34 - 104 Megaloblastic Anemias

104 Megaloblastic Anemias

Unexpected low O2 saturation by pulse oximetry (SpO2) with nor mal O2 saturation of arterial blood is occasionally seen in rare hemo globin variants with clinical phenotypes. Asymptomatic patients with unexpectedly low SpO2 should not be subjected to unneeded cardio pulmonary investigations in search of the cause of their “hypoxemia” until the existence of a hemoglobin variant is excluded.

■ ■M HEMOGLOBINS M (met) hemoglobins are characterized by oxidation of the heme-iron from its ferrous (Fe++) to ferric (Fe+++) form. The major clinical feature of these disorders is asymptomatic cyanosis. Thirteen M hemoglobin variants have been described. In nine, the mutation involves histidine residues that interact with heme. Asymptomatic slate gray/brownish

pseudocyanosis is the main clinical finding. Spectrophotometric recording of the visible spectrum of the hemolysate is diagnostic. To distinguish M hemoglobins from methemoglobinemia due to drugs or cytochrome b5 reductase (CYB5R3) deficiency, potassium cyanide (KCN) can be added to the hemolysate; methemoglobin-containing blood will turn red, but KCN has no effect on M hemoglobin. Treat ment is not needed. ■ ■UNSTABLE HEMOGLOBINS Sometimes referred to as congenital Heinz body hemolytic anemias, some mutations result in a hemoglobin tetramer that is unstable and precipitates intracellularly. One-hundred-fifty-six such variants have been described and are often a result of a new mutation that affects the tertiary or quaternary structure of the molecule. The most com mon class of mutations introduce a proline residue in the α helix or a polar amino acid into the interion of the molecule. Heinz bodies are intraerythrocytic precipitates that are detectable as dark globular aggregates after staining with a dye such as brilliant cresyl blue. Three unstable hemoglobins are the most common. Hemoglobin Köln (β99 val-met) has been found in multiple families, Hb Hasharon (α47 asphis) is found in Ashkenazi Jews, and Hb Zurich (β63 his-arg) is suscepti ble to oxidant drug-induced hemolysis. Unstable variants present with nonspherocytic hemolytic anemia, but presentation is highly variable. The associated disease is usually mild and does not require transfusion. Heating blood to 50°C or incubation with isopropanol precipitates unstable hemoglobins but must be done with careful controls. Some variants can be detected by HPLC. PART 4 Oncology and Hematology ■ ■HEMOGLOBINS WITH HIGH OXYGEN AFFINITY AND LOW OXYGEN AFFINITY Rare mutations in areas involved in the R-T transition, at critical inter faces between globin chains of the tetramer that reduce the affinity for 2,3-bisphosphoglycerate, or present in the heme pocket account for most of these variants. High O2 affinity hemoglobins (103) outnumber low O2 affinity variants (48). Isolated erythrocytosis in the

absence of splenomegaly suggests the presence of a high O2 affinity hemoglobin. High O2 affinity hemoglobin variants shift the hemoglobin-O2 disso ciation curve leftward, causing a low P50 and thereby stimulating eryth ropoiesis. Many of these variants are due to new mutations. The clinical course is benign, and phlebotomy because of erythrocytosis is usually not required. Early diagnosis is important to forestall unnecessary diagnostic procedures and therapeutics such as cardiac catheteriza tion to exclude congenital heart disease or treatment for polycythe mia vera. Low O2 affinity variants often present with cyanosis. Their hemoglobin-O2 dissociation curve is right-shifted with high P50. HPLC might reveal the presence of a hemoglobin variant. Treatment is often not necessary. ■ ■ACQUIRED DISORDERS OF HEMOGLOBIN CO binds hemoglobin with high affinity forming carboxyhemoglobin. Carboxyhemoglobin levels can be accurately measured by co-oximetry of arterial blood. Standard pulse oximeters cannot accurately make this measurement. Some newly developed pulse oximeters can measure both carboxyhemoglobin and methemoglobin. Bound CO inhibits the transport of O2; the hemoglobin-O2 binding curve is left-shifted. Acute and chronic CO intoxication, caused by occupational exposure

and other sources of incomplete combustion of hydrocarbons, presents with headache, altered mental status, and other constitutional symp toms. High-flow O2 via facemask is the preferred treatment; criteria have been developed to guide the use of hyperbaric O2. Acquired methemoglobinemia and methemoglobinemia due to deficiency of CYB5R3 are more common than the M hemoglobins. CYB5R3 is required for the reduction of methemoglobin by NADH. Affected individuals with “toxic” methemoglobinemia can be cyanotic and symptomatic. As in carboxyhemoglobinemia, O2 transport is reduced and reflected by the left shift in the hemoglobin-O2 binding curve. CYB5R3 deficiency usually affects only erythrocytes (type I), causing a mild disorder; when all cells are affected (type II), a severe disease results. Intravenous methylene blue is the preferred treatment in symptomatic patients with acquired methemoglobinemia and 40–60% methemoglobin. The usual dose is 1–2 mg/kg. Alternative treatment with ascorbic acid is preferable in people who are glucose-6-phosphate dehydrogenase deficient. Methylene blue interferes with co-oximetry, reducing the value of co-oximetry for monitoring treatment. Many drugs and chemicals can induce methemoglobin in the absence of CYB5R3 deficiency. Dapsone and topical anesthetics such as benzocaine are the most common offending agents. ■ ■FURTHER READING
Frangoul H et al: Exagamglogene autotemcel for severe sickle cell disease. N Engl J Med 290:1649, 2024. Hardouon G et al: Sickle cell disease: From genetics to curative approaches. Ann Rev Genomics Hum Genet 24:255, 2023. Leonard A et al: Gene therapy for hemoglobinopathies: Beta-thalassemia, sickle cell disease. Hematol Oncol Clin North Am 36:769, 2022. Locatelli F et al: Defining curative endpoints for sickle cell disease in the era of gene therapy and gene editing. Am J Hematol 99:430, 2024. Piel FB et al: Defining global strategies to improve outcomes in sickle cell disease: A Lancet Haematology Commission. Lancet Haematol. 10:e633, 2023. Pinto VM et al: Management of the aging beta-thalassemia transfusiondependent population: The Italian experience. Blood Rev 38:100594, 2019. Pinto VM et al: Management of the sickle cell trait: An opinion by expert panel members. J Clin Med 12:3441, 2023. Ribeil J-A et al: An integrated therapeutic approach to sickle cell disease management beyond infancy. Am J Hematol 98:1087, 2023. Sheth S et al: Management of luspatercept therapy in patients with transfusion-dependent β-thalassaemia. Br J Haematol 201:824, 2023. Taher AT et al: Beta-thalassemia. N Engl J Med 384:727, 2021. A. Victor Hoffbrand

Megaloblastic Anemias The megaloblastic anemias are a group of disorders characterized by the presence of distinctive morphologic appearances of the developing red cells in the bone marrow. The marrow is usually hypercellular, and the anemia is based on ineffective erythropoiesis. The cause is usually a deficiency of either cobalamin (vitamin B12) or folate, but megaloblastic anemia may occur because of genetic or acquired abnormalities that affect the metabolism of these vitamins or because of defects in DNA synthesis not related to cobalamin or folate (Table 104-1). COBALAMIN Cobalamin (vitamin B12) exists in a number of different chemical forms. All have a cobalt atom at the center of a corrin ring. In nature, the vitamin is mainly in the 2-deoxyadenosyl (ado) form, which is located in mitochondria. It is the cofactor for the enzyme L-methylmalonyl

TABLE 104-1 Causes of Megaloblastic Anemia Cobalamin deficiency or abnormalities of cobalamin metabolism

