REVIEW ARTICLE

FOLIC ACID, VITAMIN B_{12} AND ANÆMIA

II—MICROBIOLOGICAL ASPECTS

III—FOLIC ACID AND VITAMIN B_{12} IN MEGALOBLASTIC ANÆMIA

II—MICROBIOLOGICAL ASPECTS*

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Studies with microorganisms† have made a considerable contribution to research and development work in this field from three aspects :—(*a*) the quantitative estimation of the two B-group vitamins involved, (*b*) the production of the two substances in quantity sufficient for fundamental research on their chemical properties and possibly even bulk production pending chemical methods, and (*c*) fundamental studies on their function in cell metabolism which may eventually throw light on the basic biochemical lesions in anæmia. All three of these applications spring from the fact that comparative studies of the nutrition of organisms of widely different types (mammals, birds, insects, protozoa, fungi, bacteria) show a common requirement for substances of the vitamin B group ; it is likely therefore that these substances play their part in basic cell reactions which are common to all cells. A given organism may not have an essential nutritional requirement for a certain factor but this normally means that it is able to synthesise it for itself—the requirement in metabolism still exists. In those cases in which it is known, the function in metabolism of members of the vitamin B group has proved to be that of coenzyme or prosthetic group of enzymes concerned in key cell reactions. This has not been demonstrated with folic acid and B_{12} but it may form a useful working hypothesis.

Microbiological Assay‡

Since several microorganisms require the presence of either folic acid or vitamin B_{12} in the growth medium, and since there is a quantitative relationship between dosage and growth-response, it has been possible to estimate these substances in this way ; it must be stated however that these two microbiological assays (especially that of B_{12}) have proved more difficult in practice than those for other substances. Thus the organisms (*Lactobacilli*) most commonly used in the assay of vitamin B_{12} are micro-aerophilic whilst the factor appears not to be required for purely anærobic growth. The quantitative response may therefore depend on fine control of the degree of aeration and also on the quantity of reducing agents present.

The advantages of a microbiological assay method are mainly (*a*) that the criterion is a simple one (amount of growth or some dependent

* Based on a paper read at the British Association for the Advancement of Science Annual Meeting, Newcastle, 1949.

† See references 1, 2, and 3.

‡ See references 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15.

function such as acid production), (*b*) that results may be obtained rapidly, often in less than 24 hours, and (*c*) that cells relatively deficient in factor for use as inoculum may readily be obtained by growth on partially deficient media. There are, of course, also disadvantages. In the case of folic acid, for example, conjugated forms exist in natural materials which are available sources to the animal (which has enzymes for liberating the free factor) but not to the test microorganism. With vitamin B_{12} it is known that other red substances exist in liver concentrates which support the growth of the assay organism but whose possible activity in pernicious anæmia is not yet known. It is necessary with both factors to bear in mind the possibility that the microbe may respond to unknown precursors of the vitamin (*i.e.*, be able to complete the synthesis) whilst the animal cannot; the reverse may also be true. Such difficulties have been encountered with other vitamins.

The fact that rapid microbiological assays were available certainly considerably aided the purification, isolation and synthesis of pteroylglutamic acid (synthetic "folic acid") by the Lederle workers; it also played an important part in the isolation of crystalline B_{12} by one of the two teams (Merck) who were engaged on the problem.

Microorganisms as Sources of Folic Acid and Vitamin B_{12}§

It was mentioned above that when organisms do not require added growth factors they are often found to synthesise them. Sometimes the amount synthesised appears to be in excess of cell requirement and the organism may prove to be a comparatively rich source of the factor. The chemical degradation studies which led to the synthesis of pteroylglutamic acid were carried out with a crystalline folic acid conjugate isolated from the culture fluid of an unspecified *Corynebacterium*. Again, there is a recent report of the isolation of crystalline vitamin B_{12} from a strain of *Streptomyces griseus*; in view of the small amount present in liver and the already undoubted complexity of chemical structure, this might well be an important industrial source.

Function in Metabolism¶

Although immediate practical considerations may be met by the isolation and sufficient purification of factors such as folic acid and vitamin B_{12}, it is clear that for a full understanding of the disorders which follow their absence it is necessary to know their precise function in cell metabolism; the observed clinical symptoms may not be primary. Although this problem is not yet solved for the two substances under consideration, work on microorganisms is providing valuable initial clues. Such work has been of considerable importance in working out the function of several other members of the vitamin B group. Briefly, the present evidence suggests that both folic acid and vitamin B_{12} may be involved in some way in the biosynthesis of the desoxyribonucleic acids which are key constituents of the nuclear material of cells.

