of the causes of variation.

§ 37 At the beginning of the present century several discoveries were made that proved of the greatest

importance for the ultimate isolation and identification of vitamin D. First, it was found that rickets could be cured not only by sunlight, but also by the rays from a quartz mercury-vapour lamp, without any dietary or medicinal treatment whatever. The effect of sunlight was used to explain a number of previously known facts—the *relative* frequency of rickets in sunny and in cloudy climates, in town and in country conditions, and the fact that, according to certain schools of thought, exercise could cure rickets. Clouds and smoke apparently cut out from sunlight the rays really effective in curing or preventing rickets; exercise, whether of children or experimental animals, was frequently taken in bright sunlight.

How it was that a non-dietary treatment, such as irradiation with ultra-violet "light" from sun or lamp, could have the same effect as the oral administration of a fish-liver oil, remained for some time completely baffling. The key to the mystery was found almost simultaneously by two investigators, but in the meantime another advance of great value had been made, mainly in the laboratories of United States scientists.

§ 38 As soon as the Swedish scientists, Holst and Frölich, had succeeded in producing experimental scurvy in guinea-pigs, which they did in 1907, there was quite a craze for producing other experimental deficiency diseases in suitable animals. Avian polyneuritis in pigeons was the laboratory equivalent of human beri-beri, and a similar condition, though of more complex origin, was produced in rats.

The production of experimental rickets in dogs was a fairly simple matter, but there were many objections, apart from purely sentimental and humane ones, to the use of dogs for this work. Fortunately, therefore, for dog-

loving scientists (as they almost all are), in laboratories at Johns Hopkins and Columbia Universities, at Baltimore and New York respectively, more or less simultaneously and quite independently, a way was found to produce experimental rickets in rats.

The conditions for its production were, however, not identical with those apparently concerned in human rickets. Rats do not need any vitamin D at all, *provided* that their diet is adequate in calcium and phosphorus. Such a vitamin-D-free diet does not, in fact, allow rats to develop quite normal bones, but it certainly does not give them rickets. If, however, the phosphorus of the diet is brought down to a very low level, without any corresponding lowering of the dietary calcium, then—provided vitamin D is absent from the diet—the rats develop a condition of experimental rickets extraordinarily like that found in the rachitic human infant.

§ 39 Like human rickets, this condition can be cured and prevented by cod-liver oil administration *or* by irradiation with ultra-violet rays from sun or lamp; like human rickets, it is accompanied by a marked lowering of the blood phosphorus, and sometimes by a distinct fall in blood calcium. As in human rickets, its prolongation leads to the development of wide cartilaginous zones at the growing points of bones, where normally lime salts—especially calcium phosphate and carbonate—would be deposited; this failure in calcification produces, as in human rickets, the characteristic softening, swelling, malformation and finally fracture of the bones, and especially of the long bones of legs and arms.

Experimental rickets in rats can also be prevented and cured by restoring to the diet its deficient phosphorus—a procedure probably without effect on human rickets,

which can apparently develop on a diet adequate in its calcium and phosphorus content, but lacking in vitamin D. In other words, more technical, but more economical, experimental rickets in rats is very similar in pathology to clinical rickets in man, but it may well be different in etiology.

§ 40 This etiological difference between experimental rat rickets and clinical human rickets has certain important practical consequences. It means that we must argue only with caution from the nature, causes and cure of the one to those of the other; we can *use* rat rickets as a means of detecting and measuring vitamin D, for it can be cured by vitamin D, just like human rickets. But we must be careful not to be led astray by the pathological resemblances into overlooking the etiological differences.

Experimental rickets in rats is, as we have seen, due essentially to a shortage of phosphorus in the food, as a result of which there is a shortage in the blood-stream, which ultimately expresses itself in abnormalities of bone formation. Rickets in the human infant—and its adult analogue, osteomalacia—is due rather to shortage of vitamin D itself, whereby the calcium *and* phosphorus in the food, however abundant, are not taken into the blood-stream, again with disastrous effects on the bones. The causes are different, but the results are the same.

Owing to this difference, rat rickets can be cured without adding any vitamin D at all to the rachitogenic—rickets-producing—diet of the rat. The direct addition of phosphate will produce the same result as the administration of vitamin D: it will cure or prevent the experimental rickets of rats. So will ammonium chloride, of which the acidic nature enables it to free phosphoric acid from combination in the food and tissues, so

that it acts like phosphate added to the diet. Conversely, the presence in the diet of unavailable, because unassimilable, phosphorus tends to produce rickets in rats; the phosphorus in certain cereals—especially oatmeal—is present in this form. These considerations do not appear to apply to man. Phosphates and ammonium chloride are ineffective in curing or preventing rickets in the human patient; oatmeal will not produce it, for the presence or absence of vitamin D is here the determining factor.

§ 41 It must not be forgotten, however, that rickets is comparatively rare, if not actually unknown, in breast-fed babies. Rat tests have not shown human milk as an exceptionally rich source of vitamin D. It is, of course, always possible that only those mothers who are themselves adequately endowed with vitamin D can suckle their young successfully. Some authorities, however, favour the view that the calcium and phosphorus in breast milk are present in an optimum condition for the human infant, thereby reducing the need for vitamin D to a minimum. If this is true, it affords further argument for caution in arguing from the rat as an experimental animal to man as a clinical entity.

Nevertheless, rat rickets is invaluable as a means of testing for the presence of vitamin D. The investigator can control the amount of free or available phosphorus in the rats' diet and make it almost as low as he likes; if, in spite of this, a product is given to the rachitic rat and cures its rickets, then that product *must* contain vitamin D. That is why the discovery of how to produce rat rickets was of epoch-making importance.

It soon led to very rapid advances. First, as has already been stated, it made it possible to confirm the similar effects of ultra-violet light and cod-liver oil.

Next, it led to the distinction between the vitamin A and the anti-rachitic vitamin of cod-liver oil, already discussed in §§ 11, 12, 13 and 14 of this Section. It soon became clear that, except for small amounts in egg-yolk and summer milk and butter, vitamin D occurred only in fish-liver oils. It was obvious that most people must be relying on solar irradiation for their protection from rickets or osteomalacia—a precarious protection in smoke-dimmed industrial cities. Rickets, even if only in its milder forms, is still to-day far from having been eliminated among the child populations of our manufacturing towns.

