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## Discussion

Dr. F. Bergel (Roche Products, Ltd., Broadwater Road, Welwyn Garden City, Herts.), opener: It is proposed to touch only on one point, namely, the *specificity* of chemical constitution and activity of the B group of vitamins, especially for mammals. I should like to draw attention to differences which exist in this respect between the major B vitamins, vitamin $B_1$ and $B_2$ and nicotinic acid, and the minor ones such as pantothenic acid and pyridoxin, which might explain why only three vitamin B deficiency diseases are so far known in man. This division into major and minor vitamins was first suggested by R. J. Williams (1942) and R. R. Williams (1942).

Vitamin $B_1$ is chemically highly specific. In a recent paper Buchman and Richardson (1945) showed that any change of the hydroxyethyl group in the thiazole ring went hand in hand with loss of activity. Bergel and Todd (1937) demonstrated several years ago that, in addition to this group, the 4-amino-group in the pyrimidine nucleus, and the 2-hydrogen in the thiazole, and the methylene group connecting the two rings, are essential.

A similar state of affairs is found with the $B_2$ vitamins. Karrer (1939) showed that even slight alterations in the molecular structure of riboflavin caused loss of vitamin activity. It seems that the 6:7-methyl groups, the ribityl side chain, and a free hydrogen in the alloxan part, are essential.

Even the simple molecule of nicotinic acid appears to possess specificity at least in the 3-carboxyl group and the nitrogen containing ring system. A second carboxyl group as in quinolinic acid and the change of the

pyridine, to the closely related pyrazine, ring system is not accompanied by complete loss of activity according to Bills, McDonald and Spies (1939) and Spies, Walker and Woods (1939), but trigonelline is inactive.

On the other hand, the minor B vitamins such as pantothenic acid (vitamin $B_3$) and pyridoxin (vitamin $B_6$) possess a lower order of specificity. For instance, Jürgens and Pfaltz (1944) and Burlet (1944) showed that pantothenic alcohol appears to be twice as active for mammals as the acid, whereas its microbiological activity for *Lactobacillus casei* is nil. I am informed by Miss A. M. Copping and Dr. J. S. D. Bacon of the Lister Institute that they found a complicated derivative of pantothenic aldehyde, prepared by the Research Department of Roche Products, Ltd., to be still distinctly active for rats, possessing about half the potency of the *d*-acid. If we consider in addition the potency reported for the $\beta$-hydroxymethyl analogue, we come to the conclusion that the pantothenic series shows an order of specificity lower than that of the major B vitamins.

Several speakers today referred to various pyridoxin compounds such as pyridoxal, pyridoxamine, 4-pyridoxlactone and pyridoxin itself as being active in spite of their difference in constitution.

From the above considerations the following speculation is submitted: The greater chemico-biological specificity of the major B vitamins is a contributory factor to their being connected with the classical deficiency diseases, in addition, of course, to the facts that they are less abundant, more essential as co-enzymes and less stable, than the minor B vitamins.

In contrast, the fact that most of the minor B vitamins have so far not been linked up with clinical symptoms of deficiency diseases in man, can be explained by their being less specific and submitting to certain alterations in their molecule without losing their potency. This still leaves the possibility, as mentioned by Dr. Harris, that they are only secondary accessories and act mainly by stimulating the intestinal microflora of those organisms which need the major B vitamins first and foremost.

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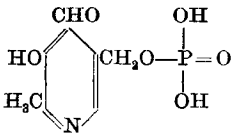
Mr. A. L. Bacharach (Glaxo Laboratories, Ltd., Greenford, Middlesex): The principle suggested by Dr. Knight, that the behaviour of possible intermediates in the biosynthesis of essential metabolites could throw light on the path of the actual biosynthesis, presumably works both ways. In that event the failure of the thiazole pyrophosphate, for example, to replace co-carboxylase as nutrient for a particular organism would afford presumptive evidence that the biogenesis of the latter did not involve synthesis from the former.

Dr. Bergel has pointed out that replacement of the ribitol residue in riboflavin by other sugar alcohol residues destroys its biological activity. May this not be because, for steric or other reasons, other residues cannot undergo the phosphorylation essential for prosthetic association with the specific protein apo-enzymes? It might be of interest to phosphorylate such riboflavin analogues *in vitro* and test the biological activity of the resulting phosphate.

Dr. E. F. Gale (Biochemical Laboratory, Cambridge): A group of workers in Cambridge has been studying the production of amines by bacteria. Up to the present, six enzymes have been isolated in a cell-free and partially purified state; each of these enzymes is specific for the decarboxylation of a single amino-acid to the corresponding amine. Of the six enzymes, four have been split into specific protein portions and a common co-enzyme portion called "co-decarboxylase". These four enzymes are all highly sensitive to inhibitors which act as CO-fixatives. The co-enzyme has a wide distribution in nature being found in all living cells tested, whether bacteria, yeasts, or animal or plant tissues (Baddiley and Gale, 1945; Epps, 1944, 1945; Gale, 1940, 1, 2, 3, 1941; Gale and Epps, 1944, 1, 2; Taylor and Gale, 1945). While a co-decarboxylase concentrate was being worked up from yeast, and its properties studied, Bellamy and Gunsalus (1944) announced that streptococci which normally produced tyrosine decarboxylase were unable to produce an active enzyme when grown in a medium deficient in pyridoxin. After the demonstration by Snell (1944) that the pseudopyridoxin factor in tissues consists of pyridoxal and pyridoxamine, Gunsalus and Bellamy (1944) and Gunsalus, Bellamy and Umbreit (1944), found that the factor missing in their pyridoxin deficient organisms was pyridoxal, as the inactive organisms could be activated towards tyrosine by its addition. In work based on this observation, pyridoxal has been synthesized and tested for activity with the apo-enzymes of the four co-decarboxylase enzymes, the decarboxylases of lysine, arginine, ornithine and tyrosine. Pyridoxal itself is not active as co-decarboxylase but an active substance is produced if pyridoxal is treated with thionyl chloride followed by silver dihydrogen phosphate. The preparation, which is presumably pyridoxal phosphate with the formula shown below, is able to combine with and activate the four apo-enzymes to the same extent as the co-decarboxylase prepared from yeast.




