Nutrition and Anaemia

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Introduction

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Introduction

The state of an individual with regard to any particular haemopoietic nutrient may be conveniently divided into five arbitrary stages represented diagrammatically in figure 1. Healthy subjects living on a good diet will have "normal stores" of nutrients which, even in the complete absence of dietary intake, will be sufficient to meet the needs of the body for a period of time (this time depends both on the nutrient and the size of the store). "Excess stores" may be defined as the accumulation of a nutrient to such an extent as to cause biochemical and/or clinical abnormality. This only occurs with iron since there is no known ill effect of large amounts of folate or vitamin B₁₂.

"Stage I deficiency" produces no clinical signs and no biochemical abnormalities, but the ability of the individual to meet increased demands, or to withstand nutrient deprivation, is decreased below that of healthy well fed individuals. As the deficiency becomes more marked serum concentrations of the nutrient fall below the normal range. This may be termed "stage II deficiency". At this stage biochemical abnormalities and clinical symptoms and signs may develop before anaemia can be detected. Finally, with more severe and prolonged deficiency, the erythropoietic tissue is unable to maintain a normal haemoglobin concentration and anaemia develops—this may be termed "stage III deficiency". Nutritional anaemia is thus seen to be the end result of a deficiency, of increasing severity, of one or more haemopoietic nutrients.

The precise definition of "normal" as applied to nutrient stores, serum concentration of nutrients, or haemoglobin concentration, presents considerable difficulties. In a community of healthy well fed individuals measurements of any of these parameters will give a range of values which can be plotted as a frequency distribution curve. Such a curve may have a normal, log-normal or skew distribution (1). When deficiencies are prevalent in a community such frequency distribution curves will be shifted to the left, but there will be no single value which will distinguish all normal from all deficient individuals. This may be illustrated by reference to haemoglobin concentrations. In each
individual there is a homeostatic mechanism which controls the haemoglobin concentration. If the individual is free of any disease and has adequate nutrition, this haemoglobin concentration may be taken as "normal" for that person and

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**Figure 1.** Diagrammatic representation of body stores of haematopoietic nutrients. Excess stores—biochemical and/or clinical abnormalities present; stage I deficiency—stores reduced but no biochemical or clinical abnormalities; stage II deficiency—biochemical and/or clinical abnormalities present but no anaemia; stage III deficiency—anaemia.
any fall below this as “anaemia”. The normal haemoglobin values of individuals, in a healthy population, when plotted as a frequency distribution will form a normal distribution about a mean (curve I, figure 2). In a population where anaemia is universal the frequency distribution curve of haemoglobin values will be shifted to the left as indicated by curve II, figure 2. In actual practice, in most communities, there is likely to be a mixture of anaemic and non-anaemic subjects. So that the frequency distribution curve of haemoglobin concentration will be compounded of one or more populations (curve III, figure 2). In such a community there will be no single haemoglobin concentration which will completely separate “anaemic” from non-anaemic individuals (2, 3). Ideally, therefore, the haematologic status of communities should be defined by means of the frequency distribution of haemoglobin concentrations. However, from the clinical point of view, it is desirable to define some arbitrary haemoglobin concentration below which anaemia may be considered to exist. The values recommended by the World Health Organization (4) for different groups living at sea level are shown in table 1. In subjects resident at higher altitudes these values should be increased (5). Statistical considerations similar to the above apply to all other measurements such as serum and tissue iron, serum iron binding capacity, serum, red cell and tissue folate and serum and tissue vitamin B₁₂ concentrations. In no case can any figure separate all normal from all abnormal concentrations, but for the sake of convenience an arbitrary dividing line between “normal” and “abnormal” is adopted.
### Table 1

*Haemoglobin concentration below which anaemia is likely to be present, in subjects living at sea level, as recommended by the World Health Organization (4).*

<table>
<thead>
<tr>
<th></th>
<th>g/100 ml</th>
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<tbody>
<tr>
<td>Children 6 months to 6 years</td>
<td>11</td>
</tr>
<tr>
<td>Children 6—14 years</td>
<td>12</td>
</tr>
<tr>
<td>Adult males</td>
<td>13</td>
</tr>
<tr>
<td>Adult females, non-pregnant</td>
<td>12</td>
</tr>
<tr>
<td>Adult females, pregnant</td>
<td>11</td>
</tr>
</tbody>
</table>

A number of substances are necessary for normal haemopoiesis, but the following discussion is confined to the ones of major clinical and public health importance, namely iron, folate and vitamin B₁₂.

**Iron**

**Occurrence**

Iron is an essential component of haemoglobin and also of myoglobin and iron containing enzymes. It is widely prevalent in animal and vegetable foods, largely in organic form. The proportion of dietary iron which can be absorbed (the bioavailability) varies widely according to the type of food. In general, the bioavailability is greatest from foods of animal origin and least in plant foods (6, 7).

**Requirements**

Iron is continually lost from the body in urine, faeces, sweat and desquamation of the skin. Long term studies of the loss of radio-iron from the body show that the basal losses in men are about 1.0 mg/day (8). In women, due to their smaller body size, the basal losses, exclusive of menstrual losses, are of the order of 0.8 mg/day. The average menstrual blood loss is about 43 ml per period. Spread over the whole cycle, this represents an iron loss of about 0.5 mg/day. To cover the losses of 95% of normal women an allowance of 2.0 mg/day for menstruation alone or 2.8 mg/day for total losses is needed (9). During pregnancy, the iron requirements are increased due both to the iron needed by the foetus and that needed by the mother on account of the increased maternal blood volume.
NUTRITION AND ANAEMIA

The daily iron intakes recommended by the World Health Organization (4) in different groups of individuals, and with different types of diets, are shown in table II.

