

REVIEW ARTICLE

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Drug-Induced Megaloblastic Anemia

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MORE THAN 50 YEARS AGO, VICTOR HERBERT FIRST DESCRIBED THE concept that defective nucleoprotein synthesis, attributable to various causes, results in the development of megaloblastic anemia.¹ Megaloblastic anemia is characterized by the presence of a hypercellular marrow with large, abnormal hematopoietic progenitor cells with a characteristic finely stippled, lacy nuclear chromatin pattern. These abnormal progenitor cells, or megaloblasts, were first described by Paul Ehrlich in 1880. Leukopenia and thrombocytopenia are frequently present. Although the marrow is hypercellular, many of the cells die within it in a process called ineffective erythropoiesis. Megaloblastosis usually results from a deficiency of vitamin B₁₂ (cobalamin) or folic acid, or a deficiency in their metabolism; however, any interference with the synthesis of purines, pyrimidines, or protein may result in megaloblastosis.²

Megaloblastic maturation is the morphologic result of any biochemical defect that causes a slowing of DNA synthesis. The hallmark of this megaloblastosis is nuclear-cytoplasmic dissociation; the nucleus remains immature in appearance while the cytoplasm matures more normally. This dissociation, which is the result of DNA synthesis that is retarded relative to normal RNA and protein synthesis, is manifested in the marrow and other proliferating tissues in the body by large cells containing a large nucleus with a diffuse and immature-appearing chromatin content, surrounded by normal-appearing cytoplasm.³ However, a high mean corpuscular volume does not necessarily imply a diagnosis of megaloblastic anemia. A high mean corpuscular volume is noted also in cases of alcohol abuse, hypothyroidism, aplastic anemia, myelodysplasia, and any condition in which the reticulocyte count is considerably elevated (such as in hemolytic anemia); it may also be a congenital finding.

Since it was first described in 1849 by Thomas Addison,⁴ megaloblastic anemia has been attributed to both congenital (uncommon) and acquired (common) problems. It is most frequently related to vitamin B₁₂ deficiency due to defective absorption, folic acid deficiency due to malnutrition, or both. However, because of the correction of most of the dietary causes of vitamin B₁₂ and folate deficiency, drug-induced megaloblastic anemia has become a more prominent cause of megaloblastic anemia. The drugs that may cause this condition are commonly used in clinical practice, and their effects on DNA synthesis pathways may be underappreciated (Table 1).

A number of biochemical processes in DNA synthesis are vulnerable to inhibition by drugs, but among the most important of these is the new synthesis of thymidine. Figure 1 shows the structure of nucleotides and their associated terminology. Thymidine is a component of DNA but not RNA, and it is present in cells in rate-limiting amounts. The other nucleotides tend to be present in excess. Thymidine can be salvaged from the turnover of DNA, but the main source is the addition of a methyl group to the 5-position of the pyrimidine ring to convert

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deoxyuridylate to deoxythymidylate (Fig. 2). This methylation process depends crucially on folate and vitamin B₁₂.

Drugs cause megaloblastic anemia by impairing the cellular availability or use of folic acid or vitamin B₁₂. This may occur because of interference with the absorption, plasma transport, or delivery of folate or vitamin B₁₂, competition for reducing enzymes, end-product inhibition of cofactor-mediated reactions, or physical destruction of the vitamins (Fig. 1).²

Small amounts of vitamin B₁₂ are required on a daily basis (1 to 2.5 μg). Because folate food fortification tends to obscure the hematologic consequences of vitamin B₁₂ deficiency, the early effects of drugs that interfere with vitamin B₁₂ may be neurologic complications rather than the development of clinically significant anemia.⁵ These neurologic manifestations can be modest or more dramatic, with the development of myelopathy, neuropathy, optic atrophy, and neuropsychiatric and, rarely, autonomic disturbances such as bladder or erectile dysfunction. A discussion of these neurologic problems and their biochemical basis is beyond the scope of this article, but they have been reviewed in detail by other authors.⁵⁻¹⁰ Clearly, such potentially devastating drug effects underscore the need for the clinician to be alert to them.

DRUGS THAT ALTER PURINE
METABOLISM, PYRIMIDINE
METABOLISM, OR BOTH

In both purine and pyrimidine synthesis (Fig. 2), the methyl group is donated by 5,10-methylene tetrahydrofolate. The consequences of inhibition of pyrimidine synthesis are more dangerous to the cell than inhibition of purine synthesis. Thymidylate synthase converts deoxyuridylate to thymidylate by transferring the methyl group from methylene tetrahydrofolate, and in the process it yields dihydrofolate.

In order for the thymidylate synthase reaction to continue, the folate must reacquire a methyl group to donate. The first step is to regenerate tetrahydrofolate from dihydrofolate. This is accomplished through the action of dihydrofolate reductase. Tetrahydrofolate is then converted to 5,10-methylene tetrahydrofolate through the ac-

Table 1. Drugs That Cause Megaloblastic Anemia.