§ 42 The next advance came from an almost simultaneous discovery by two more American scientists, Steenbock in Wisconsin and Hess in New York. Both of these workers found that rachitogenic diets would lose their ability to produce rickets if they themselves were submitted to the intense ultra-violet radiation of the mercury arc. This discovery opened the road that led directly to our present knowledge of the anti-rachitic vitamins, and incidentally to increased knowledge over the much wider field of sterol chemistry in general.

For now the hunt was up. Immediate attempts were made to find out if any one particular ingredient of the food was responsible for the effect. It was. It was the fatty part of the food that developed under the influence of ultra-violet radiation the same anti-rachitic properties as had hitherto been found mainly in fish-liver oils. It did not seem to matter much what fat or oil was concerned—the commoner vegetable oils like olive oil and pea-nut oil, or the traces of fat present in such cereals as maize and wheat; all of them became anti-rachitic when irradiated. Moreover, the rat's skin—even if the rat had previously been given rickets—

developed the same anti-rachitic properties. Further, an oil activated by irradiation could cure the experimental rickets of rats if it were rubbed into its skin! Here at last was a reconciliation between the disputant schools—those who said ultra-violet light cured rickets, and those who said that some undiscovered constituent of fish-liver oil did so. Both were right. But the fat of the rat's skin—and presumably that of human beings also—becomes anti-rachitic on irradiation, and thus renders the animal independent of external sources of vitamin D.

§ 43 The inquisitive minds of chemists did not, naturally, let matters rest here. The activatable portion of food—*pro-vitamin D*, as we now call it—had been traced down to its fatty constituent; in what part of the fat was it to be found? First the glycerides were absolved; the whole of the pro-vitamin was in the unsaponifiable matter. (See Chapter VI, especially §§ 26 *et. seq.*) Next it was traced to the sterol portion of the unsaponifiable matter. Cholesterol from animal fats and phytosterol from vegetable oils were both found to be rendered very strongly anti-rachitic by irradiation. For some time people thought that these sterols themselves might well be the pro-vitamins they were looking for. Some samples of cholesterol could be given an anti-rachitic activity—for human infants, as well as for rats—one thousand times that of a good cod-liver oil, so that this belief was by no means unreasonable. But the pure anti-rachitic vitamin was to turn out to be nearly a thousand times more active again!

After much laborious research it was found possible to purify cholesterol even further than had so far been done, and to obtain samples that could *not* be made anti-rachitic on irradiation. Moreover, one of the ways of

doing this was actually to irradiate cholesterol, and to separate from it a small oily fraction—about one two-hundredth part of the cholesterol or less—which was proportionately more anti-rachitic than the irradiated cholesterol itself before the separation. When the unchanged cholesterol was recovered, ultra-violet light would no longer make it anti-rachitic.

§ 44 It seemed, therefore, quite clear that cholesterol itself could not possibly be pro-vitamin D. But there were some very interesting clues to suggest what was. First, the method of freeing cholesterol from its accompanying traces of pro-vitamin (apart from the procedure of *destroying* the pro-vitamin by actual irradiation) was such as to suggest that the pro-vitamin would be a substance more easily reduced than cholesterol. Secondly, its leech-like adherence to cholesterol was an indication, if nothing more, that it might be chemically allied to the sterols, if not even actually an already known sterol.

The third clue was perhaps the most important. It was known with considerable accuracy which ultra-violet radiations were most effective in making cholesterol anti-rachitic; they were the ones with a wave-length somewhere near 300 mμ (millimicrons—that is, millionths of a millimetre). Now physical chemists had established a very important principle—important to have established experimentally, even though it sounded rather obvious once it had been proclaimed! It was this: any particular wave-length of the spectrum, visible or invisible, that is capable of causing a particular chemical compound to react *must* be absorbed by that compound. From this it followed that the compound must show in its spectrum an *absorption band* exactly at the wave-length of the chemically effective radiation. Consequently activatable

cholesterol *must* show an absorption band near 300 mμ. Spectroscopic examination not only revealed the presence of this band, but also showed that the band gradually disappeared during irradiation, and that the intensity of the band was roughly proportional to the degree of anti-rachitic activity that could be conferred on any sample of cholesterol. Purified cholesterol that remained unaffected by irradiation showed no absorption band whatever at 300 mμ.

§ 45 All these clues suggested certain tentative conclusions. Pro-vitamin D, it was foretold, would turn out to be a sterol, or something very closely related to a sterol, of the more easily reducible kind; it should have an extremely intense absorption band near 300 mμ, since the traces of it in activatable cholesterol (certainly under 1 per cent.) gave to that substance an easily observed band.

It is to Windaus of Göttingen that must go the credit of putting one and one and one together and making three. Many years ago Tanret, a French chemist, had separated a new sterol from the oil of ergot of rye. This he had called ergosterol. Windaus found that ergosterol absorbed very strongly indeed between 260 and 294 mμ, actually showing four separate absorption bands in this region. When ergosterol was irradiated with ultra-violet light consisting mainly of radiations within those limits, several changes took place. The original bands disappeared, and were replaced by a single band near 270 mμ. This also disappeared as irradiation went on. At an appropriate moment during the existence of the 270 mμ band, the irradiated ergosterol was found to have a staggeringly high anti-rachitic activity, some 20,000–50,000 times that of cod-liver oil, and some 100 times that of the most active irradiated cholesterol.

It certainly seemed as if the matter were now settled; following upon a masterly alternation of experimental work and logical deduction, we were now in possession of an anti-rachitic substance immeasurably more potent then anything previously known. Clinical trial soon established its beneficial action on the rachitic infant and its ability to prevent rickets when no other form of treatment, dietary or environmental, was used. Had we, at last, got possession by indirect means of the pure vitamin that existed in cod-liver oil only as a relatively insignificant contaminant?

§ 46 For a time there was a lull on this vitamin front. Irradiated ergosterol was a waxy-looking, semi-solid mass, from which unchanged ergosterol could be fairly easily removed. This operation left over a yellowish-brown resin of which the anti-rachitic activity was about twice that of the crude irradiated material before removal of unchanged ergosterol.

Windaus and his colleagues and a quite independent group of workers at the National Institute of Medical Research in Hampstead set themselves the task of separating this resin into various parts, in the belief that it was a mixture of several substances and in the hope that one of these substances would turn out to be a solid, crystalline, pure anti-rachitic vitamin. They were right, and both teams succeeded, almost simultaneously, though by different routes, in isolating from irradiated ergosterol the substance now almost universally called calciferol (as suggested by the English team), but still given by some German workers Windaus's name "vitamin D_2" (vitamin D_1 was a less pure crystalline substance, a mixture of calciferol with something else, separated by Windaus a few weeks earlier; the subscripts 1 and 2 are, however, generally preserved for historical reasons).

