MEGALOBlastic ANAEMIAS OF GASTROINTESTINAL ORIGIN

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THERE exist two known anti-megaloblastic substances, vitamin B₁₂ and folic acid. Both of these are absorbed from the small intestine and the body is dependent on their dietary supply to prevent abnormal changes in hæmopoiesis. Deficiencies of these compounds may arise in a number of ways, but one important group arises from abnormalities of the small intestine interfering with normal absorptive mechanisms.

Absorption

The efficient absorption of vitamin B₁₂ is known to depend on the formation of a complex with an unspecified material, intrinsic factor. The mechanism through which absorption is actually promoted and the problem whether intrinsic factor is simultaneously absorbed or left in the small bowel remain obscure, but the site of absorption is well delineated and appears to be in the terminal ileum (McIntyre, Sachs, Krevans and Conley, 1956; Cox, Meynell, Cooke and Gaddie, 1958; Booth and Mollin, 1959). Thus loss of intrinsic factor, as in pernicious anæmia, after total gastrectomy and sometimes after partial gastrectomy (Badenoch, Evans, Richards and Wits, 1955; Maclean, 1957) or loss of the terminal ileum, either by surgical resection or disease, inevitably leads to malabsorption of vitamin B₁₂.

It appears that calcium ions may be necessary for efficient absorption of the vitamin B₁₂-intrinsic factor complex (Gräsbeck, Kantero and Siurala, 1959) and it is possible that the removal of calcium ions by fatty acids may partly impair vitamin B₁₂ absorption in steatorrhæa, but little is known yet about their role. If calcium ions were an important factor, then malabsorption of vitamin B₁₂ in pancreatic steatorrhæa would be expected to occur, and this has been shown (Halstead, Lewis, Hvolbolt, Gasseter and Swenseid, 1956; Frost, Goldwein and Kaufman, 1957; McIntyre and others, 1956). However, megaloblastic anæmia has never been

described secondary to pancreatic disease (Mollin, 1959).

Even less is known about the absorption of folic acid. In the diet much of the folic acid exists in conjugated form, and this has been shown to be less well absorbed than the unconjugated form (Jandl and Lear, 1956). Whether this difference is due to preferential absorption of the unconjugated form or difficulty in deconjugating, or both, is unknown, as is the part played by digestive enzymes.

The site of absorption of folic acid is also not known with certainty. From a study of patients with Crohn's disease affecting various parts of the small intestine Cox and others (1958) and Doig and Girdwood (1960) found malabsorption of folic acid was most frequent when the disease affected the jejunum. Resection of sufficient ileum to result in malabsorption of vitamin B₁₂ does not as a rule lead to malabsorption of folic acid (Chanarin, Anderson and Mollin, 1958a; Cox and others, 1958; Doig and Girdwood, 1960). However, there is no conclusive evidence that the absorption of folic acid is confined to the proximal small intestine.

The bacterial flora of the gut play a rather mysterious role in the absorption of vitamin B₁₂ and folic acid. A normal flora which is probably confined only to the terminal part of the ileum is not likely to influence normal absorption, but in many diseases of the small intestine bacteria may flourish where they do not normally exist. Since some bacteria show an avid utilization of vitamin B₁₂ (Burkholder, 1952; Doig and Girdwood, 1960), it is possible that a profuse growth would compete with the body for the available vitamin leading to a deficiency. Certainly the response of some of the disorders to be considered to broad-spectrum antibiotics lends support to such a theory. Most bacteria actually synthesize vitamin B₁₂ and folic acid, but since these organisms are mainly confined to the large bowel, their synthetic

products are not available for absorption. This topic has been well reviewed by French (1961).

Results of Malabsorption

Malabsorption of vitamin B₁₂ and folic acid inevitably leads to depletion of the body stores of these vitamins. The sequence of changes in the development of vitamin B₁₂ deficiency is better known than in that of folic acid, because pure vitamin B₁₂ deficiency occurs after total gastrectomy and in pernicious anaemia. The speed of onset of symptoms and signs will obviously depend on the size of the body store when absorption begins to fail. For some reason the sequence of changes is quicker in pernicious anaemia in relapse than after total gastrectomy and Mollin (1959) suggests that this may be due to the fact that patients with pernicious anaemia are not infrequently incompletely repleted with vitamin B₁₂. Paulson and Harvey (1954) followed up 80 patients after total gastrectomy. Six of them survived long enough to develop megaloblastic anaemia two to seven years (average 4.25 years) after the operation.

