There is evidently scope for speculation on the possible contribution made to human nutrition by micro-organisms, particularly those proliferating in the gut. The interrelationships of bacterial activities and the nature of the diet are obviously complex and we may be only at the beginning stage of what may prove ultimately to be a very important aspect of nutrition. An illustration of the importance of these changes is found in a recent publication of Krehl and Elvehjem (1945), who concluded that intestinal synthesis of folic acid was a factor influencing the ultimate recovery, when given nicotinic acid, of dogs which had been deprived of it. Another interesting example is furnished by a recent claim of Milhorat and Bartels (1945) that inability to synthesize a complex of vitamin E with inositol in the human gut is responsible for the development of muscular dystrophy. We can agree with Mitchell and Hamilton (1929) in a way which, perhaps, they did not fully appreciate at the time when they wrote: "It is a matter of first importance in practical nutrition to determine the effect of different foods and classes of foods on the intestinal flora".

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The Vitamin B_2 Complex and Anaemia

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It is not proposed to deal in detail with all the different types of anaemia that have been produced by a deficiency of the components of the vitamin B_2 complex in man and experimental animals; a brief summary will indicate the field that has been covered. A deficiency of riboflavin produces a microcytic, hypochromic anaemia in dogs (Spector, Maas, Michael, Elvehjem and Hart, 1943), and in monkeys (Waisman, 1944) but doubtful and contradictory results have been obtained in human subjects. A pyridoxin deficiency produces a microcytic, hypochromic anaemia in pigs which is not haemolytic in origin (Cartwright, Wintrobe and Humphreys, 1944). A vitamin B_c deficiency produces in chicks a macrocytic anaemia which may be either hyperchromic (Hogan and Parrott, 1940), or hypochromic (Campbell, Brown and Emmett, 1944), and a deficiency of the folic acid fractions gives varying results. The effects of diets deficient in nicotinic acid will be discussed later.

The aspect of the problem of the relationship of the vitamin B_2 complex to anaemia to be dealt with in this paper is that concerned with nutritional macrocytic anaemia, its relation to Addisonian pernicious anaemia and the much disputed question of its aetiology. It is commonly stated in text books of haematology and in current teaching that nutritional macrocytic anaemia is due to a deficiency of Castle's extrinsic factor and that the blood picture, which is indistinguishable from that of Addisonian pernicious anaemia, is due to the resulting deficiency of the liver principle curative in true pernicious anaemia. This statement is based on Castle's work which postulates two factors, the first an intrinsic factor, present in normal human gastric juice but missing in that of pernicious anaemia subjects, which, though not yet isolated, has many of the characteristics of an enzyme, and the second, an extrinsic factor, also unidentified, associated in its distribution with protein of good biological value and with the vitamin B_2 complex. These two factors are thought to react in the stomach or small intestine, the resulting principle, also unidentified, being absorbed in the intestine, and stored as the anti-pernicious anaemia factor in the liver. If, therefore, nutritional macrocytic anaemia is due to a deficiency of the extrinsic factor, and if the explanation of the formation of the liver principle given above is correct, then nutritional macrocytic anaemia should be cured by liver extracts, however given, that are known to be potent in pernicious anaemia. Broadly speaking, in uncomplicated cases of nutritional macrocytic anaemia, this is not the case and another possible explanation of the aetiology of this anaemia will be put forward below.

Before, however, the work on which this alternative explanation is based is dealt with in detail, it is desirable to consider the relationship of the vitamin B_2 complex to cell enzyme systems. It is possible that all members of this complex are vital parts of enzyme systems essential for the conversion of food into energy (Robinson, 1944) and that they are, therefore, essential for cellular metabolism. The lack of any one member may interfere with cellular metabolism in such a manner that any system of cells, for example the actively metabolizing early erythroblasts, may be unable to function normally. This failure of function may produce the same pathological picture, though the essential aetiological factor, the missing part of the enzyme system, may vary from case to case. Stannus (1944) has already discussed the importance of these enzyme systems in relation to deficiency of riboflavin and other allied deficiencies.

