

Dr. Prescott recounted in opening the discussion. It seems to me, however, doubtful whether, in view of the smallness of the dose used by the former authors and of the shortcomings of existing methods, their finding is at all conclusive.

Dr. Prescott's comment on the value of milk in preventing pellagra despite its low content of nicotinic acid reminds me of a communication made to the Biochemical Society (Platt and Glock, 1942), in which a condition called African scurvy was discussed. This could be cured by the addition of milk or meat to the African diet. It is my opinion that this African scurvy was probably superimposed on an insufficiency in the diet of vitamins of the B group, in particular of nicotinic acid or its amide.

*To Dr. Brockington:* Alcoholic drinks of the type of Kaffir beer contain, apart from alcohol, considerable amounts of all B vitamins. As such beverages become more sophisticated these vitamins are removed; in European beer, for example, yeast and other solid matter is filtered off, and only factors like riboflavin, present in solution, remain. Distilled liquors do not contain any of these factors.

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Dr. L. Wills replied:

*To Dr. Kodicek:* I have only seen one case of macrocytic anaemia in which vitamin C was the limiting factor. The patient made a dramatic cure on large doses of the vitamin but relapsed later in spite of continued treatment.

*To Mr. Edwards:* All the autolysed yeast extracts were prepared from brewer's yeast. The extracts made from non-autolysed yeast were from either brewer's or distiller's yeast.

*To Mr. Bacharach:* I have considered the possibility of the excretion into the gut of fractions given parenterally but as such excretion does not occur in the stomach and probably not in the duodenum, two areas where the intrinsic factor is supposed to react with the extrinsic, I think this possibility highly improbable.

### Chairman's Summing Up

Dr. L. J. Harris (Dunn Nutritional Laboratory, Cambridge): In trying to summarize some of the main impressions left by today's meeting, one's most outstanding impression, perhaps, is the extent and importance of the recent developments in our knowledge of the B<sub>2</sub> vitamins. Together, these substances now constitute an impressive, and not inconsiderable chapter in the joint sciences of biochemistry and nutrition.

#### *Chemical Structures*

It will be useful for summing up to return once again to the table shown at the beginning of the meeting (p. 82), with the object especially of noting the gaps in knowledge which still remain. Column 1 indicates whether

or not the structural formula is known for each of the various B vitamins. It is obvious that a notable distance has already been covered in that direction since, in no less than nine cases, there is precise knowledge of the chemical constitution. It is perhaps instructive to recall that in four instances the substance was already familiar to chemists, and its structure established, before it was recognized as a vitamin; this was true of nicotinamide, *p*-aminobenzoic acid, inositol, and choline. Only relatively late in their chemical history were these compounds found to possess the biological properties entitling them to be described as vitamins. Perhaps one might make so bold as to venture a "tip", based on these past experiences; workers attempting to ascertain the chemical nature of some still unidentified vitamin might well first consider testing the effect on their animals of a great variety of known substances!

In another instance, that of riboflavin, the substance happened to be already known, although its structure still remained to be worked out, when it was unexpectedly found to possess vitamin activity.

In the remaining four instances, vitamins B<sub>1</sub>, B<sub>6</sub>, pantothenic acid and biotin, the structure was determined only after the vitamin had been isolated as a new substance not previously known to chemists. In some of these instances the molecular structure was then found to possess certain distinctly novel features. For example, vitamin B<sub>1</sub> contains a new ring system, thiazole, hitherto quite unknown in biochemistry.

So much for the vitamins whose structures are now established. Little is yet known about the chemical nature of folic acid and of what I have ventured to call the "grass juice complex". Ignorance is equally great about the chemistry of the "extrinsic factor" for pernicious anaemia, and of other anti-anaemia factors, including those concerned in the prevention of tropical macrocytic anaemias.

I fully agree with Professor Peters that there are no doubt many other vitamins still waiting to be added to the list of the round dozen or so of known "B" vitamins enumerated in Table 2. There are more things in heaven and earth than are yet dreamt of in our chemical philosophy!

