The Recognition of Vitamin B12 and Folate Deficiency.

By S. J. Baker

Wellcome Research Unit,
Christian Medical College Hospital,
Vellore, India.

Vitamin B12 and folate are both necessary for the maintenance of health. With increasing understanding of the roles of these substances in intermediary metabolism, and with the ready availability of both vitamin B12 and folic acid in pure form for therapy, the early recognition of deficiency states is becoming increasingly important.

**Deficient intake**

Vitamin B12 is present in many animal products such as liver, meat, fish, eggs and milk. Folate is present in liver, green vegetables and some fruits and nuts and to a smaller extent in milk. However, folate in the diet is fairly heat labile, and excessive cooking may destroy much of the original activity present in the uncooked food.

The daily requirement of infants and children for these vitamins has not been extensively studied. Infants with vitamin B12 deficiency anaemia will respond well to a daily oral dose of 0.1μg of vitamin B12 by mouth, and infants with folic acid deficiency anaemia associated with kwashiorkor will respond to a daily oral dose of 25μg of folic acid. The daily requirements of both vitamins are therefore presumably less than these amounts. In adults the daily requirement for vitamin B12 is probably in the region of 0.5μg and for folate in the region of 500μg.

After birth, individuals are dependent on dietary sources for the supply of both vitamin B12 and folate. If the daily requirements are not provided deficiency will ultimately develop. In infants dietary deficiency of vitamin B12 may arise in purely breast-fed children suckled by B12 deficient mothers. Goats' milk, boiled cows' milk and unsupplemented processed milks have a low folate content, and children fed on these may develop folate deficiency. The exact incidence of megaloblastic anaemia varies in different published series, presumably depending largely on the folate content of the staple diet of the population concerned. In southern India 61% of children with kwashiorkor had megaloblastic anaemia or developed it during protein repletion. All except two of these were due to folate deficiency.

In older children and adults ignorance, poverty, vegetarianism, and food faddism may lead to insufficient intake of one or other vitamin.

**Diseases of the stomach**

Even in the presence of normal dietary intake deficiency states may develop when there are gastrointestinal diseases resulting in defective absorption. For normal vitamin B12 absorption intrinsic factor produced by the parietal cells of the stomach is necessary. In rare cases histologically normal parietal cells fail to produce intrinsic factor. Usually reduction of intrinsic factor secretion sufficient to interfere with vitamin B12 absorption is associated with atrophic gastritis, gastric atrophy or gastric resection. Gastric atrophy occurs classically in pernicious anaemia where there is an associated high
Incidence of intrinsic factor and parietai cell antibodies are demonstrable in the serum. It may also occur in chronic gastritis,77, 84, 106 diverticulosis,78 fish tapeworm infestation,100 non-tropical sprue,12 and tropical sprue,16 and these diseases may produce a syndrome resembling classical pernicious anaemia.

After total gastrectomy, vitamin B₁₂ deficiency will invariably supervene provided the patient lives long enough. The time necessary for the development of frank vitamin B₁₂ deficiency will vary according to the level of the body stores at the time of the operation, but may be anything from three to five years.89, 101 Partial gastrectomy, depending on its extent, may produce varying degrees of intrinsic factor deficiency. In the Polya type of operation vitamin B₁₂ absorption may also be interfered with because of the "blind loop" formed by the gastrojejunostomy. The incidence of vitamin B₁₂ deficiency after partial gastrectomy in a Western community may be as high as 13%21, 80 and in areas of poor nutrition is almost certainly higher. Diseases of the stomach per se do not interfere with folic acid absorption, but by causing anorexia they may decrease the dietary intake of folic.

Diseases of the small intestine

Diseases of the small intestine may interfere with vitamin B₁₂ or folic absorption. Fish tapeworm infestation produces vitamin B₁₂ deficiency, at least partly by competitive uptake of vitamin B₁₂ by the worm.164 Although the worm has been found in a number of countries, most cases of megaloblastic anaemia associated with worm infestation have been reported from Scandinavian countries.

Anatomical lesions of the small intestine such as ileal resections,19, 74 diverticulosis,25, 28 strictures11, 28 short circuits and blind loops14, 21 frequently cause interference with vitamin B₁₂ absorption, either by removal of the ileum where vitamin B₁₂ is normally absorbed, or by bacterial contamination of the gut producing interference with vitamin B₁₂ absorption. In those cases where bacterial contamination is involved the condition may often be relieved or sometimes cured by chemotherapy.