The theory that is considered in this paper is that nutritional macrocytic anaemia is due to a deficiency in the diet of a factor or factors, at present unidentified, belonging to the vitamin B_2 complex and that this factor does not react with the intrinsic factor to form the liver principle in the manner usually described; in other words, it is not an essential part of the liver principle though it is necessary for the proper functioning of this principle. Napier (1939) also is of the opinion that the factor operative in nutritional macrocytic anaemia is not identical with Castle's extrinsic factor. The identity of the liver principle and its manner of functioning are unknown, so that it is impossible to correlate its mode of action with that of the factor under discussion, but it is possible that the latter acts as part of the cell enzyme system discussed above. The evidence for these speculative statements is reviewed below.

In the uncomplicated case of nutritional macrocytic anaemia, as seen in Bombay (Wills, 1934) where malaria and pellagra were relatively uncommon diseases, it was found that cases did not respond to purified liver extracts such as Anahaemin (B.D.H.) and Examen (Glaxo) in doses that would have been more than adequate in an uncomplicated case of pernicious anaemia, whereas they did respond to relatively small doses of crude liver extract, such as Campolon, or to large doses of marmite (Wills and Evans, 1938). The liver extracts were given parenterally, marmite by mouth. Comparison of the response shows that in a typical case of Addisonian pernicious anaemia a single dose of 4 ml. of Anahaemin caused an excellent reticulocyte response and a rise of over one million red cells, whereas 2 ml. daily of the same preparation for a period of 12 days was completely inactive in the nutritional anaemia, though 2 ml. of Campolon, given daily for 10 days, produced an excellent response. These findings have been confirmed by many writers, though in Calcutta Napier (1939) has reported good results from large doses of Anahaemin in some of his cases, and Foy and Kondi (1939) report that in Macedonia Anahaemin in very large doses was curative in pregnant women suffering from this anaemia but was completely inactive, as were other liver preparations, even in enormous doses, in men suffering from the same condition. Moore, Vilter, Minnich and Spies (1944), in a series of cases of pellagra with nutritional macrocytic anaemia found that purified liver extracts were very active but consider that until the extrinsic factor and the liver principle have been isolated it is impossible to decide whether these cases have the same aetiology as the Bombay cases.

An examination of the findings mentioned above is of considerable interest. In Macedonia all Foy's cases, as also many of Napier's cases in Calcutta, were suffering from life-long malaria as well as from a macrocytic anaemia; the spleen in all cases was enormously enlarged and the whole reticulo-endothelial system hypertrophied, and it is possible that in such circumstances it is the liver and the liver principle itself that are at fault. But this factor apart, Foy's results are not very convincing, since the doses of Anahaemin were enormous and might possibly contain, in very small quantities, other factors than the liver principle active in pernicious anaemia, since the purity of Anahaemin is only relative. Foy's case reports show poor responses, other than the rise in the reticulocytes, except in one case which, for 10 days before starting treatment with Anahaemin, had received a liver extract, free of the pernicious anaemia principle but a rich source of most of the vitamin B_2 components. No explanation is given of his failure with male patients.

The pellagra cases of Moore *et al.* (1944) are different; the response to purified liver extract was dramatic, but, as the authors point out, there was a deficiency of intrinsic factor as well as poor absorption in many of the cases, and the reported cases had been treated by high protein diets before receiving the liver extract and had in some cases shown a response to this preliminary treatment. To summarize, the clinical evidence shows that uncomplicated cases of macrocytic anaemia in which it can be assumed that gastric function and intestinal absorption are unimpaired and the reticulo-endothelial system undamaged, fail to respond to the highly purified liver extracts unless these are given in such enormous doses that it is probable that other factors

present as impurities become operative, since to date the liver principle has not been isolated and all extracts contain impurities. The uncomplicated cases, and many of the complicated too, respond to relatively small doses of crude liver extracts, both by mouth and parenterally and to autolysed yeast extracts, all good sources of the vitamin B_2 complex.