(see Tables 104-3, 104-4) Folate deficiency or abnormalities of folate metabolism (see Table 104-5) Therapy with antifolate drugs (e.g., methotrexate) Independent of either cobalamin or folate deficiency and refractory to cobalamin and folate therapy: Some cases of acute myeloid leukemia, myelodysplasia Therapy with drugs interfering with synthesis of DNA (e.g., cytosine arabinoside, hydroxyurea, 6-mercaptopurine, azidothymidine [AZT]) Orotic aciduria (responds to uridine) Thiamine-responsive coenzyme A (CoA) mutase. The other major natural cobalamin is methylcobalamin, the form in human plasma and in cell cytoplasm. It is the cofactor for methionine synthase. Minor amounts of hydroxo cobalamin are also present to which methyl- and ado-cobalamin are converted rapidly by exposure to light. ■ ■DIETARY SOURCES AND REQUIREMENTS Cobalamin is synthesized solely by microorganisms. Ruminants obtain cobalamin from the foregut, but the only source for humans is food of animal origin, for example, meat, fish, and dairy products. Vegetables, fruits, and other foods of nonanimal origin are free from cobalamin unless they are contaminated by bacteria. A normal Western diet con tains 5–30 µg of cobalamin daily. Adult daily losses (mainly in the urine and feces) are 1–3 µg (~0.1% of body stores), and because the body does not have the ability to degrade cobalamin, daily requirements are also about 1–3 µg. Body stores are of the order of 2–3 mg, sufficient for 3–4 years if supplies are completely cut off. ■ ■ABSORPTION Two mechanisms exist for cobalamin absorption. One is passive, occur ring equally through buccal, duodenal, and ileal mucosa; it is rapid but extremely inefficient, with <1% of an oral dose being absorbed by this process. The normal physiologic mechanism is active; it occurs through the ileum and is efficient for small (a few micrograms) oral doses of cobalamin, and it is mediated by gastric intrinsic factor (IF). Dietary cobalamin is released from protein complexes by enzymes in the stomach, duodenum, and jejunum; it combines rapidly with a salivary glycoprotein that belongs to the family of cobalamin-binding proteins known as haptocorrins (HCs). In the intestine, the HC is digested by pancreatic trypsin and the cobalamin is transferred to IF. IF (gene at chromosome 11q13) is produced in the gastric parietal cells of the fundus and body of the stomach, and its secretion parallels that of hydrochloric acid. Normally, a vast excess of IF is available. The IF-cobalamin complex passes to the ileum, where IF attaches to a specific receptor (cubilin) on the microvillus membrane of the enterocytes. Cubilin also is present in yolk sac and renal proximal tubular epithelium. Cubilin appears to traffic by means of amnionless (AMN), an endocytic receptor protein that directs sublocalization and endocytosis of cubilin with its ligand IF-cobalamin complex. The cobalamin-IF complex enters the ileal cell, where IF is destroyed. After a delay of about 6 h, the cobalamin appears in portal blood attached to transcobalamin (TC) II. Between 0.5 and 5 µg of cobalamin enter the bile each day. This binds to IF, and a major portion of

biliary cobalamin normally is reab sorbed together with cobalamin derived from sloughed intestinal cells. Because of the appreciable amount of cobalamin undergoing entero hepatic circulation, cobalamin deficiency develops more rapidly in individuals who malabsorb cobalamin than it does in vegans, in whom reabsorption of biliary cobalamin is intact. ■ ■TRANSPORT Two main cobalamin transport proteins exist in human plasma; they both bind cobalamin—one molecule for one molecule. One an HC, also known as transcobalamin (TC) I, is closely related to other

cobalamin-binding HCs in milk, gastric juice, bile, saliva, and other fluids. The gene TCNL is at chromosome 11q11-q12.3. These HCs differ from each other only in the carbohydrate moiety of the molecule. TC I is derived primarily from the specific granules in neutrophils. Normally, it is about two-thirds saturated with cobalamin, which it binds tightly. TC I does not enhance cobalamin entry into tissues. Glycoprotein receptors on liver cells are involved in the removal of TC I from plasma, and TC I may play a role in the transport of cobalamin analogues (which it binds more effectively than does IF) to the liver for excretion in bile.

The other major cobalamin transport protein in plasma is transco balamin, also known as TC II. The gene is on chromosome 22q11-q13.1. As for IF and HCs, there are nine exons. The three proteins are likely to have a common ancestral origin. TC II is synthesized by liver and by other tissues, including macrophages, ileum, and vascular endothelium. It normally carries only 20–60 ng of cobalamin per liter of plasma and readily gives up cobalamin to marrow, placenta, and other tissues, which it enters by receptor-mediated endocytosis involving the TC II receptor and megalin (encoded by the LRP-2 gene). The TC II cobalamin is inter nalized by endocytosis via clathrin-coated pits; the complex is degraded, but the receptor probably is recycled to the cell membrane as is the case for transferrin. Export of “free” cobalamin is via the ATP-binding cas sette drug transporter alias multidrug resistance protein 1. CHAPTER 104 FOLATE ■ ■DIETARY FOLATE Folic (pteroylglutamic) acid is a yellow, crystalline, water-soluble substance. It is the parent compound of a large family of natural folate compounds, which differ from it in three respects: (1) they are partly or completely reduced to dihydrofolate (DHF) or tetrahydrofolate (THF) derivatives, (2) they usually contain a single carbon unit (Table 104-2), and (3) 70–90% of natural folates are folate-polyglutamates. These usually have a chain of four to six glutamate moieties rather than one, as in the monoglutamate folic acid. The whole family is known as folate or vitamin B9. Megaloblastic Anemias Most foods contain some folate. The highest concentrations are found in liver, yeast, spinach, other greens, and nuts (>100 µg/100 g). The total folate content of an average Western diet is 400−500 µg daily, but the amount varies widely according to the type of food eaten and the method of cooking. Folate is easily destroyed by heating, particu larly in large volumes of water. Total-body folate in the adult is ~10 mg, with the liver containing the largest store. Daily adult requirements are 100–200 µg, and so stores are sufficient for only 3–4 months in normal adults, and severe folate deficiency may develop rapidly. ■ ■ABSORPTION Folates are absorbed rapidly from the upper small intestine. The absorption of folate polyglutamates is less efficient than that of mono glutamates; on average, ~50% of food folate is absorbed. Polyglutamate forms are hydrolyzed to the monoglutamate derivatives either in the lumen of the intestine or within the mucosa. All dietary folates are converted to 5-methyl-THF (5-MTHF) within the small intestinal mucosa before entering portal plasma. Monoglutamates are actively transported across the enterocyte by a proton-coupled folate trans porter (PCFT, SCL46A1). This is situated at the apical brush border and is most active at pH 5.5, which is about the pH of the duodenal and jejunal surface. Genetic mutations of this protein underlie heredi tary malabsorption of folate (see below).

Pteroylglutamic acid at doses

> 400 µg is absorbed largely unchanged and converted to natural folates in the liver. Lower doses are converted to 5-MTHF during absorption through the intestine. About 60–90 µg of folate enter the bile each day and are excreted into the small intestine. Loss of this folate, together with the folate of sloughed intestinal cells, accelerates the speed with which folate defi ciency develops in malabsorption conditions. ■ ■TRANSPORT Folate is transported in plasma; about one-third is loosely bound to albumin, and two-thirds are unbound. In all body fluids (plasma, cerebrospinal fluid, milk, bile), folate is largely, if not entirely, 5-MTHF

TABLE 104-2 Biochemical Reactions of Folate Coenzymes COENZYME FORM OF FOLATE INVOLVED REACTION Formate activation THF −CHO Generation of 10-formyl-THF Purine synthesis Formation of glycinamide 5,10-Methylene-THF −CHO Formation of purines needed for DNA, RNA synthesis, but reactions probably not rate-limiting ribonucleotide Formylation of aminoimidazole 10-Formyl (CHO)THF carboxamide ribonucleotide (AICAR) Pyrimidine synthesis Methylation of deoxyuridine 5,10-Methylene-THF −CH3 Rate limiting in DNA synthesis Oxidizes THF to DHF Some breakdown of folate at the C-9–N-10 bond monophosphate (dUMP) to thymidine monophosphate (dTMP) Amino acid interconversion Serine-glycine interconversion THF =CH2 Entry of single carbon units into active pool Homocysteine to methionine 5-Methyl(M)THF −CH3 Demethylation of 5-MTHF to THF; also requires cobalamin, flavine adenine dinucleotide, ATP, and adenosylmethionine Forminoglutamic acid to glutamic THF −HN−CH= acid in histidine catabolism Abbreviations: DHF, dihydrofolate; THF, tetrahydrofolate. PART 4 Oncology and Hematology (the monoglutamate form). Three types of folate-binding protein are involved. A reduced folate transporter (RFC, SLC19A1) is the major route of delivery of plasma folate (5-MTHF) to cells. Two folate recep tors, FR2 and FR3 embedded in the cell membrane by a glycosyl phosphatidylinositol anchor, transport folate into the cell via receptormediated endocytosis. The third protein, proton-coupled folate trans porter (PCFT), transports folate at low pH from the vesicle to the cell cytoplasm. The reduced folate transporter also mediates uptake of methotrexate by cells. ■ ■BIOCHEMICAL FUNCTIONS Folates (as the intracellular polyglutamate derivatives) act as coenzymes in the transfer of single-carbon units (Fig. 104-1 and Table 104-2). Two of these reactions are involved in purine synthesis and one in pyrimi dine synthesis necessary for DNA and RNA replication. Folate is also a coenzyme for methionine synthesis, in which methylcobalamin is also involved and in which THF is regenerated. THF is the acceptor of single carbon units newly entering the active pool via conversion of serine to glycine. Methionine, the other product of the methionine synthase reaction, is the precursor for S-adenosylmethionine (SAM), the universal methyl donor involved in >100 methyltransferase reac tions (Fig. 104-1). During thymidylate synthesis, 5,10-methylene-THF is oxidized to DHF. The enzyme DHF reductase converts this to THF. The drugs methotrexate, pyrimethamine, and (mainly in bacteria) trimethoprim inhibit DHF reductase and so prevent formation of active THF coen zymes from DHF. A small fraction of the folate coenzyme is not recy cled during thymidylate synthesis but is degraded at the C9-N10 bond. BIOCHEMICAL BASIS OF MEGALOBLASTIC ANEMIA The common feature of all megaloblastic anemias is a defect in DNA synthesis that affects rapidly dividing cells in the bone marrow. Con

ditions that give rise to megaloblastic changes have in common a disparity in the availability of the four immediate precursors of DNA or a block in their condensation to form DNA. The four precursors are the deoxyribonucleoside triphosphates (dNTPs)—dA(adenine) TP and dG(guanine)TP (purines), dT(thymine)TP, and dC(cytosine) TP (pyrimidines). In deficiencies of either folate or cobalamin, con version of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP), the precursor of deoxythymidine triphos phate (dTTP) (Fig. 104-1) fails. This occurs because folate is needed as the coenzyme 5,10-methylene-THF polyglutamate for conversion of dUMP to dTMP. The availability of 5,10-methylene-THF is reduced in either cobalamin or folate deficiency. Because of the shortage of one

SINGLE CARBON UNIT TRANSFERRED IMPORTANCE or more precursor, DNA replication from multiple origins along the chromosome is slower than normal during mitosis, and the incomplete replicons fail to join up with resulting single-stranded DNA breaks. An alternative and less likely theory for megaloblastic anemia in cobalamin or folate deficiency is misincorporation of uracil into DNA because of the accumulation of deoxyuridine triphosphate (dUTP) at the DNA replication fork as a consequence of the block in conversion of dUMP to dTMP. ■ ■COBALAMIN-FOLATE RELATIONS Folate is required for many reactions in mammalian tissues (Table 104-2).