Mechanism of Action and Agent	Type of Medication or Indication
Modulates purine metabolism	
Azathioprine	Immunomodulator
Mycophenolate mofetil	Immunomodulator
Thioguanine	Antineoplastic agent
Mercaptopurine	Antineoplastic agent
Cladribine	Antineoplastic agent
Fludarabine	Antineoplastic agent
Pentostatin	Antineoplastic agent
Methotrexate	Immunomodulator, antineoplastic agent
Allopurinol	Xanthine oxidase inhibitor
Interferes with pyrimidine synthesis	
Cytosine arabinoside	Antineoplastic agent
Gemcitabine	Antineoplastic agent
Capecitabine	Antineoplastic agent
Hydroxyurea	Antineoplastic agent
Methotrexate	Antineoplastic agent
Mercaptopurine	Immunomodulator, antineoplastic agent
Fluorouracil	Antineoplastic agent
Trimethoprim	Antibacterial agent
Nitrous oxide	Anesthetic agent
Gadolinium (paramagnetic metal ion)	Contrast agent in magnetic resonance imaging
Leflunomide	Immunomodulator in patients with psoriasis or rheumatoid arthritis
Teriflunomide	Immunomodulator in patients with multiple sclerosis
Decreases absorption of folic acid	
Alcohol	
Aminosalicic acid	For tuberculosis and inflammatory bowel disease
Birth-control pills	Hormones
Estrogens	Hormones
Tetracyclines	Antibiotic
Ampicillin and other penicillins	Antibiotic
Chloramphenicol	Antibiotic
Nitrofurantoin	Urinary antiseptic
Erythromycin	Antibiotic

Table 1. (Continued.)

Mechanism of Action and Agent	Type of Medication or Indication
Aminopterin	Antineoplastic agent, immunosuppressive agent
Phenobarbital	Antiseizure agent
Phenytoin	Antiseizure agent
Quinine	Antimalarial agent
Chloroquine	Antimalarial agent
Primaquine	Antimalarial agent
Artemether lumefantrine	Antimalarial agent
Sulfadoxine–pyrimethamine	Antimalarial agent
Glutethimide	Hypnotic sedative
Has folate analogue activity	
Methotrexate	Immunomodulator, antineoplastic agent
Pemetrexed	Antineoplastic agent
Raltitrexed	Antineoplastic agent
Proguanil	Antineoplastic agent
Pyrimethamine	Antimalarial agent
Trimethoprim	Antibacterial agent
Decreases absorption of vitamin B₁₂	
Cycloserine	For tuberculosis and psychiatric conditions
Isoniazid	For tuberculosis
Metformin	For diabetes and prediabetes
Aminosalicic acid	For tuberculosis and inflammatory bowel disease
Colchicine	For gout, familial Mediterranean fever, and Behçet's disease
Neomycin	Antibiotic
Histamine ₂ -receptor antagonists (H ₂ blockers)	
Proton-pump inhibitors	
Increases excretion of vitamin B₁₂	
Sodium nitroprusside	
Destroys vitamin B₁₂	
Nitric oxide	
Has unknown mechanism	
Arsenic	
Benzene	
Sulfasalazine	
Asparaginase	

tion of serine hydroxymethyl transferase. If dihydrofolate is not reduced and methylated, the cell is starved of thymidylate, and DNA synthesis slows. It is this crucial role of dihydrofolate reductase in thymidine nucleotide biosynthesis that makes it a target for antineoplastic therapy.¹¹ This pathway is also targeted in antibacterial therapy, especially by sulfa drugs.

Purine and pyrimidine antagonists or analogues are commonly used in the treatment of cancers, as immune antagonists, and as antiviral agents. Inhibitors of thymidylate synthase are called suicide substrates because they irreversibly inhibit the enzyme. Molecules of this class include fluorouracil and 5-fluorodeoxyuridine. Both are converted within cells to 5-fluorodeoxyuridylate, which then inhibits thymidylate synthase.¹¹⁻¹³

Antimetabolites, which masquerade as a purine or a pyrimidine, inhibit DNA synthesis by preventing these substances from becoming incorporated into DNA during the S phase of the cell cycle. Purine synthesis inhibitors include a number of commonly used drugs (Table 1): azathioprine, an immunosuppressant agent used in organ transplantation, autoimmune disorders, and inflammatory bowel disease; mycophenolate mofetil, an immunosuppressant agent used to prevent rejection in organ transplantation that inhibits purine synthesis by blocking inositol monophosphate dehydrogenase; methotrexate, a direct inhibitor of dihydrofolate reductase that indirectly inhibits purine synthesis by blocking the metabolism of folic acid; and allopurinol, which is used to treat hyperuricemia because it inhibits the enzyme xanthine oxidoreductase.