Experimental work carries the story a step further. It is possible by feeding monkeys on a diet based on one in common use among sufferers from nutritional macrocytic anaemia to produce a similar anaemia which does not respond to purified liver extracts though it reacts immediately to crude liver extracts given orally or parenterally and to yeast extracts

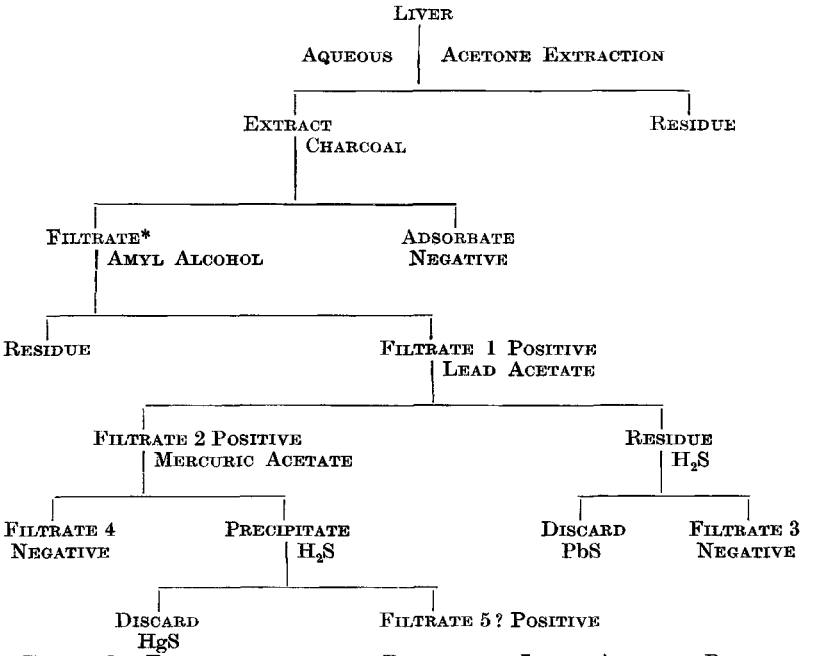

FIGURE 1. FRACTIONATION OF THE PRINCIPLE IN LIVER, ACTIVE IN PREVENTING NUTRITIONAL MACROCYTIC ANAEMIA IN MONKEYS.

* The filtrate from the charcoal extraction was kindly supplied by Glaxo Laboratories, Ltd.

given by mouth and also parenterally, which is very significant for, if the extract acts parenterally, it should not require an intrinsic factor to activate it (Wills, Clutterbuck and Evans, 1939).

An attempt was made, first with the assistance of the late Dr. Clutterbuck and then with Wing Commander Macrae of the Lister Institute, to determine the nature of the curative factor in these yeast and liver preparations. Vitamin B_1 was excluded from the start as it had no curative effect, and its inclusion in the diet did not prevent the development of the anaemia. The vitamin B_2 components were tested by exclusion and by tests with those of the pure substances which were available at the time the experiments were made. The method of preparation of some of the extracts used and their activity are shown in Figure 1. The charcoal

extraction would remove the bulk of the nicotinic acid and riboflavin present in the original liver extract and the rest of the riboflavin would be held with the lead acetate precipitation; further this member of the vitamin B_2 group was excluded by a direct trial, 1 mg. being given daily to an anaemic monkey with negative results. Pyridoxin also was inactive as was shown by direct trials, a dose of 1 mg. daily given by injection failing to produce a remission, and by the negative results obtained with the eluate factor (filtrate 3). Pantothenic acid, Macrae's filtrate factor (filtrate 4), also was entirely inactive. Nicotinic acid was largely removed by the treatment of the liver extract by charcoal, and the lead acetate filtrate which was active contained only a small proportion of the nicotinic acid present in the original liver. This small residue passed into the mercuric acetate filtrate which was inactive. Nicotinic acid is, therefore, excluded as the curative factor in the experimental anaemia.* Finally, when the outbreak of war brought the work to an end, a trial was being made of the insoluble fraction obtained after treatment of the lead acetate filtrate with mercuric acetate (filtrate 5). This trial gave inconclusive but suggestive results in the two monkeys tested.