Only two reactions in the body are known to require cobalamin. Methylmalonyl-CoA isomerization requires adocobalamin, and the methylation of homocysteine to methionine requires both methylco balamin and 5-MTHF (Fig. 104-1). This reaction is the first step in the pathway by which 5-MTHF, which enters bone marrow and other cells from plasma, is converted into all the intracellular folate coen zymes. The coenzymes are all polyglutamated (the larger size aiding retention in the cell), but the enzyme folate polyglutamate synthase can use only THF, not 5-MTHF, as substrate. In cobalamin deficiency, 5-MTHF accumulates in plasma, and intracellular folate concentra tions fall due to failure of formation of THF, the substrate on which folate polyglutamates are built. This has been termed THF starvation, or the methylfolate trap. This trap also occurs at the polyglutamate level with accumulation of the methyl form at the expense of the other one-carbon forms. This theory explains the abnormalities of folate metabolism that occur in cobalamin deficiency (high serum folate, low cell folate, positive purine precursor aminoimidazole carboxamide ribonucleotide [AICAR] excretion) (Table 104-2) and also why the anemia of cobala min deficiency responds to folic acid in large doses, which overcome the methylfolate trap (Fig 104-1). CLINICAL FEATURES Many symptomless patients are detected through the finding of a raised mean corpuscular volume (MCV) on a routine blood count. The main clinical features in more severe cases are those of anemia. Anorexia is usually marked, and weight loss, diarrhea, or constipation may be present. Glossitis, angular cheilosis, a mild fever in more severely anemic patients, jaundice (unconjugated), and reversible melanin skin hyperpigmentation also may occur with a deficiency of either folate or cobalamin. Thrombocytopenia sometimes leads to bruising, and this may be aggravated by vitamin C deficiency or alcohol in malnourished patients. The anemia and low leukocyte count may predispose to infec tions, particularly of the respiratory and urinary tracts. Cobalamin

Methylated product (e.g., methylated lipids, myelin basic protein, DOPA, DNA) GSH Pyruvate S-Adenosylhomocysteine (SAH) Cysteine

Cystathionine Cystathionine synthase vitamin B6 Homocysteine Methionine Cell Tetrahydrofolate 5-Methyl tetrahydrofolate 5,10-Methylenetetrahydrofolate reductase 5, 10-Methylene tetrahydrofolate 5-Methyl tetrahydrofolate (monoglutamate) Deoxyuridine monophosphate Folic acid Folic acid Plasma FIGURE 104-1 The role of folates in DNA synthesis and in formation of S-adenosylmethionine (SAM), which is involved in numerous methylation reactions. DHF, dihydrofolate; GSH, glutathione. (Reproduced with permission from AV Hoffbrand et al [eds]: Postgraduate Haematology, 5th ed. Oxford, UK, Blackwell Publishing, 2005.) deficiency has also been associated in a few studies with impaired bactericidal function of phagocytes and with osteoporosis. Neurologic Manifestations Cobalamin is needed for the myelin ation of the central nervous system. Its deficiency may cause a bilateral peripheral neuropathy or degeneration (demyelination) of the cervical and thoracic posterior and lateral (pyramidal) tracts of the spinal cord and, less frequently, of the cranial nerves and of the white matter of the brain. Optic atrophy and cerebral symptoms including dementia, depression, psychotic symptoms, and cognitive impairment may be prominent. Anosmia and loss of taste may occur. Magnetic resonance imaging (MRI) may show the “spongy” degeneration of the cord. The patient, more frequently male, typically presents with pares thesias, muscle weakness, or difficulty in walking but sometimes may present with dementia, psychotic disturbances, or visual impairment. Loss of proprioception and vibration sensation is usually present with positive Romberg and Lhermitte signs. Gait may be ataxic with spas ticity (hyperreflexia). Autonomic nervous dysfunction can result in postural hypotension, impotence, and incontinence. Long-term nutritional cobalamin deficiency in infancy leads to poor brain development and impaired intellectual development. In infancy, feeding difficulties, lethargy, and coma may be noted. Convulsions

Substrate Methyltransferases S-Adenosylmethionine (SAM) THE METHYLATION CYCLE ATP Polyglutamate synthase

- glutamates Methionine synthase methylcobalamin DHF reductase Serine Glycine CHAPTER 104 Purines Formate Dihydrofolate 10-Formyl tetrahydrofolate DNA CYCLE (CELL REPLICATION) Megaloblastic Anemias Deoxythymidine monophosphate and myoclonus have been described. An important clinical problem is the nonanemic patient with neurologic or psychiatric abnormalities and a low or borderline serum cobalamin level. In such patients, it is necessary to try to establish whether significant cobalamin deficiency is present, for example, by careful examination of the blood film for macrocytosis or hypersegmented neutrophils (see below), tests for pernicious anemia (PA) by serum gastrin level and antibodies to IF or parietal cells, and serum methylmalonic acid (MMA) measurement. A trial of cobalamin therapy for at least 3 months will usually also be needed to determine whether the symptoms improve. The biochemical basis for cobalamin neuropathy remains obscure. Its occurrence in the absence of methylmalonic aciduria in TC II deficiency suggests that the neuropathy is related to the defect in homocysteine-methionine conversion. Accumulation of S-adenosyl homocysteine in the brain, resulting in inhibition of transmethylation reactions, has been suggested. Folate deficiency has been suggested to cause organic neurologic disease, but this is uncertain, although methotrexate injected into the cerebrospinal fluid may cause brain or spinal cord damage. Psychiatric disturbance, as discussed above, is common in both folate and cobalamin deficiencies. This, like the neuropathy, has been attributed to a failure of the synthesis of SAM, which is needed in

methylation of biogenic amines (e.g., dopamine) as well as that of pro teins, phospholipids, and neurotransmitters in the brain (Fig. 104-1).

■ ■GENERAL TISSUE EFFECTS OF COBALAMIN AND FOLATE DEFICIENCIES Epithelial Surfaces After the marrow, the next most frequently affected tissues are the epithelial cell surfaces of the mouth (with glos sitis), stomach, small intestine, and respiratory, urinary, and female genital tracts. The cells show macrocytosis with increased numbers of multinucleate and dying cells. The deficiencies may cause cervical smear abnormalities. Complications of Pregnancy The gonads are also affected, and infertility is common in both men and women with severe deficiency of either vitamin. Maternal folate deficiency has been implicated as a cause of prematurity, and both folate and cobalamin deficiencies have been implicated in recurrent fetal loss and neural tube defects. Neural Tube Defects Folic acid supplements at the time of con ception and in the first 12 weeks of pregnancy can reduce by ~80% the incidence of neural tube defects (NTDs) (anencephaly, meningomyelo cele, encephalocele, and spina bifida) in the fetus. Most of this protec tive effect can be achieved by taking folic acid, 0.4 mg daily, before and at the time of conception. PART 4 Oncology and Hematology The incidence of cleft palate and harelip also can be reduced by prophylactic folic acid. No clear simple relationship exists between maternal folate status and these fetal abnormalities, although for NTDs, it has been established that the lower the maternal folate, the greater is the risk to the fetus. NTDs also can be caused by antifolate and antiepileptic drugs. An underlying maternal folate metabolic abnormality has also been postulated. One abnormality has been identified: reduced activity of the enzyme 5,10-methylene-THF reductase (MTHFR) (Fig. 104-1) caused by a common C677T polymorphism in the MTHFR gene. In one study, the prevalence of this polymorphism was found to be higher than in controls in the parents of NTD fetuses and in the fetuses them selves: homozygosity for the TT mutation was found in 13% of cases compared with 5% of control subjects. The polymorphism codes for a thermolabile form of MTHFR. The homozygous state results in a lower mean serum and red cell folate level compared with control subjects, as well as significantly higher serum homocysteine levels. Tests for muta tions in other enzymes possibly associated with NTDs, for example, methionine synthase and serine–glycine hydroxymethylase, have been negative. Serum cobalamin levels are also lower in the sera of mothers of NTD infants than in controls. In addition, maternal TC II receptor polymorphisms are associated with increased risk of NTD births. How ever, no studies have been undertaken that show that dietary fortifica tion with cobalamin reduces the incidence of NTDs. Cardiovascular Disease Children with severe homocystinuria (blood levels ≥100 μmol/L) due to deficiency of one of three enzymes (methionine synthase, MTHFR, or cystathionine synthase; Fig. 104-1) have vascular disease, for example, ischemic heart disease, cerebrovas cular disease, or pulmonary embolus, as teenagers or in young adult hood. Lesser degrees of raised serum homocysteine and low levels of serum folate and homozygous inherited mutations of MTHFR have been found to be associated with cerebrovascular, peripheral vascular, and coronary heart disease and with deep vein thrombosis. Prospective randomized trials of lowering homocysteine levels with supplements of folic acid, vitamin B12, and vitamin B6 against placebo over a 5-year period in patients with vascular disease or diabetes have not, however, shown a reduction of first event fatal or nonfatal myocardial infarction, nor have these supplements reduced the risk of recurrent cardiovascu lar disease after an acute myocardial infarct. Meta-analysis showed an 18% reduction in strokes. The benefit for stroke prevention has been confirmed by a large (>20,000 subjects) randomized prospective study in hypertensive subjects in China. This showed a significant reduction in the first incidence of stroke in subjects receiving enalapril and folic acid compared to enalapril alone. The