It can be estimated that the body stores of vitamin B₁₂ amount to about 1 mg., which on values for daily requirement of about 1 to 2 µg would account for the latent interval of about two to three years before the onset of anaemia.

In course of time vitamin B₁₂ deficiency leads to megaloblastic anaemia often associated with leucopenia and even thrombocytopenia. Subacute combined degeneration may complicate this deficiency regardless of its cause.

Pure dietary folic acid deficiency in adults was reported for the first time (Knowles, Prankerd and Westall, 1961) in patients fed, perforce, on artificial diets. The stores of folic acid became sufficiently depleted to give abnormal histidine tests after four to six weeks. At this time there were no haematological or bone marrow changes. After 10 months one of the patients was still not anaemic (Hb. = 15 g./100 ml.). These observations are in keeping with those of Najjar and Holt (1943) and Najjar and Barrett (1945), who fed human volunteers on diets containing 13 to 17 µg. per day of folic acid. After 18 months there were no haematological abnormalities. Stores of 'folic acid-like' substances have been estimated at about 5 to 10 mg. (Girdwood, 1959) with a daily requirement of 5 to 10 µg. (Knowles and others, 1961). Folic acid deficiency eventually leads to identical haematological changes as vitamin B₁₂ deficiency, but neurological sequelae do not occur.

The role of vitamin B₁₂ and folic acid deficiency in preserving the integrity of the mucous membranes of the gastrointestinal tract is much debated. Evidence is accumulating that deficiency of either

may lead to malabsorption (Lambert, Prankerd and Smellie, 1961).

Detection of Deficiency and Measurement of Absorption

The measurement of absorption of vitamin B₁₂ and folic acid can only be done effectively by the use of radioactive isotopes incorporated into the vitamin. With such material physiological quantities of these substances can be incorporated into the diet. Measurements of absorption after giving loading doses impose abnormal concentration-gradients throughout the absorbing surface of the small intestine and cannot be regarded as sensitive tests for defective absorption, though they are undoubtedly a guide to intestinal function.

Radioactive vitamin B₁₂ and folic acid are now easily obtainable. The former, labelled with ⁵⁸Co, can be estimated by readily available apparatus. Folic acid labelled with tritium requires a liquid scintillation counter (Anderson, Belcher, Chanarin and Mollin, 1960).

It should be borne in mind that the demonstration of impaired absorption does not necessarily imply a body deficiency of that compound. For this assessment other tests are available. In the case of vitamin B₁₂, estimation of the serum level is a reliable means of assessment of body stores (Spray and Witts, 1958), but in the case of folic acid serum measurements have not yet proved wholly successful (Herbert, Wasserman, Frank, Pasher and Baker, 1959; Baker, Herbert, Frank, Pasher, Hutner, Wasserman and Sobotka, 1959; Baker, Herbert, Frank, Pasher, Sobotka and Wasserman, 1960; Niewig, Faber, de Vries, Stenfort Kroese, 1954); and as a result various indirect procedures have been introduced to assess body stores of folic acid. The urinary excretion of folic acid after a test dose might be expected to be proportional to the degree of tissue saturation, but, whilst this is roughly true, there is sufficient overlap between normals and abnormals to make the test unsatisfactory (Cox, Meynell and Cooke, 1961). Chanarin, Anderson and Mollin (1958b) showed that the rate of clearance from the plasma of an intravenous dose of folic acid was proportional to the degree of folic acid deficiency, but it also seems to be proportional to the degree of marrow activity and therefore not specific; the results of this test before and after treatment with small doses of folic acid which have not been reported would be of interest.