It is, by the way, extremely gratifying to note that the rivalry between these two groups, culminating in their separate isolation of calciferol, was concluded in an atmosphere of cordial co-operation; they not only communicated their methods to one another, but they also exchanged specimens, and confirmed the identity of each other's crystalline by-products. It was a fitting end to a magnificent chapter in international biochemistry. But it was not the end of the whole story.

§ 47 It must never be forgotten that at every stage in this long chain of research the cumbersome, inaccurate, lengthy and expensive procedure of animal tests had to be used to check the work of the chemists. Moreover, there were no Standard Preparations available in those early days; the only way of finding out anything quantitative was simply to compare one product with another. Actually, the fundamental principles of bio-assay, as discussed in the last chapter, were very little appreciated then, and workers often deceived themselves by comparing results obtained in different laboratories, with different stocks of animals and under considerably different conditions of test. We have only quite recently come to realize how much of the quantitative data reported at that time must be discounted and regarded as, indeed, little more than qualitative. In spite of this handicap—an extremely serious one, too—the work went on, because it soon became evident that the relationship

$$\left(\text{ergosterol} \xrightarrow[\text{irradiation}]{\text{ultra-violet}} \text{vitamin D} = \text{calciferol}\right)$$

simply would not cover all the facts.

§ 48 Crude solutions of irradiated ergosterol and, later, calciferol itself were very carefully assayed as soon

as the International Standard Preparation came into existence. The former showed activities varying from a relatively low figure up to 5,000,000 units per gram (or 10,000,000 units, allowing for the unchanged ergosterol present); the latter was found to have a constant value of 40,000,000 international units per gram. Subsequent work has entirely confirmed this figure, which is now internationally accepted as the true anti-rachitic activity of calciferol.

It will be remembered that this statement has a simple and definite meaning. It means that a sample of *pure* calciferol, free from all non-anti-rachitic contaminants, will have an effect on the experimental rickets of rats exactly 40,000 times that of the International Standard Preparation, which contains by definition 1000 international units per gram.

The non-calciferol portions of irradiated ergosterol were found to be entirely devoid of anti-rachitic activity. One of them has been obtained in a pure crystalline state, and is as physiologically inert as non-irradiated ergosterol itself. Others have been found to have a marked toxic action on rats and mice when administered in very high doses, and this is also true of calciferol itself. But the other toxic substances in crude irradiated ergosterol (called tachysterol and toxisterol) are entirely without the intense anti-rachitic action of calciferol. It follows that, for a given intensity of anti-rachitic activity, calciferol will be several times less toxic to rats and mice than irradiated ergosterol.

§ 49 Calciferol was slowly becoming accepted as the only known anti-rachitic substance, in spite of the scepticism of certain clinicians. A good deal of this scepticism must be put down to that subconscious primitive Nature-worship that still seems to persist in

many laymen and some doctors and causes them to maintain with imperturbable obscurantism that "Nature knows best," and probably still to prefer that their aspirin should be made from "natural" oil of wintergreen instead of from "synthetic" coal-tar salicylic acid! But evidence of a very different order, based on carefully controlled experiments, became available when Massengale and Nussmeier threw a most unwelcome spanner into the works.

Their discovery depended on the fact that chickens need some form of anti-rachitic treatment; otherwise they develop "leg-weakness" and die. Examination of the bones of chickens lost in this manner shows their condition to be in many respects similar to the bones of rachitic rats and human infants. It has been known for years that cod-liver oil is very effective as a prevention of this condition in chickens. The two American scientists found, however, that irradiated ergosterol was quite astonishingly ineffective. Their work has been repeated a number of times—always with results that were qualitatively the same, though there was a good deal of quantitative difference between the results obtained in different laboratories. We now know that differing experimental conditions were largely responsible for these quantitative differences.

Let us put the matter, nevertheless, in numerical form. Suppose we assay a sample of cod-liver oil, using the accepted method—that is, comparing its effect on rats with that of the International Standard Preparation—and suppose we find it comes out at just 100 international units to the gram. We should then find that 2 grams a day were amply sufficient to protect a newly-hatched chick from leg-weakness; that means that the prophylactic dose of vitamin D for this chicken is 200

units per day—or less. Now we will make up a solution of calciferol in oil so that every gram contains 100 International Units; this figure is, of course, based upon assays of calciferol carried out on rats in the same way as the cod-liver oil assays. The solution, since calciferol contains 40,000,000 units per gram, will obviously contain $\frac{1}{400,000}$ gram of calciferol in every gram. Now, if calciferol is the same as the vitamin D in cod-liver oil, this calciferol solution should be *exactly* as effective as the cod-liver oil containing 100 units per gram; 2 grams of the calciferol solution should completely prevent any signs of chicken-rickets or "leg-weakness." Actually not twice or ten times the amount will do this; as much as fifty times may be required, or even more. We cannot, of course, give chicken 50 grams of the particular oil solution; we have to increase the amount of calciferol in solution, and feed stronger and stronger solutions until we reach one that is effective. Actually the most effective treatment with calciferol never equals the most effective treatment with cod-liver oil in chickens, as judged by the amount of calcium and phosphorus laid down in their growing bones.

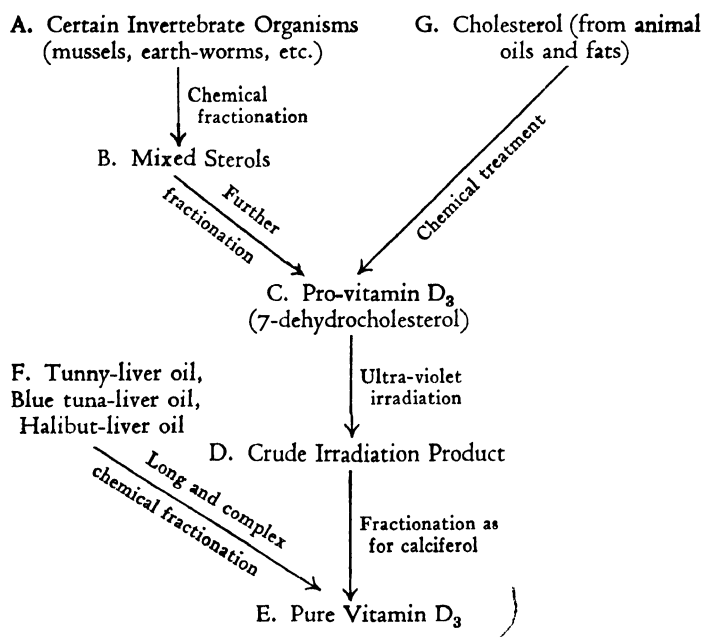
§ 50 Attempts were made to show that this curious ineffectiveness of calciferol was apparent, and not real; that the chicken needed the vitamin A of the cod-liver oil, as well as its vitamin D, for bone formation and freedom from leg-weakness. The attempts failed. *No* other explanation than one fits the facts; the vitamin D in cod-liver oil is *not* calciferol. Moreover, chickens have a curious insensitivity to calciferol, and this insensitivity is not shared by the rat. Such a deduction from the experimental facts leads at once to the very pertinent question, What about the human species? Do infants

behave, *vis-à-vis* their anti-rachitic vitamin, more like chickens than like rats? Must we abandon the use of calciferol, with its fallacious appearance of 40,000,000 units to the gram, and return to the mild but effective, if unpleasant, oil from the cod's liver?