effect was especially marked in the subjects commencing the prospective trial with the lowest serum

folate levels. Venous thrombosis has been reported to be more frequent in folate-deficient or cobalamin-deficient subjects than in controls and to occur at unusual sites such as cerebral venous sinuses. This tendency was ascribed to raised plasma homocysteine levels in folate or cobala min deficiency, but no evidence exists that folic acid or cobalamin supplements reduce the prevalence of venous thrombosis. Cognitive Decline Association between low serum folate or cobalamin levels and higher homocysteine levels with the development of decreased cognitive function and of dementia in Alzheimer's disease has been reported. A meta-analysis of randomized, placebo-controlled trials of homocysteine-lowering B-vitamin supplementation of indi viduals with and without cognitive impairment, however, showed that supplementation with vitamin B12, vitamin B6, and folic acid alone or in combination did not improve cognitive function or slow cognitive decline. It is unknown whether prolonged treatment with these B vitamins can reduce the risk of dementia in later life. Malignancy Prophylactic folic acid in pregnancy has been found in some but not all studies to reduce the subsequent incidence of acute lymphoblastic leukemia (ALL) in childhood. A significant negative association has also been found with the MTHFR C677T polymor phism and leukemias with mixed lineage leukemia (MLL) transloca tions, but a positive association was found with hyperdiploidy in infants with ALL or acute myeloid leukemia or with childhood ALL. A second polymorphism in the MTHFR gene, A1298C, is also strongly associated with hyperdiploid leukemia. Various positive and negative associations are noted between polymorphisms in folate-dependent enzymes and the incidence of adult ALL. The C677T polymorphism is thought to lead to increased thymidine pools and "better quality" of DNA synthesis by shunting one-carbon groups toward thymidine and purine synthesis. This may explain its reported association with a lower risk for colorectal cancer. Most but not all studies suggest that prophy lactic folic acid also protects against colon adenomas. Other tumors that have been associated with folate polymorphisms or status include follicular lymphoma, breast cancer, and gastric cancer. A meta-analysis of 50,000 individuals given folic acid (0.5–40 mg daily) or placebo in cardiovascular (n = 10) or colon adenoma pre vention (n = 3) trials found that folic acid supplementation did not significantly increase or decrease the overall incidence of cancer or of any site-specific cancer during a weighted average scheduled treatment duration of 5.7 years. Because folic acid may "feed" tumors, it prob ably should be avoided in those with established tumors unless severe megaloblastic anemia due to folate deficiency is present. HEMATOLOGIC FINDINGS ■ ■PERIPHERAL BLOOD Oval macrocytes, usually with considerable anisocytosis and poikilo cytosis, are the main feature (Fig. 104-2A). The MCV is usually >100 fL unless a cause of microcytosis (e.g., iron deficiency or thalassemia trait) is present. Some of the neutrophils are hypersegmented (more than five nuclear lobes). There may be leukopenia due to a reduction in granulocytes and lymphocytes, but this is usually >1.5 × 109/L; the platelet count may be moderately reduced, rarely to <40 × 109/L. The severity of all these changes parallels the degree of anemia. In a non anemic patient, the presence of a few macrocytes and hypersegmented neutrophils in the peripheral blood may be the only indication of the underlying disorder. ■ ■BONE MARROW In a severely anemic patient, the marrow is hypercellular with an accu mulation of primitive cells due to selective death by apoptosis of more mature forms. The erythroblast nucleus maintains a primitive, fine chromatin appearance despite maturation and hemoglobinization of the cytoplasm. The cells are larger than normoblasts, and an increased number of cells with eccentric lobulated nuclei or nuclear fragments may be present (Fig. 104-2B). Giant and abnormally shaped meta myelocytes

and enlarged hyperpolyploid megakaryocytes are charac teristic. In severe cases, the accumulation of primitive cells (“blasts”)

B A FIGURE 104-2 A. The peripheral blood in severe megaloblastic anemia. B. The bone marrow in severe megaloblastic anemia. (Reprinted from AV Hoffbrand et al [eds]: Postgraduate Haematology, 5th ed. Oxford, UK, Blackwell Publishing, 2005; with permission.) may mimic acute myeloid leukemia, whereas in less anemic patients, the changes in the marrow may be difficult to recognize. The terms intermediate, mild, and early have been used. The term megaloblastoid is best avoided. It has been used to describe cells with both immatureappearing nuclei and defective hemoglobinization refractory to folic acid or cobalamin therapy, especially seen in myelodysplasia. ■ ■CHROMOSOMES Bone marrow cells, transformed lymphocytes, and other proliferating cells in the body show a variety of chromosomal changes, including random breaks, reduced contraction, spreading of the centromere, and exaggeration of secondary chromosomal constrictions and over prominent satellites. Similar abnormalities may be produced by antimetabolite drugs (e.g., cytarabine, hydroxyurea, thioguanine, and methotrexate) that interfere with either DNA replication or folate metabolism and that also cause megaloblastic appearances. ■ ■INEFFECTIVE HEMATOPOIESIS Unconjugated bilirubin accumulates in plasma due to the death of nucleated red cells in the marrow (ineffective erythropoiesis). Other evidence for this includes raised urine urobilinogen, reduced hapto globins, positive urine hemosiderin, and raised serum lactate dehydro genase. A weakly positive direct antiglobulin test due to complement only can lead to a false diagnosis of autoimmune hemolytic anemia. CAUSES OF COBALAMIN DEFICIENCY Cobalamin deficiency is usually due to malabsorption. The only other cause is inadequate dietary intake. ■ ■INADEQUATE DIETARY INTAKE Adults Dietary cobalamin deficiency arises in vegans who omit meat, fish, eggs, cheese, and other animal products from their diet. The largest group in the world consists of Hindus, and it is likely that many millions of Indians are at risk of deficiency of cobalamin on a nutritional basis. Subnormal serum cobalamin levels are found in up to 50% of randomly selected, young, adult Indian vegans, but the defi ciency usually does not progress to megaloblastic anemia since the diet of most vegans is not totally lacking in cobalamin and the enterohe patic circulation of cobalamin is intact. Dietary cobalamin deficiency may also arise rarely in nonvegetarian individuals who exist on grossly inadequate diets because of poverty or psychiatric disturbance.

CHAPTER 104 Megaloblastic Anemias Infants Cobalamin deficiency has been described in infants born to severely cobalamin-deficient mothers. These infants develop mega loblastic anemia at about 3–6 months of age, presumably because they are born with low stores of cobalamin and because they are fed breast milk with low cobalamin content. The babies have also shown growth retardation, impaired psychomotor development, and other neurologic sequelae. MRI shows delayed myelination and brain atrophy. ■ ■GASTRIC CAUSES OF COBALAMIN MALABSORPTION See Tables 104-3 and 104-4. Formerly, the pathogenesis of cobalamin malabsorption was distin guishable based on the results of a Schilling test in which a radioac tive form of cobalamin was administered orally and its appearance in the urine was a sign of absorption. Radioactive cobalamin is no longer available, and Schilling tests are no longer performed. Other approaches to the differential diagnosis of cobalamin malabsorption are now employed. Pernicious Anemia PA, the dominant cause of severe cobalamin deficiency in Western countries, may be defined as a severe lack of IF due to gastric atrophy. It is a common disease in northern Europeans but occurs in all countries and ethnic groups. It is more frequent in people of African than Asian ancestry. The

overall incidence is about

TABLE 104-3 Causes of Cobalamin Deficiency Sufficiently Severe to Cause Megaloblastic Anemia NUTRITIONAL VEGANS Malabsorption Pernicious anemia Gastric causes Congenital absence of intrinsic factor or functional abnormality Total or partial gastrectomy Intestinal causes Intestinal stagnant loop syndrome: jejunal diverticulosis, ileocolic fistula, anatomic blind loop, intestinal stricture, etc. Ileal resection and Crohn's disease Selective malabsorption with proteinuria Tropical sprue Transcobalamin II deficiency Fish tapeworm

