Vitamins of the B Group.

THE TILDEN LECTURE.

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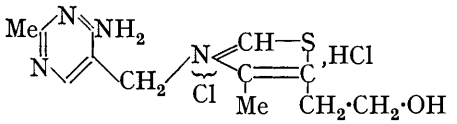
To present a clearly defined picture of the vitamins of the B group is a matter of considerable difficulty. Such difficulty must always exist where our knowledge of a subject is still incomplete, but in the case of these vitamins it is magnified by the serious confusion in nomenclature which has for long existed and by the vagueness of the term " vitamin B ". In the absence of any simple and concise definition of the term we must seek for its origin and endeavour, before proceeding to a discussion of its constituents, to get some reasonably clear idea of its significance and scope. Historically the vitamin B group originated in the " water-soluble B " of McCollum and Davis (1915). These workers showed that rats reared on a synthetic diet would only grow normally if there were added two accessory factors " fat-soluble A " and " water-soluble B ". This water-soluble B, or vitamin B as it came to be called on the adoption of the term vitamin for accessory food factors in general, was obtained from a variety of materials, *e.g.*, rice polishings, yeast. As these were known to contain Eijkmann's antineuritic factor (the original " vitamine " of Funk), vitamin B was prematurely identified with that factor. It would not be possible within the limits of this lecture to review the many researches which gradually built up evidence for the view that vitamin B was not one substance but a complex mixture one of whose components was the antineuritic factor henceforth to be known as vitamin B_1. The initial subdivision was of course into B_1 (thermolabile) and B_2 (thermostable), and it was really at this time (1926) that serious confusion originated. Had vitamin B_2 proved a single substance, matters would have been simple, but the realisation that it too was a mixture, coupled with an attempt to retain the " vitamin B " nomenclature, simply led to chaos which is only now being cleared up. Thus, for example, some workers named one of the components of the thermostable factor vitamin B_2 (riboflavin), others applied to the factor the term vitamin B_2 complex or vitamin B complex, and others erroneously identified the component promoting rat growth with that which prevents human pellagra. The use of the word " complex " to describe what is after all only a mixture of vitamins is most unfortunate, but the term, like " vitamin," has become so embedded in the literature that it is unlikely to be abandoned until all the component substances have been severally isolated and named; it would seem preferable meantime, however, to substitute for it, as far as possible, the term " vitamins of the B group ". It should also be borne in mind that to-day the materials most frequently used as sources of the B vitamins are yeast and liver and that the group is usually taken to include factors necessary not only for rats but also for other animals and birds. The latter point is readily understood when it is remembered that the vitamin requirements of animals vary according to species—dogs, for example, do not require to have vitamin C added to their diet.

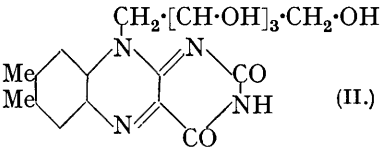
In reviewing the present position of a subject so large and so complicated in development as the vitamin B group, only the barest outline can be given and no attempt will be made either to treat the various aspects chronologically or to present in detail all the experimental evidence. At the present time the well-known members of the vitamin B group are aneurin, riboflavin, nicotinic acid, pyridoxin and pantothenic acid, all of which have been structurally identified and synthesised. To complete the list one must also take into account certain other biological entities not yet isolated in pure form, and also the probability that inositol, biotin, *p*-aminobenzoic acid and choline must be included. Doubts still exist in some quarters as to the inclusion of the latter substances, largely as a result of the vagueness of the term vitamin B and the diversity of experimental techniques employed in biological testing. It may simplify our discussion if we deal first with the main features of the well-established members of the group.

1. *Aneurin (Vitamin B_1, Thiamine).*

Aneurin has the distinction of being the substance for which the name " vitamine " was originally introduced by Funk (1912). As the factor whose absence from diet leads to the development of beri-beri it was the first vitamin to be identified biologically and the first to be isolated in a pure state as its hydrochloride (I) (Jansen and Donath, 1926). During the decade following its isolation many workers contributed to the elucidation of its structure; prominent among these was R. R. Williams, whose brilliant investigations played a major part. Final confirmation of the structure of aneurin was provided by complete synthesis (Williams, Cline, and Finkelstein; Todd and Bergel; Andersag and Westphal). Details of the chemical investigations need not be repeated here. To-day aneurin finds wide clinical application not only in the prevention and cure of beri-beri but also in many commoner but less acute manifestations of deficiency, *e.g.*, peripheral neuritis and gastro-intestinal disturbances.

