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THE VITAMIN B COMPLEX

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PREFACE

MANY excellent books on vitamins have already been published, but so rapid is the expansion of knowledge in this field that constant revision is necessary to keep them up to date. The task of assimilating new knowledge becomes progressively more difficult with each year that passes and a complete survey must necessarily become more and more voluminous. In the absence of some method of regularly revising existing monographs, the scientific worker must keep himself informed of new developments by a close study of the original literature, abstracts or periodical summaries such as the *Annual Reports* of the Chemical Society, or he must be content to rely on the publication of occasional reviews or symposia in which particular aspects of the subjects are discussed.

Hitherto, no book devoted exclusively to a study of the vitamin B complex has been published. Yet there is much to be said in favour of thus restricting the field of inquiry. Perhaps the strongest argument is the impossibility of adequately surveying the whole group of vitamins within the compass of one volume. And what more natural, in this event, than to confine the study to the group of water-soluble vitamins now known as the vitamin B complex which, after all, contains all the newly characterised vitamins ? Apart from a historical connection, the members of this group have little in common with the fat-soluble vitamins or with vitamins C and P, whereas nearly all of them have a strong family likeness, resembling one another closely in their distribution in foodstuffs, in their biological effects on animals, plants, insects and micro-organisms, and in their biochemical functions. One of the main objects of this book is to stress this close relationship, although the mode of treatment adopted, that is, the discussion of each vitamin in turn, tends perhaps to stress the distinctive characteristics of each rather than their similarities.

I first became interested in the vitamin B complex about the year 1935 or 1936, when the synthesis of vitamins B_1 and B_2 was reported, and I have maintained this interest ever since. I have followed closely every development in this field and have myself been engaged in the isolation, production and assay of the most important members of the complex and, in recent years, I have been particularly concerned with the relation between the B vitamins and chemotherapy. My qualifications for writing this monograph are therefore an intimate association with various aspects of the vitamin B complex, a conviction that it forms a group of substances of outstanding biological importance, and, I must confess, an urge to systematise the heterogeneous, rather untidy, array of data, so that others, not so intimately acquainted with the field, may have an over-all picture of what the vitamin B complex is and why it is of such significance in human and animal nutrition and in the economy of micro-organisms.

The task has not been an easy one, but I hope I have succeeded in presenting a coherent story in a form that others will find useful. I have tried to include all that is essential, and exclude all that is non-essential, but I am certain that my choice will not always meet with approval, especially as the subject is of interest to such a large number of specialists in so many branches of pure and applied science—chemists, zoologists, physiologists and bacteriologists, clinicians, nutritionists and agriculturists. Obviously it is impossible to give in one book all the information that workers in these diverse fields require, and it is to assist those who wish to have more detailed information in any particular field that I have included, at the end of each section, references to the original literature.

The story of the vitamin B complex, as will be evident in the pages that follow, has been compiled from many sources, some having no obvious connection with human nutrition. It would not be surprising, in view of the paramount importance of these substances in the metabolism of all living organisms, if further fascinating discoveries remain to be made, with consequences of perhaps even greater significance than any we have so far witnessed. I hope that this monograph may help to sustain the interest of research workers in these important substances.

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CONTENTS

CHAPTER I

INTRODUCTION

Research workers are usually so busy piling brick on brick on the edifice of human knowledge that there is never time for them to stand back and survey what has been built and how it has been done.

NATURE, *Sept.* 20, 1947.

THE WAR OF 1939-45 focused attention on the vital importance of food and nutrition, and showed how vulnerable are the food supplies of a large industrialised nation to enemy attack. The weapon of blockade was used alongside weapons of offence, and precautions against starvation were given the same high priority as air-raid precautions. It was fortunate indeed for this country—the most dependent of all the Western nations on external sources of food supplies—that so much was known concerning the nutritional value of foodstuffs. This knowledge enabled substitutes to be found for foods in short supply; it enabled a sound rationing system to be built up which, although reducing food intake well below the level considered by nutritionists to be optimal, prevented any serious symptoms of malnutrition developing in the population as a whole, and gave to "priority" classes—children, expectant and nursing mothers and certain types of manual workers—a generous allowance of special foodstuffs to take the additional strain of growth, pregnancy, lactation and heavy work; it assisted in the development of new methods of preserving and storing foodstuffs to reduce to a minimum the loss of food value; and it helped to determine what foodstuffs should be selected to ensure the best possible use of the limited shipping space available for bringing imports into this country.

