

Editors: Humes, H. David; DuPont, Herbert L.; Gardner, Laurence B.; Griffin, John W.; Harris, Edward D.; Hazzard, William R.; King, Talmadge E.; Loriaux, D. Lynn; Nabel, Elizabeth G.; Todd, Robert F.; Traber, Peter G.

Title: *Kelley's Textbook of Internal Medicine, 4th Edition*

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Chapter 243

MEGALOBLASTIC ANEMIAS

RALPH CARMEL

Megaloblastic anemia is a very characteristic disturbance of hematopoiesis that gives rise to large cells (macrocytosis) combined with nuclear abnormalities. All blood cell lines are affected—indeed, all dividing cells, such as skin and intestinal cells, show the same defect.

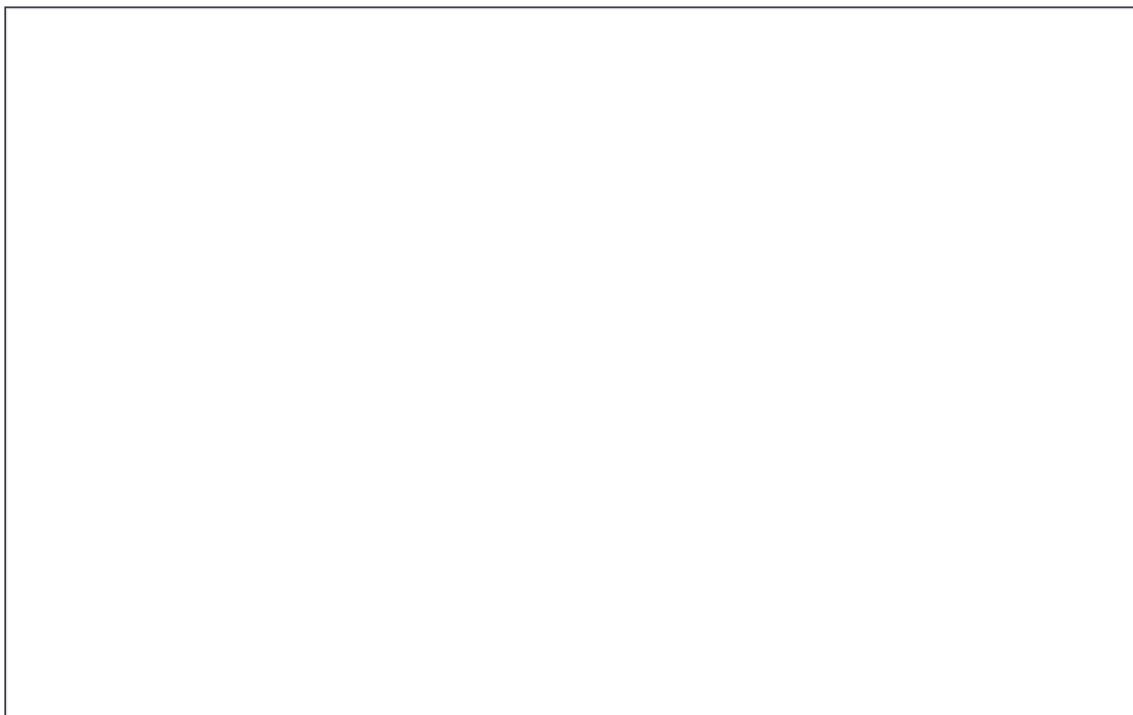
Several pathologic processes can produce megaloblastic anemia, but the most common by far are deficiencies of folate or cobalamin (vitamin B₁₂). These vitamin deficiencies are being identified with increasing frequency in patients who have little or no anemia, due, in part, to more sophisticated diagnostic approaches and earlier recognition.

NORMAL BIOCHEMISTRY AND PHYSIOLOGY

FOLATE

Biochemistry

Folic (pteroylglutamic) acid becomes metabolically active only upon reduction to tetrahydrofolic acid (THF) by dihydrofolate reductase (Fig. 243.1, reaction 1). Moreover, active folates have up to seven γ -carboxyl-linked glutamic acid side chains. This polyglutamated state enhances folate binding to enzymes and thus folate's coenzyme activity. It also is the usual intracellular form of folate because the highly polar polyglutamate side chain promotes cellular retention of the folate. Folates in the plasma are largely monoglutamates.



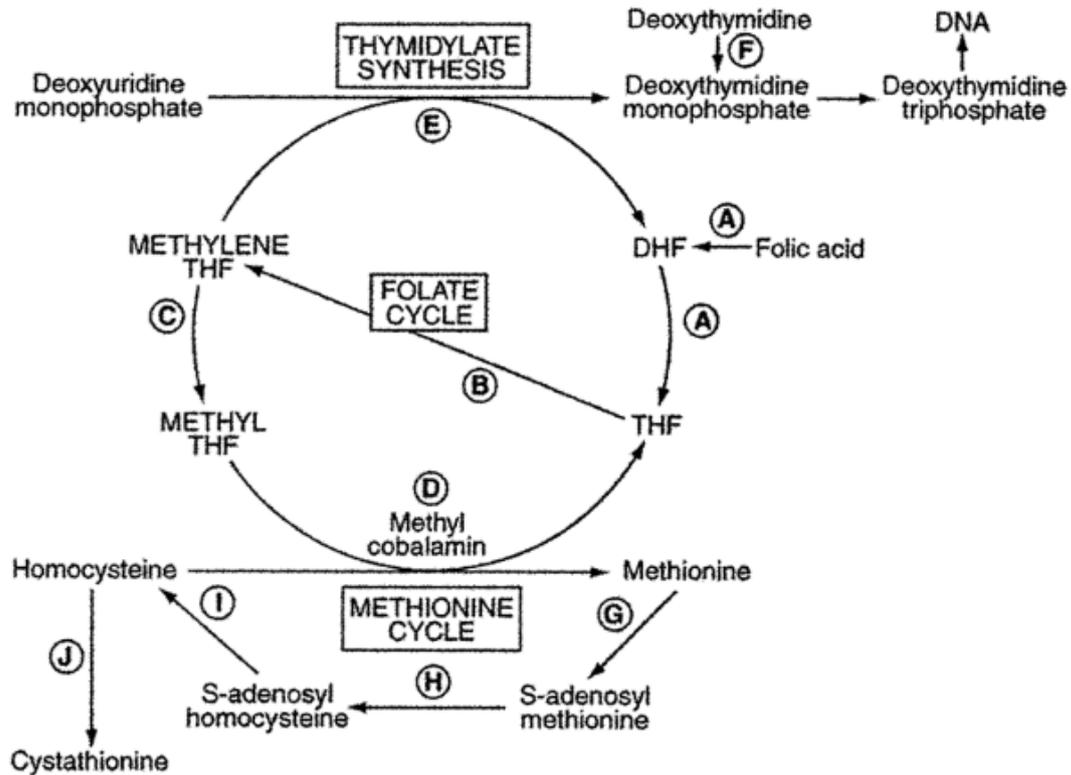


FIGURE 243.1. Folate metabolism and its interrelations with DNA synthesis (*top*) and cobalamin and homocysteine (*bottom*). This diagram includes only selected folate pathways in human beings. 1, Two steps in the reduction of folic acid and dihydrofolate to tetrahydrofolate (THF), mediated by dihydrofolate reductase. 2, The generation of 5, 10-methylene THF, coupled to serine-glycine metabolism. 3, Reaction mediated by 5, 10-methylene THF reductase; this reaction is augmented when methionine and S-adenosylmethionine production falls, as might occur in cobalamin deficiency, and thus potentiates the “methyl THF trap”. 4, Reaction mediated by methionine synthase (homocysteine:methionine methyltransferase); it requires methylcobalamin and is the focal point of the methyl THF trap hypothesis. 5, *De novo* synthesis of thymidine, mediated by thymidylate synthase. 6, Salvage pathway of thymidylate synthesis, mediated by thymidine kinase. 7, In addition to its remethylation to methionine (reaction 4), homocysteine is also metabolized to cystathionine; the initial reaction in the transsulfuration pathway is mediated by cystathionine β -synthase and pyridoxal phosphate.