Pyrimidine synthesis inhibitors are also used in active moderate-to-severe rheumatoid arthritis and psoriatic arthritis. For example, leflunomide inhibits T-cell responses and induces a shift of CD4 T cells from the type 1 helper T (Th1) cell (proinflammatory) to type 2 helper T (Th2) cell subpopulation. This process results in a beneficial effect in diseases in which T cells play a major role in the initiation and propagation of inflammation.¹⁴ Both leflunomide and its metabolite teriflunomide, which is approved for use in multiple sclerosis, inhibit dihydroorotate dehydrogenase.^{14,15}

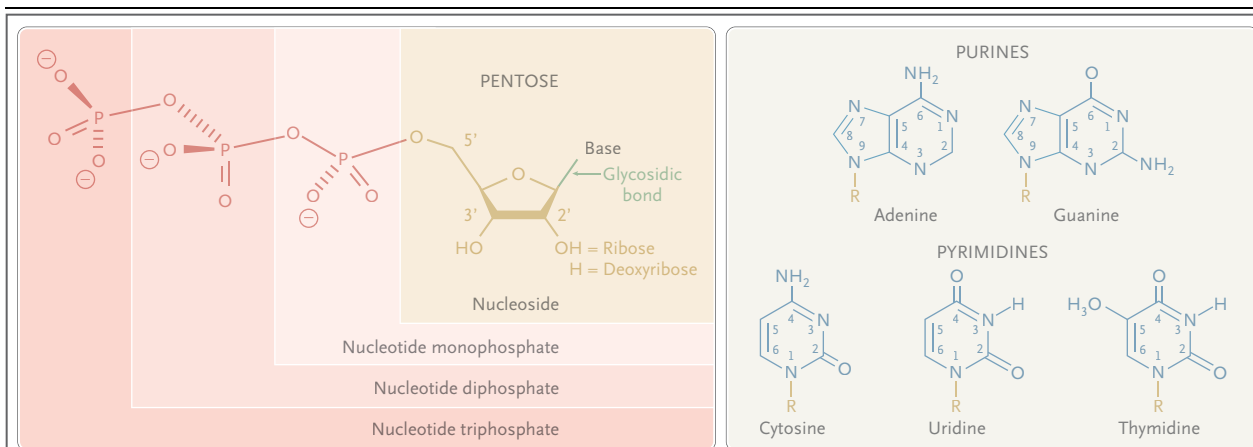


Figure 1. Nucleosides.

A nucleoside is a base plus a sugar, and the sugar can be either a ribose or deoxyribose. If the sugar is a ribose, then the purine bases are called adenine or guanine, depending on the position of the 2-amino group, and the pyrimidines are called cytosine, uridine, and thymidine. When the sugar is a deoxyribose, the purine nucleosides are called deoxyadenosine and deoxyguanosine and the pyrimidines are called deoxycytidine, deoxyuridine, and deoxythymidine. When a phosphate group is added, the structures become nucleotides and the bases are called adenylate, guanylate, cytidylate, uridylate, and thymidylate, respectively. These names refer to monophosphate nucleotides. If two phosphate groups are attached, they are referred to as diphosphate nucleotides and are called adenosine diphosphate, guanosine diphosphate, and so forth. If three phosphates are present, they are triphosphates.

Nitrous oxide, an anesthetic gas that has become increasingly popular for use as a recreational drug, may cause megaloblastic anemia by blocking the conversion of vitamin B₁₂ from the reduced to the oxidized form. In the cytoplasm, methionine synthase requires the reduced form of vitamin B₁₂ (methylcobalamin) to convert homocysteine to methionine. In contrast, in the mitochondria, the oxidized form of vitamin B₁₂ (5'-deoxyadenosylcobalamin) converts methylmalonyl-coenzyme A (CoA) to succinyl CoA. Thus, in the mitochondria, nitrous oxide will inhibit the activity of methylmalonyl CoA mutase, leading to the impairment of methylation reactions and DNA synthesis.¹⁶⁻¹⁹

INHIBITORS OF RIBONUCLEOTIDE REDUCTASE

Although they are not as ubiquitous as drugs that interfere with DNA synthesis, cytosine arabinoside, hydroxyurea, and gemcitabine inhibit the function of ribonucleotide reductase. This inhibition blocks the conversion of cytidine diphosphate or triphosphate to its corresponding deoxyribonucleotides. Cytosine arabinoside — once it is rapidly phosphorylated to its active metabolite, 5-triphosphate cytosine arabinoside — inhibits DNA polymerase. After its incorpora-

tion into DNA or RNA, it may also inhibit RNA polymerase.

DRUGS THAT INTERFERE WITH ABSORPTION OF FOLIC ACID

Folic acid (pteroylglutamic acid) cannot be synthesized in humans, so it must be obtained in the diet, where its major sources are green leafy vegetables, citrus fruits, liver, and whole grains. Dietary folates (5-methyltetrahydrofolate and formyltetrahydrofolate) are readily transported across the intestinal membranes. Vitamin B₁₂-dependent methionine synthetase converts 5-methyltetrahydrofolate to tetrahydrofolate, the form of folate that is required for nucleotide biosynthesis.