**Table II**

*Daily iron intakes for different groups of individuals, according to their basic diet, as recommended by the World Health Organization (4).*

<table>
<thead>
<tr>
<th>Iron required (mg)</th>
<th>mg of Iron needed in diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Animal Foods less than 10% of calories</td>
</tr>
<tr>
<td>Infants 0-4 months</td>
<td>0.5</td>
</tr>
<tr>
<td>Infants 5-12 months</td>
<td>0.7</td>
</tr>
<tr>
<td>Children 1-12 years</td>
<td>1.0</td>
</tr>
<tr>
<td>Boys 13-16 years</td>
<td>1.8</td>
</tr>
<tr>
<td>Girls 13-16 years</td>
<td>2.4</td>
</tr>
<tr>
<td>Menstruating women</td>
<td>2.8</td>
</tr>
<tr>
<td>Men and post meno-pausal women</td>
<td>0.9</td>
</tr>
</tbody>
</table>

*Breast feeding assumed to be adequate.

**Stages of iron nutrition**

The iron stores in normal man have been quantitated both by repeated venesection (10) and by isotopic dilution techniques (11). It has been estimated that normal Western 70 Kg man has between 3 and 4 grams of iron in his body, of which about 1000-1500 mg can be considered as storage iron (12). Stores in Western females may be of the order of 800 mg (11). In clinical practice the best indication of the extent of the body iron stores is the amount of stainable iron in the bone marrow (or, though tissue is less readily available, in the liver). It has been shown that the best histological indicator of storage iron, in marrow
specimens, is the diffuse blue staining material seen in the macrophages in preparations stained with potassium ferrocyanide (13, 15). On the basis of the grading of Hausman and Kuse (15) subjects with “normal” iron stores will have ++ stainable marrow iron or more.

Excessive iron stores, seen classically in haemochromatosis, may affect all the organs of the body, but especially vulnerable are the liver, pancreas, heart and skin. The precise point of division between “normal” and excessive iron stores can be arbitrarily defined in a variety of ways, but the most commonly employed is a plasma iron concentration of above 200 µg/100 ml together with complete or almost complete saturation of transferrin (16, 17). Stainable iron may be found in tissues where it is not usually seen, such as cells desquamated from the urinary tract, and in skin, gastric and jejunal mucosal biopsies. Marrow and liver specimens will show large amounts of stainable iron, though the exact point of distinction between “high normal” and excessive amounts is impossible to define on histological grounds.

Stage I iron deficiency, which is equivalent to the “prelatent iron deficiency” of Heinrich (18), can be recognised by a reduction (less than ++ on the scale of Hausman and Kuse) or absence of stainable iron in the bone marrow (and liver) while the serum iron remains normal. Heinrich (18, 19) has shown that during stage I deficiency the intestinal absorption of a test dose of radioactive ferrous sulphate is increased. The mechanism by which this is brought about is not known.

As the iron stores are further depleted, stage II iron deficiency (equivalent to “latent iron deficiency” of Heinrich) is reached which is characterised by a reduction in serum iron concentration below 60 µg %, a fall in the percent saturation of transferrin and a total absence of stainable tissue iron. At this stage koilonychia may develop (20) and be the first clinical sign of iron deficiency. Dysphagia, often associated with a post-crioid web, has also been ascribed to iron deficiency, but the evidence in support of this is at best inconclusive (21). Angular stomatitis and glossitis are frequently considered to be manifestations of iron deficiency, but the explanation of these lesions is obscure (22) and in a study of 1000 pregnant women in southern India (23) there was no correlation between these lesions and indices of iron nutrition.

Stage III iron deficiency (“manifest deficiency” of Heinrich) is characterised by the additional presence of iron deficiency anaemia.

Pathogenesis

Excessive iron stores may result from a primary disorder of iron metabolism as in idiopathic haemochromatosis; from prolonged increased dietary intake as in the South African Bantu, or, rarely, from excessive medicinal intake or multiple repeated transfusions in subjects with chronic aplastic or haemolytic anaemia.

Deficiency of iron may arise from inadequate intake or decreased availability of iron in the diet, defective absorption, or excessive demands such as in chronic haemorrhage or in pregnancy, or a combination of any of these.

Inadequate intake of iron may occur in association with general malnutrition, as in Kwashiorcor (24) or food faddism. Most often, however, it occurs in subjects who otherwise appear to have adequate nutrition and as such is especially
prevalent in tropical areas. This is probably associated with the poor bioavailability of iron in the predominantly vegetarian diets of these areas. It has also been suggested that phosphates and phytates in the diet may interfere with iron absorption due to formation of insoluble complexes (25, 26, 27). However the situation is complex and experiments carried out, for example, sodium phytate and a test dose of iron, do not necessarily parallel the fate of food iron (29) and further studies are urgently needed.

Diffuse small intestinal disease, such as coeliac disease, has been shown to interfere with iron absorption (29). Tropical sprue and tropical enteropathy may also have a similar effect, though this has yet to be proved.

Chronic haemorrhage, such as that caused by hookworm infestation or menorrhagia, is a common cause of increased iron requirement, and in subjects living on a marginal iron intake, may precipitate severe iron deficiency. In pregnancy it has been estimated that the total extra amount of iron required is in the region of 400 mg (12). It is therefore not surprising that many unsupplemented pregnant women show some evidence of iron deficiency.

Prevalence

The prevalence of excessive iron stores is highest in the South African Bantu where it appears to be associated with a high iron content of the diet and iron contamination from cooking and fermentation vessels (30). In the West it has been estimated that idiopathic haemochromatosis is seen about once in 20,000 hospital admissions (17). In areas where there is a high prevalence of iron deficiency, subjects with excessive iron stores will be found only rarely. Thus in eighteen years’ consultant practice in a referral hospital (20,000 admissions per year) the author has seen only one Indian with excessive iron stores and that was due to idiopathic haemochromatosis.

