Nutritional Macrocytic Anemia

Bernard A. Cooper

Division of Hematology/ Medical Oncology, Royal Victoria Hospital, Montreal, Quebec, Canada; and Department of Medicine, McGill University, Montreal, Quebec, Canada

Most cases of nutritional macrocytic anemia are caused by megaloblastic change in the bone marrow, producing macrocytic erythrocytes. In rare situations, anemia with large erythrocytes may be secondary to other nutritional deficiencies, such as (a) hypothyroidism produced by iodine deficiency or (b) the macrocytosis associated with young erythrocytes (reticulocytosis) observed in premature infants deficient in tocopherol. The latter will not be considered further in this review. We shall equate nutritional macrocytic anemia with deficiency of folate, cobalamin (vitamin B₁₂), or sometimes other nutrients which induce significant biochemical or clinical abnormalities in affected individuals. In subjects with simultaneous deficiency of iron and these vitamins, the megaloblastic abnormalities and the large erythrocytes (macrocytosis) are usually masked by the deficiency of iron, and appear only when the iron deficiency is corrected.

FOLATE

All human cells require folate metabolism to form purines, convert deoxyuridylate to thymidylate, resynthesize methionine from homocysteine, and perform a variety of other reactions (Fig. 1) (1,2). Folate is a vitamin in humans and cannot be synthesized. It functions as a coenzyme in folate-dependent reactions, receiving and donating single-carbon fragments at several levels of oxidation. For many (but not all) of its reactions, folate must be polyglutamylated (Fig. 2), with its long hydrophilic tail increasing its affinity for enzymes requiring it (3–5). In cells which are deficient in folate, folate-dependent reactions slow down, and the cell may be damaged by shortage of required products or by toxicity of accumulated substrates. In all mammalian cells except hepatocytes, methionine synthesis from homocysteine requires folate as a methyl donor (Fig. 1) and reduced cobalamin (vitamin B₁₂) as methyl acceptor and donor. In subjects with significant deficiency of either vitamin, homocysteine (or its products homocystine and various disulfides) accumulates in plasma and may be excreted in the urine (6).
FIG. 1. Folate metabolism. deU is deoxyuridylate; AICA and AICAR represent aminoimidazole carboxamide and its ribotide, two intermediates in de novo synthesis of purines. The circled C is the single carbon with oxidation level indicated as H for methenyl, HH for methylene, HHH for methyl, and HO for formyl.

COBALAMIN (VITAMIN B₁₂)

This coenzyme functions in many reactions in microorganisms, but in man it has been proved to function only as a coenzyme in methionine synthesis (see above) and in the intramitochondrial conversion of methylmalonyl coenzyme A to succinyl CoA in the catabolism of the odd-chained fatty acids, propionate, and...
methylmalonate. As would be expected, in subjects deficient in cobalamin, methylmalonate may accumulate in the plasma and may be excreted in the urine (7). Cobalamin crosses cell membranes inefficiently, and all organisms requiring it utilize cobalamin binders which may be part of the cell wall or membrane (bacteria, etc.) or may be soluble and bind the cobalamin in the gut (gastric intrinsic factor) or plasma (transcobalamin II) (8–10). These soluble binders effect transport of vitamin bound to them by endocytosis, utilizing specific receptors on cells, releasing cobalamin in lysosomes, and permitting its transport into the cytoplasm or across the gut by undefined transport mechanisms (11–15).

EFFECTS OF DEFICIENCY

In adults, severe deficiency of folate or cobalamin may cause megaloblastic anemia, which involves the following: anemia; inadequate numbers of leukocytes or platelets; impaired maturation of the cells of the mouth, tongue or esophagus, leading to sore mouth, sore tongue or difficulty in swallowing; and other gastrointestinal abnormalities causing poor appetite and weight loss. It may also cause depressive reactions or other psychological disorders.

