40 Tetrahydrofolate, Vitamin B12, And *S*-Adenosylmethionine

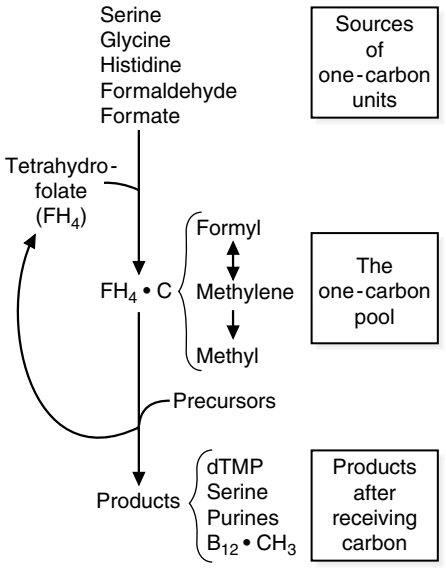


Fig. 40.1. Overview of the one-carbon pool. FH_4•C indicates tetrahydrofolate (FH_4) containing a one-carbon unit that is at the formyl, methylene, or methyl level of oxidation (see Fig. 40.3). The origin of the carbons is indicated, as are the final products after a one-carbon transfer.

Groups containing a single carbon atom can be transferred from one compound to another. These carbon atoms may be in a number of different oxidation states. The most oxidized form, CO_2, is transferred by biotin. One-carbon groups at lower levels of oxidation than CO_2 are transferred by reactions involving tetrahydrofolate (FH_4), vitamin B12, and S-adenosylmethionine (SAM).

Tetrahydrofolate: *Tetrahydrofolate, which is produced from the **vitamin folate**, is the primary one-carbon carrier in the body. This vitamin obtains one-carbon units from serine, glycine, histidine, formaldehyde, and formate (Fig. 40.1). While these carbons are attached to FH_4 they can be either oxidized or reduced. Because of this, folate can exist in a variety of chemical forms. Once a carbon has been reduced to the methyl level (methyl-FH_4), however, it cannot be re-oxidized. Collectively, these one-carbon groups attached to their carrier FH_4 are known as the **one-carbon pool**. The term folate is used to represent a water-soluble B-complex vitamin that functions in transferring single-carbon groups at various stages of oxidation.*

*The one-carbon groups carried by FH_4 are used for many biosynthetic reactions. For example, one-carbon units are transferred to the pyrimidine base of deoxyuridine monophosphate (**dUMP**) to form deoxythymidine monophosphate (**dTMP**), to the amino acid **glycine** to form **serine**, to precursors of the purine bases to produce carbons C2 and C8 of the **purine ring**, and to **vitamin B12.***

Vitamin B12: *Vitamin B12 is involved in two reactions in the body. It participates in the rearrangement of the methyl group of L-methylmalonyl-CoA to form succinyl-CoA, and it transfers a methyl group, obtained from FH_4, to homocysteine, forming methionine.*

S-adenosylmethionine (SAM): *SAM, produced from methionine and adenosine triphosphate (ATP), transfers the methyl group to precursors forming a number of compounds, including creatine, phosphatidylcholine, epinephrine, melatonin, methylated nucleotides, and methylated DNA.*

*Methionine metabolism is very dependent on both FH_4 and vitamin B12. **Homocysteine** is derived from methionine metabolism and can be converted back into methionine by using both methyl-FH_4 and vitamin B12. This is the only reaction in which methyl-FH_4 can donate the methyl group. If the enzyme that catalyzes this reaction is defective, or if vitamin B12 or FH_4 levels are insufficient, homocysteine will accumulate. Elevated homocysteine levels have been linked to cardiovascular and neurologic disease. A vitamin B12 deficiency can be brought about by the lack of **intrinsic factor**, a gastric protein required for the absorption of dietary B12. A consequence of vitamin B12 deficiency is the accumulation of methyl-FH_4 and a decrease in other folate derivatives. This is known as the*