Iron deficiency is the most prevalent of all nutritional deficiencies and occurs on a global scale (31). Among countries for which data is available India appears to have the highest prevalence. A collaborative study of the concentration of nonhaem iron in liver samples from different parts of the world showed lowest values in samples from India followed closely by those from New Guinea (32). In a smaller series Routhe and Agarwal (33), in Delhi, found extremely low levels of nonhaem iron in both the liver and the spleen of subjects dying from acute trauma. Comparative histochemical study also showed that livers from subjects in India had less stainable iron than any other population group studied (34). In Vellore, India, studies of 1000 unsupplemented pregnant women at term showed stainable iron in the bone marrow in only 2% of 740, indicating the virtual absence of iron stores (35). 97% of all the women had serum iron levels below 60 μg % (mean 31 μg) and were therefore at least in stage II deficiency. 57% were anaemic (haemoglobin below 11 g per 100 ml). In so far as this anaemia was largely due to iron deficiency these women were in stage III deficiency. In another study of a stratified random population sample in a village community 20 miles from Vellore, the prevalence of stage II deficiency varied from 19–93% in different groups (35). This indicates that, even apart from pregnancy, there is a very high prevalence of iron deficiency in this part of India.

367
The precise reasons for this high prevalence are not clear. The mean daily intake of iron provided by the average Indian diet has been variously estimated at between 15 and 30 mg per day (36, 37). In the above mentioned villages the intake of iron was calculated by measuring the diet as eaten, by the individual weight method, and directly determining the iron content of the various foods by chemical analysis. The mean daily iron intake for adult males was 29 mg and for females 27 mg. However the diets are almost entirely vegetarian and therefore there will be relatively poor absorption of this dietary iron. Iron losses from chronic hookworm infestation may aggravate the iron deficiency, but iron deficiency is common even in the absence of hookworm infestation (23). It is used to be considered that iron losses in sweat were an important contributory cause of iron deficiency in the tropics, but studies of body iron losses with Fe\textsuperscript{59} labelled iron have shown that subjects working in hot humid environments have losses of the same order as people in temperate climates (8).

Significance

In the past numerous symptoms such as easy fatigueability, palpitations, anorexia, headache and irritability have been ascribed to stage I and II iron deficiency (without anaemia). However controlled studies (38, 39, 40) indicate that these symptoms are in fact unrelated to the state of iron nutrition.

There is nevertheless a little evidence that stage I and II iron deficiency may have some effect on health, apart from the increased liability to develop iron deficiency anaemia. Ericson (41), in a double blind trial of apparently healthy elderly subjects (aged 58-71) found that oral supplementation with 120 mg of iron per day for three months resulted in an increase in physical work capacity as measured by performance on a bicycle ergometer, but was without measurable effect on haemoglobin concentration. This study seems to suggest that work capacity may be influenced by the amount of available storage iron in the body.

In a ten year follow-up of a group of subjects, Waters et al (42) found higher death rates in those having a low initial serum iron concentration. The authors, however, are careful to point out that the two are not necessarily causally related—a hypothesis which could only be tested by a prolonged double blind trial with iron and placebo supplementation.

In view of the high prevalence of iron deficiency, further studies to assess its effects on factors such as work capacity, growth and development, morbidity and mortality are urgently needed. If further studies confirm the adverse effects, it will have to be decided, especially in those communities where there is a very high prevalence of deficiency, whether the effects are sufficiently serious to warrant corrective action as a public health measure.

Folate

Occurrence

Folate is the generic term given to compounds of pteroyl acid with glutamic acid. Folic acid, as employed therapeutically, is pteroylmonoglutamic acid. In the body there are a number of different forms of folate necessary for inter-
mediary metabolism. The present knowledge of the major metabolic functions of folate has been well reviewed by Stockstad and Koch (43).

Folates are widely present in foods such as meat, dairy products, vegetables, fruits and cereals. The folates in food are heat labile and tend to be destroyed by prolonged cooking. In most foods the majority of the folate is present in the form of polyglutamates (44). During the process of absorption, which occurs in the upper small intestine, these polyglutamates are probably largely converted to the monoglutamate form by the action of conjugase (gamma-glutamyl peptidase) in the intestinal cells (45).

Bacteria isolated from the small intestine of some individuals have been shown to be capable of synthesising folate (46) but what contribution, if any, such folate makes to the body’s requirements is not clear.

Requirements

The precise availability of different forms of food folate is not yet known. Herbert (47) maintained adults on a diet containing only about 5 µg of assayable folate (L. casei assay, with previous conjugase treatment) and demonstrated that, with a 50—100 µg supplement of pteroylglutamic acid, serum folate levels fell but remained above the lower limit of normal. On the basis of this study Herbert recommended a minimal daily intake in the region of 50 µg of pteroylglutamic acid for adult females. However the experiment was only carried out for 6 weeks and if continued longer, a bigger supplement may have been needed to maintain serum levels in the normal range.

The intakes recommended by the World Health Organization (4) are shown in table III expressed as total dietary folate—i.e., including that which is “free”—available to L. casei and that which is “bound” or unavailable to L. casei—i.e., the polyglutamate forms. The apparent big discrepancy between these recommendations and Herbert’s is partly accounted for by the uncertainty of our knowledge regarding the availability of the different forms of dietary folate. Clearly more information is needed on this subject. It seems probable that the minimal adult daily requirement will be shown to be somewhere between these two extremes.

Stages of folate nutrition

There is no evidence to suggest that large amounts of folate are harmful, or indeed can accumulate, so that there is no state comparable with the excess stores of iron.

The normal body stores of folate are difficult to quantitate. Some studies have been done on the folate content of liver specimens obtained either by biopsy or at post mortem. These studies have been summarised by Chanarin (48). In subjects with normal serum folate and normal FIGLU tests the hepatic folate ranged from 4 to 10 µg/gram. However these studies were done on highly selected populations and may not reflect the normal hepatic folate in the community.