The main metabolic function of folate involves one-carbon unit transfers. Several amino acid conversions require folate; an important one is the methylation of homocysteine to methionine, with 5-methyl THF as a cosubstrate for methionine synthase (Fig. 243.1, reaction 4). Folate is also involved in purine synthesis. The clinically most apparent role, however, is in the methylation of deoxyuridine monophosphate to thymidine monophosphate by thymidylate synthase, which requires 5,10-methylene THF (Fig. 243.1, reaction 5). It is the compromise of this reaction in folate deficiency that leads to megaloblastic anemia. Precisely how the compromised reaction does so is unclear, because the diminished thymidylate synthesis appears to be compensated by the salvage pathway (Fig. 243.1, reaction 6), but there may be different thymidylate pools. Moreover, the deoxyuridine excess, along with the thymidylate insufficiency, promotes misincorporation of uracil in place of thymidine into DNA.

Nutrition, Absorption, and Metabolism

Folate derives its name from the leafy vegetables that are rich in it; many other foods, such as meat, dairy products, cereals, and flour, are also good sources. To avoid deficiency, adults normally require about 0.2 mg of folate daily. Total body stores vary but are about 10 mg. Thus, daily turnover is 1% to 2% of stores.

Food folate, largely reduced and usually methylated, is labile. For example, cooking fish, frying meat, or boiling

vegetables can decrease the folate content of these foods by 50% to 90%. Even simple storage can diminish the folate content of some foods. Such information must be considered in assessing the adequacy of a patient's dietary intake. Moderate fortification

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with folate of all cereal-grain products was mandated by the Food and Drug Administration in 1998.

Dietary folate is absorbed after its polyglutamate side chain is converted by folate conjugase to the monoglutamate form, which more readily crosses membranes. Most nonmethylated folate is also converted to methyl THF during absorption. Medicinal folic acid is absorbed to a large extent by a different process than is food folate. The efficiency of absorption depends on the form of folate, but usually more than 60% is absorbed; the process is an active, saturable one, and it is most efficient in the jejunum.

Absorbed methyl THF enters cells for use or is recycled to the liver for storage or enterohepatic recirculation by way of bile. Most folate in the blood at any time, therefore, represents recently absorbed folate and is largely methyl THF.

Two folate-binding protein activities exist in plasma. One involves low-affinity, nonspecific binding promoted by various proteins. The second is a specific protein with a high affinity for mostly oxidized rather than reduced folates and a low binding capacity. The physiologic roles of these proteins are uncertain. The nonspecific binder may prevent renal loss of folate.

Folate is ultimately degraded, and some of it may be lost through the urine. Biliary loss appears to be relatively minor.

COBALAMIN

Biochemistry

Cobalamins are corrinoids (compounds that contain a porphyrin-like corrin ring with a central cobalt atom) that have a 5,6-dimethyl benzimidazole side chain. The major physiologic cobalamins are hydroxocobalamin, methylcobalamin, and 5'-deoxyadenosyl cobalamin. By International Union of Pure and Applied Chemistry-International Union of Biochemistry nomenclature, *vitamin B₁₂* refers specifically to cyanocobalamin, a cobalamin with a cyanide moiety attached to its central cobalt, a form found only in medicinal preparations.

Cobalamins are known to take part in only two reactions in humans. First, 5'-deoxyadenosyl cobalamin is the coenzyme for methylmalonyl CoA mutase in the isomerization of methylmalonyl CoA to succinyl CoA, as part of mitochondrial propionate metabolism. Methylmalonic acidemia is a useful indicator of cobalamin deficiency.

The second reaction involves methylcobalamin as a cofactor in the methylation of homocysteine to methionine, in which methyl THF is converted to THF (Fig. 243.1, reaction 4). This key role has many important ramifications. Cobalamin deficiency impairs the demethylation of methyl THF, thus inducing a block in folate metabolism. This forms the basis of the "methyl THF trap" hypothesis. The resulting depletion of 5,10-methylene THF, needed for reaction 5 in Fig. 243.1, explains why cobalamin deficiency leads to megaloblastic anemia. It also explains why therapy with folic acid, by temporarily bypassing the block, can transiently correct the megaloblastic anemia of cobalamin deficiency (Fig. 243.1, reactions 1, 2 and 5). Probably related to the increase of the largely monoglutamated methyl THF, cellular accumulation of folates also becomes impaired in cobalamin deficiency; tissue folate levels, including red cell folate, fall while serum levels rise. Another ramification of the trap is that hyperhomocysteinemia and partial methionine deficiency can result, along with impaired generation of S-adenosylmethionine, a universal methyl donor; these changes are now thought to have many important consequences.