Methyltetrahydrofolate is required for the conversion of methionine to S-adenosylmethionine (SAM). Thus, when folate levels are low, SAM is depleted, resulting in a reduction in the methylation of cytosine in DNA. The consequences of this reduced DNA methylation include enhanced gene transcription and DNA strand breaks; these are key factors leading to many adverse effects, including the possibility of malignant transformation.

Conversely, since folate acts as a cofactor that is regenerated in a cyclic manner, any drug that

blocks the completion of this cycle (Fig. 2) will result in the accumulation of one of the metabolites of the vitamin in an unusable form, giving rise to a megaloblastic anemia. In this way, vitamin B₁₂ deficiency leads to an accumulation of 5-methyltetrahydrofolate, which leads to megaloblastosis that is, on a peripheral-blood smear, indistinguishable from that associated with folic acid deficiency. The interrelationship of folic acid and vitamin B₁₂ metabolism in this cyclical pathway is informative in determining the treatment that is required to correct the problem of either folic acid or B₁₂ deficiency.¹⁰

Thus, any drug that interferes with the intracellular concentration of folic acid, its intracellular conversion to its appropriate metabolites, or both can lead to megaloblastic anemia. Drugs may cause a perturbation in the intracellular concentration by decreasing intestinal absorption, decreasing transport and delivery to cells, decreasing transport across cell membranes, decreasing cellular retention (which includes increased excretion), increasing destruction, and increasing the requirement for folic acid. Some drugs affect the conversion or use of folic acid by interfering with the availability of vitamin B₁₂ or interfering with the enzymes involved in the conversion of folic acid to its appropriate metabolites.^{2,5,20,21}

Many drugs interfere with the absorption or proper distribution of folic acid. These include alcohol, antiseizure agents, contraceptive drugs, and antibiotics (Table 1).

Alcohol is associated with the development of megaloblastic anemia because of a low-folate diet in persons with alcoholism and because of an inhibition of intestinal absorption, metabolic use, and hepatic uptake and storage of folate.^{22,23} Alcohol is not thought to act through the dihydrofolate reductase pathway. Rather, the likely effect of alcohol is on the intestinal mucosa, where it can interfere with both vitamin B₁₂ and folate absorption. The effect on vitamin B₁₂ absorption may be due to direct toxic effects on the gastric mucosa that cause interference with the production of intrinsic factor.²² Ethanol also has a direct effect on the maturation of hematopoietic progenitor cells in the marrow. This effect may be due to the inhibition of a specific enzyme, 10-formyl-tetrahydrofolate dehydrogenase, as shown in studies in animals.²⁴

The mechanism by which folate absorption is affected by the use of oral contraceptives re-