Bakerman, Silverman and Daft (1951) noted a glutamic acid precursor in the urine of folic acid-deficient rats which was later shown to be formiminoglutamic acid (FIGLU) (Borek and Waelsch, 1953; Seegmiller, Silverman, Tabor and Mehler, 1954). FIGLU is an intermediate in histidine

metabolism and by giving a suitable dose of histidine it has been shown that the excretion of FIGLU can be increased (Tabor, Silverman, Mehler, Daft and Bauer, 1953), thus providing a suitable laboratory test for folic acid deficiency (Luhby, Cooperman and Teller, 1959a and b; Knowles, Prankerd and Westall, 1960).

Using this test we have found the following results in 105 cases of steatorrhœa:

	Abnormal test	Normal test
Idiopathic steatorrhœa ..	46	2
Postgastrectomy steatorrhœa ..	6	18
Regional ileitis ..	9	8
Pancreatic steatorrhœa ..	0	6
Jejunal diverticulosis ..	2	0
Secondary to liver disease ..	1	1
Reticulosis of the small intestine ..	1	1
Scleroderma affecting upper jejunum ..	0	1
Diagnosis uncertain ..	1	2
	66	39
	105	

Diseases of the Small Intestine

Idiopathic Steatorrhœa, Celiac Disease

Idiopathic steatorrhœa probably comprises a group of diseases for which there is no satisfactory name. Many alternatives (adult celiac disease, gluten-induced enteropathy, primary malabsorption syndrome) have been suggested, but none has met with universal approval. Gluten-sensitivity seems to be a common cause in adults and even more so in children.

Megaloblastic anaemia is much less common in children than in adults. Sheldon (1958) gives an incidence of 9%. It is known to be much commoner in adults, but exact figures are scarce. Cooke, Peeney and Hawkins (1953) found macrocytic anaemia in two-thirds of 100 patients; 49 of the 100 had bone marrow examinations and 17 had definite megaloblastic changes, 11 were entirely normal and the other 21 were intermediate.

The anaemia may be due to deficiency of iron, folic acid or vitamin B₁₂. A dimorphic blood picture with evidence of macrocytosis and hypochromia is very suggestive of malabsorption.

Serum vitamin B₁₂ levels are low and absorption of labelled vitamin B₁₂ is reduced below normal in approximately half the patients which have been studied, though figures vary from one group of worker to another (Mollin, Booth and Baker, 1957; Spray and Witts, 1958; Meynell, Cooke, Cox and Gaddie, 1957; Halstead, Lewis and Gasseter, 1956; Frost and others, 1957; Oxenhorn, Estren, Wasserman and Aldersberg, 1958; Turnbull, 1954; Callender and Evans, 1955; Doig and Girdwood, 1960).

Absorption of folic acid has been reported to be reduced in over 90% of patients (Chanarin and others, 1958a; Cox and others, 1958; Doig and Girdwood, 1960). In all these studies large doses of folic acid were used (3 to 5 mg.), doses which are 15 to 25 times greater than the total average daily intake; it may well be that these tests are really measuring the absorptive capacity of the gut rather than the physiological absorption of folic acid. Such tests are, however, useful measures of intestinal function. Anderson and others (1960) used more physiological doses of labelled folic acid and found malabsorption in nine out of 13 patients as measured by faecal excretion.

Folic acid deficiency, when assessed by the excretion of an excess of FIGLU after a histidine load, appears to be at least as common as malabsorption when measured by the folic acid load tests (Knowles, 1961).

Tropical Sprue

This is a most interesting condition with many unexplained features. The geographical distribution of tropical sprue suggests that it may be related to dietary habits. Thus it is common in Cuba and Puerto Rica, but rare in neighbouring Jamaica. It occurs in Hong Kong, but is uncommon in Singapore. It is common in India, but almost absent from Africa and the Pacific (Gardner, 1958). French (1955, 1961) has suggested that this may be related to the consumption of unsaturated fatty acids in the sprue areas. Tropical sprue can occur within a few weeks of arrival in an endemic area and in patients in an apparently excellent state of nutrition, suggesting, in the early stages at least, that malnutrition is not an important factor. Jejunal biopsy changes have been noted within 10 days of the onset of diarrhoea (Butterworth, C. E., personal communication to Gardner, 1958).