In patients deficient in vitamin B₁₂, a specific neurological abnormality may occur, characterized by degeneration of some peripheral nerves and of selected columns of fibers in the spinal cord, defective cerebral function leading to dementia, or degeneration of optic nerves causing blindness. Evidence has been published indicating similar effects from severe deficiency of folate (16,17), but such an association has not been proved. Sterility has been described in patients deficient in cobalamin (18), and some evidence has been published suggesting that mothers deficient in folate may have abnormal pregnancies (fetal loss, neural tube defects) (19,20).

Most of the symptoms described above may also affect children who are deficient in these vitamins. In children, deficiency may also cause failure to thrive, growth retardation, poor cerebral development, and death (21).

FOLATE DEFICIENCY

Recognition of Deficiency

The introduction of assays for measurement of folate in plasma and erythrocytes permitted identification of patients in whom megaloblastic anemia was caused by folate deficiency. Serum and erythrocyte folate levels of patients with megaloblastic anemia caused by folate deficiency are readily differentiated from those of normal subjects (22,23).

Serum (or plasma) folate decreases rapidly when folate absorption is decreased, and in many patients with general illness, serum folate levels are in the range associated with deficiency (<3 ng/ml, <7 nM) (24). Erythrocyte folate levels decrease during folate deficiency because folate-deficient erythrocytes replace those produced
before deficiency. In subjects with "deficient" concentrations of plasma folate for 2-3 months, the concentration of folate in erythrocytes decreases into the range observed in deficient patients (<150 ng/ml, <350 nM). Because megaloblastic anemia develops only after 3-4 months of folate deficiency in previously replete subjects (24), "deficient" erythrocyte folate levels are expected in patients with megaloblastic anemia due to deficiency of folate. In patients with severe folate deficiency of short duration [e.g., pregnancy, alcohol intake, in intensive care units (25,26)], megaloblastic anemia may precede decrease of erythrocyte folate into the "deficient" range. In addition, many subjects with "deficient" concentrations of folate in erythrocytes cannot be shown to have metabolic abnormalities caused by deficiency of folate. These observations indicate that the association of low erythrocyte folate level with clinical deficiency is fortuitous, and not the cause of the deficiency.

Folate deficiency may be recognized by demonstrating megaloblastic anemia, multilobed neutrophils (27-29), or homocysteine in serum (6), excretion of FIGlu (formiminoglutamate in urine), or changes in these or in erythrocyte volume after therapy with folate (30). When serum and erythrocyte folate levels are determined in groups of apparently normal subjects, "deficient" values are found in some. Of patients with "deficient" values of serum and erythrocyte folate, only a minority can be proved to have folate deficiency by the former criteria.

**Folate Intake and Nutrition**

Folate is widely distributed in foods, and is selectively concentrated in a few (e.g., liver, kidney, spinach) (2). Food folate deteriorates during heating (cooking) or storage, and is leached from foods which are cooked in large volumes of fluid. Folate is present in breast milk, is transferred by the placenta (31) into the developing fetus, and is present in high concentration in fetal plasma (32,33). In the body, folate is stored in the liver, but the major route of utilization of hepatic folate appears to be by secretion into the bile and reabsorption from the gut (34). Most of the folate in plasma is free, or loosely associated with albumin. Most of the folate in plasma is composed of folate which has been recently absorbed from the diet or from folate stored in the liver and secreted in the bile. The folate in plasma is the source of intracellular folate, and so intracellular folate is usually proportional to plasma folate during the period of accumulation of folate into the cells (25). The folate in erythrocytes can be used as a measure of the average plasma folate during the period of formation of the erythrocyte. In the mature erythrocyte, polyglutamyl 5-methyl tetrahydrofolate accumulated during blood formation remains bound to hemoglobin and is stable for most of the life (~100 days) of the cell. Folate can be depleted from the well-nourished human within 3 months, and sooner if alcohol is taken with a diet poor in folate (34-36).

Assays of folate intake have varied because of the many technical difficulties of assaying folate in food (37), destruction of folate during cooking and storage, and the possible interactions of dietary factors such as pH and conjugase inhibitors or
conjugases in certain foods ingested with the folates. Most of the folate in raw food is in the polyglutamyl form. Pure polyglutamyl folate is hydrolyzed to monoglutamyl in the intestine and is effectively absorbed by a pH-sensitive transport system which appears to be at least partially dependent on folate binding proteins on intestinal cells (38).