Although hostilities have now ceased, the importance of the science and technology of nutrition remains as great as ever, for a large proportion of the world's population is underfed. Even in this country we were until recently subsisting on a diet only just adequate for ordinary activity. For some sections of the population, particularly adolescents, it is probably less than adequate. Sir Jack Drummond has stated in a monograph published by the Royal Institute of Chemistry (1948) that adolescents "are often the first among the population to reveal signs of inadequate feeding. In Western Europe in 1940-45 that was true, and it is also true that this 'red light' is showing here today. Many of these young people who were well nourished for most of the war period are not gaining weight today as they should; some are even losing weight." If this is a picture of the state of nutrition in this country today, what is the picture like in less fortunate countries? The problem of adequately feeding the world's population has by no means ceased to exist with the cessation of hostilities. Indeed, now is the time to examine the problem afresh in the light of the vast experiment carried out in this country between 1939 and 1945. The theories of nutritionists were then put to the test in a way that had not previously been possible, and as a result many widely-accepted generalisations had to be modified.

In order to feed everyone properly it is obviously necessary to know the nature of the substances present in food, how much of each is needed to maintain a certain level of activity, and how much is present in the foods commonly consumed. Investigations carried out during the last forty years have gone a long way towards supplying complete information on these points and, as already stated, this was used in formulating the food policy of this country during the 1939-45 war. With the additional information accumulated during the war and since, we have an even more complete picture of what is necessary for proper nutrition. What is now lacking is the machinery for applying this knowledge to rid the world once and for all of the spectre of famine.

The foundations of the science of nutrition were laid during the nineteenth century, when Liebig demonstrated that foods consisted of three main elements—proteins, carbohydrates and fats—and Voit and his colleagues showed that carbohydrates were burnt in the body to produce energy; that proteins were used for building up the tissues of the body; and that fats provided a reserve of food on which the body could draw in an emergency. It is difficult to say when this simple concept came to be recognised as inadequate as a basis for assessing the importance of different foodstuffs, for even in the eighteenth century sailors knew that scurvy could be prevented by lime-juice and fresh vegetables, while in 1885 a Japanese admiral, Takaki, eliminated beriberi from the Japanese navy by improving the sailors' diet. Perhaps the most significant date is the year 1897, in which Dr. C. Eijkman, a Dutchman employed in his country's colonial service in Java, began to study beriberi, a common disease of the tropics, which had hitherto been attributed to a bacterial infection. He noticed that hens in the prison yard suffered from a kind of leg weakness similar to the paralysis of beriberi from which the prisoners themselves were suffering. If anyone else had noticed this similarity, they had drawn from it the obvious conclusion that the hens had caught the infection from the men! Eijkman, however, made a urther observation; he noticed that when the food of the hens was inadvertently changed from polished rice, on which the prisoners were fed, to unmilled rice the paralysed hens recovered. This suggested to him that beriberi was in some way connected with food and not with infection. Forthwith, Eijkman began to experiment, and found that he could induce paralysis in hens by feeding them on polished rice and could then cure the paralysis by adding rice polishings to their diet. His colleague, Grijns, subsequently showed that beans also prevented paralysis in birds, and that an extract of beans or rice polishings cured both paralysed birds and beriberi patients. The factor thus shown to be present in these materials was later known as vitamin B. Thus a fourth dietary essential—vitamins—was added to the three elements—carbohydrate, fat and protein—recognised by the nineteenth-century nutritionists. The main materials for building this particular " edifice of human knowledge " were now available, but the story of how it is being erected—for it is not yet finished—is a long and complicated one. It has been built, like any other house, brick by brick and plank by plank. Sometimes progress has been rapid and one individual or, more often, a team of workers has contributed several courses to the brickwork. Sometimes, indeed, the building has assumed a distinctly lop-sided appearance with one wing completed almost before the foundations of another have been laid.