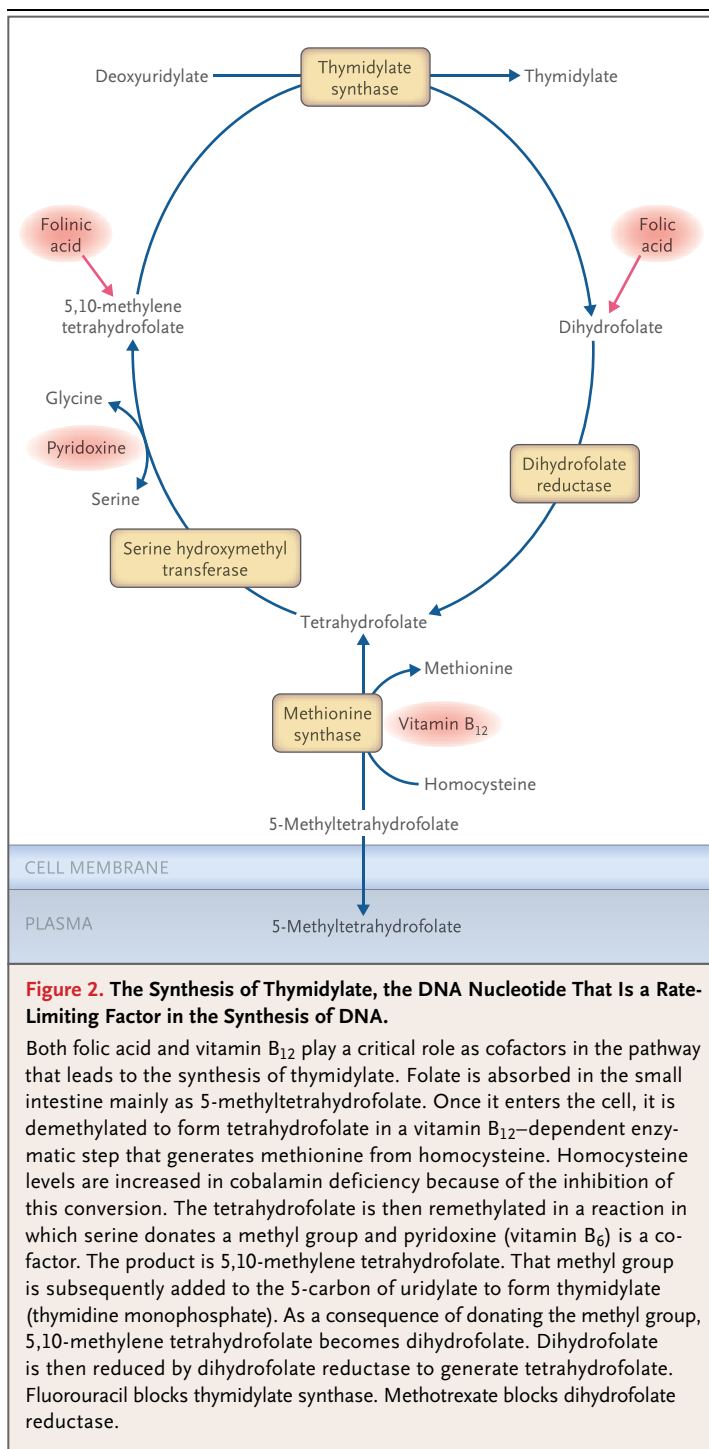


Figure 2. The Synthesis of Thymidylate, the DNA Nucleotide That Is a Rate-Limiting Factor in the Synthesis of DNA.

Both folic acid and vitamin B₁₂ play a critical role as cofactors in the pathway that leads to the synthesis of thymidylate. Folate is absorbed in the small intestine mainly as 5-methyltetrahydrofolate. Once it enters the cell, it is demethylated to form tetrahydrofolate in a vitamin B₁₂-dependent enzymatic step that generates methionine from homocysteine. Homocysteine levels are increased in cobalamin deficiency because of the inhibition of this conversion. The tetrahydrofolate is then remethylated in a reaction in which serine donates a methyl group and pyridoxine (vitamin B₆) is a cofactor. The product is 5,10-methylene tetrahydrofolate. That methyl group is subsequently added to the 5-carbon of uridylate to form thymidylate (thymidine monophosphate). As a consequence of donating the methyl group, 5,10-methylene tetrahydrofolate becomes dihydrofolate. Dihydrofolate is then reduced by dihydrofolate reductase to generate tetrahydrofolate. Fluorouracil blocks thymidylate synthase. Methotrexate blocks dihydrofolate reductase.

mains controversial. The use of contraceptives results in a partial inhibition of intestinal deconjugation of polyglutamyl forms of folic acid.^{25,26} This may explain why folic acid levels are usually normal in women who receive contraceptives, and it implies that absorption remains

adequate until some additional clinically important problem with absorption or dietary deficiency is superimposed on the use of the contraceptive.

Phenytoin and other anticonvulsant agents have also been associated with the development of megaloblastic anemia. However, a key difference between women who use birth-control pills and persons who receive phenytoin and other antiseizure medications is that folate levels are noted to be low in people who receive phenytoin. Phenytoin does not seem to have any effect on the folate metabolism pathways, nor does it appear to affect the excretion of folate.²⁷ However, most antiseizure medications increase hepatic microsomal enzyme activity, and it is believed that this increase in activity may result in an increase in the use of folic acid, thus leading to a decrease in serum folate levels. Similarly, antiseizure drugs may enhance hepatic detoxification enzymes, thus causing an increased breakdown of folic acid.

Antiseizure medications are also associated with a considerable decrease in the intestinal absorption of folic acid. Folate uses an active transport mechanism in the intestinal mucosa, as evidenced by the fact that some forms of folate, such as methyltetrahydrofolate, are absorbed more readily than others. However, since the various antiseizure medications are distinctly different from one another, it is unlikely that all these drugs would have a similar direct effect on the intestinal mucosa that would result in a decrease in active absorption of folic acid. Rather, it would appear that the effect is through a secondary action such as “solvent drag” (movement of folate across the cell membrane by bulk transport following the movement of water rather than being facilitated by ion channels or cellular pumps), sodium exchange, or an effect on intestinal ATPase.²⁷ Again, the probable reason that anticonvulsants are not a more common cause of megaloblastic anemia is that the gastrointestinal tract has a vast excess capacity for the absorption of important nutrients such as folic acid. Thus, some added compromise to absorption or significant diminution in the intake of folic acid is necessary for anemia to become an overt problem in patients. The addition of more folic acid to the patient’s diet will probably prevent or correct the problem.