Most studies in Western countries have indicated a mean dietary folate intake of 150–200 µg of total folate per day, of which about half is polyglutamyl (21). Balance studies by Milne et al. (39) have indicated that in two normal volunteers ingesting 150 µg of food folate per day, serum folate decreased to plateau between 4 and 7 ng/ml and erythrocyte folate to 250–350 ng/ml. In these two subjects, this intake was clearly adequate to maintain folate levels above the range in which clinical deficiency is observed. In 10 unpregnant women studied for 92 days on measured diets (40), serum folate decreased if intake was less than 200 µg/day of food folate. Calculation of the mean folate intake in Canada which probably reflected the intake which maintained serum and erythrocyte folate at the upper limit of the range of deficiency, suggested that the minimum intake is about 70 µg/day. Mean folate intakes from diets reported from Denmark, Ireland, Israel, Sweden, United Kingdom, and the USA have ranged from 50 to 270 µg/day, with most studies reporting 150 µg/day or more (21,41).

Folate Deficiency in Populations

Folate sufficiency in populations may be evaluated by the following: (a) evidence of malfunction of folate-dependent reactions in subjects, including evaluation of polylobe counts, bone marrow morphology, and measurement of plasma homocysteine or other metabolic products, and (b) evidence that folate supplements alter a measurable variable in a population tested, including hemoglobin, MCV, growth rate of children, perinatal morbidity, etc. In the absence of these, conclusions must be drawn based on assays of serum and erythrocyte folate, which measure sufficiency but not deficiency. Studies of women in Benin (42), marathon runners in the UK (43), children in Gambia, and pregnant women in Burma showed no benefit from folate therapy in excess of the benefit of treatment of iron deficiency. On the other hand, certain subgroups may benefit from folate supplements (44-49):

1. *Pregnant women.* Supplementation of pregnant women with folate reduces the frequency of megaloblastic bone marrow morphology, has been shown to increase total mass of erythrocytes in late pregnancy, and may increase gestation duration and birth weight of infants.

2. *Premature infants.* Supplements have had no effect on variables measured at age 1 year, but earlier benefits have not been disproved.

3. *Subjects with general defects in nutrition.* Patients with inherited defects in certain metabolic processes (Lesch–Nyhan syndrome, certain amino acidurias) may require more folate than does the normal population.

4. *Elderly patients.* Elderly patients requiring institutional support may have a
variety of nutritional deficiencies, and may have pernicious anemia as well. Free-living elderly patients appear not to require supplements. Subjects with chronic intake of alcohol or antiepileptic drugs appear to have enhanced probability of developing true deficiency of folate.

5. **Hemolytic anemia.** Even transient folate deficiency is not tolerated by patients with chronic hemolytic anemia and should be prevented with folate supplements.

6. **Neural tube defects.** In women who have borne infants with neural tube defects the risk of recurrence may be reduced by folate supplementation during the periconceptional period.

7. **Women on the contraceptive pill or patients taking phenytoin.** Such women have a greater risk of folate deficiency than does the general population and may benefit from folate supplements. For patients, taking phenytoin, a constant folate dose is recommended because folate may reduce phenytoin absorption from the gut.

8. **Lactating women.** Such women may benefit from folate supplements.

**FOLATE REQUIREMENTS**

To maintain serum folate above the deficient range requires 50–100 μg/day of folic acid added to a folate-free diet. Patients with folate-deficient megaloblastic anemia have responded to total folate administration of 50–100 μg/day, most of which was folic acid. A patient with folate deficiency maintained on a diet assayed to contain 200 μg/day of total folate responded completely. The WHO recommendations (based on much data) of 3 μg/kg/day of food folate for adults (21,50), with supplements for pregnant and lactating women and infants, appears adequate. It is probable that a smaller intake will prevent folate deficiency, since if the above-listed mean intakes are correct, a substantial proportion of the population without evidence of folate deficiency ingests less than this. Based on measurement of folate catabolites in urine, daily intake of about 100 μg of folate may be adequate (51).