Stage I folate deficiency has not been well documented. The factors which govern the distribution of folate between tissues and serum are not understood. Several studies have been undertaken in rats. Grossowicz et al (49) found that when rats were put on a folate deficient diet, folate concentrations in liver and
kidney fell before those in the blood, but they do not report on serum levels. Spector and Metz (50) fed rats on a maize diet which was low in both folate and vitamin B₁₂. With this they found that within 6 weeks hepatic folate was greatly reduced (30% of normal) but serum folate remained unchanged or rose. However, when vitamin B₁₂ supplements were given with the maize diet the reverse occurred—liver folate concentration was unchanged or only slightly reduced, but serum levels fell by about 50%. Ch samen et al (51) found that in rats on a folate deficient diet, liver folate declined much more rapidly than the serum folate, but there is no record of the vitamin B₁₂ status of the animals.

In man, when tritiated folate is injected there is an increased excretion of 5-methyltetrahydrofolate in the urine which is not labelled. This has been interpreted as demonstrating a fairly rapid exchange between the circulating labelled folate, derived from the injection, and the unlabelled tissue folate (52). Nevertheless Chanarin (48) reports a man with a very low hepatic folate concentration without other evidence of folate deficiency i.e., stage I deficiency where the serum folate was apparently not in equilibrium with the tissue folate. In the experiment conducted by Herbert (39) when dietary folate supplies were reduced to about 5 μg/day, the serum folate concentration began to fall within the first week and reached low levels within 2 weeks. The red cell folate concentration fell sometime after the serum levels, but folate probably does not enter or leave the mature red cells, so when folate depletion/repletion is occurring, red cell folate will not be a good index of body stores. Whether or not the concentration in the other tissues fell detectably before the fall in serum concentration occurred is not known. So in this experiment stage I deficiency, if it occurred at all, presumably only lasted for a few days.

The normal range of serum folate concentration depends to some extent on the assay and the laboratory performing the assay. The criteria recommended

<table>
<thead>
<tr>
<th>Micrograms total folate</th>
<th>Infants 0-6 months</th>
<th>Infants 7-12 months</th>
<th>Children 1-12 years</th>
<th>Men and women</th>
<th>Pregnant women</th>
<th>Lactating women</th>
</tr>
</thead>
<tbody>
<tr>
<td>40-50</td>
<td>120</td>
<td>200</td>
<td>400</td>
<td>800</td>
<td>600</td>
<td></td>
</tr>
</tbody>
</table>

TRENDS IN HEMATOLOGY

Table III

Daily folate intake for different groups of individuals as recommended by the World Health Organisation (4).

370
by the World Health Organization (31) for the interpretation of serum folate assays using L. casei as the test organism are as follows: 6-20 ng/ml, normal range; 3-5.9 ng/ml, probable deficiency and less than 3 ng/ml, deficiency. This suggests that a concentration below 6 ng/ml can be taken as the dividing line between stage I and stage II deficiency. In stage II deficiency, as the severity of the deficiency increases there will be an increased excretion of formiminoglutamic acid in the urine (54), hypersegmentation of the polymorphs will occur, liver and red cell folate concentrations will fall and ultimately the developing red cells in the marrow will show recognisable megaloblastic change before the onset of anaemia. In this stage glossitis, hyperpigmentation of the skin (55) and minor alterations in the morphology of the jejunal mucosa may also occur (30, 57).

Symptoms of stage II deficiency are not well defined. Sleeplessness, irritability, forgetfulness and depression are said to occur in acute stage II folate deficiency (53, 58) but a double blind trial is needed to substantiate this clinical impression. In those with more chronic folate deficiency such symptoms are not usually present.

Finally, if the deficiency persists stage III deficiency is reached as anaemia develops. In a healthy volunteer living on about 5 μg of L. casei assayable food folate, the change from presumably normal stores to stage III deficiency took 19 weeks to develop (53).

Pathogenesis

Deficient intake of folate is most often associated with artificial feeding, poverty, food fadism or excessive loss of folate due to prolonged cooking. Processed weaning foods, unless supplemented, are often low in folate. Goat's milk is also low in folate and infants fed exclusively on this milk are particularly liable to develop a folate deficient megaloblastic anaemia which has been termed "goat's milk anaemia" (59). Children suffering from protein calorie malnutrition frequently have a megaloblastic anaemia, due to concomitant dietary folate deficiency (24).

Defective absorption of folate may occur, very rarely, as a congenital defect (90) and may also develop after extensive intestinal resection (61, 62). More commonly defective absorption is the result of diffuse intestinal disease, such as coeliac disease (63) or tropical sprue (64, 65, 66). The precise way in which these later two diseases interfere with folate absorption is not clear. It may be that it is at the stage of the conversion of the polyglutamates in the food to the monoglutamate form (45) but this has not yet been demonstrated. Other factors such as decreased appetite and increased faecal losses also contribute to the low serum folate of tropical sprue (67, 68). For a fuller discussion of folate deficiency in tropical sprue see Wellcome Trust Volume (68).

Increased requirements for folate occur during periods of rapid growth e.g., in premature infants (69) and during pregnancy and lactation (70). Other conditions associated with increased folate requirements are haemolytic anaemias, skin diseases, infections and neoplastic diseases.

Folate responsive megaloblastic anaemia may occur in patients receiving anticonvulsant drugs such as diphenylhydantoin, primidone and barbiturates (71) but the precise mechanism involved has not been fully elucidated. The anti-
malarial drug, pyrimehtamine, is a folate antagonist, but in the usually employed doses, does not lead to clinically evident folate deficiency (72).

Prevalence

Accurate data on the prevalence of folate deficiency in the community is even less than that relating to the prevalence of iron deficiency. What little information is available suggests that although not as common as iron deficiency, it is also global in its occurrence (31).