This book is concerned with only one aspect of the story of nutrition, and does not even set out to tell the story of all the vitamins, but only the story of those water-soluble vitamins which we now call the vitamin B complex. Progress in this field has been so rapid, and so much information has accumulated in recent years, that a complete review of all that is known about the vitamins would fill more than one volume. Besides, the story of the vitamin B complex is a coherent one and the pattern which it follows gains in clarity when this group of vitamins is considered apart from the other factors of nutritional importance. This review of the vitamin B complex is an attempt, in the words of the quotation at the heading of this chapter, " to stand back and survey what has been built and how it has been done ", even though the building is still surrounded by scaffolding and the workers are still actively engaged in completing various parts of it.

Recent research has made it more and more evident that the members of the vitamin B complex, although chemically diverse, constitute a group of biologically related substances, responsible for effecting transformations of fundamental importance to the life of organisms ranging in complexity from men to bacteria. They are, in fact, some of the building blocks around which the fabric of all living structures is built. It is to emphasise this one-ness of function that a book dealing with the vitamin B complex alone appeared to be desirable.

As already stated, the existence of vitamin B_1 or, as it is now called in this country, aneurine or, in the U.S.A., thiamine, was first demonstrated by feeding experiments on birds and human beings suffering from a deficiency disease. The same method was used for four other members of the vitamin B complex. The biological importance of nicotinic acid, for instance, was discovered as the result of Goldberger's study of pellagra in negroes and "poor whites" in the Southern States of the U.S.A. and his subsequent experiments on humans and dogs. Riboflavine was similarly identified as a vitamin necessary for the growth of rats, pyridoxine as a factor that cured a dermatitis in rats, and pantothenic acid as a factor that cured a dermatitis in chicks. Up to this point, the isolation of the several members of the vitamin B complex had followed an invariable routine—first, the observation that an experimental animal developed characteristic symptoms when maintained on a certain type of purified diet, then the discovery that an extract of some foodstuff, more often than not yeast or liver, would cure the symptoms, and finally attempts to purify the factor using the deficient animal for following the progress of the purification steps. With pantothenic acid, however, events took a different course and one that had an important influence on the subsequent history of vitamin science.

It had been observed in 1901 by a Belgian microbiologist, E. Wildiers, that certain yeasts failed to develop on a medium made up of purified constituents, but that they grew satisfactorily when an extract of yeast was added. He concluded that these organisms required for their growth a factor derived from living cells, and he gave this hypothetical factor the name "bios". Many years later it was shown that bios was not one single substance, but a mixture of several substances. Various components were shown to be identical with aneurine, riboflavine, nicotinic acid and pyridoxine. Thus, the substances that stimulated the growth of yeasts proved to be the same as those that stimulated the growth of animals. In other words, the bios complex and the vitamin B complex, if not actually identical with one another, showed considerable overlap. One member of the bios complex, not at that time identified with any member of the vitamin B complex, was a substance to which the name pantothenic acid had been given. Concentrates of this substance prepared from liver showed chemical properties similar to those of the filtrate factor that cured chick dermatitis, and an interchange of specimens by the workers concerned showed that pantothenic acid cured dermatitis in chicks, whilst the filtrate factor stimulated the growth of yeast. Shortly afterwards the identity of the two substances was established by degradation and synthesis. Here indeed was striking confirmation that the bios complex and the vitamin B complex had much in common and that substances essential for the growth of unicellular organisms were also necessary for the growth of their multi cellular relatives. The clue thus provided was followed up, with spectacular results. Another member of the bios complex is a substance called biotin, which has a demonstrable biological activity in extremely high dilution. When biotin was administered to animals suffering from so-called vitamin H deficiency the symptoms disappeared, and thus another link was forged between the two groups. In almost exactly the same way folic acid, a factor essential for the growth of certain bacteria, was shown to be identical with a factor, termed vitamin B_c, necessary for the well-being of chicks. In this instance, however, there exists a group of at least four closely related factors exhibiting similar biological properties—a folic acid complex within the vitamin B complex. Other examples of the identity of growth factors for higher and lower organisms are provided by *p*-aminobenzoic acid and inositol, both of which are essential growth factors for many bacteria and yeasts and also essential vitamins for certain species of animals.

The discovery that *p*-aminobenzoic acid was a growth factor for bacteria led to another discovery of great theoretical significance although not apparently of great practical importance. The discovery of the growth-promoting properties of *p*-aminobenzoic acid followed the observation that it counteracted the antibacterial action of sulphanilamide, to which it is structurally analogous. This led to a theory that bacterial growth may be inhibited by the addition to the medium of a substance which antagonised the growth-promoting action of an essential metabolite.