**CONCLUSION**

Evaluation of folate sufficiency in population groups should involve objective measures of deficiency, including poly-lobe counts and plasma homocysteine concentration to permit recognition of deficiency and to determine if measurements of serum and erythrocyte folate permit calculation of such risk. The benefits of folate supplementation in selected groups at risk for such deficiency should be evaluated.

**COBALAMIN DEFICIENCY**

Cobalamin is synthesized in nature by bacteria and fungi and is required by these and by animals but not by higher plants. It is therefore present in animal products
but absent from plant foods. Vegetarian animals (rabbits, bats) obtain the required cobalamin from fecal droppings. Cobalamin is stored by hepatocytes, and is lost from the body with a half-time of about 400 days. Depletion thus requires a prolonged period of poor intake or absorption.

Recognition of Deficiency

Cobalamin Concentration in Plasma

Cobalamin can be measured in serum or plasma. Most of it is bound tightly to haptocorrin (transcobalamin I), a glycoprotein which is synthesized by leukocytes and has a long (~10 days) half-life in plasma. This bound cobalamin does not participate in metabolic reactions. Despite these considerations, there is a rough correlation between hepatic stores of cobalamin and the concentration of cobalamin in plasma or serum.

When assays for cobalamin were first described, the concentration of cobalamin in serum was shown clearly to differentiate patients with severe megaloblastic anemia due to cobalamin deficiency from normal subjects. In almost all patients with severe megaloblastic anemia secondary to cobalamin deficiency, the concentration of cobalamin in plasma or serum was less than 100 pg/ml (78 pM), and usually less than half of this concentration. In the majority of normal subjects (95%), cobalamin was present in serum or plasma at a concentration between 200 and 800 pg/ml (about 160–625 pM). As additional tests for cobalamin deficiency were developed, including correction of macrocytosis, anemia, multilobation of neutrophils, and raised levels of methylmalonate or total homocysteine in plasma following treatment with cobalamin, it became apparent that in some patients with severe deficiency, serum cobalamin concentration was not very low. This fact was obscured for a period by the widespread use of unreliable assays for cobalamin in plasma. It is possible that in as many as 40% of all patients with deficiency of cobalamin sufficient to cause serious illness, the concentration of cobalamin in serum or plasma may not be less than 100 pg/ml, and in 3% to 5% it may be indistinguishable from that of normal subjects (52). In addition, in only a minority of subjects with cobalamin concentrations in plasma between 50 and 100 pg/ml can a significant deficiency of cobalamin be proved, or illness be shown to result.

In patients with severe deficiency of folate, the concentration of cobalamin in plasma may be subnormal (53). This will become normal over a period of days after folate treatment is begun.

In summary, although most subjects with very low concentrations of cobalamin in plasma can be shown to be deficient in cobalamin and will benefit from cobalamin therapy, the relationship of cobalamin concentrations between 50 pg/ml and the lower limit of the “normal range” to deficiency or to the state of body stores remains statistical and cannot be used as the single measure of cobalamin sufficiency.
COBALAMIN REQUIREMENTS

The cobalamin intake required to prevent deficiency has been calculated to be 0.1–1.0 μg/day depending on whether the estimate is based on the minimum quantity required to treat deficiency, the quantity measured in duplicate meals in selected populations in which serious illness was infrequent, or the quantity calculated to match turnover and to maintain hepatic cobalamin at 500 μg (370 nmol) (the quantity measured in a small number of subjects with pernicious anemia in whom clinical manifestations of the disease have not appeared, but in whom deficiency of cobalamin may produce illness [21]). Absorption of cobalamin from the gut is variable in different subjects, and it would be expected that the concentration of cobalamin required in tissues to maintain normal physiology would also vary between subjects. It is thus probable that the quantity in the diet which is required to maintain the health of a population would be greater than the amounts given above.