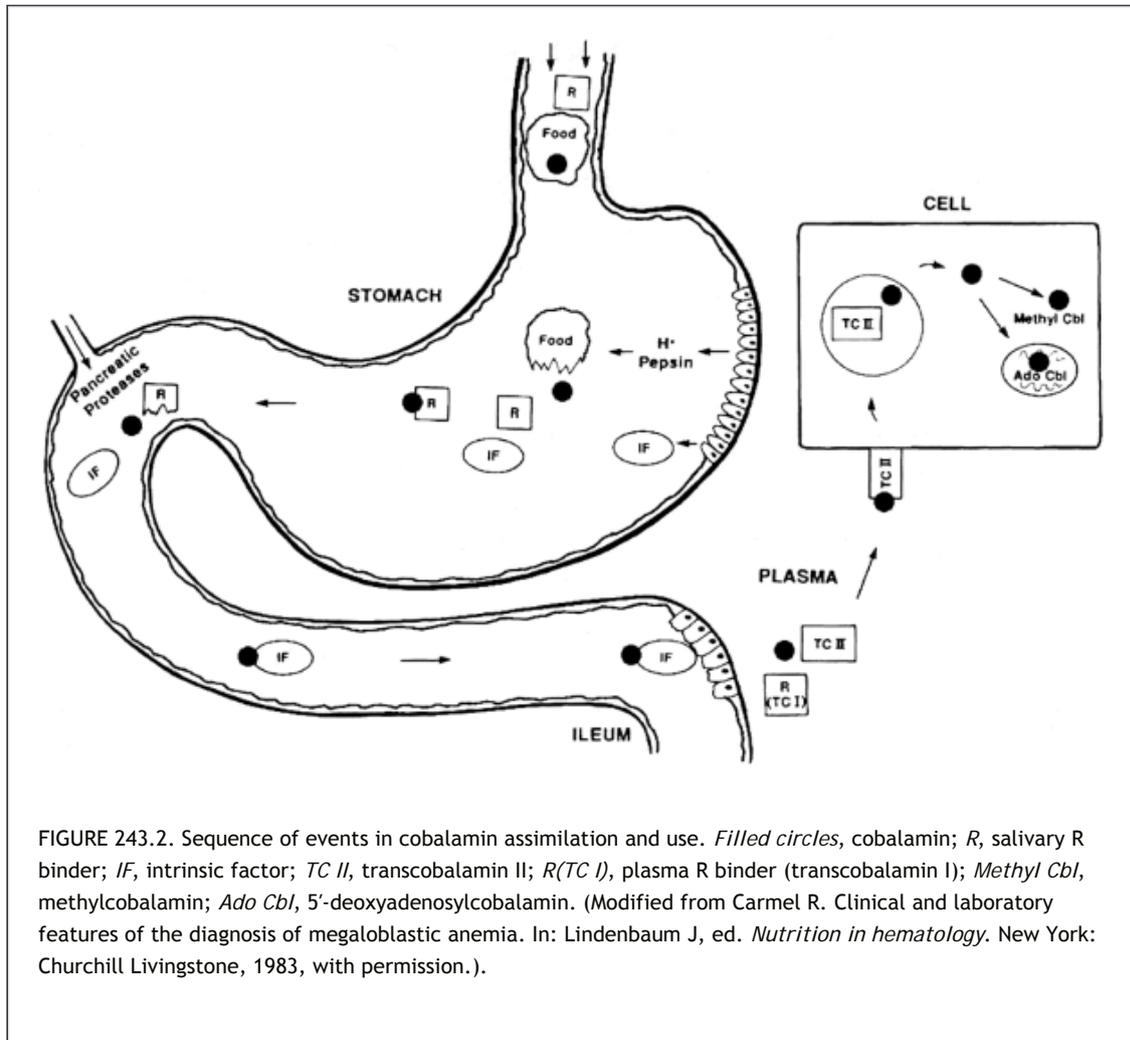
Nutrition, Absorption, and Metabolism

Cobalamin is synthesized by bacteria. Humans usually acquire it secondhand from animals that have ingested these bacteria

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and stored the cobalamin. The best food sources are meats, seafood, and products of animals, such as dairy products and eggs. Vegetables and fruits are poor sources, as are cereals, rice, flour, and nuts. The daily cobalamin requirement is thought to be 1 to 5 µg, and the usual North American diet provides more than that. Because of cobalamin's specific, saturable absorption, no more than 1 to 2 µg is absorbed in any one meal. Total body stores are estimated at 2 to 10 mg. Thus, daily turnover is only about 0.1% of stores.

Cobalamin assimilation follows the sequence of events in Figure 243.2. Ingested vitamin is split from its food binding by pepsin at a low pH. It is then bound by one of two binding proteins. The ultimately important binder is intrinsic factor. This glycoprotein is secreted by the gastric parietal cell in great excess over requirements. The other binding protein, R binder or haptocorrin, is secreted by glandular epithelial cells, such as those in the salivary gland. This glycoprotein is a less specific binder than intrinsic factor in that it also binds nonfunctional corrinoids. R binder binds cobalamin preferentially in the stomach at low pH but is then degraded in the jejunum by pancreatic proteases. Its cobalamin, thus released, is then bound by intrinsic factor.



Considerable enterohepatic recirculation of cobalamin occurs by way of biliary R binder, whose cobalamin is presumably released by pancreatic protease activity and transferred to intrinsic factor.

The intrinsic factor-cobalamin complex attaches to specific receptors for intrinsic factor on ileal epithelial cell membranes. These receptors are particularly numerous in the distal ileum. The complex is internalized, after which the cobalamin is exported from the ileal cell into the circulation.

In the bloodstream, cobalamin is bound by either of two binding proteins. Transcobalamin II binds cobalamin as it exits from the ileal cell and very quickly delivers it to all cells by way of specific receptors for transcobalamin II on their membranes. Without this binder, cellular uptake of cobalamin is inadequate. After internalization, the cobalamin becomes available as a coenzyme in the cytoplasm or mitochondria. On the other hand, transcobalamin I, the plasma representative of the R binder family, carries circulating cobalamin with a half-life of 9 to 10 days, without delivering it to cells. As a result, most measurable cobalamin in the plasma is normally attached to it, but its function is unknown; desialylated transcobalamin I may ultimately be involved nonspecifically in the hepatobiliary clearance of the vitamin.

The binding proteins are of additional interest in their own right. Plasma levels of transcobalamin I and its bound cobalamin are consistently elevated in chronic myelogenous and acute promyelocytic leukemias and sometimes in

other conditions. Plasma transcobalamin II behaves like an acute phase reactant.

Various nonfunctional corrinoid analogues are found in the blood. Their source is unknown, but intestinal bacteria synthesize them and some multivitamin preparations are rich in them. Their role, deleterious or otherwise, is also uncertain.

ETIOLOGY

COBALAMIN (VITAMIN B12) DEFICIENCY STATES

The disorders presented here follow the assimilation sequence for cobalamin in Figure 243.2. Because of the large body reserves of cobalamin, virtually all disorders take several years to produce clinically obvious deficiency. Increasingly often now, they are diagnosed before the symptoms of deficiency appear.

Dietary Insufficiency

The great margin of safety provided by the 1,000: 1 ratio of body stores to daily requirements, combined with unimpaired reabsorption of biliary cobalamin, explains why dietary insufficiency rarely causes cobalamin deficiency in the United States. Cobalamin deficiency does not occur in general malnutrition such as among homeless persons with alcoholism or malnourished patients with renal failure or cancer. For a deficiency to develop, one must be a particularly strict vegetarian for many years, usually avoiding eggs and dairy products not to mention cobalamin supplements. Even patients who meet all the criteria for dietary insufficiency usually have coexisting malabsorptive disorders that explain their deficiency on closer examination.

Nevertheless, marginal cobalamin status may well be common in vegetarians. Even if megaloblastic anemia is rare, low serum cobalamin levels have been described in several surveys. The one type of patient who often exhibits overt signs of deficiency, especially neurological signs, is the infant born to and nursed by a vegan mother; interestingly, the mother usually has no symptoms.

Disorders of the Stomach

Pernicious Anemia

Pernicious anemia is the most common cause of clinically overt cobalamin deficiency. Despite its hematologic name, it is really a gastroenterologic entity defined by malabsorption of cobalamin due to failure of the stomach to secrete intrinsic factor. One can have pernicious anemia without being anemic.

Classical, or Addisonian, pernicious anemia features severe atrophic gastritis in which autoimmune phenomena are prominent. The gastritis often spares the antrum (type A gastritis) and gastrin levels usually are elevated. Achlorhydria is universal, but the crucial defect is loss of intrinsic factor secretion by the parietal cell. Although there is still no evidence that they are pathogenic, circulating antibodies to the H⁺,K⁺-ATPase of parietal cells (55% to 90% of cases) and the cobalamin-binding site of intrinsic factor (55% to 75% of cases) are often present.

Pernicious anemia is typically a disease of the elderly person of northern European ancestry but is found in all races. Moreover, pernicious anemia can occur in younger people; occurrence in the third or fourth decade of life is especially common among black women. When the disease occurs in children or teenagers, it is called *juvenile pernicious anemia* (not to be confused with congenital pernicious anemia, a hereditary disorder without atrophic gastritis). Pernicious anemia shows a slight female preponderance.

The gastric defect is irreversible. The vitamin deficiency that it produces, however, is easily reversed and does not recur as long as maintenance therapy with cobalamin is given. A small source of risk for increased mortality is the predisposition to gastric cancer, estimated at two to five times that of the general population. An initial endoscopy is generally favored but the value of aggressive screening programs with periodic endoscopic examination is not established. Gastric carcinoids may be even more common but usually have a benign course. Other diseases that coexist with pernicious anemia more often than expected include autoimmune endocrinopathies (hypothyroidism, hyperthyroidism, diabetes mellitus, hypoparathyroidism, Addison's disease) and various immunologic disorders (hypogammaglobulinemia, [in which gastric cancer occurs particularly often], myasthenia gravis, vitiligo, autoimmune hemolytic anemia, and immune thrombocytopenic purpura). Of these, thyroid disease is by far the most common and may precede, follow, or coexist with pernicious anemia. Iron deficiency also is common in patients with pernicious anemia.