Folate transport in the blood is facilitated by

a carrier protein.²⁰ Aspirin may reduce the binding of folate to its serum protein carrier. Similarly, phenytoin and other anticonvulsants that bear structural resemblances to folate may cause a decrease in serum folate levels by reducing the transport of folate.²⁷ Finally, and again because of the structural similarities between anticonvulsants and folic acid, the therapeutic effects of some of these drugs have been thought to be due partially to their folate analogue activities. Of note, phenytoin and other anticonvulsants have been noted to cause immunosuppression and even myelosuppression. In addition, administration of folate in persons with seizures has been reported to increase the incidence of seizures in those persons, whereas low folate states have been associated with improved seizure control.²⁷

DRUGS THAT INTERFERE WITH
THE METABOLISM OF FOLIC ACID

Drugs that are commonly termed folate analogues lead to a break in the important cyclic pathway in which folic acid is critically involved in returning dihydrofolate to tetrahydrofolate (Fig. 1). Many folate analogues have been synthesized for a variety of therapeutic purposes. Most commonly, they are used in the treatment of malignant diseases such as leukemia, as well as various solid tumors, including lung, bladder, breast, and head and neck cancers, mesothelioma, and sarcomas. Common to each of these drugs is the ability to bind to the enzyme dihydrofolate reductase, thus inhibiting the reduction of dihydrofolate to tetrahydrofolate. In this way, a true deficiency of reduced folic acid (i.e., a deoxidized form of folic acid caused by dihydrofolate reductase in the metabolism of dihydrofolate to tetrahydrofolate, which involves the use of NADPH as an electron donor) is produced.

Clinically, this folate analogue–generated defect can be corrected by administration of reduced folic acid in the form of folinic acid (10-formyl-tetrahydrofolate), since the reduced folic acid is beyond the block to the pathway caused by the folate analogue. The use of folinic acid should be considered prophylactically in patients who receive high doses of methotrexate. Folinic acid therapy should be initiated 24 hours after the administration of methotrexate in order to offset the adverse effects of methotrexate

on the more rapidly dividing progenitor cells of the gastrointestinal tract and bone marrow.

Pemetrexed, an folate analogue that inhibits multiple enzymes involved in purine and pyrimidine synthesis (thymidylate synthetase, dihydrofolate reductase, glycinamide ribonucleotide formyltransferase, and 5-aminoimidazole-4-carboxamide), is commonly used in the treatment of various solid tumors, including mesothelioma, lung, colon, breast, and head and neck cancers. The use of folinic acid can ameliorate the toxic effects of pemetrexed without diminishing its efficacy.²⁸⁻³⁰

Other uses of folate analogues are based on the fact that the dihydrofolate reductase of various species shows varying affinities for folate antagonists. Thus, a folate analogue, such as trimethoprim, was developed to treat various bacterial infections, and pyrimethamine was developed to treat protozoan infections.³¹

Trimethoprim is a structurally remote analogue of folic acid. It was developed because of biochemical evidence that its structural alterations produce a compound that maximally binds to the bacterial form of dihydrofolate reductase with minimal binding to the mammalian enzyme. Trimethoprim binds to a different dihydrofolate reductase epitope than methotrexate and interferes with the human enzyme only under unusual circumstances (e.g., in the case of human immunodeficiency virus infection when other DNA synthesis inhibitors are also used).³¹

Trimethoprim and pyrimethamine are often combined with sulfonamides. Sulfonamide is an antagonist of para-aminobenzoic acid, a folate precursor in microorganisms, but not in humans.³¹ Megaloblastic changes may develop in patients receiving trimethoprim and pyrimethamine because of the inhibition of DNA synthesis. Folinic acid completely reverses this effect without interfering with the antibacterial properties of the drugs.

DRUGS THAT DECREASE THE ABSORPTION OF VITAMIN B₁₂

The absorption of vitamin B₁₂ from food is shown in Figure 3. Animal products (meat, fish, chicken, and dairy products) are the only natural source of vitamin B₁₂ (cobalamin), which is synthesized in bacteria. Cobalamin is a coenzyme in the interactions of only two enzymes in mammals.

One of them, methylmalonyl-CoA mutase, is important in the catabolism of fatty acids in mitochondria; the other is methionine synthetase.¹⁰