### *Vitamin Action*

In considering the mode of action of the B vitamins, one of the most salient points emerging from Dr. Quastel's (1946) paper is the variety of reactions which vitamin B<sub>1</sub> can catalyse. Pyruvic acid can undergo at least four types of transformation under the influence of vitamin B<sub>1</sub>, namely, decarboxylation, oxidation, degradation and condensation. Apart from pyruvic acid, various other related substances including certain aliphatic acids and related keto-acids, not to mention glucose and ethyl alcohol, also can act as the substrate. In an endeavour to introduce some measure of unity into this puzzle, one is tempted to speculate whether decarboxylation at some stage may not be the key to all of these reactions, although, admittedly, this theory may well prove to be an over-simplification in some respects.

In the case of riboflavin and nicotinic acid, on the other hand, there is in fact a unifying point, in that both these substances function as hydrogen carriers by virtue of a nitrogen atom which can undergo a change in its valency. These vitamins are concerned as successive links in a chain of hydrogen carriers, in the same way that cytochrome is the carrier of

oxygen. It will be admitted that this picture provides an intellectually satisfying concept, more so indeed than is the case with vitamin B<sub>1</sub> as far as its mode of action is at present understood. But, as I have said, I suspect that there may still be lacking some essential clue relating to the intimate function of vitamin B<sub>1</sub> as a co-enzyme for so many apparently diverse reactions.

Dr. Gale (1946) has emphasized the important point that vitamin B<sub>6</sub> in the form of pyridoxal is concerned in the decarboxylation of amino-acids; its possible role in transaminations also has been pointed out. All that can be said of biotin, pantothenic acid and choline is that a start has been made, in recent months almost, in exploring their biochemical mode of action; no doubt knowledge will continue to grow at a rapidly accelerating rate for some time to come until the principal gaps are filled in.

### *The Newer Vitamins*

All these recent advances are the more remarkable when it is recalled that only about 30 years ago many scientists were still disbelieving the new "vitamine theory" as it then was. Indeed it is less than 20 years since the chemical identity of a vitamin was established for the first time, when ergosterol was identified as a precursor of vitamin D. Within the B group, the development is even more recent, since it is only about ten years since the first of the B<sub>2</sub> vitamins, riboflavin, was identified, and now almost a dozen of them have been clearly characterized.

To turn next to the newest of the B<sub>2</sub> vitamins discussed by Mr. Robinson (1946), namely, folic acid and the "grass juice complex", including vitamins B<sub>c</sub> and M, xanthopterin and the growth factors for *Lactobacillus casei* and *Streptococcus faecalis* R, it is evident that there is still some uncertainty concerning their relationship one to another, and their respective properties and functions. The complicated picture is, however, showing distinct signs of beginning to be clarified.

### *New Methods of Study*

An important recent development which has been barely touched on today is the possibility of a new technique in producing vitamin deficiencies experimentally by administration of the appropriate anti-vitamins. To take one example, 3-acetyl-pyridine will produce symptoms of nicotinic acid deficiency when administered to mice (Woolley, 1945).

Yet another technique for studying vitamin action, for which I think a promising future can be predicted, is by the use of labelled elements.

A third, and equally new departure is by the use of bacteriostatic agents to prevent the symbiotic synthesis of vitamins by the micro-organisms of the alimentary tract. In this way vitamin deficiencies may be precipitated which would not otherwise occur under a given set of conditions, or in a particular species.

### *"Major" and "Minor" Vitamins*

Dr. Bergel (1946) raised the interesting point whether some vitamins are more essential and others less essential, whether some should be considered as "minor" and others as "major". One possible explanation may be that, in some instances at least, the "minor" vitamins are what Dr. Kodicek and I in our opening paper (p. 81) ventured to term "secondary

accessories", that is they are needed by the intestinal microflora in order that the latter may flourish and, in doing so, synthesize other vitamins needed by the host. This theme will be mentioned again later.