Postgastrectomy State

Cobalamin deficiency develops in 15% to 30% of patients who undergo subtotal gastrectomy. Loss of intrinsic factor can occur as a result of atrophic gastritis in the remnant stump. Bacterial overgrowth in the upper intestine may also contribute to the malabsorption. However, malabsorption limited to food-bound cobalamin is much more common and is discussed in the following section. Iron deficiency occurs frequently and can mask the hematological changes of cobalamin deficiency; folate deficiency may also coexist.

Other Gastric Diseases

Cobalamin deficiency can result from extensive infiltrative disease of the stomach and the attendant loss of intrinsic factor. Deficiency has also been described after gastric stapling or bypass for obesity.

The concept that atrophic gastritis leads to cobalamin malabsorption and deficiency only if intrinsic factor secretion is lost (pernicious anemia) is no longer tenable. Patients who have lost only acid and pepsin secretion absorb free cobalamin adequately. However, they may not always be able to liberate, and thus absorb, cobalamin from food or attached to protein, a defect identified only by modifications of the standard absorption test. This form of malabsorption has been described in patients with achlorhydria associated with gastritis, patients who have undergone subtotal gastrectomy or vagotomy, and patients taking acid-suppressive drugs. Food-cobalamin malabsorption accounts for 25% to 40% of cases of low cobalamin levels where the Schilling test result, which measures only absorption of free cobalamin, is normal.

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Hereditary Disorders

Congenital pernicious anemia produces cobalamin deficiency because of a selective failure to secrete intrinsic factor or the secretion of an abnormal intrinsic factor. The stomach is otherwise morphologically and functionally intact. No autoimmune phenomena are seen, and the patient is not at increased risk for gastric cancer. Although the patients usually come to medical attention in the first few years of life, some are first diagnosed in adolescence or even adulthood.

Disorders of the Intestinal Lumen

Bacterial Contamination of the Intestine

The small intestine can become overgrown with bacteria that take up cobalamin. This bacterial overgrowth can result from blind loops, large diverticula, strictures, or disorders of bowel motility. Increased bacterial counts in the upper intestine may be seen in patients with gastric achlorhydria but are of uncertain significance. Bacterial contamination can produce cobalamin deficiency before any other intestinal symptoms or signs are apparent. Treatment includes antibiotic therapy, but unless the underlying cause of the bacterial overgrowth can be reversed, recurrence is common.

Tapeworm Infestation

Diphyllobothrium latum avidly takes up cobalamin. The greater the number of parasites, the more likely there is to be cobalamin deficiency. The major source of the tapeworm is poorly cooked fish from infested lakes. The disorder is rare in the United States.

Pancreatic Insufficiency

Pancreatic insufficiency produces an abnormal cobalamin absorption test result in about half of patients. The cause is the unavailability of pancreatic proteases to degrade the R binder to which cobalamin initially binds in the stomach. As a result, less vitamin becomes available for binding to intrinsic factor. Nevertheless, deficiency rarely develops, perhaps because pancreatic replacement therapy usually is given before cobalamin deficiency supervenes.

Gastrinoma

The Zollinger-Ellison syndrome can produce cobalamin malabsorption. The low intestinal pH inhibits intrinsic factor attachment to ileal receptors.

Disorders of the Ileum

Acquired Diseases Involving the Ileum

Nearly any disease involving enough of the ileum, especially the distal ileum, can produce cobalamin malabsorption. Cobalamin deficiency can be the first manifestation of tropical sprue, which may appear long after emigration from the endemic area and is often combined with folate deficiency. Loss of ileum due to surgical resection or diversion (e.g., the Kock pouch) or due to damage after pelvic radiotherapy can also produce malabsorption. Low serum cobalamin levels occur in 15% to 25% of patients with AIDS, and ileal malabsorption can be demonstrated in some of these cases.

Drugs, Toxins, and Nutritional Deficiency

Alcohol and many drugs (e.g., neomycin, biguanides, colchicine, slow-release potassium chloride, cholestyramine, and para-aminosalicylic acid) can impair cobalamin absorption. Absorption tests can become abnormal within a week or two of beginning drug therapy. Actual cobalamin deficiency is uncommon, however, because the drugs are rarely taken long enough to deplete stores.

Cobalamin deficiency itself sometimes produces megaloblastic changes in intestinal epithelial cells. The transient ileal malabsorption of cobalamin (i.e., even when exogenous intrinsic factor is supplied in the Schilling test), seen in 40% to 75% of patients with pernicious anemia, has been attributed to this phenomenon. The malabsorption normally reverses with cobalamin therapy after a few days or weeks. When the ileal malabsorption takes several months to reverse, it seems more plausible to implicate coexisting small bowel disorders.

Hereditary Disorders

In congenital malabsorption of cobalamin (Imerslund-Gräsbeck syndrome), malabsorption is limited to cobalamin only. The genetic defect is thought to involve the ileal receptor for intrinsic factor, cubilin, which is also found in kidney tubular cells. Mild proteinuria occurs in nearly all cases. Deficiency of the vitamin usually appears in the first few years of life but is sometimes delayed until the second decade. Malabsorption also accompanies hereditary transcobalamin II deficiency. This seems to be due to the inability of absorbed cobalamin to leave the ileal cell.

Disorders of Transport, Metabolism, and Utilization

Nitrous Oxide Exposure

Nitrous oxide inactivates cobalamin by oxidizing cob(I)alamin and inactivates methionine synthase (Fig. 243.1, reaction 4). Repeated exposure produces megaloblastic anemia and neurologic symptoms, with the latter often predominating. Cobalamin levels are usually normal and methylmalonic acidemia cannot always be demonstrated, so that the diagnosis of cobalamin abnormality can be difficult to make unless a history of exposure is elicited. Transient exposure during routine anesthesia produces no clinical sequelae unless a coexisting cobalamin deficiency has been overlooked.

Transcobalamin II Deficiency

Congenital deficiency of this crucial transport protein produces a life-threatening cellular depletion of cobalamin early in life. Serum cobalamin levels are usually, but not invariably, normal.

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Occasional cases may not be diagnosed until the second decade of life, but these usually are patients who had been misdiagnosed and inadequately treated earlier. Curiously, neurologic disturbances are not initially prominent in most patients. However, some patients have developed such deficits, perhaps as a result of inappropriate folate therapy.

R Binder (Transcobalamin I) Deficiency

Because most cobalamin circulates in the blood attached to transcobalamin I, absence of the binder produces low serum levels of the vitamin even though cellular levels are adequate. The binder appears to have no other role in cobalamin metabolism, and these patients are nondeficient and asymptomatic (although one patient had unexplained, progressive myelopathy and dysarthria). The patients usually are discovered by accident because of an unexplained low serum cobalamin level. Absence of R binder in both blood and secretions such as saliva establishes the diagnosis. Deficiency of lactoferrin, which, like transcobalamin I, originates in neutrophil specific granules,

coexists in some cases.