(I.)





(II.)

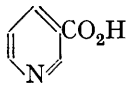
2. *Riboflavin (Lactoflavin, Vitamin B_2, Vitamin G).*

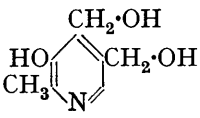
After it had been definitely established that vitamin B could be separated into a thermolabile and a thermostable component (Smith and Hendrick, *U.S. Publ. Health Reports*, 1926, **41**, 201) efforts were made by many workers to isolate the thermostable factor affecting rat growth, but although by 1930 potent concentrates had been obtained, the recognition that riboflavin was at any rate a part of it came from a rather different angle. In 1933 Kuhn, György, and Wagner-Jauregg (*Ber.*, 1933, **66**, 1034) isolated in pure form a pigment from whey to which they gave the name lactoflavin (now generally called riboflavin in nutritional work). As a result of researches by Kuhn and Karrer and their collaborators the pigment was shown to have structure (II) and its synthesis was effected. Riboflavin was recognised to be a component of the thermostable part of vitamin B, *i.e.*, it was the rat growth factor " vitamin B_2 " (Kuhn, György, and Wagner-Jauregg, *loc. cit.*). Riboflavin was also obtained from liver, yeast, and many other materials and was found to be a constituent of the " yellow enzyme " isolated from yeast in 1932 by Warburg and Christian.

3. *Nicotinic Acid (Pellagra-preventive or P.P. Factor).*

Although nicotinic acid (III) was obtained from an extract of rice polishings by Funk in 1911, it was not until 1937 that it was recognised as the pellagra-preventive factor, a member of the vitamin B group. It had been suggested as early as 1915 by Goldberger and his collaborators that diet played a major rôle in the development of pellagra, but it was not until it was found that the thermostable portion of vitamin B had pellagra-preventive properties (*ca.* 1926) that serious search for this factor began. For a number of years great confusion reigned, largely as a result of failure to distinguish between human pellagra and outwardly similar forms of dermatitis in rats and chicks. Success came ultimately when Elvehjem, Madden, Strong, and Woolley (*J. Amer. Chem. Soc.*, 1937, **59**, 1767) showed that nicotinic acid would cure blacktongue in dogs, a condition analogous to pellagra, and isolated nicotinamide from liver extracts. Immediately following this work nicotinic acid was successfully applied in the treatment of pellagra in human beings, and similar conditions in pigs and monkeys were cured by the same means. The pellagra-preventive factor is thus usually considered to be nicotinic acid. It may well be that it is simply the precursor of the amide or some other more labile derivative synthesised from it in the body. It is, of course, true that pellagrins are usually found to suffer from a multiple vitamin deficiency, but nicotinic acid deficiency is certainly responsible for their characteristic symptoms. In passing it may be noted that rats do not require to have nicotinic acid present in their diet. The pellagra-like conditions which are seen in rats

and chicks when reared on certain B deficient diets are due to lack of other vitamins mentioned below.

(III.) 

 (IV.)

$HO{\cdot}CH_2{\cdot}CMe_2{\cdot}CH(OH){\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CH_2{\cdot}CO_2H$ (V.)

4. *Pyridoxin* (*Vitamin B_6, Adermin*).

The experiments of György (1934—1936) showed that " rat pellagra " or " rat dermatitis," a condition at one time confused with human pellagra, could be cured by a factor present in yeast to which he gave the name vitamin B_6. This factor was subsequently shown to be identical with the " yeast eluate factor " of Macrae and co-workers. Vitamin B_6 or pyridoxin (IV) was isolated almost simultaneously by a number of investigators in 1938 (Lepkovsky, György, Kuhn and Wendt, Keresztesy and Stevens, Ichiba and Michi) and in the course of a year its structure was elucidated and it had been synthesised (Harris and Folkers, *Science*, 1939, **89**, 347; Kuhn, Westphal, Wendt, and Westphal, *Naturwiss.*, 1939, **27**, 469). As yet insufficient data are available to permit an estimate of the clinical value of this vitamin, but animal experiments suggest that it may be of some importance. Deficiency in animals does not merely cause dermatitis; it produces convulsive fits very similar to epilepsy in pigs (Chick, Macrae, Martin, and Martin, *Biochem. J.*, 1938, **32**, 2207) and other animals, and dogs deprived of it show a rather typical microcytic anæmia (Fouts, Helmer, Lepkovsky, and Jukes, *J. Nutrition*, 1938, **16**, 197).