TABLE 104-4 Malabsorption of Cobalamin May Occur in the Following Conditions but Is Not Usually Sufficiently Severe and Prolonged to Cause Megaloblastic Anemia Gastric causes Simple atrophic gastritis (food cobalamin malabsorption) Zollinger-Ellison syndrome Gastric bypass or bariatric surgery Use of proton pump inhibitors Intestinal causes Gluten-induced enteropathy Severe pancreatitis HIV infection Radiotherapy Graft-versus-host disease Deficiencies of cobalamin, folate, protein,? riboflavin,? nicotinic acid Therapy with colchicine, para-aminosalicylate, neomycin, slow-release potassium chloride, anticonvulsant drugs, metformin,a cytotoxic drugs Alcohol aIt is now thought that metformin lowers serum vitamin B12 level by lowering the level of transcobalamin I. PART 4 Oncology and Hematology 120 per 100,000 population in the United Kingdom (UK). The ratio of incidence in men and women among whites is ~1:1.6, and the median age of onset is 70–80 years, with only 10% of patients being <40 years of age. However, in some ethnic groups, notably blacks and Latin Americans, the age at onset of PA is generally lower. The disease occurs more commonly than by chance in close relatives and in persons with other organ-specific autoimmune diseases, for example, thyroid diseases, vitiligo, hypoparathyroidism, type 1 diabetes, and Addison's disease. It is also associated with hypogammaglobulinemia, premature graying or blue eyes, and persons of blood group A. An association with human leukocyte antigen (HLA) 3 has been reported in some but not all series and, in those with endocrine disease, with HLA-B8, -B12, and -BW15. Life expectancy is normal in women once regular treatment has begun. Men had in earlier decades a slightly subnormal life expectancy as a result of a higher incidence of carcinoma of the stomach than in con trol subjects, but current data on their life expectancy are unavailable. Gastric output of hydrochloric acid, pepsin, and IF is severely reduced. The serum gastrin level is raised, and serum pepsinogen I levels are low. Gastric Biopsy A single endoscopic examination is recommended if PA is diagnosed. Gastric biopsy usually shows atrophy of all layers of the body and fundus, with loss of glandular elements, an absence of parietal and chief cells and replacement by mucous cells, a mixed inflammatory cell infiltrate, and perhaps intestinal metaplasia. The infiltrate of plasma cells and lymphocytes contains an excess of CD4 cells. These are directed against gastric H/K-ATPase. The antral mucosa is usually well preserved. Helicobacter pylori infection occurs infrequently in PA, but it has been suggested that H. pylori gastritis occurs at an early phase of atrophic gastritis and presents in younger patients as iron-deficiency anemia and in older patients as PA. H. pylori is suggested to stimulate an autoimmune process directed against pari etal cells. It has been suggested that H. pylori infection is replaced, in some individuals, by the autoimmune process. Serum Antibodies Two types of IF immunoglobulin G antibody may be found in the sera of patients with PA. The "blocking," or type I,

antibody prevents the combination of IF and cobalamin, whereas the "binding," or type II, antibody prevents attachment of IF to ileal mucosa. Type I occurs in the sera of ~55% of patients, and type II in 35%. IF antibodies cross the placenta and may cause temporary IF deficiency in a newborn

infant. Type I antibody has been detected rarely in the sera of patients without PA but with thyrotoxicosis, myxedema, Hashimoto’s disease, or diabetes mellitus and in relatives of PA patients. IF antibodies have also been detected in gastric juice in ~80% of PA patients. These gastric antibodies may reduce absorption of dietary cobalamin by combining with small amounts of remaining IF. Patients with PA also show cell-mediated immunity to IF.

Parietal cell antibody is present in the sera of almost 90% of adult patients with PA but is frequently present in other subjects. Thus, it occurs in as many as 16% of randomly selected female subjects age

> 60 years. The parietal cell antibody is directed against the α and β subunits of the gastric proton pump (H+, K+-ATPase). ■ ■JUVENILE PERNICIOUS ANEMIA This usually occurs in older children and resembles PA of adults. Gastric atrophy, achlorhydria, and serum IF antibodies are all present, although parietal cell antibodies are usually absent. About one-half of these patients show an associated endocrinopathy such as autoimmune thyroiditis, Addison’s disease, or hypoparathyroidism; in some, muco cutaneous candidiasis occurs. ■ ■CONGENITAL INTRINSIC FACTOR DEFICIENCY OR FUNCTIONAL ABNORMALITY An affected child usually presents with megaloblastic anemia in the first to third year of life; a few have presented as late as the second decade. The child usually has no demonstrable IF but has a normal gastric mucosa and normal secretion of acid. The inheritance is auto somal recessive. Parietal cell and IF antibodies are absent. Variants have been described in which the child is born with IF that can be detected immunologically but is unstable or functionally inactive, unable to bind cobalamin or to facilitate its uptake by ileal receptors. ■ ■GASTRECTOMY After total gastrectomy, cobalamin deficiency is inevitable, and pro phylactic cobalamin therapy should be commenced immediately after the operation. After partial gastrectomy, 10–15% of patients also develop this deficiency. The exact incidence and time of onset are most influenced by the size of the resection and the preexisting size of cobalamin body stores. ■ ■FOOD COBALAMIN MALABSORPTION Failure of release of cobalamin from binding proteins in food is responsible for this condition, which is more common in the elderly. It is associated with low serum cobalamin levels, with or without raised serum levels of MMA and homocysteine. Typically, these patients have normal cobalamin absorption, as measured with crystalline cobala min, but show malabsorption when a modified test using food-bound cobalamin is used. It is usually due to mild forms of atrophic gastritis or therapy with proton pump inhibitors. Bariatric surgery is likely to be an increasing cause of this form of cobalamin malabsorption and defi ciency. The frequency of progression to severe cobalamin deficiency and the reasons for this progression are not clear. ■ ■INTESTINAL CAUSES OF COBALAMIN MALABSORPTION Intestinal Stagnant Loop Syndrome Malabsorption of cobala min occurs in a variety of intestinal lesions in which there is coloniza tion of the upper small intestine by fecal organisms. This may occur in patients with jejunal diverticulosis, entero-

> anastomosis, or an intestinal stricture or fistula or with an anatomic blind loop due to Crohn’s dis ease, tuberculosis, or an operative procedure. Ileal Resection Removal of ≥1.2 m of terminal ileum causes malabsorption of cobalamin. In some patients after ileal resection, particularly if the ileocecal valve is incompetent, colonic bacteria may contribute further to the onset of cobalamin deficiency. Selective Malabsorption of Cobalamin with Proteinuria (Imerslund’s Syndrome; Imerslund-Gräsbeck Syndrome; Congenital Cobalamin Malabsorption; Autosomal Reces sive Megaloblastic Anemia; MGA1) This autosomal recessive disease is the most common cause of megaloblastic anemia due to cobalamin deficiency in infancy in Western countries. More than 200 cases have been reported with familial clusters in Finland, Norway, the Middle East, and North Africa. The patients secrete normal amounts of IF and gastric acid but are unable to absorb cobalamin. In Finland, impaired synthesis, processing, or ligand binding of cubilin due to

inherited mutations is found. In Norway, mutation of the gene for AMN has been reported. Other tests of intestinal absorption are nor mal. Over 90% of these patients show nonspecific proteinuria, but renal function is otherwise normal, and renal biopsy has not shown any con sistent renal defect. A few have shown aminoaciduria and congenital renal abnormalities, such as duplication of the renal pelvis. Tropical Sprue Nearly all patients with acute and subacute tropi cal sprue show malabsorption of cobalamin. This may persist as the principal abnormality in the chronic form of the disease, when the patient may present with megaloblastic anemia or neuropathy due to cobalamin deficiency. Absorption of cobalamin usually improves after antibiotic and, in the early stages, folic acid therapy. Fish Tapeworm Infestation The fish tapeworm (Diphylloboth rium latum) lives in the small intestine of humans and accumulates cobalamin from food, rendering the cobalamin unavailable for absorp tion. Individuals acquire the worm by eating raw or partly cooked fish. Infestation was common around the lakes of Scandinavia, Germany, Japan, North America, and Russia. Megaloblastic anemia or cobalamin neuropathy occurs only in those with a heavy infestation. Gluten-Induced Enteropathy Malabsorption of cobalamin occurs in ~30% of untreated patients (presumably those in whom the disease extends to the ileum). Cobalamin deficiency is not severe in these patients and is corrected with a gluten-free diet. Severe Chronic Pancreatitis In this condition, lack of trypsin is thought to cause dietary cobalamin attached to gastric non-IF (HC) binder to be unavailable for absorption. It also has been proposed that, in pancreatitis, the concentration of calcium ions in the ileum falls below the level needed to maintain normal cobalamin absorption. HIV Infection Serum cobalamin levels tend to fall in patients with HIV infection and are subnormal in 10–35% of those with AIDS. Mal absorption of cobalamin not corrected by IF has been shown in some, but not all, patients with subnormal serum cobalamin levels. Cobala min deficiency sufficiently severe to cause megaloblastic anemia or neuropathy is rare. Zollinger-Ellison Syndrome Malabsorption of cobalamin has been reported in the Zollinger-Ellison syndrome. It is thought that there is a failure to release cobalamin from HC binding protein due to inactivation of pancreatic trypsin by high acidity, as well as interference with IF binding of cobalamin. Radiotherapy Both total-body irradiation and local radiotherapy to the ileum (e.g., as a complication of radiotherapy for carcinoma of the cervix) may cause malabsorption of cobalamin. Graft-Versus-Host Disease This commonly affects the small intestine. Malabsorption of cobalamin

due to abnormal gut flora, as well as damage to ileal mucosa, is common. Drugs The drugs that have been reported to cause malabsorption of cobalamin are listed in Table 104-4. However, megaloblastic anemia due to these drugs is rare. It has been suggested that metformin lowers serum cobalamin by lowering TC I level rather than causing malab sorption of cobalamin. ■ ■ABNORMALITIES OF COBALAMIN METABOLISM Congenital Transcobalamin II Deficiency or Abnormality