Patients with coeliac disease or non-tropical sprue often develop megaloblastic anaemia.17, 79 Most commonly this is due to folic acid deficiency,26 but vitamin B₁₂ deficiency may also occur due to malabsorption of vitamin B₁₂.4, 23, 32

In tropical sprue megaloblastosis due to vitamin B₁₂ and/or folic acid deficiency occurs in a high proportion of cases. O'Brien and England10 report an incidence of 100%. In South India we have found an incidence varying from 60% to 80%.72 Vitamin B₁₂ deficiency in tropical sprue is most probably related to defective vitamin B₁₂ absorption. 2, 9, 10, 11 This absorptive defect is not corrected by intrinsic factor administration, but in some cases may be corrected by antibiotics.87, 31, 84 Folic acid deficiency is also common in tropical sprue and may frequently be the major deficiency.14, 84 The reasons for the development of folic acid deficiency are not well documented. Defective diet may play a part in some cases, but folic acid deficiency may develop even when the patients are taking a good diet. Defective folate absorption has been advanced as a possible cause, but in absorption studies using a balance technique with tritiated folic acid, evidence of folate malabsorption is rare. Similar results have been obtained using a double label technique.8 In preliminary studies excessive folic acid losses of plasma folate have been found in association with diarrhoea,73 and this may be an added factor in producing folate depletion.

Defective intestinal transport of vitamin B₁₂ which is not influenced by intrinsic factor, may be seen in the rare syndrome of familial relapsing megaloblastic anaemia and proteinuria.18, 88

Pregnancy

The increased nutritional demands imposed by the fetus make pregnant women particularly susceptible to deficiency states. This may be further aggravated by associated alterations in dietary intake. The commonest deficiency in pregnancy in most parts of the world is that of iron, but folate deficiency is also very common. The incidence of deficiencies in pregnancy varies widely, presumably depending on differences in the basic diet. In one study in South India, 54% of pregnant women in the third trimester had a megaloblastic bone marrow.97 In a current study* in this laboratory of 825 subjects 48% had evidence of folate deficiency as shown by serum folate levels of 4μg/ml or less and a further 30% had levels between 4 and 6μg/ml.

* Supported by the World Health Organisation.

Haemolytic anaemias and neoplastic diseases

Chronic haemolytic anaemias, and other conditions associated with prolonged over-production of cells such as leukaemias, lymphomas, etc., may lead to a relative folate deficiency and frank megaloblastic change in the developing red cells.15, 60, 92

Other diseases

Patients with cirrhosis of the liver, especially when associated with alcoholism,35, 58, 60 and patients with Addison's disease48 appear to be prone to develop folate deficiency.

Infections of various types may also precipitate folate deficiency. This is particularly so in children57 in whom gastrointestinal and respiratory infections are the ones most commonly associated with folate deficiency.14

In patients with thyroid disease folate deficiency58 and vitamin B₁₂ deficiency8 have both been described.

Drug administration

Megaloblastic anaemia occasionally occurs in patients receiving anticlotting drugs—especially diphenylhydantoin, primidone and phenobarbitone. This anaemia is usually associated with low serum folate levels and responds to folic acid.59 The antimalarial drug pyrimethamine may also produce a folate deficiency megaloblastic anaemia.12

Para-aminosalicylic acid administration has been shown to interfere with vitamin B₁₂ absorption and prolonged administration of this drug may be associated with lowered serum vitamin B₁₂ levels.14
Symptoms and Signs of Vitamin B₁₂ and Folate Deficiency States

Anaemia

The most commonly recognised clinical symptoms and signs of vitamin B₁₂ and folate deficiency are those associated with anaemia. If the anaemia is severe there may also be signs of haemolysis such as mild icterus and splenomegaly.

The tongue

Glossitis is commonly an early symptom in both vitamin B₁₂ and folate deficiency, but the incidence of glossitis varies widely in different series and in the same series with different observers. In Indians, patchy hyperpigmentation of the tongue may be seen in some cases of vitamin B₁₂ and folate deficiency. It is most commonly seen on the dorsal surface of the tongue (Fig. 2), but may also be seen on the ventral surface and around the gum margins and in the buccal mucosa. The hyperpigmentation can be observed to disappear with treatment. Similar hyperpigmentation may also occur as a congenital anomaly, and in other conditions associated with generalised hyperpigmentation such as Addison’s disease of the suprarenals.

The skin

Hyperpigmentation of the skin may be seen in cases of megaloblastic anaemia in Indians, and may be a presenting symptom of this disorder. It is often associated with vitamin B₁₂ deficiency, but can also occur in pure folate deficiency and need not necessarily be associated with anaemia. The hyperpigmentation is most marked on the exposed parts and those subjected to pressure. It is usually maximal on the dorsum of the hands, over the terminal phalanges and the interphalangeal and metacarpophalangeal joints (Fig. 3). The nail bed itself may also be involved leading to pigmented nails. Sometimes there is a patchy “geographical” type of hyperpigmentation seen on the hands and feet. The hyperpigmentation is due to the excessive deposition of melanin in the basal-cell layers of the skin.