5. *Pantothenic Acid.*

The occurrence of a dermatitis resembling pellagra in chicks reared under conditions of controlled deficiency is due, as has already been mentioned, not to nicotinic acid deficiency but to the lack of a further vitamin of the B group which has been in the past called the " chick anti-dermatitis vitamin " or " filtrate factor ". Although it proved very elusive—indeed it has not yet been isolated from natural sources in a pure condition—sufficiently potent concentrates were obtained by 1939 for Jukes (*J. Biol. Chem.*, 1939, **129**, 225) and Woolley, Waisman, and Elvehjem (*ibid.*, p. 573) to identify it with the substance known as pantothenic acid (V). This acid was discovered and named by R. J. Williams and his collaborators (*J. Amer. Chem. Soc.*, 1933, **55**, 2912), who recognised it as an acidic water-soluble substance of very widespread distribution which was an important growth factor for yeast. Investigations by these workers over a period of years, although they did not lead to the isolation of a really pure specimen, did enable them to deduce the essential features of its structure. The accuracy with which these features were deduced is remarkable and the account of R. J. Williams' experiments makes fascinating reading. The final work leading to complete structural determination and synthesis was carried out in association with the team of workers at the Merck Research Institute which has been prominently associated with work on several of the vitamins of the B group (Williams and Major, *Science*, 1940, **91**, 246; Stiller, Keresztesy, and Finkelstein, *J. Amer. Chem. Soc.*, 1940, **62**, 1779; Stiller, Harris, Finkelstein, Keresztesy, and Folkers, *ibid.*, p. 1785).

Parallel with investigations on the chick anti-dermatitis vitamin a number of workers were searching for the factor or factors in liver or yeast filtrates (*i.e.*, filtrates from fuller's earth adsorbates) which stimulated the growth of rats receiving as supplements aneurin, riboflavin, nicotinic acid and pyridoxin; in its absence growth was very small. The complexity of investigations on this topic cannot be described here; a fairly recent account has been given by Lepkovsky (*Ann. Rev. Biochem.*, 1940, **9**, 400). The results, however, can be briefly summarised. The rat growth factor in liver filtrates is composite in nature, one component (factor α) being extracted from acid aqueous solutions by amyl alcohol or ether, the other (factor β) remaining in the aqueous phase under these conditions (El Sadr, Hind, Macrae, Work, Lythgoe, and Todd, *Nature*, 1939, **144**, 73). Factor α has been shown to be identical with pantothenic acid and to be biologically distinct from factor β

(Lythgoe, Macrae, Stanley, Todd, and Work, *Biochem. J.*, 1940, **34**, 1335). Factor β has not yet been isolated, although experiments to that end are in progress.

In addition to factor β there is evidence from various sources for the presence of at least one further rat growth factor in liver. This missing factor, to which my colleagues and I apply the term factor γ (Lythgoe *et al.*, *loc. cit.*), is present in acid autoclaved extracts of whole liver. Recent unpublished experiments by Dr. Lythgoe in my laboratory have shown that factor γ is not identical with biotin, although crude concentrates of factor γ usually contain biotin. This observation is of considerable interest in view of recent work on the so-called vitamin H. This name was given by György (*Z. ärtzl. Fortbildung*, 1931, **28**, 377, 417) to the factor which prevents or cures " egg-white injury," a pellagra-like condition arising in rats to which excess of egg-white is fed. The identity of vitamin H with biotin has recently been established (du Vigneaud, Melville, György, and Rose, *Science*, 1940, **92**, 384). We have here an apparent anomaly in that the work on vitamin H suggests that biotin is indispensable for the rat whereas the work on factor γ indicates that it has no growth effect. The most probable explanation is that biotin is normally synthesised in adequate amount by the bacterial flora of the rat, an explanation which is perhaps supported by the relatively high concentration of biotin found in the intestine. Biotin ought on this basis, perhaps, to be included among the members of the vitamin B group, although it may be that most animals will only suffer from a deficiency when this arises through the intervention of some external agent (*e.g.*, in egg-white injury).

