Cameron Prize Lectures

ON

THE PRESENT POSITION OF THE VITAMIN PROBLEM.

DELIVERED IN THE UNIVERSITY OF EDINBURGH,
JUNE 27TH AND 28TH, 1923,

BY

F. GOWLAND HOPKINS, D.Sc., F.R.S.,
PROFESSOR OF BIOCHEMISTRY IN THE UNIVERSITY OF CAMBRIDGE.

LECTURE I.

I FIND that the Cameron Prize which I have had the honour of receiving from the University of Edinburgh is awarded for contributions to the advance of practical therapeutics. I cannot claim, however, to have had any influence whatever upon the progress of this branch of knowledge, save perhaps in a very humble degree with respect to deficiency diseases and their prevention and cure by dietetic adjustments. Modern developments tend more and more to make dietetics a branch of therapeutics, and part at least of this tendency is due to the discovery and study of dietetic essentials which we have agreed to call vitamins. Lecturing, then, as the honoured recipient of a prize possessing the associations just mentioned, I find that to take vitamins as my subject is almost a logical necessity. Otherwise, I think, I would not have chosen just the present moment for a discussion of these elusive substances. I have some faith that we are near the moment when the constitution of one or another of them will be known. Then, indeed, both lecturer and audience might find greater pleasure in considering them.

Since the necessity of postulating the existence of factors in diet of which the concentration is very small, but the importance very great, became evident (and there can be no doubt of that necessity), much work has been done in the endeavour to discover what may be the nature of these factors. That end has not yet been reached, and as a consequence all discussion must suffer from definite limitations. But the real and objective existence of vitamins must not be doubted, and I feel the more tempted to express here and now a strong opinion on this point because it is in Scotland, and I think, save very early, in Scotland alone, that doubts about their existence—or perhaps I ought to say doubts about their practical importance in dietetics—have arisen.

When I recall certain published statements and certain oral expressions of opinion which have emanated from two important northern centres of scientific activity, I almost feel that I have crossed the border to accept a challenge!

It may be, and it is not surprising, that in certain quarters the importance of the subject has been exaggerated or its significance distorted. Unhappily, it seems that no line of genuine scientific study which bears in any way upon the food of mankind can escape attention and misrepresentation at the hands of the quack, the trade advertiser, and the less informed press. The intelligent public is made tired of facts before they have time to become fully established. Moreover, as in other subjects of inquiry calling for a technique which is apparently simple, amateur investigators have been active in this domain, and not all that has been written about it is sound. It would, however, be, I think, disastrous if those who have no time to appraise the evidence as a whole should receive any general impression that it is other than conclusive.

As you well know, the last century closed with a consensus of opinion on the part of students of nutrition that the main determinant of dietetic efficiency was the energy value of the food consumed. The view tended to crystallize into the form of Rubner's law of isodynamic equivalence. One foodstuff might, it was taught, with unimportant limitations be replaced by another so long as the total energy supplied was undisturbed. The only foodstuffs calling for serious consideration—it was also taught—were proteins, fats, carbohydrates, and mineral salts. Such

was orthodoxy even ten years ago. The change which has come over our views concerning nutrition may be sufficiently described as one involving a greater recognition of the qualitative factor in diets; a recognition which, of course, leaves the importance of quantitative factors quite undisturbed.

In a limited sense the law of isodynamic equivalence is true and indisputable. Because the body as a physical system is subject to the first law of thermodynamics the energy it gives out must be proportional to the energy liberated from its food, whether this liberation occurs on physiological lines or not. But the body is not a heat engine. Its machinery and its fuel are not to be sharply distinguished. Energy must be liberated in various forms, in the right places, and with due regard to certain sequences. Much of this organization is due to the influence within the body of the chemical agents which we call hormones. It may be that we can best define the substances we are considering by thinking of them as exogenous hormones—a term due, I think, to Sir Archibald Garrod.

Even the classical teaching of the latter part of the nineteenth century had to reckon with the specific nutrient properties of protein, but there was a tendency to minimize the importance even of these. It is now clear that protein is not the only dietary constituent with highly specific functions, and we know, moreover, that it is not true to say that a quantitative sufficiency of proteins, fats, carbohydrates, and minerals makes a diet physiological. The long dominant assumption that it did so always lacked a proper basis, because that sufficiency had never been tested on pure materials. The actual fact is that a dietary consisting of pure protein, pure fat, pure carbohydrate, and a fully efficient salt supply may be well digested, normally absorbed, and duly oxidized in the body of an animal, while wholly failing to support it. This highly significant fact is firmly based upon experimental results which are easy to obtain and always reproducible. Not less significant is the complementary fact that such a diet can be made efficient by additions which are quantitatively almost infinitesimal.