The commonest cause of folate deficiency in any community is undoubtedly pregnancy and lactation. The serum and red cell folate concentrations of all unsupplemented pregnant women tend to fall as pregnancy advances (73). The reported prevalence of folate deficiency among hospitalised pregnant women, as measured by serum and red cell folate levels, and the prevalence of megaloblastic changes in the bone marrow, varies widely. Thus the prevalence of serum folate below 6 ng/ml in pregnant women at term ranges from 16% in Poland (31) to 95% in England (73). Megaloblastic change in the developing red cells, due to folate deficiency, has been reported to occur in from 3% (74) to 75% (75) of all unsupplemented pregnant women at term.

In Latin America 60% of pregnant women had a serum folate of below 6 ng/ml (2). In southern India, in a hospitalised population, 60% of unsupplemented pregnant women at term had a megaloblastic bone marrow and 73% had a serum folate of less than 6 ng/ml (23). These prevalence figures from developing countries are lower than some of those from England. Available evidence therefore suggests that a high prevalence of folate deficiency in pregnancy is a universal phenomenon and not one related simply to the economically underprivileged (67).

In some areas of the tropics where tropical sprue is prevalent this may be a common cause of folate deficiency but in other areas it does not appear to cause folate deficiency. In Singapore (66), of 45 expatriates with tropical sprue, all had low serum folate (<6 ng/ml) and 90% had concentrations below 3 ng/ml. In southern India (64), over a 7 year period, out of 259 patients with tropical sprue studied in hospital, 71% had a serum folate concentration below 6 ng/ml and 90% below 3 ng/ml. During the same period, apart from pregnancy, only 10 cases of folate deficiency apparently due to defective folate intake were seen. On the other hand, in Haiti (76), folate deficiency is uncommon even in subjects with sprue.

Significance

The significance of stage I and II folate deficiency has not been widely studied. Premature children run a greater risk of developing folate deficiency than full term children (69). Berland et al (71) studied two groups of low birthweight infants (<300 g) one untreated and one given supplementary folic acid (i.m. injection 100 µg x 14 for the first month of life). The supplemented group showed less fall in serum and red cell folate during the first nine months of life, and the unsupplemented group showed an increased incidence of hypersegmented polymorphs. There was however no other significant difference between the two groups, either in haemoglobin or other haematological parameters, or in growth and development. In this group of infants there would therefore seem to have
been no valid reason for advising folate supplementation. The infants were initially in stage I deficiency and the unsupplemented group moved into stage II deficiency but this did not affect any other parameters of growth or development. It is possible that if a group of low birthweight infants born to mothers with a lower folate intake had been studied, the results may have been different and such a study needs to be carried out.

Experiments to prevent the occurrence of stage I and II deficiency during pregnancy have been carried out by several investigators. Willoughby and Jewell (78) found that a daily oral supplement of 330 μg of folic acid prevented the fall in whole blood folate which occurred in normal unsupplemented women during pregnancy. The same results were obtained by Hansen and Rybo (79) and Chanarin et al. (80) using a supplement of 100 μg. These results are in contrast to those reported by Lowenstein et al. (81) who gave pregnant women 500 μg daily after the 29th week and found that 18% still had subnormal serum and/or erythrocyte folate concentrations at the end of the 38th week and three out of 112 patients had megaloblastic changes in the bone marrow. It is possible that the dietary folate intake of the women studied by Lowenstein et al. was less than that of the women in the other studies. However, even when supplementation reduces the prevalence of low-serum folate concentrations, the beneficial effects, if any, on maternal and child health have yet to be conclusively established. Claims have been made that folate deficiency in pregnancy may be associated with an increased prevalence of a variety of obstetric conditions such as abruptio placentae, abortion, foetal malformation, still birth, neonatal death, low birth weight, prematurity, toxaemia and postpartum haemorrhage. Nevertheless most of these relationships are not adequately established (82) and a reduction in their prevalence following folate supplementation has not been demonstrated. Baumslag et al. (83) showed that Bantu women given iron and folic acid during pregnancy had children of higher birthweight than those given iron alone. However it is not clear whether this is a direct or indirect effect. Yusufi et al. (28) found a relationship between maternal haemoglobin and birthweight, but no relationship between serum folate concentration and birthweight and Baumslag et al. do not appear to have taken maternal haemoglobin concentrations into account.

In many parts of the world folate supplements are given routinely to pregnant women. This may be reasonable, even if unnecessary, where cost is no problem. But in developing countries where health budgets are limited, it is essential firstly to determine the full extent of morbidity arising from folate deficiency and secondly the cost and practicability of eliminating this deficiency. Only then can rational decisions be made regarding the desirability or otherwise of supplementation programmes.

Vitamin B₁₂

Occurrence

Vitamin B₁₂ consists basically of a cobalt containing corrin ring attached to a nucleotide. The common pharmaceutical products are cyanocobalamin or hydroxocobalamin in which a—GN or—OH group respectively are bound to
the cobalt atom. The naturally occurring forms are chiefly coenzymes in which a deoxyadenosyl or methyl group is attached to the cobalt atom. The metabolic functions of vitamin B₁₂ are not fully understood. Present knowledge in this area is well reviewed by Barker (84). The haematological effects of deficiency are most probably due to a block in the folate cycle, since vitamin B₁₂ coenzyme is necessary for the conversion of N₅ methyl-tetrahydrofolate to tetrahydrofolate. The biochemical basis of the neurological defects associated with vitamin B₁₂ deficiency are not at all understood.