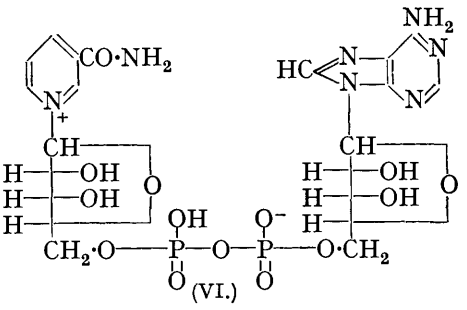
Of the various substances so far mentioned, biotin (Kögl and Tönnis, *Z. physiol. Chem.*, 1936, **242**, 43) and pantothenic acid are perhaps better known as essential growth factors for yeast and other micro-organisms. The same kind of function can be allotted to other members of the B group, and this leads to a consideration of the important relationship between vitamin B and " bios ". Wildiers (*Cellule*, 1901, **18**, 313) observed that, though yeast would not grow appreciably in a solution of sugar, ammonium tartrate and the mineral salts of yeast ash, prolific growth could be obtained by adding a small amount of a sterile decoction of yeast, and he introduced the term " bios " to describe the unknown growth-stimulating factor or factors in such yeast decoctions. It would be out of place to trace here the development of " bios," but the investigations of Lash Miller, R. J. Williams, Kögl and many others have led to the recognition of a number of its constituents. Of the well-established members, aneurin, pyridoxin, biotin, pantothenic acid and inositol may be mentioned. Closely related to bios investigations are those on growth factors for other micro-organisms in which, in addition to the above, riboflavin, nicotinic acid or its amide, *p*-aminobenzoic acid, and choline (all of which occur in vitamin B extracts) have been found essential in certain cases.

The fact thus apparent that the vitamins so far discussed are also essential growth factors for micro-organisms, taken in conjunction with the common sources of bios and the vitamin B group, suggests strongly that the two are really identical. It is worthy of note that this idea was expressed by R. J. Williams as early as 1919 (*J. Biol. Chem.*, 1919, **38**, 465). On this basis we might expect to find that inositol and *p*-aminobenzoic acid, both known as growth factors for micro-organisms, may function as vitamins for animals, and quite recently evidence has been obtained that this is indeed the case. Woolley (*Science*, 1940, **92**, 384) has shown that inositol prevents or cures alopecia in rats, and the evidence of Ansbacher (*Science*, 1941, **93**, 164) suggests that deficiency of *p*-aminobenzoic acid may be the reason for the greying of hair (achromotrichia) often observed in B-deficient rats. Similarly, evidence from various sides suggests that choline plays a definite part in rat nutrition.

When it is remembered that certain members of the vitamin B group have been shown to have a definite function in higher plants and in insects, it becomes obvious that the substances we are discussing must play a vitally important part in life processes. What that part is we do not know with certainty in all cases, but evidence as to function is available in the case of certain members. In 1937 Lohmann and Schuster (*Biochem. Z.*, 1937, **294**, 188) isolated in crystalline form the co-enzyme (co-carboxylase) of the yeast enzyme carboxylase, which catalyses the decarboxylation of pyruvic acid, and showed it to be

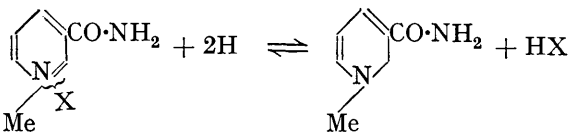
aneurin pyrophosphate; this structure was subsequently confirmed by synthesis (Tauber, *J. Amer. Chem. Soc.*, 1938, **60**, 730). It has since been found that aneurin pyrophosphate plays a similar rôle in micro-organisms (Lipmann) and in animal tissues (Peters) as a component of pyruvic oxidases. In animals it appears that the aneurin absorbed from foodstuffs is phosphorylated in the body.

Co-enzyme function also applies in the cases of nicotinic acid and riboflavin. The original discovery of Harden and Young (*Proc. Roy. Soc.*, 1906, *B*, **125**, 171) that a thermostable dialysable component co-zymase (co-enzyme I or co-dehydrase I) of yeast was essential to fermentation has been followed by much work directed to its isolation and identification. Largely through the work of v. Euler and Myrbäck on co-zymase and of Warburg and his school on the very similar co-enzyme II from mammalian red blood corpuscles it is now possible to assign a tentative formula to co-zymase (VI) (v. Euler and Schlenk, *Z. physiol. Chem.*, 1937, **246**, 64). Co-zymase is thus described as diphosphopyridine nucleotide (DPN) and co-enzyme II has a similar structure except in so far as it contains three phosphoric acid residues, being consequently known as triphosphopyridine nucleotide (TPN).



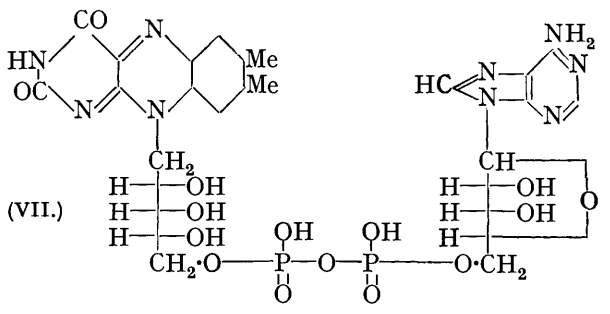
(VI.)