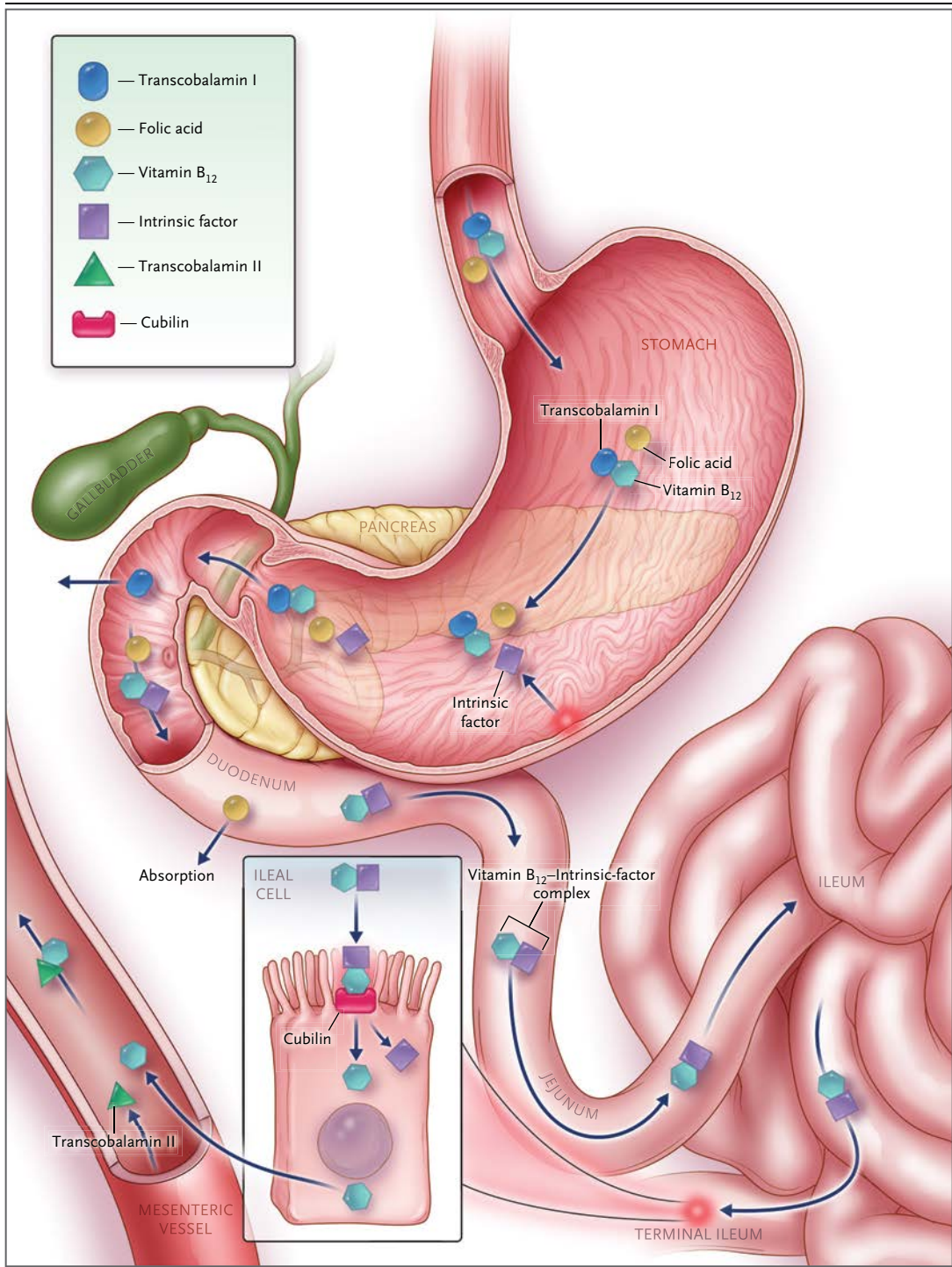
After ingestion, cobalamin is bound to haptocorrin, a glycoprotein related to plasma transcobalamin I, a member of the cobalamin-binding protein family, which is present in the saliva and other gastrointestinal juices. Haptocorrin is degraded by gastric enzymes and acid, after which the released cobalamin binds to intrinsic factor. This reaction is favored by an alkaline pH.¹⁰

In the terminal ileum, intrinsic factor attaches to a receptor, cubilin, which is located on the microvillus membrane and facilitates receptor-mediated transport of cobalamin in a neutral pH environment with the presence of calcium ions. Cubilin regeneration requires the presence of a protein, amnionless, and is thought to require a third protein, megalin, which stabilizes the cubilin–amnionless complex. In the process of cobalamin transport into ileal cells, intrinsic factor is degraded by lysosomal enzymes, and the free cobalamin in the plasma becomes bound to one of two major cobalamin-binding proteins, either transcobalamin I or transcobalamin II.¹⁰

This transcobalamin–cobalamin complex is transported across cellular membranes in the liver and other organs by two related receptors that belong to the low-density lipoprotein–receptor gene family, CD320 and renal Lrp2, or megalin.³²⁻³⁴ It is also filtered in the kidney, where the major portion is excreted, while some is reabsorbed by kidney cells and is secreted back into the plasma.