#### *Universality of Vitamin Action*

From Dr. Knight's (1946) review on micro-organisms a large measure of unity begins to be apparent in the conception of vitamin action, since the most diverse types of cells come into the picture in a relatively orderly manner, not those only of mammals, but of insects, plant roots, moulds and bacteria. No mention has been made today of the relation of vitamins to plant hormones, or "auxins". Yet some of these are certainly the same substances which have been discussed here as vitamins.

#### *Fermentation and Nutrition*

Drs. Platt and Webb (1946) drew attention to the significance of fermentation in relation to vitamin studies. Its importance to human beings is only beginning to be realized. As was made clear, vitamins may be synthesized by fermentation, in the food before it is ingested, or in the gastro-intestinal canal afterwards. It appears that the amount of certain vitamins thus synthesized may be of real significance, and may thus have a possible, practical importance in the prevention of vitamin deficiency. Here is an almost new field open for study. One aspect of the problem which seems to have been somewhat overlooked is as to how far the vitamins synthesized by micro-organisms in the intestine are capable of being absorbed by the animal organism. It should not be forgotten that if the vitamins so synthesized are inside the bacterial cells, little or no absorption will take place, in the absence of extensive autolysis.

Quite surprisingly, Dr. Platt has shown us how this phenomenon of fermentation may invade even the territory of medical jurisprudence. It appears, from what he said, that there may occur a change of bread to wine *in vivo*. If carbohydrates can serve in this way as the unexpected precursor of large amounts of alcohol appearing in the blood, it may be necessary to reconsider whether after all "beer is best".

#### *The B Vitamins and Human Nutrition*

To turn finally to more clinical considerations, the greatest gaps in our knowledge seem to be those relating to the human requirement of some of these newer vitamins, and the uncertainty whether their absence causes deficiency diseases in man. It is interesting to recall that the two B vitamins which are most obviously concerned in the prevention of human deficiency diseases, aneurin and nicotinamide, were first made known through observations on man of beriberi and pellagra. Experiments on beriberi and pellagra in animals followed only later. Now the position is reversed; the newest B vitamins are being studied intensively in animals, and perhaps their relation to human disease will be demonstrated later.

There seem to be at least four possible approaches to this question of the human needs for these newer vitamins and to the problem of the so-called "minor" as distinct from the "major" vitamins:

(1) It is possible that the corresponding deficiency disease occurs naturally in man but has not yet been recognized. In this case some of

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the obscure dermatoses and other conditions seen, for example, in Africa and elsewhere may yet claim attention as vitamin deficiencies.

(2) Some of these vitamins may be only "secondary accessories", needed by the intestinal microflora for the synthesis of other vitamins. In other circumstances they may be themselves synthesized by these micro-organisms.

(3) If this is true, one way of studying such deficiencies in man would be to provide the appropriate anti-vitamins in the diet. Certain anti-vitamins, as is already known, occur naturally in some food products; such is the factor in fish which antagonizes aneurin.

(4) A second method of attack, as already indicated, is by the administration of bacteriostatic drugs notably those of the sulphonamide class.

By the use of the two last mentioned new methods of study in human subjects it is not unlikely that new vitamin deficiencies may come to light.

#### *The B Vitamins and Anaemia*

The main conclusion to be drawn from Miss Wills' (1946) account of her work is that the dietary factor preventive of tropical macrocytic anaemias is not identical with Castle's "extrinsic factor". This is in keeping with the findings reached by Castle himself in his most recent paper (Watson and Castle, 1945). Dr. Wills' work throws some doubt on the very existence even of the extrinsic factor, although I think she was rightly cautious on that point. At any rate, we have as yet no idea of its chemical nature.

#### *General Conclusions*

Looking over what has been said, I would have no hesitation in concluding, as a last general comment, that the most obvious and striking gap at present is our ignorance of whether some seven or eight of the newer B vitamins are needed by man and, if so, what the symptoms are which appear in their absence, and what is their biochemical mode of action.

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