All naturally occurring vitamin B₁₂ is ultimately of bacterial origin. Ruminant animals have bacteria in their rumen which can manufacture vitamin B₁₂ provided the diet contains cobalt. This vitamin is then passed on to the stomach and distal small intestine where it is absorbed and finally it is stored in the tissues. The bacteria in the large intestine produce considerable amounts of the vitamin, but this is unavailable to the host because little or no absorption of the vitamin can occur from the large intestine. Many animals nevertheless make use of this material by practising coprophagia. Man, having rejected the practice of coprophagia, has to obtain his vitamin B₁₂ largely from animal sources such as meat, milk, eggs and fish. Plant foods often contain some assayable vitamin B₁₂, but this presumably represents contamination from soil bacteria. Similar contamination of drinking water may occur, especially when the source is an unprotected tank or well. The average south Indian villager lives on a largely vegetarian diet and eats little or no animal protein, yet the daily intake of vitamin B₁₂ as measured by Euglena gracilis assay is of the order of 0.4 µg per day, which comes largely in association with rice, pulses, vegetables and drinking water (85). This presumably represents bacterial contamination of these commodities and may be a refined form of coprophagia. Unlike folic acid, vitamin B₁₂ is relatively heat stable and little is lost in cooking.

The ingested vitamin is freed from the food by the digestive processes, and combines with intrinsic factor, which is secreted by the parietal cells of the stomach (86). The intrinsic factor—vitamin B₁₂ complex passes distally and is taken up by the brush borders of the ileal cells. The vitamin then passes through the cell and enters the portal blood stream, where it is bound to a transport protein, transcobalamin II (87), and finally it enters the body stores.

Requirements

The daily requirements of the vitamin are not known with accuracy. It appears that the 0.3–0.4 µg present in vegetarian diets is enough to maintain health of adults (85) though perhaps not enough to maintain stores at optimal levels. The intakes recommended by the World Health Organization are shown in table IV. It should be noted however, that these intakes are virtually impossible to achieve on an unsupplemented vegetarian diet, yet since most vegetarians maintain adequate health, these recommended intakes are clearly higher than necessary.

Stages of B₁₂ nutrition

As with folate, large amounts of vitamin B₁₂ do not produce any harmful effects, there is therefore no question of there being excess stores. It might be
TABLE IV

<table>
<thead>
<tr>
<th></th>
<th>Micrograms vitamin B₁₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants 0-12 months</td>
<td>0.3</td>
</tr>
<tr>
<td>Children 1-3 years</td>
<td>0.9</td>
</tr>
<tr>
<td>Children 4-9 years</td>
<td>1.5</td>
</tr>
<tr>
<td>Children over 10 and adults</td>
<td>2.0</td>
</tr>
<tr>
<td>Pregnant women</td>
<td>3.0</td>
</tr>
<tr>
<td>Lactating women</td>
<td>2.5</td>
</tr>
</tbody>
</table>

assumed that it is advantageous to have as large a store of the vitamin as possible, because the larger the store, the longer the body can withstand periods of vitamin B₁₂ deprivation. On the other hand, it has been shown that the larger the stores the greater the daily losses of the vitamin (14), so there is to some extent a built-in regulatory mechanism. Attempts have been made to quantitate the stores of vitamin B₁₂ both by estimating the content of tissues, obtained ante-mortem or post-mortem, by microbiological assay and also, by radiisotopic dilution techniques. Non-vegetarian Western man, who lives on a diet rich in animal protein, may consume in the region of 30 µg of the vitamin per day (88). Such individuals have been estimated to have total body stores in the region of 2 mg (range 0-95 to 4.3) (89) to 5 mg (range 3.5—10.9) (90). The vegetarian, who lives on a vitamin B₁₂ intake of less than one microgram per day, may be assumed to have smaller body stores, however there are no published estimates of the total body vitamin B₁₂ of such individuals.

It has been estimated that the liver contains some 66—70% of the total body store (91, 92). Further, since liver tissue is fairly readily available for assay, the concentration of the vitamin in the liver has often been employed as an index of body stores. Mean values for liver vitamin B₁₂ in Western non-vegetarian subjects range from 700-2100 ng/g wet weight of liver (48). On the other hand in a series of 96 south Indian subjects, dying after non haematological illnesses of less than one month's duration, the mean liver vitamin B₁₂ was 425 ng/g wet weight (range 46-1200 ng/g) (93). This suggests that south Indians have lower body stores than their Western counterparts. If the latter are taken as having normal stores then, the majority of southern Indian subjects must be considered to be in stage I deficiency or more.

The normal range of serum vitamin B₁₂ concentration depends to some extent on the method of assay and on the laboratory concerned. Using Euglena
assay it has been suggested that concentrations below 80 pg/ml be considered as indicating definite deficiency and below 140 pg/ml as indicating probable deficiency (94, 31). The figure of 140 pg/ml of serum would thus appear to be a convenient dividing line between stage I and II deficiency. When for any reason vitamin B₁₂ supplies to the body are interrupted, the rapidity with which stage II deficiency is reached will depend on the extent of the body stores.

In Caucasians in Singapore, who developed vitamin B₁₂ malabsorption due to tropical sprue, serum vitamin B₁₂ concentrations remained normal during the first two to three months of illness (66), whereas in southern India low levels were found in 25% of patients during the first month of illness (64).

In stage II deficiency biochemical abnormalities may also develop. These include increased excretion of aminomimidazole carboxamide in the urine (95), a positive FIGLU test (96) and an increased excretion of methylmalonic acid in the urine (97). Hyperpigmentation of the skin and mucousae may also occur (98). In the latter part of this stage changes may be found in the peripheral blood such as macrocytosis of the red cells and hypersegmentation of the polymorphs. In the bone marrow alterations may also be seen in the morphology of the developing red cells and white cells before there is any fall in haemoglobin concentration.

The nervous system appears to be particularly vulnerable to vitamin B₁₂ deficiency, although the precise metabolic role of the vitamin in maintaining the integrity of the nervous system is not understood. Clinically apparent nervous system involvement is usually a sign of severe vitamin deficiency. This may occur in the absence of anaemia (especially if there is a high intake of folate) but usually it is associated with a greater or lesser degree of anaemia i.e. it is most often a manifestation of stage III deficiency. Several major vitamin B₁₂ deficiency neurological syndromes may be recognised. Infants with vitamin B₁₂ deficiency show apathy, developmental retardation or regression, pyramidal tract involvement, extrapyramidal lesions with tremors and involuntary movements and extensive cortical changes as shown by electroencephalographic abnormalities (99).