Infants with TC II deficiency usually present with megaloblastic anemia within a few weeks of birth. Serum cobalamin and folate levels are normal, but the anemia responds to massive (e.g., 1 mg three times weekly) injec tions of cobalamin. Some cases show neurologic complications. The pro tein may be present but functionally inert. Genetic abnormalities found include mutations of an intraexonic cryptic splice site, extensive deletion, single nucleotide deletion, nonsense mutation, and an RNA editing defect. Malabsorption of cobalamin occurs in all cases, and serum immunoglob ulins are usually reduced. Failure to institute adequate cobalamin therapy or treatment with folic acid may lead to irreversible neurologic damage.

Congenital Methylmalonic Acidemia and Aciduria Infants with this abnormality are ill from birth with vomiting, failure to thrive, severe metabolic acidosis, ketosis, and intellectual disability. Anemia, if present, is normocytic and normoblastic. The condition may be due to a functional defect in either mitochondrial methylmalonyl-CoA mutase or its cofactor adocobalamin. Mutations in the methylmal onyl-CoA mutase are not responsive or are only poorly responsive to treatment with cobalamin. A proportion of infants with failure of adocobalamin synthesis respond to cobalamin in large doses. Some children have combined methylmalonic aciduria and homocystinuria due to defective formation of both cobalamin coenzymes. This usually presents in the first year of life with feeding difficulties, developmental delay, microcephaly, seizures, hypotonia, and megaloblastic anemia.

Acquired Abnormality of Cobalamin Metabolism: Nitrous Oxide Inhalation Nitrous oxide (N_2O) irreversibly oxidizes meth ylcobalamin to an inactive precursor; this inactivates methionine synthase. Megaloblastic anemia has occurred in patients undergoing prolonged N_2O anesthesia (e.g., in intensive care units). A neuropathy resembling cobalamin neuropathy has been described in dentists and anesthetists who are exposed repeatedly to N_2O. Methylmalonic acid uria does not occur as adocobalamin is not inactivated by N_2O. CHAPTER 104 CAUSES OF FOLATE DEFICIENCY (Table 104-5) ■ ■NUTRITIONAL Dietary folate deficiency is common except in countries that fortify their diet with folic acid. In most patients with folate deficiency, a nutritional element is present. Certain individuals are particularly prone to have diets containing inadequate amounts of folate (Table 104-5). In the United States and other countries where fortification Megaloblastic Anemias TABLE 104-5 Causes of Folate Deficiency Dietarya Particularly in: old age, infancy, poverty, alcoholism, chronic invalids, and the psychiatrically disturbed; may be associated with scurvy or kwashiorkor Malabsorption Major causes of deficiency Tropical sprue, gluten-induced enteropathy in children and adults, and in association with dermatitis herpetiformis, specific malabsorption of folate, intestinal megaloblastosis caused by severe cobalamin or folate deficiency Minor causes of deficiency Extensive jejunal resection, Crohn’s disease, partial gastrectomy, congestive heart failure, Whipple’s disease, scleroderma, amyloid, diabetic enteropathy, systemic bacterial infection, lymphoma, sulfasalazine (Salazopyrin) Excess utilization or loss Physiologic Pregnancy and lactation, prematurity Pathologic Hematologic diseases: chronic hemolytic anemias, sickle cell

anemia, thalassemia major, myelofibrosis Malignant diseases: carcinoma, lymphoma, leukemia, myeloma Inflammatory diseases: tuberculosis, Crohn’s disease, psoriasis, exfoliative dermatitis, malaria Metabolic disease: homocystinuria Excess urinary loss: congestive heart failure, active liver disease Hemodialysis, peritoneal dialysis Antifolate drugsb Anticonvulsant drugs (phenytoin, primidone, barbiturates), sulfasalazine Nitrofurantoin, tetracycline, antituberculosis (less well documented) Mixed causes Liver diseases, alcoholism, intensive care units aIn severely folate-deficient patients with causes other than those listed under Dietary, poor dietary intake is often present. bDrugs inhibiting dihydrofolate reductase are discussed in the text.

of the diet with folic acid has been adopted, the prevalence of folatedeficient megaloblastic anemia has dropped dramatically and is now restricted to high-risk groups with increased folate needs. Nutritional folate deficiency occurs in kwashiorkor and scurvy and in infants with repeated infections or those who are fed solely on goats’ milk, which has a low folate content.

■ ■MALABSORPTION Malabsorption of dietary folate occurs in tropical sprue and in gluteninduced enteropathy. In the rare congenital recessive syndrome of selec tive malabsorption of folate due to mutation of the PCFT, there is an associated defect of folate transport into the cerebrospinal fluid, and these patients show megaloblastic anemia, which responds to physiologic doses of folic acid given parenterally but not orally. They also show intellectual disability, convulsions, and other central nervous system abnormalities. Minor degrees of malabsorption may also occur after jejunal resection or partial gastrectomy, in Crohn’s disease, and in systemic infections, but in these conditions, if severe deficiency occurs, it is usually largely due to poor nutrition. Malabsorption of folate has been described in patients receiving sulfasalazine (Salazopyrin), cholestyramine, and triamterene. ■ ■EXCESS UTILIZATION OR LOSS Pregnancy Folate requirements are increased by 50% daily in a normal pregnancy, partly because of transfer of the vitamin to the fetus but mainly because of increased folate catabolism due to cleavage of folate coenzymes in rapidly proliferating tissues. A dietary folate intake of 600 µg daily is recommended. Megaloblastic anemia due to folate deficiency is prevented by prophylactic folic acid therapy. It occurred in 0.5% of pregnancies in the UK and other Western countries before pro phylaxis with folic acid, but the incidence is much higher in countries where the general nutritional status is poor. During lactation, folate requirements are increased about 25 and a dietary intake of 500 µg of folate daily is advised. PART 4 Oncology and Hematology Prematurity A newborn infant, whether full term or premature, has higher serum and red cell folate concentrations than an adult. However, a newborn infant’s demand for folate has been estimated to be up to 10 times that of adults on a weight basis, and the neonatal folate level falls rapidly to the lowest values at about 6 weeks of age. The falls are steepest and are liable to reach subnormal levels in premature babies, a number of whom develop megaloblastic anemia responsive to folic acid at about 4–6 weeks of age. This occurs particu larly in the smallest babies (<1500 g birth weight) and those who have feeding difficulties or infections or have undergone multiple exchange transfusions. In these babies, prophylactic folic acid should be given. Hematologic Disorders Folate deficiency frequently occurs in chronic hemolytic anemias, particularly in sickle cell disease, autoim mune hemolytic anemia, and congenital spherocytosis. In these and in other conditions of increased cell turnover (e.g., myelofibrosis, malig nancies), folate deficiency arises because it is not completely reutilized after performing coenzyme functions. Inflammatory Conditions Chronic inflammatory diseases such as tuberculosis, rheumatoid arthritis, Crohn’s disease, psoriasis, exfolia tive dermatitis, bacterial endocarditis, and chronic bacterial infections cause deficiency by reducing the appetite and increasing the demand for folate.

Systemic infections also may cause malabsorption of folate. Severe deficiency is virtually confined to the patients with the most active disease and the poorest diet. Homocystinuria This is a rare metabolic defect in the conversion of homocysteine to cystathionine. Folate deficiency occurring in most of these patients may be due to excessive utilization because of com pensatory increased conversion of homocysteine to methionine. Long-Term Dialysis Because folate is only loosely bound to plasma proteins, it is easily removed from plasma by dialysis. In patients with anorexia, vomiting, infections, and hemolysis, folate stores are particularly likely to become depleted. Routine folate pro phylaxis is now given.