Dogs as well as monkeys develop a macrocytic anaemia on diets deficient in the vitamin B_2 complex (Miller and Rhoads, 1933). Handler and Featherston (1943), using different blacktongue producing diets, deficient in nicotinic acid, produced in dogs, which had been treated with sodium chloride for the relief of their blacktongue symptoms, a macrocytic hyperchromic anaemia; these dogs had normal gastric secretion. It was shown that the anaemia was not due to inanition or iron deficiency and that it did not respond to liver extract but did respond to nicotinic acid. It is pointed out that in the macrocytic anaemia of pellagra nicotinic acid is generally given as well as liver extracts. It is argued from these results that, since the only known function of nicotinic acid is its synthesis to the pyridine nucleotides and its subsequent role in cell metabolism, it is possible that the rapidly maturing erythroblasts, with their high metabolic activity, suffer from a relative lack of co-zymase in the earliest stages and so fail to develop, a macrocytic anaemia resulting as a consequence.

In the monkey anaemia another component of the vitamin B_2 complex is probably at fault. A few cases of human, nutritional, macrocytic anaemia conditioned by a deficiency of nicotinic acid, in the absence of signs of pellagra, have been reported (Ahmad, 1944; Fuchs and Wisselwick, 1939), but such cases are rare.

Recently Castle, Ross, Davidson, Burchenal, Fox and Ham (1944), using untreated cases of pernicious anaemia, have shown that crude casein is an active source of extrinsic factor but that purified casein, supplemented by all the known fractions of the vitamin B_2 complex, is completely inactive. These authors, however, still regard the extrinsic factor as a thermostable component of the vitamin B_2 complex, as yet unidentified.

* I am indebted to Mr. Emery of Glaxo Laboratories, Ltd., for these results with nicotinic acid.

Summary

Clinical and experimental work suggests that nutritional macrocytic anaemia is not due to a lack of Castle's extrinsic factor, if by this is meant a substance that reacts with the intrinsic factor to form the liver principle. In uncomplicated cases in men and monkeys, which have received no previous treatment with fractions of the vitamin B_2 complex, highly purified liver extracts are inactive. The deficiency causing the anaemia is in some component of the vitamin B_2 complex, at present unidentified. This factor is active when given parenterally and does not, therefore, need the presence of normal gastric juice for its proper functioning. It is suggested that the condition is due to a failure of the liver principle to promote the maturation of the erythroblasts owing to a failure in cellular metabolism due to the absence of one or other members of the vitamin B_2 complex which act as essential components of cellular enzyme systems.

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Discussion

Dr. F. Prescott (The Wellcome Research Institution, 183–193 Euston Road, London, N.W.1) opener: In connexion with the biosynthesis of the B vitamins in the human gut, to which Dr. Platt referred in his paper, there is a question which until recently has been overlooked. If it is assumed that the intestinal flora of man can synthesize vitamin B_1, riboflavin and nicotinic acid, are these vitamins available to the host? A recent paper suggests that they are not. Alexander and Landwehr (1945) of Harvard have shown that the vitamin B_1 in human faeces is largely within the bodies of the bacteria and not free in the lumen of the gut. It may, therefore, not be available to the host. Furthermore, most of the vitamin B_1 is present as co-carboxylase, which cannot be absorbed as such and must first be split by dephosphorylating enzymes. It is doubtful if these are present in the large intestine. Najjar and Holt (1943) in their paper on the biosynthesis of vitamin B_1 in man assumed that the vitamin synthesized by the intestinal bacteria was available to the host