COBALAMIN INTAKE

The dietary intake of cobalamin in different populations correlates with the intake of animal products, ranging from 2.7 to 31.6 μg/g (2.0–23 nmol) in the United States to 0.25–0.5 μg/day (0.18–0.37 nmol) in vegetarian populations. An expert committee of the World Health Organization calculated that mean intake was less than 0.5 μg/day in India, Bangladesh, and many countries of tropical Africa. The frequency of macrocytic anemia or of other manifestations of cobalamin deficiency in these areas has not been measured, but megaloblastic anemia has been recognized in many immigrants from India to the United Kingdom and in subjects seeking medical attention in India (54). Nutritional anemia is uncommon in Western vegetarians. Neurological abnormalities resulting from cobalamin deficiency may be observed but are less common than one would predict.

CONCLUSION

Evaluation of cobalamin sufficiency in population groups should involve objective measures of deficiency, including poly-lobe counts and measurements of concentrations of methylmalonate and homocysteine to permit recognition of deficiency and to evaluate the frequency of significant deficiency. The benefits of cobalamin supplementation in selected groups at risk for such deficiency should be evaluated by assessing improvement in hemoglobin values, decrease in macrocytosis of erythrocytes, reduction in hyperlobation of neutrophils, alteration of concentrations of homocysteine and methylmalonate in plasma, growth of children, frequency of infection, and general health.
OTHER DEFICIENCIES

It is well established that a small number of cases of megaloblastic anemia have been observed which improved when thiamine (55) or ascorbate (2) were administered. Deficiencies of thiamine or ascorbate appear not to be important causes of nutritional macrocytic anemia, the other consequences of deficiency being much more important in the majority of patients. Whether all subjects who became deficient in these vitamins would develop megaloblastic anemia, or if the reported cases represent unusual examples of metabolism, is unknown.

REFERENCES


DISCUSSION

Dr. Adelekan: Dr. Cooper's data show that folate intakes in the range 50-75 μg/day are sufficient to maintain normal serum folate concentrations, but the WHO recommendation of 3 μg/kg/day in a 70-kg man translates to a value of 200 μg/day. Is this recommendation on the high side, and is there any additional benefit to be gained from consuming as much as this if normal red cell and folate levels can be maintained on as little as 50 μg/day?

Also, what is the significance of the low values found in population studies in individuals who are not clinically folate-deficient?

Dr. Cooper: The 3-μg/kg figure was probably the lowest the WHO could have recommended in view of the fact that the American RDA was twice this value at the time! A value of 70 μg/day is on around the 15th percentile of North American folate intakes and at this level there is little or no evidence of clinical folate deficiency, so it is probably adequate.

As far as serum folate values are concerned, every population that has been studied has contained 5-10% of individuals with low serum values but without evidence of illness. Maybe we are looking at the wrong things. It may be that these are people who have consumed little dietary folate in the recent past but who from time to time do eat folate-containing foods in sufficient amounts to prevent deficiency symptoms. This group needs to be studied further.

Dr. Adelekan: Another question is related to at-risk groups. I am thinking especially of women in the childbearing age who are on oral contraceptives. The use of the contraceptive pill is increasing in developing countries, and there is evidence that women who become pregnant soon after coming off the pill are at increased risk of developing folate deficiency. In most developing countries, efforts are being made to encourage family planning. It would be a pity if this were to result in an increase in folate deficiency during pregnancy.

Dr. Cooper: There is no doubt that women who are taking the oral contraceptive pill have lower serum folate levels. Smoking also reduces serum folate. We need to find out whether these effects are important in pregnant women in developing countries and whether supplements should be given to women on the pill.

Dr. Brabin: There was a classic study in the 1970s which showed that in American women taking oral contraceptives who had normal biochemical tests for folate there was a significant degree of cervical dysplasia which was corrected by folate supplements. Folate may have very local effects which could be important for long-term gynecological morbidity.
Dr. Cooper: Preliminary studies from the group in Alabama suggested that the frequency of carcinoma was decreased by folate supplements (1). However, Dr. Branda, from Vermont, has shown (2) that the rate of mutation in cancer cells was greater when these cells were folate-deficient, which would support the contention that folate-deficient cervical cells might be more likely to become cancerous. I agree that in women who are pregnant or who are taking oral contraceptives, more folate is required than in the general population to prevent all evidence of folate deficiency. If this means an additional glass of orange juice a day, then I think that would be justified. If, however, it requires regular folate supplements and a change in lifestyle, it is doubtful whether it could be justified without more definite evidence of a health benefit.