Now every theory, to be of any value, should enable predictions to be made, and the fulfilment of these predictions greatly strengthens the theory from which they are derived. If, then, sulphanilamide could reverse the growth-promoting effect of *p*-aminobenzoic acid, would substances chemically related to other members of the bios or vitamin B complex inhibit bacterial growth by competing with these vitamins in the metabolic processes going on within the bacterial cell? This question was asked—and answered in the affirmative—by groups of workers in Great Britain, the U.S.A. and Germany. Pyridine-β-sulphonic acid was shown to antagonise the effects of nicotinic acid, isoriboflavine those of riboflavine, desoxypyridoxine those of pyridoxine, pantoyltaurine and many other related substances those of pantothenic acid, and oxybiotin those of biotin. The use of such antagonists has thrown a great deal of light on the function of the vitamin B complex.

Another important phenomenon that has recently come to light as the result of work with sulphanilamide and related sulphonamides is that of intestinal synthesis. When certain sulphonamides, not readily absorbed from the gut, were given to experimental animals, symptoms of vitamin B complex deficiency developed, and investigation showed that the sulphonamide had checked the growth of the intestinal flora which normally synthesised certain members of the vitamin B complex. Many animals are able to utilise the vitamins thus formed and are therefore independent of external sources of supply. The phenomenon undoubtedly occurs in man, but normally only in respect of certain vitamins, and it is not known what the conditions are for stimulating the growth of the appropriate organisms in man and whether the vitamins so formed are invariably available to the host or are only available under special circumstances.

The names of those whose labours have contributed to the accumulation of the vast amount of knowledge we now possess about these substances, those who, so to speak, have " piled brick on brick on the edifice ", is legion. In some instances, a particular individual may have contributed only one little item of knowledge and then transferred his energies to other spheres. In other instances, the contribution of one individual may have extended over a period of years, indeed over a whole life-time. There are others again who have built up large schools of vitamin research and have carried out elaborate programmes of investigation as leaders of teams of specialists. There are also industrial organisations, who have used their research laboratories and development departments for the improvement of manufacturing processes and testing techniques, and who have often made discoveries of outstanding importance.

Of those who have thus contributed to the advance of vitamin science, only a few can be referred to specifically. Mention has already been made of Dr. C. Eijkman, the pioneer in the field, who showed that beriberi was a deficiency disease caused by the absence from the diet of the factor we now call aneurine or thiamine ; in 1930, a few months before his death, Eijkman was awarded the Nobel prize in recognition of his discoveries. He shared it with another pioneer of vitamin science, Sir Frederick Gowland Hopkins, one-time President of the Royal Society, who showed that the growth rate of rats maintained on a purified diet rapidly declined until the animals died, and that the addition of milk to the diet, in amounts that supplied only negligible amounts of protein and carbohydrate, checked the fall in growth and enabled the animals to live and thrive. Hopkins' classical experiments have been repeated, with appropriate modifications, by all subsequent investigators who have studied growth factors for higher animals. Another name closely associated with vitamin science is that of Casimir Funk, a Pole working at the Lister Institute, London, who in 1912 coined the word " vitamine " to describe the then mysterious factors responsible for curing deficiency diseases. He it was who brought Eijkman's work to the notice of a larger scientific public and who predicted the existence of other deficiency diseases. This prediction was fulfilled within a few years, when Dr. J. Goldberger proved, contrary to all previous opinion, that pellagra was a deficiency disease. Goldberger was appointed in 1913 by the U.S. Bureau of Public Health to investigate the outbreak of pellagra in the Southern States of the U.S.A. He was struck by the fact that nurses and doctors attending pellagra patients in an asylum never contracted the disease, and came to the conclusion that it was due to the particular diet on which the patients, invariably poor, had to maintain themselves. He proved his point, first, by adding milk and eggs to an orphanage diet and thereby eliminating pellagra from that particular institution and, secondly, by giving them diets consisting solely of deficient foods; this diet was in fact similar to that eaten regularly by thousands of poor farmers in the areas in which pellagra was endemic.