Drugs that interfere with B₁₂ absorption include aminosalicic acid, colchicine, neomycin, and metformin.^{35,36} Because of the minute amount of B₁₂ required by the body and its abundance in most diets, it is relatively rare that these agents may give rise to megaloblastic anemia. In most instances, the impaired absorption is believed to be secondary to an effect of the drug on the intestinal mucosa of the terminal ileum, where vitamin B₁₂ is absorbed. There are no reports of these drugs causing intrinsic-factor abnormalities or other problems with regard to the transport of vitamin B₁₂ across membranes and in the circulation.³⁵ The effect of metformin on vitamin B₁₂ absorption is reversible with calcium because the ileal absorption of vitamin B₁₂, as indicated previously, is a calcium-dependent process.³⁷

Another factor that may play a role in the



intestinal absorption of vitamin B₁₂ is an alteration in the intestinal pH. Since vitamin B₁₂ is a weak acid, its absorption is decreased in an alkaline environment. Phenytoin in solution has a very high pH (approximately 12), and an elevated

gastric pH has been noted in persons who have been receiving long-standing phenytoin therapy. This may partially explain why antacids such as histamine₂-receptor antagonists (H₂ blockers) and proton-pump inhibitors may, on rare occa-

Figure 3 (facing page). Absorption of Folic Acid and Vitamin B₁₂ (Cobalamin).

After ingestion, vitamin B₁₂ is bound in the mouth to haptocorrin (transcobalamin I), from which it becomes disassociated in the stomach because of the presence of gastric enzymes and acid. The haptocorrin is replaced by intrinsic factor, which is secreted by the parietal cells of the stomach. The vitamin B₁₂-intrinsic-factor complex attaches to the receptor cubilin, which is present on the surface of the epithelial cells of the terminal ileum and facilitates the absorption of the vitamin B₁₂-intrinsic-factor complex. Intrinsic factor is degraded within the ileal cells, and vitamin B₁₂ is absorbed into the bloodstream, where it becomes bound to transcobalamin II, which transports it to the various organs for DNA synthesis. Contrary to this more complex process of vitamin B₁₂ absorption, folic acid is readily absorbed in the jejunum by means of a passive process.

sions, be associated with the development of megaloblastic anemia, most usually in patients who have continued to receive these agents for 2 years or longer.³⁸⁻⁴⁰

Another mechanism may be the inhibition of intrinsic-factor production, given that proton-pump inhibitors do not act only on acid production by parietal cells but may also act on intrinsic-factor production. Thus, the continued use of such agents may be more likely to cause anemia than intermittent use, which is the recommended approach to the use of these acid-lowering agents.³⁸

MANAGEMENT

The key to managing megaloblastic anemia is determining the cause of the megaloblastosis, deciding whether the causative agent is expendable in the patient's treatment, and discontinuing the agent or switching to an alternative regimen, if possible. If the causative drug is essential to the patient's treatment and there are no acceptable alternatives, one should ensure that folate and vitamin B₁₂ intake are adequate. Both vitamins can be supplemented orally.

Regardless of the drug or the specific mechanism by which it may cause megaloblastic anemia, understanding the consequences of blocking the specific process is critical. The most important issue is recognizing the problem in the first place and relating it to the use of a drug. Physicians who administer any agent that blocks DNA synthesis should be aware of the

potential drug effect. Agents that are more potent, such as purine or pyrimidine analogues or folate antagonists, are likely to result in anemia that may occur rapidly, whereas with the use of less potent inhibitors, megaloblastic anemia may develop more slowly.

If the mechanism of action of the offending agent is not related to a deficiency in folic acid or vitamin B₁₂ absorption, the anemia will not be corrected by vitamin supplementation. Thus, because of the significant downstream effects, it can be corrected only by the removal of the agent from the therapeutic regimen or, when possible, bypassing the antagonism with folinic acid.

Perhaps a more consequential complication of vitamin B₁₂ and folate deficiency is the resultant hyperhomocysteinemia.³⁷ Homocysteine is a neurotoxic amino acid that is not found in proteins. An elevated homocysteine level has been implicated in many pathologic conditions, including cardiovascular diseases, fetal neural-tube defects, and, perhaps more questionably, in several neurodegenerative disorders such as stroke, Parkinson's disease, and Alzheimer's disease.³⁸⁻⁴¹ Elimination of homocysteine is regulated by both the transmethylation and transsulfuration pathways and is thus affected by folate, whereas the conversion of homocysteine to methionine is mediated by methionine synthase, the activity of which is regulated through vitamin B₁₂. Homocysteine also seems to play an important role in the regulation of neurogenesis and apoptosis.⁴¹⁻⁴⁶

Other effects of folate and vitamin B₁₂ deficiency are related to the fact that they play key roles as methyl donors in one-carbon metabolism. The results of methyl-donor deficiency have been noted in studies of intrauterine growth retardation. In addition, reduced Stat3 signaling targeted by miR-124 has been associated with long-term postnatal brain defects.⁴⁷ Even depression may be affected by folate deficiency owing to effects on the synthesis of neurotransmitters such as monoamine metabolites.⁴⁸

Thus, the consequences of drug-induced changes in folate and vitamin B₁₂ physiology can be substantial. Early recognition is critical for the prevention of irreversible consequences.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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