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Vitamin B_{12} and other Dietary Factors in Megaloblastic Anaemias

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The megaloblastic anaemias to which I refer fall into two groups: pernicious anaemia, in which gastric atrophy and permanent loss of Castle's intrinsic factor leads to deficient absorption of vitamin B_{12}, and non-Addisonian megaloblastic anaemias, e.g. those associated with pregnancy and intestinal disorders where a different mechanism is at work.

Pernicious anaemia

The effects of parenteral administration of vitamin B_{12} in pernicious anaemia have already been described (Ungley, 1949*a*) and I need only recall a few salient points.

Single doses of 10 μg. or more produced, on the average, a maximal reticulocyte response. A better yardstick, however, is the increase of red blood cells in 15 days. Here, 10 μg. produced a response up to the average standard of Della Vida & Dyke (1942), but we found that doubling the dose anywhere in the range from 5 to 80, or even 160 μg., produced a constant increase in response. About 80 % of the total increase of red blood cells in 15 days occurred in the first 10 days.

These findings form a useful basis for comparison, e.g. in assessing the efficacy of vitamin B_{12} in other types of megaloblastic anaemia and for comparing vitamin B_{12} from liver with a similar crystalline vitamin obtained from *Streptomyces griseus*.

Subacute combined degeneration of the cord

Maintenance doses of 10 μg. a fortnight prevented the development or exacerbation of neurological disorders in pernicious anaemia although larger doses are desirable for routine use.

More direct evidence of the efficacy of vitamin B_{12} against the neurologic manifestations of pernicious anaemia was obtained by the intensive treatment of nine fully-established cases of subacute combined degeneration of the cord (Ungley, 1949*b*). Neurological findings were assessed on a quantitative basis and scored. During a control period anaemia was counteracted in some cases by transfusion, without improvement in the neurological condition. Subsequently the weekly injection of 40 μg. vitamin B_{12} led to a gradual reduction in the total score for neurological defect. Improvement continued for about 6 months leaving, as usual, a residuum of irreversible damage in the nervous system. The proportion of irreversible damage depends chiefly on the duration of difficulty in walking. Comparison with forty-four cases treated before the war showed that vitamin B_{12} was as effective as liver extracts, whether crude or refined, given parenterally.

Cobalt-containing red pigments related to vitamin B_{12}

Free forms. Vitamin B_{12a} is an artificial product obtained chemically by hydrogenation of vitamin B_{12}. Vitamin B_{12b} is a constituent of liver extract and appears in the slow-moving red component of the chromatogram. An allied, but as yet unnamed substance, has been isolated by Lester Smith from *S. griseus*. All these factors are clinically active when administered by injection.

Comparisons between crystalline material from *S. griseus* and vitamin B_{12} from liver were made first on the basis of the increase of red blood cells in 15 days following a single dose. Seventeen patients with pernicious anaemia in relapse received single doses of 10, 20, 40 and 80 μg. On the average, the response was equal to that which would be expected from similar doses of vitamin B_{12} obtained from liver. Secondly, improvement was observed in three patients with subacute combined degeneration of the cord, but it is too early to say whether there is any quantitative difference between the effect of this substance and that of crystalline vitamin B_{12} obtained from liver. Thirdly, requirements for maintenance are being assessed. Fourthly, comparisons are being made by the double reticulocyte response method.

Bound forms. The 'animal-protein factor' is apparently the same as vitamin B_{12}, either free or bound. Information about bound forms of vitamin B_{12} is scanty. These forms are probably unavailable to bacteria and inactive by injection. When given by mouth the linkage to protein is presumably split by gastric or pancreatic digestion.

Interrelationship between vitamin B_{12} and folic acid and other nutrients.

With bacteria the needs for vitamin B_{12} can be partly met by thymidine and certain other deoxyribosides or even, under some conditions, by ascorbic acid or methionine.

In a case of pernicious anaemia the injection of 48 mg. thymidine produced a negligible response (Ungley, 1949*c*). Hausmann (1949) claims positive results with doses of 1–2 g. This is approaching the enormous doses required for thymine.

We know little about the interrelationship between folic acid and vitamin B_{12}, but three questions may be considered. The first is do patients with pernicious anaemia ever need folic acid in addition to vitamin B_{12}? Patients with pernicious anaemia with or without subacute combined degeneration of the cord can be successfully treated for indefinite periods with refined liver extracts or vitamin B_{12}. The following is an exceptional case in which vitamin B_{12} failed until needs for folic acid had been met. The patient was a man aged 55 years. Treatment for syphilis had been successfully completed some months before, otherwise the case was typical of pernicious anaemia with complete achlorhydria and megaloblastic marrow. His diet had been reasonably good. The first dose of 10 μg. vitamin B_{12} produced a very poor response. Thereafter small amounts of folic acid, 15 mg. in all, led to a satisfactory reticulocyte response and increase in red blood cells. When the haematopoietic effects had worn off and counts were falling, a second dose of the same batch of vitamin B_{12} caused a satisfactory increase in red blood cells. This case recalls that of the pigs described by Heinle, Welch & Pritchard (1948) which were so completely depleted of haematopoietic factors that folic acid failed unless liver extract was supplied, and liver extract failed unless small amounts of folic acid were supplied.