Now we know comparatively little about the synthetic mechanisms

§ See references 6, 8 and 16.
¶ See references 2, 3, 5, 7, 17, 18, 19, 20, 21, 22, 23, 24 and 25.

of the cell and only in rather few cases has it been possible to obtain such synthesis in non-viable systems. But even in the more complicated case in which growth occurs it is possible to obtain valuable information from studies of the nutrition of microorganisms. Thus if it is found that a given growth factor may be effectively replaced by larger quantities of another substance of quite different chemical type it suggests that the former is required in some catalytic system involved in the synthesis of the latter. Similarly when growth is specifically inhibited in a competitive manner by a substance which is chemically analogous to the growth factor, growth may be found to be restored by a substance of different chemical type and the same very tentative conclusions may be drawn.

Evidence of this sort indicates a function for folic acid (probably in the form of a higher compound, folinic acid, [26,27]) in the synthesis of pyrimidine, thymine (or perhaps its riboside, thymidine) and the purine bases. This view is supported by the finding that cells of *Lactobacillus casei* grown in medium partially deficient in folic acid contain only half the normal amount of desoxyribonucleic acid.

p-Aminobenzoic acid, an essential growth factor which is a moiety of the folic acid molecule, appears also to be concerned in the synthesis of certain amino-acids but it is not yet certain that this function occurs *via* the formation of folic acid.

In the case of the *Lactobacilli* which need vitamin B_{12} it has been found that the requirement can be met by various desoxyribosides, including thymidine. It is possible therefore that at least one function of vitamin B_{12} is in the synthesis of this type of structure.

It is unlikely however that vitamin B_{12} and folic acid are involved at different stages in the synthesis of some coenzyme-like factor required for desoxyriboside formation : *Lb. leichmannii*, for example, requires for growth both factors ; neither can replace the other. It therefore seems more likely on the present evidence that they are involved at separate stages of the synthetic pathway which eventually leads to desoxyribosides. Thus with *Lb. Leichmannii* the requirement for both factors can be met (though not optimally) by thymidine alone. The latter completely replaces vitamin B_{12}, but gives growth without folic acid or without both only after a lag. Finally there are organisms (*e.g.*, *Lb. bifidus*) whose need for thymidine cannot be replaced by vitamin B_{12}, which would indicate a failure in synthesis at a later stage.

It must be emphasised strongly that such inter-relationships are at present not proved and must be considered only as working hypotheses ; they may serve as a guide to further experiments which will yield further facts.

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III—FOLIC ACID AND VITAMIN B_{12} in MEGALOBLASTIC ANÆMIA*

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This is a brief survey of work done at the Royal Victoria Infirmary, Newcastle upon Tyne, in co-operation with Dr. R. B. Thompson, who performed marrow cultures, Dr. W. Walker, who followed the survival of transfused erythrocytes, and Dr. L. W. Carstairs, who intubated the small intestine. Vitamin B_{12} and vitamin B_{12C} were supplied by Dr. E. Lester Smith, and Dr. W. F. J. Cuthbertson, also of Glaxo Laboratories, was responsible for microbiological assays. A fuller account of the various investigations is being published elsewhere.

Two groups of megaloblastic anæmia will be considered:—(a) Addisonian pernicious anæmia in which there is gastric atrophy and permanent loss of Castle's intrinsic factor; (b) Non-Addisonian megaloblastic anæmias associated with pregnancy or with intestinal disorders such as stenosis or the sprue syndrome.

Parenteral Administration of Vitamin B_{12} in Pernicious Anæmia

Our findings amplify earlier reports on the effect of vitamin B_{12} given parenterally in pernicious anæmia (West[1], Ungley[2,3,4], Hall and Campbell[5], Spies, Stone, Kartus and Aramburu[6], Berk, Denny-Brown, Finland and Castle[7], Bethell, Meyers and Neligh[8], Spies, Suarez, Garcia Lopez, Milanes, Stone, Lopez Toca, Aramburu and Kartus[9], West and Reisner[10]).

Vitamin B_{12} is effective in Addisonian pernicious anæmia but in only

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