In adults the earliest neurological lesion may be a peripheral neuritis due to demyelination of the nerve fibres (100). The most widely recognised and pathognomonic lesions is subacute combined degeneration of the spinal cord. An excellent description of this disorder is given by Wilson (101). It is a popular misconception that subacute combined degeneration of the cord occurs only in patients with pernicious anaemia. It can in fact occur in any condition producing severe vitamin B₁₂ deficiency. Depression and even psychosis may occur with or without signs of cord involvement (102, 103) and finally vitamin B₁₂ deficiency may be associated with retrobulbar neuritis and optic atrophy (104, 105).

Pathogenesis

Dietary deficiency of vitamin B₁₂ may occur at any age. Infants born of vitamin B₁₂ deficient mothers start life with low body stores and, while being breast fed by the same mother, will not have an opportunity to make up for the antenatal deficiency (106). Such children start life with stage I or II deficiency and develop stage III deficiency between the 8th and 18th month of life (99). In older individuals in the West, dietary deficiency of vitamin B₁₂ may occur in strict vegetarians who eat no animal products at all. In India, pure dietary
deficiency of vitamin B₁₂ in older individuals is uncommon, since even the vegetarian diet still contains enough available vitamin to meet the body’s requirements. As already pointed out, this is probably due to contamination of food and water supply. If such subjects move to a more protected environment this intake of vitamin B₁₂ from their traditional diet may be greatly reduced and frank deficiency may develop.

Numerous conditions can interfere with the absorption of vitamin B₁₂. Disease of the stomach may lead to failure of intrinsic factor secretion and of vitamin B₁₂ absorption. The classical situation in which this occurs is pernicious anaemia (107) in which there is a genetically determined atrophy of the gastric mucosa. Other disorders, such as total gastrectomy (108), partial gastrectomy (109, 110), chronic gastritis (111, 112), tropical sprue (113), Diphyllobothrium latum (fish tape worm) infestation (114) and diverticulosis (115) may also produce, or be associated with, intrinsic factor deficiency.

The fish tape worm, in addition to producing a gastritis, causes interference with vitamin B₁₂ absorption by preferentially absorbing and retaining the dietary vitamin B₁₂, leaving insufficient amount to be passed on to the ileum for absorption by the host (116).

Rarely, hereditary vitamin B₁₂ malabsorption may occur. This usually presents as a relapsing megaloblastic anaemia with defective absorption of the vitamin which is not corrected by intrinsic factor administration (117, 118). Since ileal mucosal homogenates of subjects with this condition take up vitamin B₁₂ normally, the defect would appear to be in the intestinal cells (119).

Resection of the ileum removes the vitamin B₁₂ absorbing area of the intestine and, if extensive enough, will result in complete failure of vitamin B₁₂ absorption (120, 121). Diffuse disease of the small intestine such as coeliac disease (122, 123) and tropical sprue (114, 115) may also result in failure of vitamin B₁₂ absorption. Conditions such as short circuits, strictures (125) and diverticula (126) of the small intestine are frequently associated with vitamin B₁₂ malabsorption. This malabsorption is closely related to the stagnation of intestinal contents and the subsequent bacterial overgrowth and can be corrected, at least temporarily, by antibiotic administration (127).

Para-aminosalicylic acid (PAS) administration has been reported to interfere with vitamin B₁₂ absorption in some subjects (128) but this does not appear to be an important cause of vitamin B₁₂ deficiency, as patients on long term PAS therapy do not show a decline in serum vitamin B₁₂ concentrations (129).

Patients with chronic pancreatitis may have malabsorption of vitamin B₁₂ (121, 190). However, this does not occur in all cases of chronic pancreatitis (131) and there are no recorded cases of B₁₂ deficiency anaemia due to pancreatic disease.

Increased demands for vitamin B₁₂ occur during pregnancy and in certain disease states such as thyrotoxicosis (132, 133). However, owing to the extent of the body stores, these seldom persist for long enough to cause overt deficiency.

Prevalence

There is only scanty data on the prevalence of vitamin B₁₂ deficiency in the community. A number of studies of pregnant women have shown that serum levels fall, especially during the last trimester (134, 135, 136). This fall occurs even
in well nourished women with a high vitamin B12 intake and may not necessarily indicate a deficiency of the vitamin. Nevertheless, comparative studies of serum B12 concentrations in the last trimester of pregnancy in different countries (31), indicate a higher prevalence of low serum concentrations in pregnancy in those areas where the diet is predominantly vegetarian (table V). Presumably this difference is a reflection of the body stores in the different areas. A study among blood donors in South Africa (137) also showed a much higher prevalence of low serum levels among the “coloured” group who had a poor dietary intake and among the Indians who were largely vegetarian. However these, and most other published studies, are based on selected populations and do not necessarily reflect the status of the community as a whole.

Table V

Distribution of serum vitamin B12 concentrations in women in the last trimester of pregnancy (31).