I apologize for asking attention here to the results of an experiment which is of a kind now very familiar. I would like the fundamental facts just mentioned to be freshly in mind before I proceed.

The results in question were obtained by myself, but they represent the experience of many investigators. The lower curve in the diagram shows the average growth exhibited by ten animals placed on a synthetic diet in which the only fat constituent was a purified vegetable fat. The upper curve shows the growth of ten similar animals upon the same diet, with the sole difference that each received in addition 5 milligrams per diem of crude cod-liver oil. The animals in the former set, it will be seen, all ceased to grow. By the seventieth day six out of the ten were showing serious lesions of the eyes, and all would have soon died had not their diet been altered. Those in the second set grew well, as the curve shows, and remained in good health until the experiment ceased after seventy days. The addition which made the difference between health and ill health, and

ultimately between life and death, amounted quantitatively to one part in twenty-five hundred of the total food eaten. We now know that if a particular fraction of the oil had been given, instead of the intact oil, the amount might have been much less than this.

Such facts, abundantly confirmed as they are, surely imply that the dogmatic teaching of twenty years ago must be profoundly modified; they have indeed no inherent lack of probability which would justifiably stand in the way of their frank acceptance. We are familiar in quite other connexions with the potent influence which infinitesimal quantities of specific material may exert in biological phenomena. In the region of nutrition itself we have been learning that even micro-organisms with highly developed synthetic powers may require in their environment substances which specifically stimulate growth, and this especially when they are adjusted to parasitic habits and therefore to complex media. The animal is essentially parasitic upon the green plant, and there is no reason why its needs should be confined to the substances which the plant makes in large amount. It is sure from the work of recent years that if we wish criteria for an adequate diet we must not confine our consideration to pounds and ounces, nor, for that matter, to calories. This is a scientific certainty; whether it has practical importance is a matter for separate consideration.

I have so far spoken almost as though I were putting before you a new and unfamiliar subject. My only justification for doing so is that I feel these general observations will provide a background for the presentation of some technical details. I can in reality, of course, assume a general familiarity with the facts. I must do so, indeed, if in the short time at my disposal I am to attempt a personal estimate of the present outlook. All I can do, indeed, is to report upon certain facts established by the most recent researches which seem to make firmer the basis for a belief in the objective reality of these accessory nutrients, and to illustrate the practical importance of their study.

I can assume your acquaintance with the fact that at least three distinct substances are known to exist which conform to the only available definition of a vitamin. Each possesses, that is to say, a nutritive importance which is very great, while its actual concentration in natural foodstuffs is very small. Perhaps, for convenience of reference, I may just enumerate them, familiar though they be. They are: (1) vitamin A, originally known as the fat-soluble vitamin; (2) vitamin B, the antineuritic substance; and (3) vitamin C, the antiscorbutic factor. That we should speak of them in this non-committal way, with merely alphabetical labels, was a suggestion due to McCollum, which at present seems well justified. It is well, too, that we should adopt Drummond's suggestion and write "vitamin" instead of "vitamine," as by doing so we avoid certain unjustifiable chemical connotations in the name. In my second lecture it will become clear that the vitamins A, B, and C probably do not stand alone in their class.

It is clear that if the distribution, properties, and functions of substances are to be established, quantitative methods for their estimation are a prime necessity. It is no less clear that to find such methods in the case of substances which have not been isolated, and cannot therefore be weighed, is no easy task. Nevertheless, owing to pioneer efforts made years ago at the Lister Institute, and to the later work of many—in particular that of Osborn and Mendel, of Drummond and Zilva, and of Mottram—it may be honestly claimed that we have now a technique of sufficient accuracy in the quantitative sense for the establishment of many definite conclusions. This technique is based upon biological experimentation. It consists essentially in determining the minimal amount (or the relative amount in comparison with a standard preparation) of the material under study which will induce certain observable effects in a standard animal—the smallest amount, for instance, which, when added to a dietary previously proved not to maintain growth, will enable this normal growth to occur in a young animal; or the amount which will prevent or remove symptoms experimentally produced in an

animal by deprivation of the particular vitamin in question. I must not stop to discuss the details of technique. All cautious observers have found that such methods call for experience, and call for standardization, but all agree that reliable results are to be obtained. The number of such observers is becoming legion. Experiments which began in this country and were immediately prosecuted with great enterprise in the United States are now being carried out in almost every civilized country.

The application of the above methods is continuously increasing our knowledge of the exact *distribution* of vitamins. This assuredly, if we are to believe at all in their significance, is a subject of much practical importance, but one upon which I need not dwell.

