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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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some types of non-Addisonian megaloblastic anæmia. The response of some 50 patients with pernicious anæmia to the injection of vitamin B_{12} have been analysed. The earliest change was a maturation of megaloblasts in the bone marrow. Clinical improvement followed and the erythrocytes, platelets and leucocytes increased to normal levels. For constructing a dosage response curve reticulocyte responses proved unreliable and we used the increase of erythrocytes in 15 days. Doses were spaced logarithmically from 1·25 to 160 μg. Ten μg. was enough, on average, to give a response satisfactory according to the "average" standard of Della Vida and Dyke[11], but larger doses gave greater responses which were roughly proportional to the logarithm of the dose in the range 5 to 80 μg. (Ungley[12]). Further tests at high and low levels of dosage are necessary to complete the dose response curve which will probably be sigmoid in form, rising gradually then steeply from the threshold dose and flattening out to a horizontal level when supramaximal doses are reached.

In 21 patients followed for 6 to 18 months neurological manifestations if present were improved or unchanged, and fresh symptoms did not develop. More direct evidence of the efficacy of vitamin B_{12} against the neurological manifestations of pernicious anæmia was obtained by treating 8 established cases of subacute combined degeneration. The usual dosage was 40 μg. a week. Using a quantitative method of neurological assessment, significant improvement was demonstrated in each case. The improvement continued for about 6 months, after which a state of arrest was maintained. The degree of residual neurological defect was proportional to the duration of difficulty in walking. Comparison with 44 cases treated before the war showed that vitamin B_{12} was as effective as parenteral liver extract, crude or refined, in the treatment of subacute combined degeneration of the cord (Ungley[13]).

These findings are in agreement with other published work which has already been reviewed (Ungley[12,13]).

Vitamin B_{12} in Non-Addisonian Megaloblastic Anæmias

In 5 patients with megaloblastic anæmia of pregnancy or the puerperium single injections of 63 to 80 μg. of vitamin B_{12} had no effect (apart from a slight reticulocytosis in one case), whereas subsequently all patients responded to folic acid.

In megaloblastic anæmia with intestinal disorders the results were variable. A patient with intestinal stenosis responded quite well to 80 μg. In a patient with non-tropical sprue vitamin B_{12}, even in repeated large doses, was only partially effective; in this patient folic acid, too, was only partially effective. In a patient suffering from thyrotoxicosis with steatorrhœa, vitamin B_{12} failed completely, although subsequently there was an excellent response to small doses of folic acid.

Factors Related to Vitamin B_{12}

By a combination of microbiological assay and partition chromatography on paper Cuthbertson and Lester Smith[14] have demonstrated that

liver extracts contain at least 4 substances related to vitamin B_{12}:—(a) A fast-moving red component—vitamin B_{12} itself. (b) A slow-moving red component. (c) An unidentified substance ("Band 3"). (d) Thymidine. The slow-moving component, which consists of several compounds, has proved effective in a dose colorimetrically equivalent to 10 μg. of vitamin B_{12}. Thymidine, on the other hand, appears to be without effect, at least in a dose of 48 mg. (Ungley[4]). Since vitamin B_{12} proved just as effective as crude liver extract there is no necessity to postulate a need for multiple factors in pernicious anæmia and subacute combined degeneration.

Absorption of Vitamin B_{12} from the Alimentary Tract

Oral administration of 5 μg. daily was ineffective, whereas the same quantity with 50 ml. of normal gastric juice daily produced a satisfactory response. In another patient an excellent response was obtained by administering 50 μg. with 500 ml. of normal gastric juice in a single dose. In a third patient 40 μg. with only 150 ml. of gastric juice proved inadequate. Filtration of the gastric juice through a Seitz filter led to loss of intrinsic factor activity.

The mechanism whereby normal gastric juice facilitates absorption of vitamin B_{12} or prevents its destruction in the gastrointestinal tract remains obscure. No response followed the application of 5 μg. of vitamin B_{12} daily to the buccal mucosa, although the same quantity given by mouth with 50 ml. of gastric juice gave a good response.

To test the possibility that, even without gastric juice, vitamin B_{12} might be absorbed if protected from contact with the intestinal contents, we isolated a segment of the small intestine between two balloons on a Miller-Abbott tube. After washing to remove intestinal contents 40 μg. of vitamin B_{12} was instilled into this segment. There was no response. Subsequently there was a submaximal response to the same dose of vitamin B_{12} given orally with 150 ml. of normal gastric juice (an inadequate amount), and thereafter maximally to a single injection of 40 μg. The problem of the mechanism of absorption of vitamin B_{12} remains unsolved. A full account of this work will appear shortly[15].