The years immediately following the work of these pioneers saw few developments of scientific importance, although the empirical knowledge gained as the result of their labours was used in various parts of the world in the prevention and cure of both beriberi and pellagra. In 1926, however, events began to move more rapidly, and in that year pure crystalline aneurine was isolated. It was synthesised ten years later by Prof. R. R. Williams of Columbia University in collaboration with a group of chemists employed by Merck & Co., Rahway. A year earlier, in 1935, riboflavine had been synthesised independently by Prof. R. Kuhn of the University of Heidelberg and Prof. P. Karrer of the University of Zurich, and in 1937 nicotinic acid, known since 1867 as a chemical of no particular importance, was recognised as the pellagra-preventive factor. Pyridoxine was characterised as a vitamin in 1938 and in the following year was synthesised independently by Prof. R. Kuhn and the Merck workers who had already achieved fame in connection with the synthesis of aneurine, and who were to enhance their reputation still further by the successful synthesis of other vitamins. Shortly afterwards, they collaborated with Prof. R. J. Williams, then of Oregon State College and later of the University of Texas and brother of Prof. R. R. Williams, in studying the constitution of pantothenic acid, which they synthesised in 1940. This was followed by an investigation into the structure of biotin in collaboration with Prof. V. du Vigneaud of Cornell University; they synthesised biotin in 1943. Another name associated with biotin is that of Prof. F. Kögl of the University of Utrecht, who isolated it from egg-yolk in 1936, studied its constitution under particularly difficult conditions during the

German occupation of Holland and suggested a formula which he subsequently admitted, in the light of du Vigneaud's results, to be erroneous. Actually, Kögl's biotin is probably different from, although closely related to, du Vigneaud's, and what is believed to be the correct formula for egg-yolk biotin was suggested by Kögl in 1944. Folic acid was isolated in 1941 from spinach leaves by Prof. R. J. Williams, of pantothenic acid fame, and Dr. E. E. Snell, who had already carried out a large amount of microbiological work in connection with members of the vitamin B complex. Similar substances were subsequently isolated from yeast and liver, and synthesised in 1946 by research chemists employed by the Lederle Labs. Inc., Pearl River, New York, and the American Cyanamid Co., Bound Brook, New Jersey. The latest member of the vitamin B complex to be discovered, vitamin B_{12}, was obtained in crystalline form in 1948 by the Merck group already referred to and by Dr. E. Lester Smith of Glaxo Laboratories Ltd., Greenford.

This is the story in briefest outline of the vitamin B complex. In the chapters that follow, details are given of the isolation, chemistry, biological properties and functions of each vitamin in turn, and in a final chapter an attempt is made to show the close biological relationship that exists between these substances by indicating the different stages of metabolism in which each participates.

CHAPTER II

ANEURINE (THIAMINE)

1. HISTORICAL

Beriberi

The existence in foodstuffs of substances essential for the proper functioning of the animal organism was first recognised by C. Eijkman and H. Grijns, two Dutch medical officers working in the Dutch East Indies. They suggested that beriberi was not caused by a toxic principle or by infection, as had been supposed, but by a nutritional deficiency.

Discovery of the Vitamins

In 1911, C. Funk[1] published a series of papers describing the isolation from rice polishings of a substance capable of curing beriberi. In the following year he wrote:[2] "The deficient substances, which are of the nature of organic bases, we will call 'vitamines', and we will speak of a beriberi or scurvy vitamine, which means a substance preventing the special disease." The word "vitamine" remained in use until 1920, by which time it had become clear that only a few of these substances were organic bases. It was then proposed[3] that the name should be changed to "vitamin" with the implication that a vitamin is "a neutral substance of undefined composition".

Although several of the vitamins contain nitrogen atoms and are basic, only one or two contain the amino group, NH_2, characteristic of a primary amine. One of these is vitamin B_1, now known in this country as aneurine hydrochloride and in America as thiamine hydrochloride. At first it was called vitamin B, and it is the absence of this substance that is responsible for beriberi, which, as already mentioned above, was the first deficiency disease to be recognised as such. The condition is due to the use of polished rice as a major article of diet, the bulk of the vitamin being contained in the outer layers of the grain which are removed in the processing. The resulting rice polishings have a marked curative effect on the course of the disease and an aqueous extract possesses similar activity.