The answer is, "No, no—a million units, no!" Man is, fortunately, exactly, or almost exactly, like the rat, and quite unlike the chicken. We now know that there exist in nature at least two, and probably more than two, forms of vitamin D. It is nonsense to distinguish between cod-liver oil vitamin D and calciferol as natural and artificial, because the latter exists in nature—in certain moulds, in some samples of ergot of rye, and in the husk of the cocoa-bean—while the former has been made by methods no less, and no more, "artificial" than those used to prepare calciferol. If it were necessary to find adjectives for distinguishing between the two anti-rachitic vitamins, then it would be legitimate to talk of "animal" and "vegetable" vitamin D.

§ 51 German and Dutch scientists have been able to prepare a crystalline vitamin D_3 in two different ways, and there is good reason to believe that it is identical with the anti-rachitic vitamin of cod-liver oil. It is, like calciferol, a product of irradiation, but its pro-vitamin is not ergosterol. Indeed, we now believe that the pro-vitamin present in the cholesterol of the early workers never was ergosterol at all, but the pro-vitamin of vitamin D_3. It is a substance very closely related *both* to cholesterol *and* to ergosterol. Apparently its relation to cholesterol is what causes the corresponding vitamin D_3 to be preferred by chickens to calciferol, but its relationship to ergosterol is what causes it to be susceptible of anti-rachitic activation by ultra-violet light.

The diagram gives an indication of how vitamin D_3 has been separated or "synthesised." The vitamin has been separated in pure crystalline form from certain fish-liver oils (route F E); it has also been prepared by irradiation of its pro-vitamin, 7-dehydrocholesterol (route C D E). The pro-vitamin itself has been isolated from natural sources (route A B C) and has also been chemically prepared from cholesterol (route G C).



§ 52 So far sufficient vitamin D_3 has not been available in pure form for exhaustive laboratory and clinical trials. Sufficient is known about it, however, for it to be stated :—

1. That its anti-rachitic activity, as assayed biologically on rats, is the same as that of calciferol—namely, 40,000,000 international units per gram.

2. That its effect on chickens is the same as that of cod-liver oil, if both are administered on the basis of the same number of units; it follows from 1 above that this means in equal weights.

3. That its method of laboratory preparation by irradiation of its pro-vitamin is by a route exactly parallel to the preparation of calciferol.

4. That in clinical use for protection from rickets and the prevention of increase in severity of existing rachitic symptoms, the most careful investigation yet conducted has failed to show any detectable difference between sources of calciferol and sources of vitamin D_3.

5. That reports of the superiority of vitamin D_3 over calciferol in the cure of marked or severe rickets are based on so small a number of patients as to be nothing more than suggestive, and in any event do not indicate a difference in efficiency of more than 2 units to 1, if as much.

§ 53 We can therefore safely conclude that calciferol will continue, at any rate for some time, to be the anti-rachitic agent of choice, whether for use in medicinal preparations, or for additions, under careful control, to certain foods, especially margarine, intended for human consumption. Its efficiency as a prophylactic against rickets has been proved up to the hilt; in spite of the mumblings of sceptics and hypercritics, the ill effects of administering even enormous doses of this "artificial" vitamin to human beings still await the slightest convincing demonstration, and in the small doses used for

activating foodstuffs it is no more toxic than common salt. It is cheap and available in small or large quantities to reputable food manufacturers. There is not the slightest scientific reason, taking all these facts into consideration, why infantile rickets should not in three years be entirely eliminated from the world. Actually the daily prophylactic requirements of the whole child population in Great Britain, if supplied as calciferol at the lowest possible manufacturing price, would be about £250—that is, assuming a child population of 5,000,000 and allowing every child a *generous* prophylactic dose. The total annual cost would therefore be well under £100,000, which I can leave to the reader to evaluate in terms of bombers and tanks. The expenditure would completely and finally eradicate rickets from these islands, though of course it would have to include the cost of suitable distributive and administrative measures. Say £500,000 or even £1,000,000 annually.

Scientists have, without any doubt, tracked down and identified this vitamin. Calciferol is there, ready and available; rickets has still to be eliminated. Presumably the cost is too high.

Other substances with slight or marked anti-rachitic action are also known. They are all, however, the products of special laboratory experiments, and have not so far been found in natural products; they are, therefore, of no immediate practical interest from the standpoint of nutrition.

§ 54 By equally arduous, though very different, routes other vitamins have been obtained as pure crystalline synthetic substances. There are ascorbic acid (vitamin C), aneurin chloride (vitamin B_1) and lactoflavin, part of what used to be called vitamin B_2, now known to contain several different factors. The constitution of

vitamin A is also known; it has been separated, but not yet synthesized as a single pure substance (though that might be achieved at any moment). Of other pure substances believed to have vitamin-like action—nicotinic acid amide, hesperidine and eriodyctyol glucoside, linolic acid and so on, space will permit here nothing more than passing mention. For those, however, who are interested in the chemical nature of this very varied group of dietary essentials, our knowledge of their composition has been summarized in Table II.