I should like to stress here once again that this case is exceptional and that ordinary patients with pernicious anaemia and subacute combined degeneration can be treated for years without requiring the administration of anything more than refined liver extract or vitamin B_{12}. Does this mean that vitamin B_{12} aids in the synthesis of folic acid in man as it seems to do in chicks (Dietrich, Nichol, Monson & Elvehjem, 1949) or that there was no deficiency of folic acid in the first place?

Patients with pernicious anaemia are said to have difficulty in deriving free folic acid from conjugated forms in natural foodstuffs, many of which contain conjugase inhibitors. Even some of the responses to pure folic acid conjugates (pteroyltri- and di-glutamic acids (Wilkinson & Israëls, 1949)) were not very good, considering the large doses administered.

Nevertheless, patients with pernicious anaemia seem able to excrete in the urine as large a percentage of a loading dose of 20 mg. folic acid as normal persons. This does not suggest any deficiency of folic acid, but tests must be repeated using smaller, 'more physiological', loading doses.

The second question is why do patients with pernicious anaemia treated with folic acid alone respond at first and then sometimes progressively relapse, even if the dose is increased? Why should folic acid work at all if there is no deficiency of this substance? Is it that an excess of folic acid improves the function of traces of vitamin B_{12} still remaining in the tissues? On this hypothesis the increased tendency to involvement of the nervous system would be explained by an accelerated utilization and ultimate exhaustion of these traces of vitamin B_{12}.

Even in megaloblastic anaemias associated with the sprue syndrome prolonged administration of folic acid alone may lead to neurological disorders. This occurred in

two patients described by Davidson & Girdwood (1948) and in one of my own who developed a psychosis which responded promptly to a source of vitamin B_{12}.

The third question is can folic acid potentiate the action of small doses of vitamin B_{12}? In a patient with pernicious anaemia the daily injection of 0·5 μg. vitamin B_{12} led to a reticulocytosis. Thereafter the same daily dose of vitamin B_{12} supplemented by 100 μg. folic acid caused a second reticulocyte response. This, however, may have been due merely to summation of effects and not to a catalytic effect. In another patient the dose of vitamin B_{12} in the second period was reduced to 0·4 μg. and there was no secondary reticulocytosis. Much more work is necessary before conclusions can be drawn.

With chickens, administration of folic acid increased the stores of vitamin B_{12} in the liver and vitamin B_{12} increased the stores of folic acid (Dietrich *et al.* 1949). We do not yet know whether the same is true with man.

Absorption of vitamin B_{12} from the alimentary tract

The responses to an unnamed red crystalline factor from *S. griseus* and to vitamin B_{12} when given by mouth in the enormous dosage of 80 μg./day were poor. After a total of 1920 μg. in 24 days the increase of red blood cells was much less than would have been expected in 15 days from a single injection of 5 μg. The subsequent daily administration of only 1 μg. by injection was followed by a satisfactory rise of red blood cells (equivalent to the expected rise from a single dose of 10 μg.). In this case the amount of orally administered material necessary to produce a given response was several hundred times that that would have been required by injection.

The daily administration of 5 μg. vitamin B_{12} by mouth was ineffective, whereas the same quantity given daily for 10 days with 50 ml. of normal unfiltered gastric juice produced a satisfactory response. The rise of red blood cells in 15 days following a total quantity of 50 μg. was equivalent to the expected response from a single dose of 10 μg. by injection. In another case similar amounts of material produced a response equivalent to that expected from 20 μg. by injection. Incidentally filtration of the gastric juice through a Seitz filter led to loss of activity of intrinsic factor. In one case the administration of 40 μg. vitamin B_{12} with only 150 ml. of gastric juice was inadequate.

In another patient a single dose of 50 μg. and 500 ml. gastric juice given by stomach tube produced a good response. The increase in red blood cells in 15 days more than equalled the expected response from a single dose of 40 μg. by injection.

Further data are necessary, but the findings suggest that vitamin B_{12} given by mouth with gastric juice may be from one-fifth to four-fifths as effective as the same dose of vitamin B_{12} given by injection. Possibly the results might be even better with larger amounts of gastric juice.

Does intrinsic factor directly facilitate the absorption of vitamin B_{12} or merely prevent its destruction in the gastro-intestinal tract? The daily application of 5 μg. vitamin B_{12} to the mucous membrane of the floor of the mouth produced no haematopoietic response, whereas the same quantity given by mouth with 50 ml. gastric juice produced a good response, equivalent to the expected response to a single injection of 20 μg.