Congestive Heart Failure and Liver Disease Excess urinary folate losses of >100 µg per day may occur in some of these patients. It appears to be due to release of folate from damaged liver cells. ■ ■ANTIFOLATE DRUGS A large number of people with epilepsy receiving long-term therapy with phenytoin or primidone, with or without barbiturates, develop low serum and red cell folate levels. The exact mechanism is unclear. Alcohol may also be a folate antagonist, as patients who are drinking spirits may develop megaloblastic anemia that will respond to normal quantities of dietary folate or to physiologic doses of folic acid only if alcohol is withdrawn. Macrocytosis of red cells is associated with chronic alcohol intake even when folate levels are normal. Inadequate folate intake is the major factor in the development of deficiency in spirit-drinking alcoholics. Beer is relatively folate-rich in some coun tries, depending on the technique used for brewing. The drugs that inhibit DHF reductase include methotrexate, pyri methamine, and trimethoprim. Methotrexate has the most powerful action against the human enzyme, whereas trimethoprim is most active against the bacterial enzyme and is likely to cause megaloblastic ane mia only when used in conjunction with sulfamethoxazole in patients with preexisting folate or cobalamin deficiency. The activity of pyri methamine is intermediate. The antidote to these drugs is a reduced form of folate, folinic acid (5-formyl-THF). ■ ■CONGENITAL ABNORMALITIES OF FOLATE METABOLISM Some infants with congenital defects of folate enzymes (e.g., cyclohy drolase or methionine synthase) have had megaloblastic anemia. DIAGNOSIS OF COBALAMIN AND FOLATE DEFICIENCIES The diagnosis of cobalamin or folate deficiency has traditionally depended on the recognition of the relevant abnormalities in the peripheral blood and analysis of the blood levels of the vitamins. ■ ■COBALAMIN DEFICIENCY Serum Cobalamin This is measured by an automated enzymelinked immunosorbent assay (ELISA) or competitive-binding lumi nescence assay (CBLA). Normal serum levels range from 118–148 pmol/L (160–200 ng/L) to ~738 pmol/L (1000 ng/L). In patients with megaloblastic anemia due to cobalamin deficiency, the level is usually <74 pmol/L (100 ng/L). In general, the more severe the deficiency, the lower is the serum cobalamin level. In patients with spinal cord damage due to the deficiency, levels are very low even in the absence of anemia. Values between 74 and 148 pmol/L (100 and 200 ng/L) are regarded as borderline. They may occur, for instance, in pregnancy, in patients with megaloblastic anemia due to folate deficiency. They may also be due to heterozygous, homozygous, or compound heterozygous mutations of the gene TCN1 that codes for HC (TC I). There is then no clinical or hematologic abnormality. The serum cobalamin level is suf ficiently robust, cost-effective, and most convenient to rule out cobala min deficiency in the vast majority of patients suspected of having this problem. However, problems have arisen with commercial CBLA assays involving IF in PA patients with intrinsic antibodies in serum. These antibodies may cause false normal serum cobalamin levels in up to 50% of cases tested. Where clinical indications of PA are strong, a normal serum cobalamin does not rule out the diagnosis. Serum MMA levels will be elevated in untreated PA (see below). Folate deficiency, TC I (HC) deficiency, oral contraceptives, and multiple myeloma have all been

associated with low serum cobalamin levels that do not indicate cobalamin deficiency. On the other hand, high serum cobalamin levels are usually due to raised serum TC I lev els and can be due to the presence of liver, renal, or myeloproliferative diseases or to cancer of the breast, colon, or liver. Serum Methylmalonate and Homocysteine In patients with cobalamin deficiency sufficient to cause anemia or neuropathy, the serum MMA level is raised. Sensitive methods for measuring MMA

and homocysteine in serum have been introduced and recommended for the early diagnosis of cobalamin deficiency, even in the absence of hematologic abnormalities or subnormal levels of serum cobalamin. Serum MMA levels fluctuate, however, in patients with renal failure. Mildly elevated serum MMA and/or homocysteine levels occur in up to 30% of apparently healthy volunteers, with serum cobalamin levels up to 258 pmol/L (350 ng/L) and normal serum folate levels; 15% of elderly subjects, even with cobalamin levels >258 pmol/L (>350 ng/L), have this pattern of raised metabolite levels. These findings bring into question the exact cutoff points for normal MMA and homocysteine levels. It is also unclear at present whether these mildly raised metabo lite levels have clinical consequences. Serum homocysteine is raised in both early cobalamin and folate deficiency but may be raised in other conditions, for example, chronic renal disease, alcoholism, smoking, pyridoxine deficiency, hypothy roidism, and therapy with steroids, cyclosporin, and other drugs. Levels are also higher in serum than in plasma, in men than in premenopausal women, in women taking hormone replacement therapy or in oral con traceptive users, and in elderly persons and patients with several inborn errors of metabolism affecting enzymes in trans-sulfuration pathways of homocysteine metabolism. Thus, homocysteine levels must be care fully interpreted for diagnosis of cobalamin or folate deficiency. Tests for the Cause of Cobalamin Deficiency Only vegans, strict vegetarians, or people living on a totally inadequate diet will become cobalamin deficient because of inadequate intake. Studies of cobalamin absorption once were widely used, but difficulty in obtain ing radioactive cobalamin and ensuring that IF preparations are free of viruses has made these tests obsolete. Tests to diagnose PA include serum gastrin, which is raised; serum pepsinogen I, which is low in PA (90–92%) but also in other conditions; and gastric endoscopy. Tests for IF and parietal cell antibodies are also used, as well as tests for indi vidual intestinal diseases. Patients with atrophic gastritis may also have sufficient occult gas trointestinal blood loss to have iron deficiency as well as cobalamin deficiency. Iron deficiency may blunt the development of macrocytosis. Iron deficiency is much more common than cobalamin deficiency. In people older than age 60 years, cobalamin deficiency may accompany iron deficiency in 15–20% of cases. Thus, older patients diagnosed with iron-deficiency anemia should have cobalamin levels assessed, and those diagnosed with cobalamin deficiency should have their iron status assessed. ■ ■FOLATE DEFICIENCY Serum Folate This is also measured by a chemiluminescence immu noassay or ELISA technique. In most laboratories, the normal range is from 11 nmol/L (2 µg/L) to ~82 nmol/L (15 µg/L). The serum folate level is low in all folate-deficient patients. It also reflects recent diet. Because of this, serum folate may be low before there is hematologic or bio chemical evidence of deficiency. Serum folate rises in severe cobalamin deficiency because of the block in conversion of 5-MTHF to THF inside cells; raised levels have also been reported in the intestinal stagnant-loop syndrome due to absorption of bacterially synthesized folate. Red Cell Folate The red cell folate assay is a valuable test of body folate stores. It is less affected than the serum assay by recent diet and traces of hemolysis. In normal adults, concentrations range from 880 to 3520 µmol/L (160–640 µg/L) of packed red cells. Subnormal levels occur in patients with megaloblastic anemia due to folate deficiency but also in nearly two-thirds of patients with severe cobalamin defi ciency. False-normal results may occur if a folate-

deficient patient has received a recent blood transfusion or if a patient has a raised reticulo cyte count. Serum homocysteine assay is discussed earlier. Tests for the Cause of Folate Deficiency The diet history is important. Tests for transglutaminase antibodies are performed to confirm or exclude gluten–induced enteropathy. If positive, duodenal biopsy is needed. An underlying disease causing increased folate break down should also be excluded.

TREATMENT Cobalamin and Folate Deficiency It is usually possible to establish which of the two deficiencies, folate or cobalamin, is the cause of the anemia and to treat only with the appropriate vitamin. In patients who enter the hospital severely ill, however, it may be necessary to treat with both vitamins in large doses once blood samples have been taken for cobalamin and folate assays and a bone marrow biopsy has been performed (if deemed necessary). Transfusion is usually unnecessary and inadvisable. If it is essential, packed red cells should be given slowly, one or two units only, with the usual treatment for heart failure if present. Occasion ally, an excessive rise in platelets occurs after 1–2 weeks of therapy. Antiplatelet therapy, for example, aspirin, should be considered if the platelet count rises to >800 × 109/L. COBALAMIN DEFICIENCY It is usually necessary to treat patients who have developed severe cobalamin deficiency, as from PA, with lifelong regular cobalamin injections. In the UK, hydroxocobalamin is used; in the United States, cyanocobalamin. In a few instances, the under lying cause of cobalamin deficiency can be permanently corrected, for example, fish tapeworm, tropical sprue, or an intestinal stagnant loop that is amenable to surgery. The indications for starting cobal amin therapy are a well-documented megaloblastic anemia or other hematologic abnormalities and/or neuropathy due to the deficiency. Cobalamin should be given routinely to all patients who have had a total gastrectomy or ileal resection. Patients who have undergone gastric reduction for control of obesity or who are receiving longterm treatment with proton pump inhibitors should be screened and, if necessary, given cobalamin replacement. CHAPTER 104 Megaloblastic Anemias Replenishment of body stores should be complete with six 1000-µg IM injections of hydroxocobalamin given at 3- to 7-day intervals. More frequent doses are usually used in patients with cobalamin neuropathy, but there is no evidence that they produce a better response. Allergic reactions are rare and may require desensitiza tion or antihistamine or glucocorticoid cover. For maintenance therapy, 1000 µg hydroxocobalamin IM once every 3 months is sat isfactory. Because of the poorer retention of cyanocobalamin, pro tocols generally use higher and more frequent doses, for example, 1000 µg cyanocobalamin IM, monthly, for maintenance treatment. Because a small fraction of cobalamin can be absorbed passively through mucous membranes even when there is complete failure of physiologic IF-dependent absorption, large daily oral doses (1000– 2000 µg) of cyanocobalamin may be used in PA for replacement (especially in Canada and Sweden) and maintenance of normal cobalamin status. Sublingual therapy has also been proposed for those in whom injections are difficult because of a bleeding ten dency and who may not tolerate parenteral therapy. If oral therapy is used, it is important to monitor compliance, particularly with elderly, forgetful patients. This author prefers parenteral therapy for initial treatment for PA, particularly in severe anemia or if a neuropathy is present, and for maintenance. Treatment of patients with subnormal serum cobalamin levels with a normal MCV and no hypersegmentation of neutrophils and a negative IF antibody is, however, problematic. Some (perhaps 15%) cases may be due to TC I (HC) deficiency. Homocysteine and/ or MMA measurements may help, but in the absence of these tests and with otherwise normal gastrointestinal function, repeat serum cobalamin assay after 6–12 months may help one decide whether to start cobalamin therapy. Oral cyanocobalamin therapy with low doses (e.g., 50 µg daily) has a large role in treating patients thought to have food malabsorption of