TABLE II

Elements, Vitamins and Pro-vitamins

Designation	Name	Elements Present or Formula
Vitamin A	—	$C_{20}H_{28}OH$
Pro-vitamin A	Carotene	$C_{40}H_{56}$
Vitamin B_1	Aneurin chloride *	$C_{12}H_{17}N_4SOCl$
Vitamin B_2	Lactoflavin †	$C_{17}H_{20}N_4O_6$
	Filtrate factor	Unknown
	Eluate factor	Unknown
	Nicotinic acid (amide)	$C_6H_6N_2O$
Vitamin C	Ascorbic acid ‡	$C_6H_8O_6$
Vitamin D_2	Calciferol	$C_{28}H_{43}OH$
Vitamin D_3	—	$C_{27}H_{41}OH$
Pro-vitamin D_2	Ergosterol	$C_{28}H_{43}OH$
Pro-vitamin D_3	Dehydrocholesterol	$C_{27}H_{41}OH$
Vitamin E	Tocopherol	$C_{29}H_{48}O{\cdot}OH$
Vitamin P	Hesperidine	$C_{28}H_{34}O_{15}$
Vitamin K	—	Unknown
	Linolic acid	$C_{18}H_{32}O_2$

* Called thiamin chloride in U.S.A.
† Called riboflavin in U.S.A.
‡ Called cevitamic acid in U.S.A.

CHAPTER XVII

DEFICIENCY DISEASES

§ 55 THE term deficiency disease is generally used in a restricted sense, to describe a condition due to shortage in the body of some particular dietary constituent. Indeed, it is sometimes still further restricted to vitamin-deficiency diseases. We have already seen that nutritional anæmia is due to a shortage of iron, and red blood-cells, in the blood, following the consumption of a diet inadequate in its iron content. The "coastal sickness" of sheep is a cobalt-deficiency disease; in South Africa cattle may suffer from shortage of phosphorus if they pasture upon soils that contain too little of the element. This aphosphorosis has been responsible for immense losses.

Actually any sick condition due to the absence or shortage of dietary constituents may rightly be termed a deficiency disease. The most complex and complete deficiency disease is starvation; like other deficiency diseases, it ends fatally, unless curative measures are taken.

In this chapter we shall deal very briefly with the clear-cut vitamin-deficiency diseases, in our final section. It must, however, never be forgotten that partial vitamin deficiency, and especially partial deficiencies of more than one vitamin, are responsible for much of the general ill-defined bad health or "malnutrition" to which reference is made. There is, of course, no hard-

and-fast line, except in so far as a line is to be drawn between illness and ill-health; the division is simply one of convenience. But its arbitrary nature must always be remembered, and we must not be misled into making absolute what is a purely relative distinction.

§ 56 Beri-beri, due to deficiency of vitamin B_1, aneurin chloride, is associated with various symptoms: shortage of breath, paralysis, œdema (dropsical swellings) and numbness, with loss of sensation, especially in the hands and feet. It is prevalent amongst poor, rice-eating peoples, specially when the husks of the rice, which are known as polishings and are rich in aneurin chloride, have been removed in milling. In the experimental animal similar symptoms are observed, and the avian polyneuritis of the B_1-deficient pigeon has been much used in laboratory work on the vitamin.

§ 57 Scurvy is due to the absence of ascorbic acid (vitamin C) from the diet. It is associated with lethargy, pains in the joints, sallowness of complexion, subcutaneous hæmorrhages, sponginess of the gums, looseness of the teeth, fragility of the bones and often œdema. It has been called the "mariners' disease," for it used to be a terrible scourge on long voyages, when seamen were for weeks unable to obtain fresh fruit, vegetables and meat. We have already considered the experimental scurvy of guinea-pigs, in Chapter XV. Some animals—the rat, for example—cannot be given scurvy, and it has been shown that the rat has the power, withheld from man and many other mammals, of synthesising its own ascorbic acid.

§ 58 Rickets in human children, and osteomalacia in adults, are due to a deficiency of vitamin D—either because it is absent from the ingested food and medicines, or because the individual is not receiving effective

irradiation with the appropriate ultra-violet radiations, or for both reasons. Though, unlike beri-beri and scurvy, it is seldom directly fatal, the effect of rickets on the individual is extremely serious and may well last into adult life. Rachitic female infants may suffer from deformations of the pelvis that are later sources of grave danger to themselves and their babies during child-birth.

§ 59 Dangerous shortage of vitamin A is fortunately not common, but it was experienced rather widely during the Great War in places where dairy produce and green vegetables (or carrots) had become inaccessible. In its extreme form it is characterized by night-blindness; we now know that vitamin A forms an essential part of one of the sensitive pigments in the retina. Deficiency also involves degenerative changes in the mucous membranes of the respiratory and alimentary tracts, and also of the urino-genitary system. These tracts have normally a marked ability to resist the invasion of bacteria; in vitamin-A deficiency this lessens to vanishing-point, and the victim is likely to succumb to the consequent secondary infections.

§ 60 Pellagra is endemic in certain districts where the population exists largely on maize; parts of the United States, Italy and certain Balkan countries have been heavily afflicted with the disease. Ulceration of the mouth and a symmetrical reddening of the skin, especially after exposure to sunlight, accompany the earlier stages of the disease, which ends in death, with enteritis, general dermatitis and degenerative changes in spinal cord and nerves. Though it is curable (in the early stages) by yeast and yeast extract, neither aneurin chloride (vitamin B_1) nor lactoflavin is of any avail. It is probable, though not certain, that one of the other so far unisolated portions

of "vitamin B_2" is the curative and preventative anti-pellagra factor; more than one substance may be involved. Some quite recent research points to a fairly simple substance, nicotinic acid, as being closely connected with the pellagra problem, if not the actual vitamin factor itself.

§ 61 Shortage of the other well-defined vitamins—lactoflavin and vitamin E, for example—has not been so far considered the cause of any simple deficiency diseases. There are, however, other manifestations of vitamin shortage besides the frank deficiency diseases. The effects of inadequate vitamin supplies must be considered as part of the more general problem of inadequate diets.

SECTION V—DIET AND HUMAN HEALTH

CHAPTER XVIII

THE NECESSITY FOR THE OPTIMAL

§ 1 THE main task of this book has been accomplished; it is for the reader to judge how successfully. Its aim has been to put before him the kind of methods used in the laboratory study of nutritional problems and the kind of knowledge obtained thereby. To attempt the further task of showing how this knowledge bears on problems of human diet would require another book of at least the same length—and even then only the barest outlines could be given. Rather have I tried to put the reader in a position to apply some degree of scientific criticism to the many dietary proposals that are regularly to be found both in the lay press and in the more technical journals: if I have in any way helped to do this, I am satisfied.

Nevertheless, no scientific investigation can be properly evaluated except in relation to the circumstances that, directly or indirectly, were its origin, and to the applications that may be made of its results. In order, therefore, to round off this outline, it becomes necessary to say something about the bearing of nutritional research on the food and health of man. In so doing, I shall perforce lay myself very much open to the charge of dogmatism and even bias, foreign to the objective spirit of scientific inquiry, as there will be little space for the saving clause

and the qualifying parenthesis. In writing this concluding section, therefore, one hope will be very prominent in my mind—that the reader, when reading it, will himself apply that degree of scientific criticism to which I have tried to assist him.