These two substances, in each of which a nicotinamide residue occurs, form the prosthetic group of a large class of enzymes (pyridinoproteins) intimately concerned in carbohydrate metabolism. The co-enzymes function in the enzyme systems as hydrogen acceptors, hydrogen being removed from the substrate and the co-enzyme reduced to a dihydro-derivative; this reduction is a reversible process and on its reversibility depends the catalytic function of the enzymes. The nicotinamide residue is the part of the molecule concerned in the oxidation–reduction process, the case being quite analogous to the reversible reduction of, say, nicotinamide methiodide.



Co-enzyme II is concerned in, for example, the oxidation of hexosemonophosphate whereas co-enzyme I does not function in this reaction but in, for example, lactic and triosephosphoric acid systems. The large class of pyridinoproteins falls into two distinct groups in which the specific proteins are combined with one or other of these co-enzymes.

Intimately bound up with the functioning of co-enzymes I and II are the so-called flavoproteins, enzymes containing riboflavin derivatives as prosthetic groups. Perhaps the most widely known of these is the yellow enzyme which Warburg and Christian originally obtained in 1932 from bottom yeast; this is a conjugated protein containing riboflavin-5-phosphate as prosthetic group. A number of flavoproteins have been isolated from various sources and the prosthetic group of several (*e.g.*, Straubs' heart flavoprotein, the yeast flavoprotein of Haas, and amino-acid oxidase) has been shown to be a flavinadenine dinucleotide whose structure may be expressed by (VII).



(VII.)

Flavoproteins are concerned in the re-oxidation of dihydro-coenzymes I and II, being themselves reduced to leuco-compounds in the process. To complete the cycle the leuco-flavoproteins are re-oxidised by molecular oxygen, in certain cases directly and in others by intervention of a carrier whose nature is at present unknown but which can be replaced in the laboratory by such compounds as methylene-blue. The following scheme indicates roughly the process for the preliminary oxidation of hexosemonophosphate.

(1) Hexosemonophosphate + H_2O + co-enzyme II $\longrightarrow$ phosphohexonic acid + dihydro-coenzyme II.
(2) Dihydro-coenzyme II + flavoprotein $\longrightarrow$ co-enzyme II + dihydroflavoprotein.
(3) Dihydro-flavoprotein + O_2 $\longrightarrow$ flavoprotein + H_2O_2.

These examples show, then, the great importance of at any rate three members of the vitamin B group in the metabolism of the living cell and it seems to the writer reasonable to imagine that similar functions will be in due course assigned to the other vitamins of the group. Such a view is perhaps supported by the fact that pyridoxin, biotin, and pantothenic acid all exist in tissues in combined form. This conception of the vitamin B group as simply a storehouse of co-enzymes required for vital processes is an attractive one and, besides making the importance of the vitamins to all forms of life readily understood, it suggests many possibilities for future work and development. A few points that strike one may be briefly mentioned in conclusion. This conception may prove of service in chemotherapeutic studies both in the search for new agents and for the understanding of their mode of action. Progress has already been made in this direction following the discovery of the antagonism of *p*-aminobenzoic acid and sulphanilamide in bactericidal tests (Wood and Fildes, *Chem. and Ind.*, 1940, **59**, 133). A possible explanation of this antagonism is that both these substances compete for some specific protein functioning in an enzyme system in the bacteria (cf. Fildes, *Lancet*, 1940, i, 955 : Stamp, *ibid.*, 1939, ii, 10). From this point of view the study of the bactericidal properties of sulphur-containing analogues of nicotinamide commenced by McIlwain (*Nature*, 1940, **146**, 653) is of great interest. The production of biotin deficiency in animals mentioned above, by egg-white injury, may be related to the toxic effect of certain other proteins (*e.g.*, in snake venom) ; here again competition with a specific protein for a particular co-enzyme may be the clue.

The account which has been given of the vitamins of the B group is very incomplete and does scant justice to a multitude of sustained and brilliant researches carried out by many workers. It may, however, serve to focus some attention on the merging of several lines of chemical and biological research into one field. Knowledge of that field is still incomplete, but its further investigation promises to take us still nearer to a true understanding of at any rate some aspects of the chemistry of living matter, an understanding which must be the ultimate goal of organic chemistry.