cobalamin. Cobalamin injections are used in a wide variety of diseases, often neurologic, despite normal serum cobalamin and folate levels and a normal blood count and in the absence of randomized, double-blind, controlled trials. These conditions include multiple sclerosis and chronic fatigue syndrome/myalgic encephalomyelitis (ME). It seems probable that any benefit is due to the placebo effect of a usually

painless, pink injection. In ME, oral cobalamin therapy, despite pro viding equally large amounts of cobalamin, has not been beneficial, supporting the view of the effect of the injections being placebo only.

FOLATE DEFICIENCY Oral doses of 5–15 mg of folic acid daily are satisfactory, as suf ficient folate is absorbed from these extremely large doses even in patients with severe malabsorption. The length of time therapy must be continued depends on the underlying disease. It is cus tomary to continue therapy for about 4 months, when all folatedeficient red cells will have been eliminated and replaced by new folate-replete populations. Before large doses of folic acid are given, cobalamin deficiency must be excluded and, if present, corrected; otherwise, cobalamin neuropathy may develop as the deficiency progresses despite a response of the anemia of cobalamin deficiency to folate therapy. Long-term folic acid therapy is required when the underlying cause of the deficiency cannot be corrected and the deficiency is likely to recur, for example, in chronic dialysis or chronic hemolytic anemias. It may also be necessary in gluten-induced enteropathy that does not respond to a gluten-free diet. Where mild but chronic folate deficiency occurs, it is preferable to encourage improvement in the diet after correcting the deficiency with a short course of folic acid. In any patient receiving long-term folic acid therapy, it is important to measure the serum cobalamin level at regular (e.g., once-yearly) inter vals to exclude the coincidental development of cobalamin deficiency. PART 4 Oncology and Hematology Folinic Acid (5-Formyl-THF) This is a stable form of fully reduced folate. It is given orally or parenterally to overcome the toxic effects of methotrexate or other DHF reductase inhibitors, for example, trimethoprim or cotrimoxazole. PROPHYLACTIC FOLIC ACID Prophylactic folic acid is used in chronic dialysis patients and in par enteral feeds. Prophylactic folic acid has been used to reduce homo cysteine levels to prevent cardiovascular disease and for cognitive function in the elderly, but there are no firm data to show any benefit. Pregnancy In over 80 countries (but none in Europe), food is for tified at a level of 120–250 µg/100 g with folic acid (in grain, flour, or rice) to reduce the risk of NTDs. In all countries that have studied this, fortification has led to a lower prevalence of NTD pregnancies and births. Nevertheless, folic acid, 400 µg daily, should also be given as a supplement before and throughout pregnancy to prevent mega loblastic anemia and reduce the incidence of NTDs, even in coun tries with fortification of the diet. Most if not all the folic acid used in fortification and eaten over three meals a day will be converted dur ing absorption to 5-MTHF. This compound at the levels achieved by fortification will not correct the anemia in cobalamin deficiency. Studies in the United States suggest that there is no increase in the proportion of individuals with low serum cobalamin levels and no anemia since food fortification with folic acid. It is unknown if there has been a change in incidence of cobalamin neuropathy, but no country has reported this since mandating fortification. Data in early pregnancy show significant lack of compliance with taking folic acid supplements, emphasizing the need for food fortification. In women who have had a previous fetus with an NTD and others at high risk (e.g., diabetes, sickle cell anemia), a dose of 5 mg daily is recommended when pregnancy is contemplated and throughout the subsequent pregnancy. Infancy and Childhood The incidence of folate deficiency is so high in the smallest premature babies during the

first 6 weeks of life that folic acid (e.g., 1 mg daily) should be given routinely to those weighing <1500 g at birth and to larger premature babies who require exchange transfusions or develop feeding difficulties, infec tions, or vomiting and diarrhea. The World Health Organization currently recommends routine supplementation with iron and folic acid in children in countries where iron deficiency is common and child mortality, largely due to infectious diseases, is high. However, some studies suggest that in

areas where malaria rates are high, this approach may increase the incidence of severe illness and death. Even where malaria is rare, there appears to be no survival benefit. MEGALOBLASTIC ANEMIA NOT DUE TO COBALAMIN OR FOLATE DEFICIENCY OR ALTERED METABOLISM This may occur with many antimetabolite drugs (e.g., hydroxyurea, cytarabine, mercaptopurine, thioguanine) that inhibit DNA replica tion. Antiviral nucleoside analogues used in treatment of HIV infec tion may also cause macrocytosis and megaloblastic marrow changes. In the rare disease orotic aciduria, two consecutive enzymes in purine synthesis are defective. The condition responds to therapy with uridine, which bypasses the block. In thiamine-responsive megaloblastic ane mia, there is a genetic defect in the high-affinity thiamine transporter (SLC19A2) gene. This causes defective RNA ribose synthesis through impaired activity of transketolase, a thiamine-dependent enzyme in the pentose cycle. This defect leads to reduced nucleic acid production. It may be associated with diabetes mellitus and deafness and the presence of many ringed sideroblasts in the marrow. The explanation is unclear for megaloblastic changes in the marrow in some patients with acute myeloid leukemia and myelodysplasia. The most frequent causes of macrocytosis without megaloblastic changes are alcohol, liver disease, hypothyroidism, and pregnancy. Myelodysplasia, myeloma and other paraproteinemias, aplastic ane mia, and smoking are other causes. ■ ■FURTHER READING Berry RJ: Lack of historical evidence to support folic acid exacerbation of the neuropathy caused by vitamin B12 deficiency. Am J Clin Nutr 110:554, 2019. Bunn HF: Vitamin B12 and pernicious anemia: The dawn of molecular medicine. N Engl J Med 370:773, 2014. Del Bo C et al: Effect of two different sublingual dosages of vitamin B12 on cobalamin nutritional status in vegans and vegetarians with a marginal deficiency: A randomized controlled trial. Clin Nutr 38:575, 2019. Green R: Vitamin B12 deficiency from the perspective of a practicing hematologist. Blood 129:2603, 2017. Green R et al: Vitamin B12 deficiency. Nat Rev Dis Primers 3:17040, 2017. Hesdorffer CS, Longo DL: Drug-induced megaloblastic anemia. N Engl J Med 373:1649, 2015. Hoffbrand V: The Folate Story: A Vitamin Under the Microscope. Leicestershire, UK, Troubador Publishing, 2023. Kancherla V et al: Preventing birth defects, saving lives, and promot ing health equity: An urgent call to action for universal mandatory food fortification with folic acid. Lancet Global Health 10:e1053, 2022. Ma F et al: Effects of folic acid and vitamin B12 alone and in combina tion on cognitive function and inflammatory factors in the elderly with mild cognitive impairment: A single blind experimental design. Curr Alzheimer Res 16:622, 2019. Miller JW: Proton pump inhibitors, H2-receptor antagonists, metfor min, and vitamin B-12 deficiency: Clinical implications. Adv Nutr 9:511S, 2018. O’connor DMA et al: Low folate predicts cognitive decline: 8-year fol low–up of 3140 older adults in Ireland. Eur J Clin Nutr 76:950, 2022. Rogers LM et al: Global folate status in women of reproductive age: A systematic review with emphasis on methodological issues. Ann N Y Acad Sci 1431:35, 2018. Salinas M et al: High frequency of anti-parietal cell antibody (APCA) and intrinsic factor blocking antibody (IFBA) in individuals with severe vitamin B12 deficiency: An observational study in primary care patients. Clin Chem Lab Med 58:424, 2020. Wald NJ: Folic acid and neural tube defects: Discovery, debate and the need for policy change. J Med Screening 29:138, 2022. Zaric BL et al: Homocysteine and hyperhomocysteinaemia. Curr Med Chem 26: 2948, 2019.

Revision #1
Created 2026-01-06 16:31:57 UTC by Omar Ayman
Updated 2026-01-06 16:31:57 UTC by Omar Ayman