Treatment of the established disease may be affected by removal from the affected area, by folic acid or by antibiotics. The prognosis is usually excellent, but an occasional case reaches an apparently irreversible stage. Folic acid deficiency has not been clearly demonstrated, but most cases respond to folic acid alone, suggesting that deficiency of this vitamin, however determined, is important in keeping the disease active. Not all such patients are anaemic. There is often no evidence of vitamin B₁₂ deficiency in the early stages of the disease (Frazer, 1960; Gardner, 1958), but it is not uncommon in the later stages (Gardner, 1958).

In a proportion of patients it is necessary to give antibiotics before a full remission is obtained (French, 1961; French, Gaddie and Smith, 1956). Antibiotics alone may also induce a remission

(Keele and Bound, 1946; Foy and Kordi, 1954).

The incidence of megaloblastic anaemia varies in reports from different parts of the world, but it would seem to be inevitable if the disease is not treated or it does not undergo spontaneous remission.

Pancreatic Steatorrhœa

As previously mentioned, megaloblastic anaemia has not been recorded in this type of steatorrhœa, although absorption of vitamin B₁₂ is impaired.

Absorption of folic acid is normal (Cox and others, 1958) and there does not appear to be a deficiency in the body stores of folic acid (Knowles, 1961).

Cirrhosis of the Liver and Megaloblastic Anaemia

Although steatorrhœa may occur as a complication of cirrhosis of the liver, megaloblastic anaemia, when it occurs, is probably not due to this cause. Nutritional deficiency or hepatic failure would seem the most likely causes. In any case, it is rare.

Intestinal Strictures, Resections, Fistulae, Blind Loops and Jejunal Diverticula

Two mechanisms operate in the production of megaloblastic anaemia. First, the terminal ileum (the site of vitamin B₁₂ absorption) may be bypassed by fistulae or removed by resection. Secondly, strictures or jejunal diverticulae may give rise to blind loops of intestine. Here micro-organisms not normally found in the small intestine multiply and interfere with absorption. One of the ways they may do this is by actually competing with the intestine for vitamin B₁₂ (Doig and Girdwood, 1960; Badenoch, Bedford and Evans, 1955). Organisms have not been shown to compete for folic acid *in vitro*. Nevertheless, folic acid deficiency does occasionally arise, though it is much less common than vitamin B₁₂ deficiency. Girdwood (1960) reports a possible case and we have seen two cases where there was an abnormal excretion of FIGLU. One of these patients also had a low vitamin B₁₂ level (80 µg./ml.) and polycythæmia vera, but the other patient had no other cause for her megaloblastic anaemia. Girdwood (1960) suggests that folic acid deficiency may arise from direct bacterial irritation of the gut wall. Folic acid absorption tests using high dosage of folic acid are usually normal in these patients (Doig and Girdwood, 1960). Impaired absorption of vitamin B₁₂ has been demonstrated by Halstead, Lewis, Hvolbolt, Gasseter and Swendseid (1956), Frost and others (1957), Mollin and others (1957) and Oxenhorn and others (1958), and low serum B₁₂ levels by Spray and Witts (1958). Megaloblastic anaemia after partial gastrectomy is usually due to subsequent atrophy

of the stomach remnant, but occasionally a blind loop is the cause (Naish and Capper, 1953).

Infiltrative Diseases of the Small Intestine

In regional ileitis and jejunitis megaloblastic anaemia is occasionally observed. The mechanism operates through the formation of blind loops or fistulae, or the wall of the small intestine may be so diseased that absorption is impeded. In the first group cure may sometimes be effected by antibiotics and in the latter group corticosteroids will occasionally bring amelioration (Cooke, 1958).

Tuberculosis (Davidson, 1950) and reticulosis (Girdwood, 1960) of the small intestine are rare causes of megaloblastic anaemia.