Inborn Errors of Metabolism

Recognition of abnormalities that interfere with cellular cobalamin activity begins with the demonstration of methylmalonic acidemia, homocysteinemia, or both, but the biochemical and clinical defects vary. Some disorders with abnormality limited to the 5'-deoxyadenosyl cobalamin-mediated reaction feature only acidosis, methylmalonic acidemia, and perhaps developmental problems (as in *cbIA* and *B* mutations). Others have defective methylcobalamin activity as well (*cbIC*, *D*, and *F* mutations); these patients usually have homocysteinemia in addition to methylmalonic acidemia. Their clinical pictures have ranged from mild neurologic symptoms appearing in adolescence to severe hematologic and neurologic abnormalities terminating in death in infancy. Thrombotic complications sometimes occur. In the *cbIE* and *G* mutations, only methylcobalamin activity is impaired; homocysteinemia occurs without methylmalonic acidemia, and patients have hematologic and neurologic manifestations. The inborn errors should be considered in children who have developmental or neurologic problems as well as in children who have megaloblastic anemia. Some of the disorders may first be detected in adolescence or young adulthood.

Miscellaneous

Aging and mild cobalamin deficiency

Although the causes vary, low cobalamin levels occur in about 10% of the healthy elderly population. Most of the low levels are not accompanied by clinical symptoms or anemia but metabolic testing has shown evidence of cobalamin deficiency in 50% to 70% of cases. Food-cobalamin malabsorption explains 30% to 40% of these and pernicious anemia or ileal disease only about 10%; dietary insufficiency is rare.

FOLATE DEFICIENCY STATES

Deficiency of folate often is multifactorial in origin. In part this stems from the relative ease with which nutritional insufficiency can supervene in any illness. Alcohol and various drugs also have multiple effects on folate status that often are complicated by poor diet, coexisting disease, or the underlying illness for which the drugs are used. The disorders are presented here in the sequence of folate intake, absorption, and utilization.

Dietary Insufficiency

Poor dietary intake is the most common cause of folate deficiency. Because body stores are only about 100 times the daily requirement and because food folate is labile, significant tissue depletion can occur within a few months.

Certain factors potentiate the problem of poor or marginal intake. Chief among these is ingestion of alcohol. When combined with poor diet, alcohol use often has been implicated as the major cause of folate deficiency. Another potentiating factor can be an increased requirement for folate, which may strain body stores if the diet is marginal.

Disorders of Absorption

Diseases of the Small Intestine

Almost any condition involving enough of the upper small intestine can produce folate malabsorption. This includes sprue, infiltrative diseases, Whipple's disease, and resection of the small intestine. Bacterial contamination of the small intestine, however, does not produce folate deficiency, although it can produce cobalamin deficiency.

Drugs, Toxins, and Nutritional Deficiency

Several drugs and toxins have been associated with folate malabsorption. The chief one is alcohol (discussed in a later section). Sulfasalazine and para-aminosalicylic acid have been implicated in folate malabsorption. Malnutrition and its effects on the intestine also have been postulated to impair folate absorption.

Hereditary Disorders

The nature of the defect in congenital folate malabsorption is unknown, but it affects monoglutamate as well as polyglutamate absorption. A concurrent transport block into the cerebrospinal fluid has been implicated, perhaps explaining the frequent cerebral abnormalities these patients have in addition to their anemia.

Disorders of Transport, Metabolism, and Utilization

Alcohol

Alcohol has multiple effects on folate economy. Serum folate levels drop sharply with alcohol ingestion. Suggested mechanisms include impaired ability to form methyl THF in the liver,

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curtailed enterohepatic recirculation of folate, and increased urinary losses of folate. These putative defects are superimposed on the previously mentioned dietary insufficiency and on the possible absorptive defect. However, alcohol also can directly induce red cell macrocytosis that is not related to altered folate status and does not respond to folate therapy.

Drugs

Folate metabolism is a major target in antineoplastic therapy. Methotrexate inhibits dihydrofolate reductase (Fig. 243.1, reaction 1), thus inhibiting the generation of metabolically active THF.

Several antibiotics, such as trimethoprim, sulfamethoxazole, and pyrimethamine, inhibit dihydrofolate reductase in microorganisms, but the human enzyme is much less susceptible. Triamterene, a pteridine derivative, and sulfasalazine, which also affects two other folate-related enzymes, inhibit dihydrofolate reductase. All these drugs can, on rare occasions, directly induce folate deficiency. Nevertheless, whenever deficiency occurs, the initial focus should be to find coexisting disorders such as malnutrition or other diseases that compromise folate status.

Patients receiving anticonvulsant therapy often become folate-deficient. The mechanism is unclear, and a poor diet often contributes to the deficiency. In addition to possibly impairing absorption, hydantoin may impair cellular folate metabolism.

Acute Folate Deficiency

Acute folate deficiency has been found among some patients in intensive care units. These patients develop cytopenia and megaloblastic bone-marrow changes, but the peripheral blood appearance is often unaltered and vitamin levels are normal. The cause of this syndrome of cellular folate depletion is unknown.

Hereditary Disorders

Hereditary folate metabolic disorders are often associated with cerebral abnormalities, such as retardation or seizures; megaloblastic anemia is not always present. Methylene THF reductase deficiency may be the most common disorder (Fig. 243.1, reaction 3). It is associated with low serum folate levels due to impaired generation of methyl THF. Neurological symptoms are common, but megaloblastic anemia does not occur because methylene THF remains available for thymidine synthesis (Fig. 243.1, reaction 5). The disorder has been found among teenagers as well as among younger children.

A milder genetic mutation producing a thermolabile methylene THF reductase is very common. It produces no symptoms but can contribute to mild hyperhomocysteinemia and perhaps an increased thrombotic risk.

Increased Requirements and Losses

Low folate levels are common in pregnancy. Megaloblastic anemia sometimes ensues, especially in the last trimester. Routine prenatal folate supplementation has decreased its incidence.

Folate requirements are increased in chronic hemolytic anemias and other chronic states of increased cell turnover. However, clinical deficiency from the increased demand alone is unusual, and it is wise to search for contributing problems. Controlled studies have not demonstrated any clinical benefit from the common practice of routinely giving folate prophylactically to patients with chronic hemolysis, such as those with sickle-cell anemia. Cobalamin levels should be checked first if folate supplementation is planned.

Increased folate losses have been described in patients undergoing dialysis for renal failure, and supplementation is routinely given.

OTHER CAUSES OF MEGALOBLASTIC ANEMIA

Megaloblastic changes are sometimes caused by mechanisms unrelated to either folate or cobalamin deficiency and