Haemorrhage into the skin due to thrombocytopenia may rarely be a presenting symptom in cases of severe vitamin B₁₂ and folate deficiency. Haemorrhages may also occur in the fundus oculi.

The central nervous system

Infants with vitamin B₁₂ deficiency may develop a characteristic neurological syndrome first recognisable as a slowing of mental development, which if untreated may then proceed to developmental regression, tremors, spasticity and extensor plantar responses, and even convulsions, coma and death.

In adults, both vitamin B₁₂ and folate deficiency may present with symptoms suggestive of neurasthenia. More severe degrees of vitamin B₁₂ deficiency may result in changes either in the peripheral nerves, in the cord, or in the cerebrum. Peripheral neuritis due to vitamin B₁₂ deficiency is usually of the “glove and stocking” type. Involvement of the spinal cord may result in changes varying from a mild degree of impairment of the sense of vibration to the fully developed classical picture of sub-acute combined degeneration of the cord. Involvement of the cerebrum may produce slowing of mental processes, depression, hallucinations, psychosis and finally severe dementia. Retro-bulbar neuritis and optic atrophy may also occur.
Haematological Diagnosis

The earliest haematological sign of folate and vitamin B12 deficiency may be hypersegmentation of the neutrophils in the peripheral blood. Subsequently leucopenia and thrombocytopenia may occur. The red cells develop macrocytosis and poikilocytosis, but these changes can be masked by concurrent iron deficiency. Anemia of moderate to severe degree is a relatively late sign and indicative of moderately severe deficiency states.

The changes in the marrow vary according to the severity and duration of the deficiency. The classical 'bone-marrow' haematological hallmark of these deficiencies are the changes in the developing red cells. In the first stages the marrow may be markedly hypercellular and will contain many large primitive erythroblast cells. In severe deficiencies the abnormalities ranging from the most severe to the just abnormal may be seen. The abnormalities in the red cells may be graded according to the degree of morphological change—grade I being the just abnormal and grade IV being the grossly abnormal.

The interpretation of morphology is a subjective matter, and there may therefore be considerable differences in interpreting the minor degrees of change represented by grades I and II. In such cases the ultimate test of abnormality is to observe a change towards normality in the morphology of the nucleated red cells following specific treatment. The changes of megaloblastosis may be masked by severe iron deficiency, by defects of haemoglobin synthesis, and by the presence of B12 deficiency in the marrow, inadequate amounts of folate or indigestible fermentable foodstuffs.

The presence of megaloblasts in the bone marrow is not absolutely diagnostic of vitamin B12 or folate deficiency. They may also be present in DI-Guglielmo's disease and in cases of leukaemia, where serum vitamin B12 and folate levels are normal and where there is no response to these agents. Megaloblastosis has been reported in scurvy, but this may be caused by concomitant folate deficiency. Megaloblastosis responding to vitamin E therapy has also been reported in cases of marasmus and kwashiorkor, but the precise role of vitamin E in erythropoiesis is not yet clear.

The changes in the white cells in the marrow are in increase in size of the cells, characteristic giant metamyelocytes, giant band or 'stab' cells and large hypersegmented polymorphs or 'macropolyocytes'. In some cases of milder deficiency these changes in the developing white cells may be more obvious than those in the red cell precursors.

Assays of Vitamin B12 and Folate

The advent of microbiological assays for measuring vitamin B12 and folate concentrations has provided a sensitive method for following changes in the concentration of these substances in plasma and tissues.

A number of methods are available for the assay of vitamin B12 levels in the serum. These include Euglena gracilis var. bacillaris, Euglena gracilis Z strain, Lactobacillus-leichmannii, Echerichia coli, and Ochromonas malhamensis. Of these, E. coli is the most rapid but least specific. L. leichmannii and Euglena gracilis are probably the most widely used organisms, but Ochromonas is the most specific in its growth requirements. In addition to microbiological assay methods for vitamin B12, radioactive labelled vitamin B12 of high specific activity can be used in combination with saturation analysis technique for measuring serum levels. This is still not widely employed but may ultimately displace microbiological methods.

For the measurement of serum folate levels the organism Lactobacillus casei is used. This organism responds to a number of folate forms including N5-methyl tetrahydrofolate which is the chief folate form in serum.

In less severe cases of folate deficiency, the serum folate level in the plasma is lowered, it is probably the most reliable indicator of folate deficiency, but normal folate levels do not always rule out folate deficiency. For example in cases of kwashiorkor even in the absence of obvious infection the serum folate levels may be low and the child will have a megaloblastic anaemia which responds to folate administration.

Interpretation of serum vitamin B12 levels may be even more difficult. A low serum vitamin B12 level in the presence of a normal to high folate level is almost certainly indicative of vitamin B12 deficiency. However, with a low folate level in the blood, a low serum vitamin B12 level may also be found which rises on treatment with folic acid. In cases of liver disease there may be very high levels of vitamin B12 in the plasma, yet total body stores may be reduced. In all these instances the results of serum assays do not accurately reflect the position of the body stores.