We are learning much that is interesting concerning the *origin* of vitamins. Although the statement may ultimately have to be qualified, it may be said generally that it is to the plant cell we must look as the essential place of origin. Vitamins arise in the green plant as the result of photosynthesis, though in the case of A at least, to judge from the work of Coward and Drummond, as part of events which are independent of the chlorophyll function. The relatively great stores of A which accumulate in the livers of fish take origin from the diatoms and algae of the sea (Hjort; Coward and Drummond).

Concerning the general *chemical and physical properties* of vitamins there is one statement to be made which is of considerable practical importance. There long existed a very general belief that these substances as a class were easily destroyed by heat, and upon this was based the popular assumption that any form of cooking necessarily reduces the vitamin content of natural foods. It must not be supposed that this belief is wholly wrong, but it requires considerable modification. Three years ago I was able to establish the fact that the vitamin A is resistant to heat though very prone to destruction by oxidation. Since then various observers, in particular Hess and Zilva, have shown that the same is true of vitamin C. Zilva has further determined very carefully the conditions which make for stability in the case of the latter (the antiscorbutic) substance. He found, for instance, that de-citrated lemon juice when left slightly acid (pH 6.8) could be boiled for two hours in an atmosphere of carbon dioxide without loss of antiscorbutic potency. By boiling, however, for only one hour in a current of air the potency was greatly reduced. The process of oxidation, which in the latter case was mainly responsible for the destruction, occurs much more slowly if the fluid be rendered more acid. At pH 2.2, for instance, oxidation is found to be quite slow. In the alkaline condition vitamin C is much less stable, though in this case also it is oxidation rather than the effect of heat which determines its rapid destruction (Harden, Zilva). The vitamin B has long been known to be resistant to heat.

It follows from the facts just enumerated that if access of oxygen be avoided foodstuffs may be heated to boiling point with relatively little danger from the standpoint of vitamin destruction. It should be remembered, however, that ordinary cooking operations in no case wholly exclude oxidation.

Considerable progress is being made in our knowledge of other chemical and physical properties of the individual vitamins. This knowledge, together with a realization of the conditions which make for their stability, is notably assisting current attempts to isolate the substances. Although in no case has final success been reached, preparations of each vitamin have been obtained possessed of such potency as to indicate that they contain the active substance in a concentration enormously in excess of that in the original source.

In the case of vitamin A, for instance, several observers have been able so to fractionate cod-liver oil as to obtain a product of which much less than a milligram could serve as an efficient daily ration for a rat. Of the B vitamin Seidall has made a preparation capable of preventing polyneuritis in pigeons when administered in doses of at most 1 milligram. In spite of the high activity of such preparations the final endeavour to separate from them an active pure substance has so far proved in all

cases disappointing. Hofmeister, for instance, having made from rice meal a preparation which certainly contained vitamin B in relatively large amount, obtained from it a crystalline base, but nothing else which gave evidence of purity. The base proved quite inactive. The much earlier attempts of Casimir Funk ended in a similar way, and so twelve years ago did endeavours of my own. The pursuit of pure chemistry has never involved analytical problems of such complexity, and there exists no classical technique for their solution. Maybe we shall have to replace the familiar use of selective solvents and precipitants by some new technique. Some have felt that there may be hope in the adoption of physical methods such as electrical transport, or in the use of selective adsorbents, on the lines of Willstätter's current endeavours to isolate enzymes. Vitamins would seem to be considerably more stable than enzymes, and the work done in attempts to purify them has encouraged at any rate a faith that the problem will be solved. I have already ventured an opinion that we shall not have to wait very long before the constitution of one or other of them is known.

Much current work is being done in the endeavour to decide how vitamins produce their effect in the body. It has from the first been clear that the influence of each is exerted on specific lines. The clear differences in function demonstrated by differences in the effects of deprivation have indeed constituted the main evidence for our belief in their diversity. In studies of metabolism, however, it is well to remember that chemical events in the organism, though they may often be more or less isolated experimentally, form an organized whole. No one process in the body can be rendered abnormal without ultimate effects upon other processes. Since there is a harmonious concurrence of events in normal metabolism, and since vitamins individually affect different aspects of metabolism, it is not unlikely that an optimum supply in the food would be one in which—apart from the absolute amounts—a definite proportionality obtains among them. Some unpublished experiments of my own seem to show that within certain limits this is the case. Vitamin A and B were supplied to rats on a synthetic dietary. In one experiment each was supplied in very small quantity, but the amount of the one bore a definite ratio to the amount of the other; growth was maintained, but was subnormal. In a second experiment the absolute amounts were increased, but the ratio was undisturbed; growth was normal. Next both vitamins were given in relatively very large amounts, but with the ratio still the same; growth was again normal and the animal's health was undisturbed. When, however, in another case, animals received A in the small amount employed in the first experiment and at the same time took B in the large amount of the third experiment, they showed little or no growth and failed to flourish, though there were no acute symptoms to suggest a direct deleterious effect from the large dose of A. These experiments require extension. If the results are confirmed they will show in respect of vitamins the importance of that balance among factors which has been demonstrated by others in the case of the mineral constituents of diets, and which E. Mellanby has recently insisted upon in the case of still other constituents.