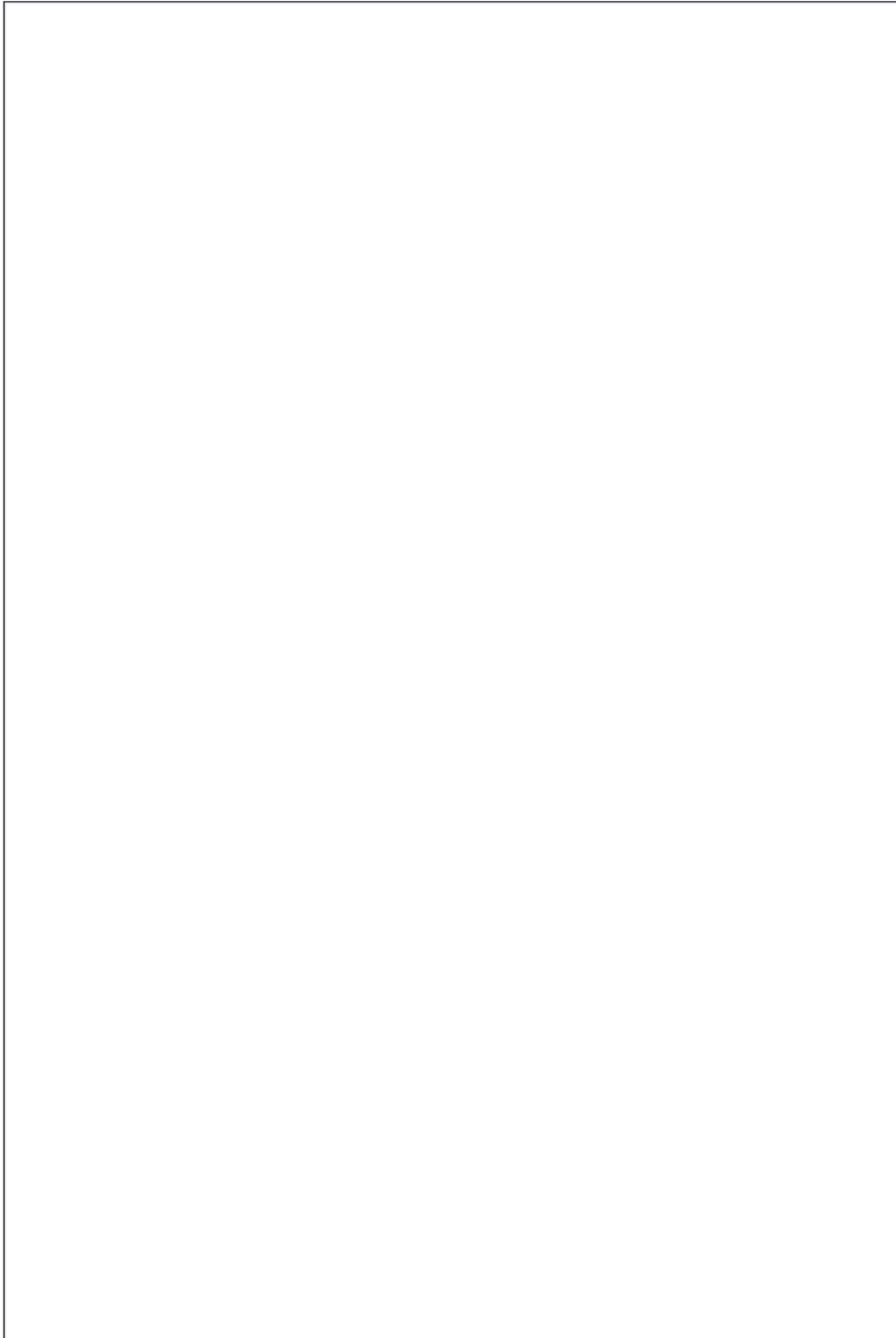
do not respond to therapy with either vitamin.

Drugs and Toxins

Drugs that impair nucleoprotein synthesis directly, for example, antineoplastic drugs like 5-fluorouracil, cytosine arabinoside, and hydroxyurea, often produce megaloblastic changes and macrocytosis. Hypersegmentation of neutrophil nuclei occurs with steroid therapy and reverses with its discontinuation; the mechanism is unknown. Arsenic poisoning does not produce truly megaloblastic changes, but the nuclear fragmentation and increased mitoses that result can be confused with megaloblastic anemia.

Primary Disorders of the Hematopoietic Stem Cells

Many primary disorders of the hematopoietic stem cells are associated with macrocytosis (Table 243.1). Some, like the myelodysplastic syndrome, may also feature erythroid maturation changes that resemble those of megaloblastic anemia. Neutrophils may have hypersegmented nuclei in some myeloproliferative diseases.



Megaloblastic Anemia

Cobalamin or folate deficiency

Drugs

Alcohol

Agents that interfere with nucleoprotein synthesis

Chemotherapeutic and immunosuppressive drugs

Zidovudine

Hematologic Disorders

Aplastic anemia

Pure red blood cell aplasia

Myelodysplastic syndrome

Myeloproliferative disease

Leukemia

Multiple myeloma

Refractory anemia

5q – syndrome

Hemolytic anemia

Nonhematologic Disease

Liver disease (usually, but not invariably, alcoholic)

Hypothyroidism

Physiologic Process

Red blood cells are normally enlarged in the first 4 wk of life

Idiopathic Factors^a

Pregnancy

Chronic lung disease, smoking

Cancer

Artifact of Electronic Cell Sizing

Cold agglutinins

Severe hyperglycemia

Hyponatremia

Stored blood

Warm antibody to red blood cells

^a Higher mean corpuscular volumes than usual (although not necessarily above normal range) have been observed in these conditions, but the mechanism is unknown and the possibility of subtle megaloblastosis exists in some cases.

TABLE 243.1. CAUSES OF MACROCYTOSIS

Hereditary Disorders Unrelated to Cobalamin or Folate

Orotic aciduria affects two enzymes in orotic acid metabolism. Among other features, severe megaloblastic anemia appears in the first year of life. The Lesch-Nyhan syndrome, a disorder of purine metabolism, sometimes produces megaloblastic anemia. Thiamine-responsive megaloblastic anemia, whose mechanism is unknown, may not produce anemia until late in childhood. Diabetes mellitus and sensorineural deafness are regular findings.

CLINICAL FINDINGS

The clinical expressions of cobalamin or folate deficiency are the same no matter what the underlying cause of the respective

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deficiency. Sometimes, however, especially in cobalamin deficiency, the only recognizable abnormality is simply a low vitamin level in the blood or metabolic evidence of insufficiency.

MEGALOBLASTIC ANEMIA

The severity of morphologic changes tends to correspond with the severity of anemia. All cells, whether precursors or mature cells, tend to be larger than normal. A nuclear-cytoplasmic asynchrony appears in the precursor cells. Erythrocytes are often oval in addition to being macrocytic, and there may be considerable poikilocytosis. Mature granulocytes have hypersegmented nuclei, and metamyelocytes and bands tend to be large with large, bizarrely shaped nuclei and abnormally fine chromatin. Hypersegmentation in granulocytes can be defined in several ways: the presence of at least one neutrophil with six or more nuclear lobes; the presence of more than 3% to 4% five-lobed neutrophils; or an increased neutrophil lobe average. Granulocytic changes occur early in deficiency and are found in nearly all cases, whereas erythroid abnormalities are sometimes partially masked (e.g., by coexisting iron deficiency) and macrocytosis may be absent.

Functionally, the anemia is primarily one of ineffective hematopoiesis. Up to 90% of the cells may die within the bone marrow. The resulting indirect hyperbilirubinemia and increased serum lactate dehydrogenase are often striking, unless the anemia is mild. Evidence of hemolysis, including low serum haptoglobin levels and hemosiderinuria, is sometimes seen but reticulocytosis does not occur.

Leukopoiesis and thrombopoiesis are also ineffective, and pancytopenia may occur. Granulocyte and platelet dysfunctions are sometimes demonstrable but rarely produce clinical manifestations.

NEUROLOGIC ABNORMALITIES

Cobalamin deficiency often produces characteristic and usually symmetric myelopathy and neuropathy. Pathologic examination shows axonal degeneration with loss of myelin. This predominates in the posterior and lateral columns in the upper thoracic spinal cord. Patchy changes may also be found in the brain. How the deficiency produces the neurologic lesions is unknown. Current evidence favors impaired homocysteine-methionine conversion with an altered S-adenosylmethionine/S-adenosylhomocysteine equilibrium as the key to the defect. Some patients have severe neurologic deficits with little or no evidence of hematologic abnormality, whereas in others the reverse is true.

The earliest symptom is usually paresthesia in the feet. Vibration and position sense become impaired. Deep tendon reflexes may be diminished. Later, ataxia and a positive Romberg's sign may appear. As the lesion progresses, the deficits ascend. Spasticity, increased deep tendon reflexes, and weakness appear as the lateral columns are involved. Ultimately, paraplegia, bladder atony, poor control of sphincters, and impotence may result.