Toxic and Hæmolytic Aspects

Not all the facts can be explained on a simple nutritional basis. Both in true pernicious anæmia and in non-Addisonian megaloblastic anæmias toxic and hæmolytic factors may play a part. Since methæmalbumin may be present in the plasma, some of the hæmolysis must be intravascular. Destruction of poorly-formed red cells is not a sufficient explanation. In collaboration with Dr. W. Walker we have followed the survival of transfused cells from normal donors. In most patients with pernicious anæmia such cells are eliminated at a normal rate, surviving about 120 days. In three cases, however, the transfused cells were rapidly destroyed. A change to a normal rate of elimination occurred after vitamin B_{12} in 2 cases and spontaneously in 1 case. In 3 patients with non-Addisonian megaloblastic anæmia associated with pregnancy or

intestinal disorders excessive hæmoylsis changed to a normal rate of elimination about 2 weeks after giving folic acid. The dramatic change in the rate of destruction after treatment suggests that vitamin B_{12} and folic acid may be concerned in detoxicating or preventing the production of a hæmolytic agent.

My colleague, Dr. R. B. Thompson, confirms the finding of Rusznyák, Löwinger and Lajtha[16] that the maturation of megaloblasts in marrow culture is accelerated by the addition of normal plasma, but inhibited by pernicious anæmia plasma. The greater the concentration of pernicious anæmia plasma the less the megaloblasts mature. This suggests active inhibition rather than mere absence of a maturation factor. Low concentrations of folic acid (1 μg./ml.) added to an inert medium cause rapid maturation of megaloblasts, but pernicious anæmia plasma antagonises this effect. The maturing effect of small amounts of normal plasma is also antagonised by the addition of pernicious anæmia plasma. Larger amounts of folic acid or of normal plasma overcome this antagonism. Cerebrospinal fluid from patients with pernicious anæmia has an effect similar to their plasma, so that the inhibiting factor is probably ultrafiltrable. The action of vitamin B_{12} on maturation of megaloblasts *in vivo* is presumably indirect, for unlike folic acid it fails to accelerate maturation *in vitro*.

Other relevant facts follow. Early lesions in the spinal cord in pernicious anæmia are spotty in distribution and are often related to vessels. They suggest the action of a substance destructive to myelin rather than a simple nutritional deficiency. The urinary excretion of certain phenolic compounds is excessive in relapse and becomes normal in remission. These phenolic substances possibly arise from the incomplete metabolism of tyrosine or from bacterial action on tyrosine in the small intestine.

The metabolism of liver slices from rats deficient in folic acid is deficient unless folic acid is added (Rodney, Swendseid and Swanson[17]) Another potentially toxic substance is indol, a product of the metabolism of tryptophane. Indol given to pigs receiving a diet deficient in vitamin B-complex produces hæmolysis and macrocytic anæmia, a result not observed in normal pigs (Rhoads, Barker and Miller[18]).

For the production of macrocytic anæmia following intestinal stenosis, loops or blind sacs, stagnation of intestinal contents and bacterial infection seem to be essential. In the rats of Watson, Cameron and Witts[19] many weeks elapsed before the animals suddenly became ill and anæmic. My tentative interpretation is that a toxic and hæmolytic factor was produced in the infected contents of the blind sac. During the latent period detoxication occurred through enzymes using folic acid and possibly vitamin B_{12}, stores of which were gradually depleted in the process. When these stores were exhausted detoxication failed, resulting in sudden illness and anæmia. Folic acid restored powers of detoxication and relieved the anæmia.

Something of the same kind may occur in the small intestine of patients with pernicious anæmia as a result of bacterial infection and alteration in food residues due to lack of gastric enzymes.

A tentative hypothesis based on these findings, some of which require confirmation, is that in megaloblastic anæmias, toxic as well as nutritional factors play a part. These are responsible for megaloblastic erythropoiesis, for some of the hæmolysis and possibly for the lesions in the spinal cord. Potentially toxic material, for example indol or a phenolic compound, arises either from bacterial action on protein metabolites in the small intestine or from a defect in intermediary metabolism of some substance such as tyrosine or tryptophane. Detoxication or a return to normal metabolism in which production of toxic material ceases, occurs through the action of enzymes using folic acid and vitamin B_{12}.

Wills Factor

Is there a hæmatopoietic factor other than vitamin B_{12} or folic acid present in whole liver and in yeast? Why should yeast extracts which appear to contain no vitamin B_{12} when tested microbiologically or in animals be effective as a source of Castle's intrinsic factor? Can the effect of yeast extract in non-Addisonian megaloblastic anæmias be explained by their content of folic acid or folic acid conjugates? In a patient with pernicious anæmia of pregnancy an alcoholic extract of yeast was effective in doses which contained less than 40 μg. of folic acid tested both microbiologically and in rats for conjugates. Moreover, the daily excretion of folic acid in the urine during the period of administration of yeast was extremely low—only 1 to 5 μg. per day. There was no secondary reticulocyte response after the subsequent administration of 2·5 mg. of folic acid daily, although the average daily excretion of folic acid in the urine rose to 700 μg. per day. The evidence is against folic acid being the cause of the hæmatopoietic response observed with yeast. Further work is necessary.

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