In the hope of discovering the seat and mode of action of individual vitamins some have studied the effects upon functions, and others the nature of the anatomical or histological changes in the organs, which result when the animal is deprived of one or another of these substances. The remarkably specific lesions in the cornea seen in animals which have lacked a supply of vitamin A have been very fully studied (Stevenson and Clark, Mori). The latest view is that the immediate effect is upon the lacrimal glands and the entire secretory apparatus of the eye. The failure of secretion leads to xerosis of the conjunctiva and eyeball, to which the destructive lesions in the cornea are secondary. Whether this be so or not there is no doubt that in avitaminosis the glandular organs are especially prone to suffer and the changes in them differ in kind and sequence from what is seen in actual starvation. McCarrison, several years ago, showed that in pigeons deprived of vitamin B, while most organs lose

weight the adrenals increase to two or three times the weight of those of control birds. This I find occurs also in the rat. Certain secretions have been said to fail when vitamin B is absent, but causal relations here have not been fully established. Hess, Abderhalden, and others have found that the oxidizing capacity of the tissues is diminished in animals which have been deprived of vitamin B, and Funk has brought forward evidence that the functions of this vitamin have some special concern with carbohydrate metabolism. In estimating the present outlook it is well to remember that studies of the kind just indicated are continuously going on. They have not yet told us what precisely is the function or mode of action of any one vitamin, but they are making to that end. The results reported by various workers seem to be reproducible and are therefore real.

There is one suggestion sometimes made in criticism of observations such as those I have been discussing which those concerned have a right to resent. I mean the suggestion that we are not exploring the properties of nutrients unrecognized before, but are carelessly engaged in removing or supplying familiar necessities—a due supply of iron, for instance, or other minerals. This implies that those devoted to vitamin studies lack common sense. An unbiased and close consideration of the technique employed will show how essentially baseless the suggestion has been, even though here and there insufficient attention may have been given to a general balance in the experimental dietaries employed.

One important aspect of the whole matter calls for final reference. Some few, while accepting the results obtained from animal experiments, feel that it is easy to exaggerate their importance in human nutrition. There is, however, nothing more characteristic of recent studies than their tendency to emphasize this importance. Species differ in the details of metabolism, and different species differ in their response to lack of vitamins; but the differences shown are superficial. They comprise variations in the actual severity of particular symptoms and in the sequence of their occurrence. Fundamentally the events are the same. We now possess actual experimental data, quite apart from the more general evidence from deficiency diseases, which link the behaviour of the rat suffering from vitamin deficiency through that of higher animals to that of human subjects. Although it be true, as is so repeatedly said, that a civilized adult community suffers no danger from any lack of vitamins it is different (this too has been frequently said) with the children in that community. If the conception of vitamins were a myth, instead of an important corrective to older views concerning nutrition, its advent would yet, in my opinion, have served a useful purpose. The discussion which it has called forth during the last ten years has assisted in directing special attention to the importance of all that comes under the head of "quality," as distinct from quantity, in the food of children, and this, in my opinion, has already greatly benefited the community.

THE REGULATING AND REFLEX PROCESS.*

BY

P. T. HERRING, M.D.,

PROFESSOR OF PHYSIOLOGY, ST. ANDREWS UNIVERSITY, AND
PHYSIOLOGIST TO THE ST. ANDREWS INSTITUTE FOR
CLINICAL RESEARCH.

PART III.—THE IMPORTANCE OF THE SYNAPSE IN THE REFLEX OR REGULATING PROCESS.

THE nerve impulse is practically invariable in strength, it being the property of the neurone to give a maximal response to any stimulus which is capable of setting it into activity. In the isolated nerve the strength of a nerve impulse can be cut down or even extinguished by subjecting the nerve to the vapour of certain anaesthetics, but it is doubtful if the strength of the anaesthetic required to do this can be attained in the body. Death would ensue much earlier from the effect of the anaesthetic

* The expense of this research was in part met by a contribution from the Medical Research Council.