<table>
<thead>
<tr>
<th>Percent of cases with serum vitamin B12 levels</th>
<th>&lt; 80</th>
<th>80-139</th>
<th>140-199</th>
<th>&gt; 200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Israel</td>
<td>2</td>
<td>6</td>
<td>36</td>
<td>56</td>
</tr>
<tr>
<td>Poland</td>
<td>1</td>
<td>7</td>
<td>21</td>
<td>71</td>
</tr>
<tr>
<td>India (Delhi)</td>
<td>49</td>
<td>31</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>India (Vellore)</td>
<td>0</td>
<td>49</td>
<td>40</td>
<td>11</td>
</tr>
<tr>
<td>Mexico</td>
<td>7</td>
<td>8</td>
<td>4</td>
<td>81</td>
</tr>
<tr>
<td>Venezuela</td>
<td>23</td>
<td>20</td>
<td>23</td>
<td>34</td>
</tr>
</tbody>
</table>

Kilpatrick and Withcy (138) conducted a survey of serum vitamin B12 concentrations in two communities in Wales. There was an overall decline in serum vitamin B12 concentration with age. Only 0.5% had a serum vitamin B12 level below 100 and most of these were in the age groups of 65 or more. In an epidemiological survey in Coventry, England, and in Wales, among subjects aged 65 years or more, Elwood et al (139) found low serum concentrations of vitamin B12 (below 150 pg/ml) in 4 and 6% of English men and women (non pregnant), 15% of Welsh men and women and 40 and 32% of Asian immigrant men and women. In a study in a southern Indian village community (35) the prevalence of serum B12 concentrations below 140 pg/ml was 10 and 13% among adult males and females (non pregnant) respectively and in Latin America the corresponding figures were approximately 9% and 7% (2).

Significance

The effects of stage I and stage II deficiencies of vitamin B12 have received little attention. In a study of 1000 pregnant women in the third trimester of
pregnancy, Yusufji et al (23) could find no evidence that low serum vitamin B₁₂ levels had any influence on maternal haemoglobin or foetal birthweight. The fact that mothers with vitamin B₁₂ deficiency may give birth to vitamin B₁₂ deficient infants with resultant infant morbidity and mortality has been documented (106). However it is not clear whether the risk of this occurring is sufficiently great to recommend the routine supplementation of pregnant vegetarian women on a public health scale.

The growth pattern of Indian village children is below that of Western children, but it is doubtful whether their low daily intake of vitamin B₁₂ (about 0.1 μg) is in any way a limiting factor. Similarly the adults who consume about 0.4 μg of the vitamin per day do not appear to suffer any adverse effects, apart from the more rapid development of stage III deficiency when absorption is interfered with or requirements are increased.

In the study of Kilpatrick and Withey (138), 27 subjects with serum vitamin B₁₂ concentrations below 100 pg/ml were followed for 3 years. In the majority there was no change in their B₁₂ concentration or haematological status. Two subjects however developed stage III deficiency during this period and were diagnosed as suffering from pernicious anaemia.

In a population study in South Wales (42), eight subjects with grade II deficiency, with serum vitamin B₁₂ concentration persistently below 100 pg/ml, remained in apparently good health and maintained a normal haemoglobin level over a ten year follow up period. In the same study no correlation was found between initial serum vitamin B₁₂ concentration and mortality trends over the subsequent ten years. Hughes et al (140) in a double blind short term trial, administered a placebo or vitamin B₁₂ 1000 μg by injection to 44 subjects aged 65 or more who had serum vitamin B₁₂ concentrations of less than 150 pg/ml. The group receiving the vitamin B₁₂ showed no advantage in the feeling of general well being as compared with the group receiving the placebo, suggesting that these low serum concentrations were not associated with any symptomatic disturbances. Unfortunately in both these studies no information is given regarding the folate status of these patients, so it is not possible to be sure whether these low serum vitamin B₁₂ concentrations really indicated low body stores of vitamin B₁₂ or were related to some degree of folate deficiency.

Although available evidence suggests that stage I or II deficiency of vitamin B₁₂ may have no adverse effects in the majority of people, a detailed long term double blind study of the effects of vitamin B₁₂ supplementation to a deficient population is desirable to confirm this.

Significance of Nutritional Anaemia

From the foregoing it is clear that the full significance of stage I and II deficiency of iron, folate or vitamin B₁₂ has yet to be established. It is usually assumed, and commonly taught, that stage III deficiency, i.e. the presence of nutritional anaemia, is disadvantageous and a normal haemoglobin concentration is optimal. There is in fact little evidence to support this assumption and some epidemiological evidence that moderate degrees of anaemia may be
advantageous (141, 142) and high haemoglobin concentrations deleterious (143, 144, 139).

Numerous symptoms have been attributed to the presence of anaemia, but controlled studies have failed to show any correlation between symptoms and even moderate anaemia with haemoglobin concentrations down to 8 g. per 100 ml (145, 146). Similarly, Elwood and Hughes (38) took a group of anemic women and treated them with placebo or iron tablets. These authors could demonstrate no beneficial effect of the rise in haemoglobin on symptomatology or psychomotor function as measured by a battery of acute tests.

The effect of haemoglobin concentration on work capacity has been studied by several groups. Ericsson (41) and Veller and Hermansen (147) could find no correlation between haemoglobin concentration and physical work capacity; however, their subjects were at most only mildly anaemic. Andersen and Barkve (148) with rather more anaemic subjects (lowest haemoglobin 7.7 g%), found a significant relationship between haemoglobin concentration and the rate of return of oxygen uptake after exercise to resting levels.

In pregnancy severe anaemia may increase the hazards of delivery by making the mother less able to withstand blood loss. Anaemic women (below 8 g) are more likely to have a premature delivery (149) and a significant correlation between foetal birth weight and maternal haemoglobin concentration has been demonstrated (23). Further, children born of anaemic mothers will have lower stores of haemopoietic nutrients (106, 150). Nevertheless, it has yet to be proven that prevention of anaemia in pregnancy provides significant benefit in terms of decreased morbidity or mortality of mother or child or improved growth and development of the infant.

In view of the high prevalence of nutritional anaemia and the limited budgets available for public health measures in developing countries it is essential to have more precise data on the detrimental effects, if any, of different grades of anaemia in the various segments of the population. When this is established, controlled trials of the best way of eliminating anaemia will have to be undertaken, and the effects of this measured in terms of morbidity and mortality. Only then will it be possible to determine whether the cost of public health action, to reduce the prevalence of nutritional anaemia, is justifiable in terms of the anticipated benefits.

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REFERENCES


390


TRENDS IN HAEMATOLOGY


382


383


304