Amyloidosis of the small intestine and Whipple's disease not infrequently lead to steatorrhœa, but so far as we are aware, megaloblastic anaemia has not been reported. Hendrix, Black-Schaffer, Withers and Handler (1950), in a general review, state that macrocytic anaemia is uncommon in Whipple's disease and report one of their four cases as having a bone marrow showing 'slight arrest of erythro- and myelo-blastic tissues'.

Fish Tape Worm

Diphyllobothrium latum, the fish tape worm, which affects particularly the population of Finland and Japan, has been directly demonstrated to have a high affinity for vitamin B₁₂. *In vivo* it competes with intrinsic factor for the vitamin, but if intrinsic factor and vitamin B₁₂ are mixed prior to ingestion there is no interference with absorption (Brante and Ernberg, 1958). Infestation with this worm is common in Finland (9 to 12% of the population, depending on the area) (von Bonsdorff, 1948), though only a small percentage of infected persons develop significant anaemia (1 in 113, 1 in 659) (Tötterman, 1944).

Diagnosis

The peripheral blood pictures in all cases of megaloblastic anaemia are often indistinguishable, showing macrocytosis and considerable deformity of the red cells. All cases of vitamin B₁₂ deficiency can show subacute combined degeneration of the cord. A most important clue to the occurrence of malabsorption in the aetiology is the presence of coincidental evidence of iron deficiency. This is absent in untreated pernicious anaemia and the rarer causes of megaloblastosis; it manifests itself in the peripheral blood by the appearance of hypochromic red cells. Other evidence of iron deficiency may be obtained from serum iron levels.

Achlorhydria as demonstrated by the augmented histamine test seems synonymous with total gastric atrophy, and if pernicious anaemia is not present it may be expected to develop (Card and Circus,

1958). However, the presence of free acid excludes pernicious anæmia except in young people (Lambert and others, 1961). Evidence of coincident folic acid deficiency is also present in the more severe cases of pernicious anæmia (Knowles and Pranker, 1961).

Ultimate diagnosis therefore rests on a combination of the histological and radiological demonstration of an abnormality in the gastrointestinal tract, together with evidence of malabsorption. Barium studies showing flocculation patterns are important, as is the demonstration of malabsorption by estimation of daily fat and urinary calcium excretion. The latter may rarely be abnormal in pernicious anæmia (Lambert and others, 1961). Final differentiation from pernicious anæmia may be achieved by studies with ^{58}Co -labelled vitamin B_{12} in which the restoration of normal absorption is demonstrated by administering vitamin B_{12} with intrinsic factor. The amount of the latter used is important, since excessive amounts may increase vitamin B_{12} absorption in idiopathic steatorrhœa (Callender and Evans, 1955).

Finally, mucosal biopsy from the jejunum and stomach is particularly useful in diagnosis as specific changes can often be detected in different disorders. Pernicious anæmia patients show complete gastric atrophy. Characteristic changes are found in the small intestine in idiopathic steatorrhœa, tropical sprue, regional ileitis and Whipple's disease. The finding of a normal, or nearly normal,

intestinal biopsy is usual in post-gastrectomy steatorrhœa, pancreatic steatorrhœa, cirrhosis and in steatorrhœa associated with blind loops and jejunal diverticulosis.

Treatment

Symptomatic treatment of megaloblastic anæmia of any cause may be effected by giving folic acid or vitamin B_{12} , depending on the deficiency.

When megaloblastic anæmia is due to intestinal causes more specific therapy is usually desirable, directed at the cause. Thus nearly all children with celiac disease and most adults with idiopathic steatorrhœa will respond to withdrawal of gluten from the diet. The excretion of FIGLU will cease in two to four months after starting on a gluten-free diet, suggesting complete replacement of the body stores of folic acid after this time (Knowles, 1961).

The treatment of tropical sprue has already been discussed.

Antibiotics will usually induce a hæmatological remission in the blind-loop syndrome, but it is interesting to note that neomycin, the most powerful intestinal antibiotic of all, does not do this (Halstead, Lewis and Gasseter, 1956). Neomycin is not absorbed, so possibly does not reach the blind loops in sufficient concentration to be effective. Surgical procedures will be required when correction of some anatomical defect is necessary.

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