Mild cerebral manifestations such as depression, memory impairment, and irritability are common. Electroencephalographic abnormalities are frequently demonstrable. Psychiatric symptoms, confusion, or dementia may appear. Rarely, the disturbances can include ophthalmoplegia and optic atrophy.

Folate deficiency does not produce the neurologic abnormalities that cobalamin deficiency does. Minor cerebral symptoms, such as irritability, memory loss and personality changes, may occur, however, and neuropathy has been described by some observers. The nature of these changes is unclear. Cerebral disturbances such as mental retardation, seizures, and psychosis are prominent in children with inborn errors of folate metabolism.

The neurologic deficits of cobalamin deficiency, unlike the hematologic ones, do not respond to folate therapy. Indeed, the risk is that they may progress unchecked while hematologic improvement occurs.

MISCELLANEOUS ABNORMALITIES

Some patients, especially those with cobalamin deficiency, may have glossitis and atrophy of the tongue papillae. Oral symptoms even predominate in some cases. Infertility has also been attributed to cobalamin deficiency. Other abnormalities that reverse with cobalamin therapy include unexplained weight loss, pigment changes in skin, hair, and nails, and low serum levels of bone alkaline phosphatase and osteocalcin.

Folate deficiency has been implicated in damage to fragile sites on chromosomes. Some studies have also described protection by folate supplementation against progression of cellular atypia to neoplasia, but the phenomenon is unclear. Periconceptual folate supplementation of the mother partially protects her fetus against neural tube defects. This effect, whose mechanism is unknown, prompted the recent fortification of all cereal-grain products with folate.

HYPERHOMOCYSTEINEMIA

Although asymptomatic per se, acquired as well as hereditary hyperhomocysteinemia may be clinically important. Epidemiologic evidence shows it to be, even when mild, a risk factor for thrombotic and arteriosclerotic disease. Studies are underway to determine if reducing homocysteine levels by vitamin therapy reduces the vascular risk. Hyperhomocysteinemia, which predominates in men and in the elderly, can be caused by folate, cobalamin, or pyridoxine deficiency (Fig. 243.1), by several enzyme deficiencies or by renal failure.

LABORATORY FINDINGS

DIAGNOSIS OF MEGALOBLASTIC ANEMIA

Classic megaloblastic anemia is easy to diagnose. The advent of electronic cell sizing has made macrocytosis instantly obvious in the routine blood cell count. Alertness to details optimizes diagnosis in the early phases of deficiency and in mild anemias, atypical presentations, and the presence of findings altered by coexisting problems. Macrocytosis typically precedes the anemia and can be invaluable in making an early diagnosis. Even a red blood cell mean corpuscular volume in the upper range of normal should be viewed with suspicion. Macrocytosis is not invariable,

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however; more than 25% of patients with pernicious anemia have normal or even low mean corpuscular volumes when first seen. Coexisting iron deficiency or thalassemia minor often is responsible for this, or the patient may simply have early deficiency or, for other, unknown reasons, may have only minimal hematologic abnormality. On the other hand, many nonmegaloblastic processes can also cause a high mean corpuscular volume (Table 243.1).

Neutrophil hypersegmentation is a sensitive index of the megaloblastic process. Hypersegmentation can also be found in nonmegaloblastic conditions, however, examples of which include myeloproliferative disease, corticosteroid therapy, and congenital hypersegmentation; it is nevertheless always advisable to demonstrate that vitamin deficiency does not coexist. The bone marrow aspirate is the court of last resort and should be examined before initiating therapy whenever the diagnosis is in doubt. However, megaloblastic changes may be subtle in mild or early deficiency, and megaloblastic erythroid changes may be blunted by coexisting iron deficiency.

Various features of the anemia can mimic other disorders. Pancytopenia is sometimes severe enough to be confused with aplastic anemia. The maturation abnormalities, particularly if accompanied by exuberant myeloid proliferation or transient red blood cell hypoplasia, have been mistaken for leukemia. Increased iron levels and abnormal sideroblasts can mimic sideroblastic anemia. The frequent hyperbilirubinemia, elevated lactate dehydrogenase, decreased haptoglobin, and hemosiderinuria may resemble primary hemolytic anemia.

DIAGNOSIS OF THE VITAMIN DEFICIENCY

For all practical purposes, megaloblastic anemia in adults is secondary to deficiency of folate, cobalamin, or both (Table 243.2). Cobalamin deficiency should also be considered in any patient with neuropathy, myelopathy, or cerebral disturbances, whether or not megaloblastic anemia is present.



	In Deficiency of		
	Cobalamin	Folate	Cobalamin and Folate
Serum cobalamin	↓	N-↓	↓
Serum folate	N-↑	↓	↓
Red blood cell folate	N-↓	↓	↓
Methylmalonic acid ^a	↑	N	↑
Homocysteine ^b	↑	↑	↑

N, normal level; ↓, decreased level; ↑, increased level.

^a Can be assayed in serum or urine.

^b Should be assayed in EDTA-anticoagulated plasma.

TABLE 243.2. LABORATORY DIAGNOSIS OF COBALAMIN AND FOLATE DEFICIENCY

Serum vitamin levels, even though they reflect body stores indirectly, are the mainstay of diagnosis. Indeed, low serum levels are sometimes the only evidence of deficiency. However, they are sometimes too sensitive (especially serum folate, which falls with poor intake within days) and at other times are falsely low (Tables 243.3 and 243.4). Although the lower the serum level, the more likely it is that a deficiency exists, even mildly decreased values require investigation. The burden of proof is always on the physician to demonstrate that deficiency is not present, given its treatability and the potentially serious consequences of failure to treat it. Serum cobalamin levels also tend to fall in folate deficiency, whereas serum folate tends to rise in cobalamin deficiency. Low levels of both vitamins may indicate combined deficiencies, especially in malabsorption syndromes.

Cobalamin Deficiency

Overt deficiency

Mild or preclinical deficiency

Latent pernicious anemia or other malabsorptive state, including food-cobalamin malabsorption

Old age^{a,b}

Vegetarianism^a

Unexplained but Sometimes Associated with Compromised Cobalamin Status

Long-term dialysis for renal failure

Hydantoin therapy

Cancer

AIDS

Presumed not to Represent Cobalamin Deficit^b

Pregnancy (last trimester)

Folate deficiency

Transcobalamin I deficiency

Multiple myeloma and Waldenström's macroglobulinemia

Aplastic anemia

Hairy cell leukemia

Oral contraceptive use

Severe iron deficiency

Artifact

Radioactive serum (e.g., after isotopic scanning procedures)

High-dose ascorbic acid ingestion

^a Only a few patients will display clinically overt evidence of cobalamin deficiency.

^b Some patients in these categories can have coexisting cobalamin malabsorption and either clinically overt or mild, preclinical deficiency.

TABLE 243.3. CAUSES OF LOW SERUM COBALAMIN LEVELS

Folate deficiency**Low serum levels apparently not reflecting overt folate deficiency**

Transiently poor dietary intake without actual folate deficiency

Sickle-cell anemia and other chronic hemolytic states

Drug use (e.g., oral contraceptives, acetylsalicylic acid, alcohol)

Idiopathic factors

Artifact

Improper handling of specimen (e.g., long storage)

Radioactive blood^a

Folate-binding protein abnormality in renal failure^a

Drugs that inhibit microbial growth (e.g., antibiotics)^b

^a Source of artifact only in radioisotopic assay.

^b Source of artifact only in microbiologic assay.