Microbiological assays may also be used for studying tissue levels of vitamin B12 and folate. Red cell folate levels have been studied by a number of investigators. They are reduced not only in cases of folate deficiency, but also in vitamin B12 deficiency. Red cell folate levels are therefore difficult to interpret in cases where there may be combined deficiencies present. In developing folate deficiency the serum level falls before the red cell level, but the red cell levels probably give a better indication of tissue folate stores and therefore of total body folate than do the serum levels.
Liver levels of vitamin B₁₂ and folate may also be measured on liver biopsy specimens. The problems associated with liver biopsy make it unlikely that the assay of liver tissue will ever become a routine investigation in the diagnosis of deficiency states. It is, however, a useful research method for obtaining an indication of body stores of these materials.

**Intermediary Metabolism**

In folate deficiency the conversion of formimino glutamic acid (figlu) to glutamic acid is impaired, and when a loading dose of L histidine is given, uracil acid and figlu are excreted in excess in the urine. However, the test is also abnormal in some cases of vitamin B₁₂ deficiency and therefore of very limited value in distinguishing the two deficiencies. Lubby and Cooperman claim that by modifying the test it can be used to distinguish the two deficiencies, but this awaits confirmation. The studies of Herbert have shown that the figlu test becomes positive only a number of weeks after the fall in serum folate levels and after the appearance of morphological changes in the peripheral blood. The test is therefore of little value in the diagnosis of early folate deficiency, or in distinguishing vitamin B₁₂ deficiency from folate deficiency.

The excretion of aminoimidazole carboxamide in the urine is raised in cases of vitamin B₁₂ deficiency and this has been suggested as a test for B₁₂ deficiency. However, a similar finding also occurs in folate deficiency and the test therefore cannot distinguish between deficiencies of the two substances.

Urinary methylmalonic and propionic acid excretion are increased in subjects with vitamin B₁₂ deficiency but not in subjects with folic acid deficiency. Most of the methods for estimating methylmalonic acid are not suitable for routine use, however two simpler methods have recently been introduced which may make the test more applicable to routine studies. If the usefulness of these methods is confirmed the test may become important in the investigation and differentiation of vitamin B₁₂ and folate deficiency states.

**Haematological Responses**

The final proof of the presence of a deficiency state of sufficient severity to cause a recognisable abnormality, is the reversion of the abnormality to normal following specific therapy. This is most easily followed in those cases where there is a significant degree of anaemia, but the method can also be applied to the clinical and metabolic manifestations of deficiency states. In anaemic subjects studies of the haematological response to physiological amounts of vitamin B₁₂ or folic acid may provide the best indication of the nature of the deficiency. In cases of pure vitamin B₁₂ deficiency the parenteral administration of 200 micrograms of folic acid daily will produce no haematological response whereas 1 μg of vitamin B₁₂ will produce a prompt response (Fig. 4). Conversely in cases of pure folate deficiency there will be no haematological response to the daily parenteral administration of 1 μg of vitamin B₁₂, but an adequate response following the administration of 100-200 μg of folic acid (Fig. 5). In some cases of mixed deficiency there may be a response to both vitamin B₁₂ and to folate when they are given one after the other (Fig. 6). In other such cases it appears as though a severe deficiency of the second therapeutic agent may prevent any haematological response to the first until the second agent is also administered—in such cases a study of therapeutic responses alone may not give a true indication of the nature of the deficiencies present.
Fig. 6. — Haematological findings in a patient with combined vitamin B12 and folate deficiency. Reticulocyte response to injection of vitamin B12 with further response to oral folate in the form of yeast and 100 mg of folic acid. Patient on low B12 low folate diet. Symbols as for Figure 4.

Other Investigations
Once the presence of a vitamin B12 or folate deficiency has been recognised, other special investigations may be necessary to establish the aetiology of the deficiency. Some of the more important tests are listed in the table.

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<td>Augmented histamine test meal</td>
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Summary and Conclusion
The recognition of vitamin B12 and folate deficiency depends on:
1. An awareness of those conditions likely to lead to deficiency states.
2. A knowledge of the clinical manifestations produced by the deficiencies.
3. An adequate morphological study of the peripheral blood and bone marrow.

The presence, severity and precise nature of the deficiency can usually be determined by microbiological assays of vitamin B12 and folate in blood and tissues, by a study of the excretion of abnormal metabolites, and by the study of therapeutic responses. Other investigations may be necessary to elucidate the precise aetiology of the deficiency.

Acknowledgments
The assistance of Mr. V. Giri and Mr. S. D. Siganori in the preparation of the figures is gratefully acknowledged.

References


Recognition of Vitamin B₁₂ and Folate Deficiency—Baker December, 1966