§ 2 I propose, then, to lay down certain general propositions which, in my opinion, would be accepted by most of those who have worked on nutritional problems in the laboratory or in the wider field of applied dietetics, or in both. First, that the results of animal experiments are applicable, with due caution, to the problems of human nutrition and health. Secondly, that inadequate feeding, in quantity and quality of food, is responsible for a vast amount of ill-health and definite disease. Thirdly, and as a direct corollary of the second generalization, that improved dietary conditions would bring incalculable improvements to the health of vast numbers of people, in " civilized " and " primitive " communities alike. Fourthly, that such improvement could to some extent be brought about by educational methods, to both children and adults, and to both rich and poor. Fifthly, that a far greater improvement would result from securing that all who serve the community by working are so remunerated as to make adequate expenditure on food possible for them all. Sixthly, that such a possibility, whether brought about by a reduction in retail prices or an increase in wage levels or both, would in fact lead to that improvement in diet necessary for a general improvement in health. In other words, food does constitute for the majority of people the first call on income, after the payment of essential overheads, like rent, heat, light, and clothing ; a large portion of any increase in real income to the poorer paid workers would quite certainly be used for buying more and better food.

§ 3 During the last few years there has been much discussion of the essential dietary needs among populations in Western industrial countries. The literature on the subject is very large and fills many pages in many journals; yet, in spite of the importance of the subject, the lack of agreement among the experts is disconcerting. Even a superficial examination, however, of their recommendations shows at once that there are certain basic causes for the disagreement. In the first place, very different methods have been used for assessing needs, and, in the second place, there has been no uniformity as to what exactly constitute the needs themselves. The dietary requirements of an individual will be largely determined by the state of health to which we desire that his diet shall raise him. Unless we define with some care the exact level of nutritional well-being at which we are aiming, it is obviously impossible to define what we mean by dietetic requirements. So it happens that proposals for adequate diets have varied, as they were bound to, according as the particular investigators have aimed at a minimum diet, a safe diet, a normal diet, a good diet, and so on.

Not the least alarming factor about most of the official or semi-official investigations into this matter, and the reports that have followed from them, has been the rarity with which they have been undertaken with the object of laying down an optimum diet. An optimum diet is the easiest to define and to make the object of a dietary scheme, and is open to few of the subjective differences of interpretation that characterize some of the other dietary levels. An optimum diet can be defined as one that, for any individual or group of individuals, cannot be improved by increasing (or diminishing) any of its constituents. By this is not, of course, meant any

single food, since most, if not all, dietary constituents may be obtained from a variety of foods. The diet must be considered as a whole; the sum total of each individual constituent must be such that it is not capable of quantitative improvement. The quantitative then passes into the qualitative; such a diet, in which each constituent is at its quantitative optimum, is also qualitatively optimal.

Now, it may be true that to define precisely an optimum diet for any one individual, let alone for a group, class, or nation, may not be possible with great accuracy, but it is certainly easier to define such a diet in the light of present-day knowledge than to define one that shall be only "minimal" or "safe." We shall, moreover, do this with the greater certainty if we always err on the generous side. Let us, then, try to lay down, as accurately as may be, what amounts of each of the known essential dietary constituents are necessary for maintaining, say, the British urban industrial worker in the best possible health that can be achieved by nutritional means alone. The fact that such a diet is immeasurably beyond the reach of the average, or even the best-paid, urban industrial worker, is not germane to the purely dietary aspect of the problem, however important it may be to the politician and—in his capacity as a private citizen—to the dietary expert himself. When a constructional firm is asked to tender for a railway bridge, it is asked to tender for a railway bridge that will do its job as well as possible, and not for one that will be just "safe" or "minimal" within the financial ability of the customer to pay. It seems unfortunate that, in matters of human construction, a different principle should be adopted.

§ 4 One of the first things to remember about an optimum diet is that there is no need to bother

about its fuel or calory content. If there is enough money to pay for enough food to contain enough of all necessary food constituents, it is quite certain that the primary call of hunger, which is the expression of the need for calories, will be easily satisfied. Even on a basis of protein alone, this is certain. We can take the protein requirements of an adult on an optimum diet as about 120 grams per day, of which at least half should consist of biologically first-class protein; the 120 grams will actually supply some 420 calories. We shall find it very difficult to make up an all-round mixed diet containing this amount of protein in less than one kilogram of food; even if 200 grams of this kilogram consist of water, we are left with about 700 grams of fat and carbohydrate or, to make very modest claims, 500 grams, after allowing a further 100 grams for mineral constituents, roughage, and other items that do not contribute to the calorific intake. Even if the carbohydrate predominates, 500 grams of mixed carbohydrate and fat must furnish at least 2000 calories; if it includes any normal proportion of fat, it will furnish 2500 calories, which, with the protein already mentioned, will make up about 3000 calories altogether. Since these calculations have been based on very modest assumptions, it is certain that this number of calories will generally be exceeded by a good all-round diet satisfying other essential criteria and containing 100–120 grams of protein.

The optimum requirements for carbohydrate can, therefore, be ignored altogether, since they will follow inevitably from an optimum consumption of protein and other less obvious essentials. The experts differ somewhat as to the optimum proportion of fat to carbohydrate in a mixed diet; it is almost legitimate to state that, provided the requirements of fat-soluble

vitamins are met, and provided there are no abnormal demands on the energy content of the diet by way of heavy manual labour, fat itself is hardly an essential dietary constituent at all. It is largely then a matter of taste how the balance between fat and carbohydrate is maintained for different individuals, and this taste will be partly conditioned by the primary physiological need for calories. A given weight of fat affords rather more than twice as many calories as the same weight of carbohydrate.

There is no need to discuss in detail the sources of protein, fat, and carbohydrate, since they are to be found in all the ordinary staple foods. It is, however, worth recalling that an adequate intake of protein—that is to say, of the *essential* amino-acids; some half of the total number of amino-acids occurring in the human body—will be most easily secured from a diet containing about 50 per cent. of its protein as animal protein, derived from meat, fish, poultry, or dairy produce; with less animal protein in a diet it is necessary to depend upon the "complementary" action of vegetable proteins for an adequate supply of all the necessary amino-acids.