(Even the subsequent daily injection of 5 μg. produced no secondary reticulocytosis and the increase of red blood cells continued at the same rate.)

We next tried to determine whether vitamin B_{12} would be absorbed from the intestine without gastric juice if we prevented contact with intestinal contents which might destroy it or render it unavailable. A segment of small intestine was isolated between two balloons of a Miller-Abbott tube. This segment was washed clear of intestinal contents and 40 μg. vitamin B_{12} were instilled. A small sample withdrawn after 1 hr. still had a high vitamin B_{12} content. There was no haematopoietic response. Later the same amount of vitamin B_{12} given orally with 150 ml. of normal gastric juice produced a reticulocyte response. The response was submaximal probably because the volume of gastric juice was too small. A maximal response followed the injection of 40 μg.

Contents aspirated from various levels of the small intestine have been assayed microbiologically by Dr W. F. J. Cuthbertson. The subjects were two untreated cases of pernicious anaemia. Some samples contained thymidine or minute amounts of vitamin B_{12}. No greater amounts were released after heat treatment or digestion with papain. The intestinal contents did not contain anything which inhibited the growth of the lactobacilli used for the microbiological assay.

The remarkably high excretion of vitamin B_{12} in the stools of patients with pernicious anaemia reported by Callender, Mallett, Spray & Shaw (1949) is not necessarily due to deficient absorption—it might equally well be due to biosynthesis of vitamin B_{12} in the colon.

Is there any interaction of vitamin B_{12} and intrinsic factor? In microbiological assays carried out last year Cuthbertson and I were surprised to find that what little vitamin B_{12} activity there was in a beef digest disappeared after incubation with normal gastric juice, whereas pernicious-anaemia gastric juice had little or no effect.

Recent work by Ternberg & Eakin (1949) seems to show that something in the gastric juice (not necessarily Castle's intrinsic factor) combines with vitamin B_{12} in vitro and renders it unavailable to bacteria. The combination seems to be quite loose. Heating the compound destroys the gastric factor and leaves the vitamin B_{12} microbiologically available. We still do not know whether this combination with a gastric factor facilitates absorption of the vitamin or merely protects it from destruction in the gastro-intestinal tract.

Megaloblastic anaemias

Those associated with pregnancy

In six cases of megaloblastic anaemia associated with pregnancy or the puerperium, the injection of vitamin B_{12} in doses of 65–80 μg. was completely ineffective except for a slight reticulocytosis in one case. The patients subsequently responded to folic acid, often in small doses, 2·5 μg. daily (Ungley & Thompson, 1950).

Those associated with intestinal disorders

Here the results are variable. In a case associated with intestinal stenosis the injection of 80 μg. vitamin B_{12} produced a good response. But even allowing for an initial fall in the first 3 days, the increase of red blood cells by the 15th day was equivalent only to the expected response from 20 μg. in a patient with Addisonian pernicious

anaemia. In a patient with idiopathic steatorrhoea the response to 80 μg. vitamin B_{12} was about equal to an injection of 5 μg. in a patient with Addisonian pernicious anaemia. In a patient with steatorrhoea associated with thyrotoxicosis, vitamin B_{12} was completely ineffective but there was a good response to folic acid. Two further cases associated with steatorrhoea are now under treatment. One showed no response to vitamin B_{12} but a good response to folic acid. The other is responding to vitamin B_{12}.

Toxic and haemolytic aspects

Certain facts suggest that the mechanism of megaloblastic anaemias cannot be explained on a simple nutritional basis. Haemolysis in megaloblastic anaemia is usually attributed to poorly formed red cells. Some of our patients show more excessive haemolysis, at least partly intravascular since methaemalbumin is present. In such patients even normal red cells transfused from donors are rapidly destroyed. Moreover, the rate of elimination (measured by Dr W. Walker using the Ashby technique of differential agglutination) becomes normal after the administration of vitamin B_{12} or folic acid.

My colleague, Dr R. B. Thompson (1950), confirms the finding of Rusznyák, Löwinger & Lajtha (1947) that the maturation of megaloblasts in marrow culture is accelerated by the addition of normal plasma but inhibited by pernicious-anaemia plasma. The greater the concentration of pernicious-anaemia 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 caused rapid maturation of megaloblasts but pernicious-anaemia plasma antagonized this effect. The maturing effect of small amounts of normal plasma is also antagonized by the addition of pernicious-anaemia plasma. These antagonistic effects of pernicious-anaemia plasma can be swamped by increasing the concentration of folic acid or by adding larger amounts of normal plasma. Cerebro-spinal fluid from pernicious anaemia has a similar effect, so that the inhibiting factor is probably ultrafiltrable.