Dr. Dallman: What are your views on the recommendations for supplement use in pregnancy? And what are the prospects for making hypersegmentation of neutrophils a more practical laboratory test for folate deficiency? Can it be automated?

Dr. Cooper: The WHO recommendation attempts to allow for the extra folate required to prevent deficiency during pregnancy. The problem is that it is not clear whether the objective is to maintain normal serum folate levels and blood morphology or to prevent clinical illness. In Canadian women on normal diets in the 1960s, bone marrow was megaloblastic in about 25% and this was largely prevented by supplementation with 200 µg of folate per day. However, there was no measurable effect on the health of the mothers or the infants. In studies with folate supplementation the frequency of megaloblastic bone marrow declines to around 3–5%, the residual incidence probably being accounted for by women who do not take their tablets, though it is possible that a few individuals need very large doses of folate to prevent megaloblastic marrow during pregnancy.

Enumeration of multilobular neutrophils as a test of megaloblastic anemia remains difficult. Since the multilobed neutrophils found in this situation are diploid and not hyperdiploid, flow cytometry measuring the amount of DNA per cell would probably not detect them.

Dr. Fomon: Vitamin B$_{12}$ deficiency in a breast-fed infant of a B$_{12}$-deficient mother is a good example of a micronutrient deficiency that develops rapidly in an infant born to and nursed by a woman who is deficient in the micronutrient. Such an infant is born with poor stores and thereafter receives an inadequate intake from his mother's milk. This problem has also been observed with vitamins A, D, and K and with iodine and selenium.

Dr. Haschke: In some European countries there has been a good deal of discussion about children who are on macrobiotic diets and who have vitamin B$_{12}$ deficiency. The population in the Netherlands has been followed during the past 5–6 years, and the data clearly show that this subgroup of the population needs supplements of iron, folate, and B$_{12}$.

Dr. Cooper: Cobalamin deficiency in vegans is of course also well known. People are at risk of folate deficiency if their diets omit any of the major food groups: vegetables, fruits, cereals, meats, and dairy products (in populations without lactase deficiency).

Dr. El-Mauhoub: I have seen in the past few years a few children with megaloblastic anemia associated with proteinuria. I believe this is now referred to as the Immerslund–Grasbeck syndrome.

Dr. Cooper: The Immerslund–Grasbeck syndrome of acquired cobalamin malabsorption is very rare. Some of the affected individuals lack the intestinal receptor for the intrinsic-factor–cobalamin complex; in others the absorptive defect is different and at present undefined. In some affected patients there is a tubular type of proteinuria, but this does not cause the cobalamin deficiency. The majority of children with this inherited condition do not have proteinuria.

Dr. Koletzko: There is a new method of assessing the duration and degree of cobalamin
deficiency which may prove useful. This is the measurement of odd-chain fatty acids in plasma lipids. Patients with reduced activity of methylmalonic mutase due to cobalamin deficiency will accumulate propionic acid and subsequently also odd-chain fatty acids, which are relatively stable. The concentration of these acids in plasma lipids and adipose tissue samples may well reflect the duration and severity of cobalamin deficiency.

Dr. Cooper: This is interesting and should be pursued. Accumulation of methylmalonate in plasma has also been shown to correlate with cobalamin status in subjects with normal renal function, and this may prove to be the most reliable screening test for cobalamin deficiency. I suspect, however, that we shall use serum total homocysteine as a screen for folate or cobalamin deficiency because it is simpler to determine. We certainly need an easy test that does not require examination of bone marrow.

Dr. Koletzko: My point about odd-chain fatty acids was that it might give a long-term perspective, like HbA1c in diabetics.

REFERENCES