§ 5 Just as it may be said with general truth, "Look after the proteins, and the calories will take care of themselves," so may it also be said, "Look after the calcium and iron, and the rest of the minerals will look after themselves." It is almost certain that small but essential amounts of copper, and possibly of other elements like manganese and cobalt, will accompany the iron in foodstuffs, if the iron is available in sufficient quantity. For an optimal diet, the daily intake of iron should be 15 milligrams a day, and it is important that a high proportion of this should be available in the easily-assimilable form know to characterize the iron of vegetable, and especially cereal, foods. By a curious natural

paradox, the iron of hæmoglobin and of most animal tissues is inefficiently assimilated. The "offals" of the animal body—that is, the liver, heart, kidney, and spleen—appear, however, to be an exception to this rule and to afford excellent sources of dietary iron; so do egg-yolk and certain vegetables, particularly spinach, as well as some fish, some nuts, and, perhaps most important of all, whole wheat. White flour is a relatively poor source of iron; the superiority of the whole grain as a source of iron is an argument that has been little exploited by the advocates of wholemeal bread. Two and a half pounds of wholemeal bread contain abut 15 milligrams of assimilable iron.

The adult requirement for calcium in an optimum diet may be put at 1 gram per day at least, probably at 2 grams. This should be mainly obtainable from dairy products, though the abandonment of the habit of crunching bones, doubtless prevalent amongst our prehistoric ancestors, may perhaps be regretted by the punctilious nutritionist. However, there are other sources of calcium, such as the outer leaves of vegetables—found to be the preferential choice of the rabbit, in this respect a better dietician than man, who prefers the "heart" of the vegetable—the pulses, egg-yolk, and, again, the invaluable germ of wheat, which is present in wholemeal flour, but absent from the white.

Although, physiologically speaking, phosphorus is perhaps an even more important element than calcium, it is safe to say that an optimum intake of calcium will automatically secure an optimum intake of phosphorus. It is also true that magnesium, an essential dietary element, follows calcium very closely, so that an adequate calcium supply is a guarantee against magnesium deficiency.

§ 6 Having disposed of the major dietary constituents and the minerals, we find that the problem of the vitamins has simplified itself very much. The use of wholemeal cereal and green leaves, as the best sources of certain indispensable minerals, automatically secures an adequate intake of ascorbic acid (vitamin C), aneurin chloride (vitamin B_1), carotene (pro-vitamin A), and vitamin E; on the other hand, abundant dairy produce, included as a source of calcium and animal protein, brings with it a corresponding intake of flavin and some of the less characterized members of the "vitamin-B_2 complex," with some vitamin A and, at any rate in summer, a certain amount of vitamin D.

A diet consisting largely of dairy produce, green vegetables, whole wheat, and meat or fish, with additions of eggs and pulses, is pretty certain to be adequate in all essential dietary constituents, with the possible exception of vitamin D. Man has evolved in such a way as to make him independent of food for his vitamin-D requirements, since he has the ability to make this for himself in bright sunlight. If the conditions of our industrial civilization make his access to bright sunlight insufficient, then he must get his vitamin D in some other form, whether he makes use of the ingenuity of the organic chemist or the courtesy of the cod-fish, whose liver is always at his disposal. We have already considered the possibility that with optimal intakes of calcium and phosphorus, which implies also an optimal ratio of the two elements, the vitamin-D requirements of man may be materially less than they are when either of the elements is being taken in sub-optimal amounts. In any event, compensatory daily doses of vitamin D are so easy to obtain that there is not the slightest excuse, even with sub-optimal quantities of

calcium and phosphorus in the diet, for the appearance of any manifestations of vitamin-D shortage in any member of a "civilized community."

§ 7 An attempt has been made in this chapter to show that the essentials of an optimal diet are so well known to us, so simple, and so easy of access that there is not the slightest justification for the existence of malnutrition, or even of sub-optimal nutrition. Any figures given here have been, admittedly, approximations; but so must all figures be, however precise the laboratory knowledge on which they are based, if they endeavour to cater for groups or communities. Even in such a simple matter as calorific requirements, all that can be said is that the total daily requirement of calories for a community containing N people is M, so that the *average* daily requirement for the individual is M/N. Actually, the requirements of the population as a whole will presumably be distributed about M/N, in accordance with the normal frequency distribution curve, though nothing at all is known about the breadth or steepness of the curve that indicates the normal variations in need for this or for any other dietary essential. But it is clear that up to half the population must require something less than M/N calories per day, while from a third to a half require more. There will certainly be no harm, from the social point of view, even though it may be slightly wasteful in times of economic stringency, if the former part of the population, through receiving M/N calories per day, receive more than their needs; on the other hand serious harm may arise if others, also receiving M/N calories per day, receive less than their needs. Indeed, those members at the upper end of the frequency distribution curve may come perilously near to starvation; how near we cannot say. Until, therefore, there is complete

freedom of choice both in quality and quantity of foodstuffs for all members of the community, when the total requirements for any one food constituent will automatically be the average requirements multiplied by the population, it is necessary to cater in such a way as to make quite sure that those persons who have the misfortune to be on the upper side of the mode shall receive optimal nourishment, even though this means that we shall be offering to those below the mode more than they require or will take. It is surely better, to paraphrase what is alleged to be the basic principle of British justice, that a dozen men shall be over-fed than that one shall starve to death.

CHAPTER XIX

THE ABSENCE OF THE OPTIMAL

§ 8 THE results of animal experiment and of medical and social investigations have made it clear that inadequate diets most need improving in certain very simple directions. They are deficient in protein—especially in "good" protein—and in some minerals, notably iron and calcium; they lack sufficient of vitamins A and B_1; they are probably low in vitamin D (especially for infants and young children), and possibly in vitamin C, lactoflavin, and other parts of the "vitamin-B_2 complex."

During the last few years many expert bodies have described "minimum," "adequate," "safe," and even "optimum" diets. Among these bodies are the Ministry of Health and the British Medical Association in this country, and one having an even wider authority—the Health Organization of the League of Nations. Their reports have been published, and can be profitably read by those with little or no technical knowledge. Indeed, their recommendations are likely to be incompletely translated into practice until responsible public opinion understands and supports them; the assent of scientists alone can be of little immediate effect.

§ 9 In the last chapter an attempt was made to lay down certain features of an optimal diet—with the qualification that this can only be done provisionally, in the light of existing knowledge. It is likely that

further advances in knowledge may point the way to still further improvements. This possibility is clearly foreshadowed by experiments carried out in one of the most famous laboratories of the United States; some striking and wholly unexpected results obtained there during the last few years have hardly received sufficient notice from students of dietetics.