TABLE 243.4. CONDITIONS ASSOCIATED WITH LOW SERUM FOLATE LEVELS

Measuring tissue levels is an ideal goal but is impractical, aside from determining red cell folate levels. Red cell levels are truer indices of folate status than serum levels and are not immediately affected by vitamin therapy. Their chief shortcomings are laboratory performance problems and the fact that low levels often occur in cobalamin deficiency as well.

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Metabolic tests are becoming increasingly useful. Elevated homocysteine levels are a helpful marker for folate or cobalamin deficiency but occur in other conditions too. High methylmalonic acid levels are relatively specific for cobalamin deficiency. Serum levels of both metabolites also rise in renal insufficiency, however. Some patients with cobalamin deficiency have abnormal metabolite levels even when their serum cobalamin levels are normal. Testing for these two metabolites is occasionally helpful in acquired vitamin deficiencies and is mandatory when considering hereditary disorders. Perhaps an even more sensitive test for both vitamin deficiencies, the deoxyuridine suppression test in bone marrow cells, which tests reactions 5 and 6 in Fig. 243.1, is still a research tool.

DIAGNOSIS OF THE UNDERLYING DISEASE

Each of the two deficiencies has a distinct differential diagnosis. In cobalamin deficiency, the most common cause is pernicious anemia; for folic acid, it is dietary insufficiency, often combined with alcohol abuse. The temptation to automatically assume the most common diagnosis must be resisted, however.

Testing of cobalamin absorption is central to the diagnostic evaluation because nearly all cobalamin deficiency in the Western Hemisphere is either of gastric or intestinal origin. This is done by measuring the absorption of a small oral dose of radioactive cyanocobalamin. Radioactivity can be measured in urine (Schilling test), plasma, or feces, or whole-body retention can be measured. Each method has its advantages. The Schilling test is the most commonly used, but it requires a complete 24-hour urine collection and normal renal function. Pernicious anemia is diagnosed by an abnormal absorption test that is corrected by retesting with an oral dose of intrinsic factor. A popular version of the Schilling test combines both of the above steps but gives falsely normal results in 30% to 40% of cases due to isotope exchange.

The presence of anti-intrinsic factor antibody in the blood is diagnostically useful; it is rarely positive in patients without pernicious anemia, but an artifact can be produced if blood is sampled within 48 hours after a cobalamin injection is given. Antibody to parietal cells, on the other hand, is specific for atrophic gastritis, not pernicious

anemia. High serum gastrin and low pepsinogen I levels occur in 80% to 90% of patients with pernicious anemia.

Malabsorption of food-bound cobalamin cannot be detected by standard absorption tests that use free cobalamin, such as the Schilling test. Absorption tests using cobalamin in scrambled egg yolk or bound to chicken serum have been devised but are still not widely available.

Diagnosing the cause of folate deficiency requires a good dietary history, evaluation for possible alcoholism, and a careful drug history. Malabsorption is responsible for 10% to 20% of cases. Because tests of folate absorption are not widely available, folate malabsorption is usually diagnosed indirectly by documenting other malabsorptive phenomena.

OPTIMAL MANAGEMENT

TREATING THE SYMPTOMS

Megaloblastic anemia responds rapidly to small doses of the appropriate vitamin. A single injection of cobalamin or one folic acid pill reverses megaloblastic erythropoiesis. A feeling of well-being

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often precedes hematologic response by days. Reticulocytosis begins within a day or two and peaks at 5 to 7 days, at which time the hemoglobin level begins rising noticeably, although hypersegmented neutrophils may persist for several weeks. With adequate treatment, the blood count should always become completely normal within 8 weeks.

Transfusion is rarely indicated, given its hazards, the well-compensated state of even severely anemic patients, and the responsiveness to vitamin therapy. If clinical findings necessitate transfusion, a unit of packed red blood cells should be given slowly and the patient reevaluated before proceeding.

Vitamin therapy can be safely deferred in most patients until the specific vitamin deficiency has been identified, but it can be started in an emergency as soon as the necessary diagnostic specimens have been obtained. The neurologic symptoms of cobalamin deficiency call for more urgent therapy, but there is no evidence that higher doses or more frequent injections are necessary. The completeness of the neurologic response is not as predictable as the hematologic response. The more extensive the neurologic involvement and the longer the symptoms have been present, the less likely it is that complete reversal will occur. Nevertheless, some degree of neurologic improvement is possible even in longstanding cases, although it may take 6 to 12 months to occur. In any case, neurologic dysfunction never progresses when therapy is given. Patients with spasticity, gait disturbances or bladder problems require early rehabilitative efforts.

TREATING THE VITAMIN DEFICIENCY

Before therapy is begun, it is essential to be sure of the diagnosis. Treatment of cobalamin deficiency with folic acid must be avoided. If both vitamins are given, proper diagnosis may become difficult if the initial test results are inconclusive. Giving both vitamins can also blur the specificity of the diagnosis in the patient's mind, if therapy is continued for long. The two main goals are to reverse the abnormalities produced by the deficiency and to prevent relapse. A third goal is to replete tissue stores and provide a reserve, although there is no evidence that fully repleted stores are essential to the patient's well-being.

Because cobalamin deficiency is usually due to malabsorption, therapy should be parenteral. Lifelong maintenance is required when the underlying disease is irreversible, as in pernicious anemia. Usual doses are 100 to 1000 µg; a smaller fraction of the 1000-µg dose, but a larger absolute amount, is retained. Cyanocobalamin is the common form of cobalamin used, but hydroxocobalamin is better retained and requires less frequent injections. Once repletion has been achieved, monthly maintenance injections can be given. However, individual requirements vary: some patients may need more frequent injections, whereas others can do with less frequent ones. Metabolic and transport blocks require very frequent injections. Oral cobalamin suffices in dietary insufficiency. It can be used even in malabsorption because a small fixed proportion is absorbed by mass action, but at least 100 µg must be taken daily, and its effectiveness must be continuously monitored.

Folate therapy can usually be given orally because most deficiencies are dietary in origin. Daily doses of 1 mg are sufficient and stores can be repleted within a few weeks. Long-term maintenance is necessary only when the underlying disorder cannot be reversed. Oral folic acid can often be absorbed normally even in malabsorption syndromes, but higher doses should be used.

TREATING THE UNDERLYING DISEASE

The underlying disease must always be identified. Several causes have their own specific therapy, such as antibiotics for bacterial contamination of the gut and gluten-free diets for sprue, and others have specific complications and prognostic implications. Vitamin therapy can be discontinued if the underlying disease has been reversed and does not recur.

COMPLICATIONS

Vitamin therapy itself has no complications (allergic reactions are rare), unless the wrong vitamin is given. Because a transient hematologic response can be obtained with folate in cobalamin deficiency while neurologic abnormalities continue to progress, this can be a serious problem. A more common occurrence is an incomplete hematologic response despite appropriate vitamin therapy. This is indicated by a blunted reticulocyte response during the first week or by failure of the blood count to become completely normal within 2 months and is almost always due to coexisting disorders. Iron deficiency often coexists and may initially be masked in untreated megaloblastic anemia, with completely normal blood and marrow tests of iron status.

Transfusion should be avoided whenever possible; volume overload is only one of the many risks involved.

Long-term complications are those related to the underlying disease itself. For example, in pernicious anemia, there are the increased risks of gastric cancer, carcinoid tumors, and immune endocrine disorders, particularly hypothyroidism.

PATIENT EDUCATION

Because cobalamin therapy usually becomes a lifelong concern, patient education is essential. Too many patients discontinue therapy once their symptoms disappear. Reinforcement and careful explanation of the nature of the underlying disease may help. Some patients can be taught to inject themselves, which saves costs and often improves compliance as well. Dietary education is essential in most cases of folate deficiency. Addressing alcohol problems is also important.

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