The action of vitamin B_{12} on maturation of megaloblasts is presumably indirect since, unlike folic acid, it fails to accelerate maturation in vitro.

Early lesions in the spinal cord in pernicious anaemia are spotty in distribution and often related to vessels. They suggest the action of a substance destructive to myelin rather than a simple nutritional deficiency. Urinary excretion of certain phenolic compounds is excessive in relapse and becomes normal after treatment with vitamin B_{12} (Abbott & James, 1950). Liver slices from rats deficient in folic acid failed to metabolize tyrosine completely until folic acid was added (Rodney, Swendseid & Swanson, 1949). Intermediary products of tyrosine metabolism include phenolic substances. Another potentially toxic substance is indol, a product of the metabolism of tryptophan. Indol fed to pigs on a diet deficient in the vitamin B complex produces haemolysis and macrocytic anaemia, a result not observed in normal pigs (Rhoads, Barker & Miller, 1938).

For the production of macrocytic anaemia resulting from intestinal loops or blind sacs, stagnation of contents and bacterial infection seem to be essential. In the rats of Watson, Cameron & Witts (1948) 60–100 days elapsed before the animals became

suddenly ill and anaemic. My tentative interpretation is that a toxic and haemolytic 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 depletion reached a certain level, detoxication failed, resulting in sudden illness and anaemia. Folic acid restored the power of detoxication and relieved the anaemia.

Something of the same kind may occur in the small intestine of patients with pernicious anaemia 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 anaemias toxic as well as nutritional factors play a part. These are responsible for megaloblastic erythropoiesis, for some of the haemolysis and possibly for the lesions in the spinal cord. Potentially toxic material, e.g. indol or phenol, arises either from bacterial action on products of protein hydrolysis in the small intestine or from a defect in intermediary metabolism of some substance such as tyrosine or tryptophan. 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}.

Yeast extracts in the treatment of anaemias

Wills's factor. Is there a 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 extrinsic factor? In pernicious anaemia yeast extracts such as Marmite have to be given in large doses, e.g. 120 g. Marmite, to produce even a moderate effect. When given in small doses, e.g. 12 g. Marmite, along with a source of intrinsic factor the effect is better and more consistent. At present this cannot be explained on the supposition that the yeast extracts contain vitamin B_{12} because none can be demonstrated either microbiologically or in animal tests.

In non-Addisonian megaloblastic anaemias, such as those associated with pregnancy and sprue, yeast extracts are sometimes effective by mouth in doses much smaller than those needed in pernicious anaemia. If the effects were due to vitamin B_{12} one would expect that injections of the yeast extract would be effective in doses 60–100 times less than the oral dose. Yet a patient with non-tropical sprue failed to respond to doses one-tenth of those successful when given by mouth.

Can the effect be due to folic acid or folic acid conjugates? In a patient with megaloblastic anaemia of pregnancy the daily dose of yeast extract that produced a good reticulocyte response contained less than 40 μg. folic acid as tested both microbiologically and in animals for conjugates. Moreover, the daily excretion of folic acid in the urine during the period of administration of the yeast was extremely low, only 1–5 μg./day. During the next period 2·5 mg. folic acid produced no secondary reticulocyte response such as one might have expected if the initial response to yeast had been due to traces of folic acid. The mean excretion of folic acid now rose to 700 μg./day. The evidence is very much against folic acid being the cause of the haematopoietic

response observed with yeast. This leads me to think that there may be a Wills's factor after all, despite the modern tendency to explain the action of yeast in terms of folic-acid conjugates. It is true that folic-acid conjugase inhibitors make microbiological assay of yeast difficult. This difficulty does not apply to assays in the rat which were used as a check in this instance.

I should like to thank my colleagues: Dr R. B. Thompson who has been responsible for marrow cultures, Dr W. Walker, who followed the survival of transfused erythrocytes, Dr L. W. Carstairs for intubation of the small intestine. In addition I should like to thank the medical, nursing and lay staff of the Hospital and Medical School and many general practitioners for their co-operation. Dr E. Lester Smith supplied the whole of the vitamin B_{12} used in this investigation and Dr W. F. J. Cuthbertson was responsible for microbiological assays. I should like to thank both these members of the Research Division of Glaxo Laboratories Ltd. for their co-operation, and Glaxo Laboratories for a research grant made to King's College which provides for the Research Fellowship now held by Dr R. B. Thompson.

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The Place of Diet in the Treatment of the Sprue Syndrome

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Clinical aspects of the sprue syndrome

In preparing this talk, I have been conscious of the fact that it has to be presented to a somewhat mixed audience, possibly including some who are uncertain as to what is meant by 'sprue'. For this reason, it is perhaps better first to say a few words about what is meant by this term.

My own clinical interest in the subject has been based on cases that I have seen in recent years in Britain, in India and Burma, and in the United States of America, but