A colony of rats had been kept going for several years—that is, for many generations—on a diet that seemed "optimal," judged by the usual criteria. The animals were healthy and lively; they produced many and large litters of viable young; they lived to what was considered for rats a ripe old age. It was decided to find out what effects, if any, could be produced by certain simple supplements to a diet that seemed already to allow the development of physically perfect rats. Green leaves and liver were the main additions; the diet itself contained abundant milk.

When these additions were made, the health of the colony was improved in almost every respect. The animals grew bigger and lived longer; more young were born; the incidence of infections was significantly reduced. The apparently perfect diet had been made more perfect! Further investigations suggest that the extra calcium and the extra lactoflavin afforded by the supplements should receive credit for a good part of the improvement.

§ 10 With the proviso, then, that conceptions of the optimum may change as it is approached, it may safely be stated that the diets of most people fall short of this level, even as it can at present be prescribed. There exists to-day the available technique for analysing any diet, not only for its contents of fat, protein, and carbohydrate—a quite simple matter for the chemist—but

also for the essential minerals and vitamins. The chemist, again, can give precise figures for calcium and iron, and these figures, along with the values for the three major constituents, are to be found tabulated in many books on "Food Values." As a result of recent work, moreover, due allowance can be made for certain losses—trimming before cooking, wastage during cooking and on the plate, and any difficulty in absorbing and digesting particular portions of the diet. All these facts are known with sufficient accuracy to make possible the comparison of any diet with the current conception of the optimum.

This can also be done for vitamin contents, with sufficient exactness for practical purposes. For vitamins A and C, for aneurin (vitamin B_1) and for lactoflavin there are chemical or physico-chemical tests that permit the expression in quantitative terms of their amounts in any foodstuffs suitably analysed, without recourse to the far more elaborate and costly methods of animal assay, though this is still required for the measurement of vitamins D and E, and for certain other less well-defined factors. Broadly speaking, however, we can now evaluate any given diet as a source of vitamins, in comparison with our accepted optimum. The vitamin values are not published in anything like so accessible a form as other food values, but they can and will be determined whenever the necessity for it is fully appreciated. There is sufficient agreement, too, about the daily optimal requirements for most of the vitamins to give practical utility to a knowledge of their amounts in various foods.

§ 11 We do not, in short, lack knowledge to improve human health by nutritional means. Nor do we need to indulge in fantastic habits of eating to obtain the best results from an optimum dietary. The order of taking foods at a meal is hallowed by custom that has,

for the most part, a sound physiological basis. In this connection, I am grateful for permission to quote from *The Lancet* of 26th June, 1937, its editorial views on certain recent proposals for making a drastic alteration in our ordinary dietary conventions.

> "Few doctors in this country can have escaped being asked at some time or another what they think of the Hay diet. The questioner is more often an acquaintance than a patient, and the question casual rather than serious; for the person who intends to follow directions given in his newspaper does not want to hear his regular medical adviser say that these are unreasonable. Dr. W. H. Hay and his business associates have shown, if nothing else, that there are a great many people who can readily be persuaded to follow such directions; with suitable assistance one simple idea can be made to go a very long way. Dr. Hay's idea—or, as some call it, bugaboo—is that carbohydrates and proteins should not be eaten at the same meal, because protein (he says) increases the gastric secretion of acid and so hinders the digestion of starch by the saliva. The delayed digestion of carbohydrate is supposed to allow fermentation to take place, and the acids (of secretion and fermentation) are absorbed into the circulation, causing almost every known ailment, and eventually death. This month Dr. Eugene Foldes of New York has demonstrated how completely experience and experiment contradict this theory. The amount of hydrochloric acid secreted after taking carbohydrates is not very different from the amount secreted after taking proteins. Even if there were a significant difference, an increased

amount of hydrochloric acid in the stomach would not wholly interrupt starch digestion; and even if it did so, fermentation in the stomach would not follow. If there were any fermentation it would be in the intestines rather than the stomach, and it would not lead to the production of significant quantities of acid; nor is there any reason to believe that the body would be unable to cope with such acids even if they formed in larger quantities. So far, then, there is no evidence to substantiate the theory. Against it are the intimate mixture of protein and carbohydrate in many natural foods, the fact that contents of the stomach ferment only where there is achlorhydria, and the comparative harmlessness of 'acids' in a ketogenic diet except in one or two well-defined conditions, like diabetic coma. The chief danger run by followers of the Hay diet is that they may reduce the protein in their daily ration unsuitably!"

I can hardly imagine a more thoroughly effective exposure of the attempt to make Hay diets while the moon shines.*

§ 12 Most "dieting" schemes tend merely to obscure the real nature of dietary deficiencies, though they occasionally bring incidental advantages. A "slimming" diet, for example, that reduces the intake of fat and carbohydrate—that is, generally, of chocolates and bread—may result in a higher consumption of foods containing

* Quite recently it has, moreover, been shown that meals of carbohydrate and fat, planned to contain minimal quantities of protein, cause considerable losses of nitrogen, presumably derived from tissue protein, that is, from muscle—surely a curious method of "slimming"!

more protein—if the consumer has the money to pay for such foods. And this may improve not only the balance of protein to fat and carbohydrate, but also the actual intake of minerals and vitamins that, we have seen, tend to fall to a low level in so many diets.

§ 13 It is known to-day—and has indeed been known empirically for a long time—that an abundance of dairy and garden produce is essential to a good dietary. But only in recent years have we learnt why. These foods—called " protective " for want of a better term—are rich sources of essential minerals and vitamins: the staple carbohydrate foods, cereals and sugar, of our vast industrial populations are often very poor sources of minerals and practically devoid of several vitamins. The poorer man's diet is doubly deficient; he cannot afford the protective foods, which contain these essential " minor " constituents, and the foods that satisfy his need for calories—expressed physiologically as simple hunger—supply him with little or none of those constituents. At the same time, these foods are lamentably short of protein, particularly of first-class protein. Man cannot live on fat and carbohydrate alone, but innumerable men are compelled to exist on diets that contain little else. If the reader thinks that this statement tends to exaggeration, let him recall for a moment peoples outside the British Isles, but not necessarily outside the British Empire.

Whatever may be the defects in the cooking and eating of most diets, these are not the most important faults. For the vast majority of the world's workers what is wanted is not mainly an altered order of courses, or even a better cook and kitchen, but simply more money. With that will inevitably come the purchase of more food by those who need it, and the rest will follow.

INDEX