The curves obtained from a study of the rate of decarboxylation of lysine and tyrosine by the lysine decarboxylase and tyrosine decarboxylase, respectively, in the presence of increasing amounts of pyridoxal phosphate are typical of such curves for apo-enzyme-co-enzyme combinations. The solubilities of the active material and of its salts are the same as those of co-decarboxylase and the synthetic material has the same unusual properties, being stable to heating for 4 hours at 100° C. in *N* NaOH but being rapidly destroyed by boiling with *N*/10 $H_2SO_4$. It is not

possible to establish the identity of co-decarboxylase and pyridoxal phosphate until these substances have both been obtained in a pure condition but the evidence strongly suggests that the co-enzyme of certain bacterial amino-acid decarboxylases is a phosphorylated derivative of pyridoxal.

The co-decarboxylase function of pyridoxal phosphate is shown in the following table.

| Apo-enzyme | $CO_2$ liberated from substrate in 5 minutes | | |
|---|---|---|---|
| | Alone µl. | + Co-decarboxylase µl. | + Pyridoxal phosphate µl. |
| *l*(−) Tyrosine decarboxylase .. .. | 2 | 80 | 81 |
| *l*(+) Lysine decarboxylase .. .. | 15 | 110 | 116 |
| *l*(+) Arginine decarboxylase .. .. | 15 | 70 | 72 |
| *l*(+) Ornithine decarboxylase .. | 22 | 89 | 91 |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Miss A. M. Copping (Lister Institute, Chelsea Bridge Road, London, S.W.1): I should like to ask Mr. Robinson whether it has been proved that pyridoxal is biologically active when tested on rats.

Dr. H. S. Baar (Children's Hospital, Birmingham): May I ask Mr. Robinson whether the co-operation of pyridoxin and folic acid applies only to the anaemia of chicks or also to the microcytic anaemia of pigs. Recent work appears to be conclusive in showing that pyridoxin is the sole factor responsible (Cartwright, Wintrobe and Humphreys, 1944).


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Dr. T. S. Work (National Institute for Medical Research, Mount Vernon, London, N.W.3): May not the difference in specificity of the two groups of B vitamins which Dr. Bergel has designated as the major and the minor vitamins be due to the different function of these two groups, the major vitamins being necessary for the functioning of the

higher forms of life which are exacting in their requirements, the minor vitamins being necessary only for the proper balance of the intestinal flora?

Dr. F. Bergel: This may well be the case. As I have mentioned before, the division of the members of the vitamin B complex into major and minor components was first suggested by the brothers Williams.

Dr. F. W. Chattaway (School of Medicine, Leeds): It is an over-simplification to suggest that vitamin $B_c$, folic acid and the *Lactobacillus casei* factor are one and the same. The following evidence supports the view that these factors are different: (1) Keresztesy, Rickes and Stokes (1943) have described the preparation from liver of a factor active for *Streptococcus lactis* R but almost inactive for *L. casei*; (2) Chattaway, Dolby, Happold, McMillan and Waters have shown in unpublished work that a folic acid concentrate is inactive for *L. casei* unless certain unknown factors are added, while Chattaway, Dolby and Happold (1944) found that a liver preparation active for *L. casei* could be split into at least two components, one of which was obtained in a form having no stimulatory effect on *S. lactis* R; (3) Williams (1944) has recently referred to the "folic acids" and apparently believes that these are multiple factors.

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Mr. F. A. Robinson gave the following replies:

*To Miss Copping*: Both pyridoxal and pyridoxamine, or a mixture of the two previously known as pseudo-pyridoxin, are as active as pyridoxin when tested on rats. Unfortunately they are far more potent than pyridoxin in stimulating the growth of certain micro-organisms, particularly *Lactobacilli*, and the results of microbiological tests of the vitamin $B_6$ content of foodstuffs may, therefore, be considerably higher than those of rat tests.

*To Dr. Baar*: I agree with Dr. Baar that from published work pyridoxin appears to be the only factor the absence of which causes anaemia in pigs. On the other hand it is highly probable that the diet used in the experiments mentioned by Dr. Baar contained folic acid and, until further tests are carried out with a diet known to be free from folic acid, no valid conclusions can be drawn as to the effect of folic acid deficiency on pigs.

## *Afternoon Session:* Chairman, Dr. L. J. HARRIS

## Fermentation and Human Nutrition

Dr. B. S. Platt and Dr. R. A. Webb (Medical Research Council Human Nutrition Research Unit, National Hospital, Queen Square, London, W.C.1)

Fermentation may, in various ways, make a contribution to human nutrition, the full significance of which has scarcely begun to be appreciated. Foodstuffs may be fermented before they are eaten, and again