***methyl-trap hypothesis**, in which, because of the B12 deficiency, most of the carbons in the FH_4 pool are trapped in the methyl-FH_4 form, which is the most stable. The carbons cannot be released from the folate, because the one reaction in which they participate cannot occur because of the B12 deficiency. This will therefore lead to a functional folate deficiency, even though total levels of folate are normal. A folate deficiency (whether functional or actual) will lead to **megaloblastic anemia** caused by an inability of blood cell precursors to synthesize DNA and therefore to divide. This leads to large, partially replicated cells being released into the blood to attempt to replenish the cells that have died. Folate deficiencies also have been linked to an increased incidence of **neural-tube defects**, such as spina bifida, in mothers who become pregnant while folate deficient.*

THE WAITING ROOM

After resection of the cancer in his large intestine and completion of a course of postoperative chemotherapy with 5-fluorouracil (5-FU), **Colin Tuma** returned to his gastroenterologist for a routine follow-up colonoscopy. His colon was completely normal, with excellent healing at the site of the anastomosis. His physician expressed great optimism about a cure of Colin's previous malignancy but cautioned him about the need for regular colonoscopic examinations over the next few years.

Bea Twelvlow, a 75-year-old woman, went to see her physician because of a numbness and tingling in her arms. A diet history indicated a normal and healthy diet, but Bea was not taking any supplemental vitamin pills. Laboratory results indicated a slight elevation of methylmalonic acid, and this led the physician to suspect a vitamin B12 deficiency. Direct measurement of serum B12 levels did indicate a deficiency, but the results of a Schilling test were normal.

The initial laboratory profile, determined when **Jean Ann Tonich** first presented to her physician with evidence of early alcohol-induced hepatitis, included a hematologic analysis that showed that Jean Ann was anemic. Her hemoglobin was 11.0 g/dL (reference range = 12–16 for an adult female). The erythrocyte (red blood cell) count was 3.6 million cells/mm3 (reference range = 4.0–5.2 for an adult female). The average volume of her red blood cells (mean corpuscular volume, or MCV) was 108 fL (reference range = 80–100), and the hematology laboratory reported a striking variation in the size and shape of the red blood cells in a smear of her peripheral blood (see Chapter 44). The nuclei of the circulating granulocytic leukocytes had increased nuclear segmentation (polysegmented neutrophils). Because these findings are suggestive of a macrocytic anemia (in which blood cells are larger than normal), measurements of serum folate and vitamin B12 (cobalamin) levels were ordered.

I. TETRAHYDROFOLATE (FH_4)

A. Structure and Forms of FH_4

Folates exist in many chemical forms. The coenzyme form that functions in accepting one-carbon groups is tetrahydrofolate polyglutamate (Fig. 40.2), generally just referred to as tetrahydrofolate or FH_4. It has three major structural components, a bicyclic pteridine ring, para-aminobenzoic acid, and a polyglutamate tail consisting

The Schilling test involves the patient ingesting radioactive (Co^{60}) crystalline vitamin B12 after which a 24-hour urine sample is collected. The radioactivity in the urine sample is compared with the input radioactivity, and the difference represents the amount of B12 absorbed through the digestive tract.

Folate deficiencies frequently occur in individuals with chronic alcoholism. A number of factors are involved: inadequate dietary intake of folate; direct damage to intestinal cells and brush border enzymes, which interferes with absorption of dietary folate; a defect in the enterohepatic circulation, which reduces the absorption of folate; liver damage causing decreased hepatic production of plasma proteins; and interference with kidney resorption of folate.

Because of the possibility of a direct toxic effect of alcohol on hematopoietic tissues in **Jean Ann Tonich**, a bone marrow aspirate was performed. The aspirate contained a greater than normal number of red and white blood cell precursors, most of which were larger than normal. The large red blood cells are called megaloblasts.

These hematopoietic precursor cells when exposed to too little folate and/or vitamin B12 show slowed cell division, but cytoplasmic development occurs at a normal rate. Hence, the megaloblastic cells tend to be large, with an increased ratio of RNA to DNA. Megaloblastic erythroid progenitors are usually destroyed in the bone marrow (although some reach the circulation). Thus, marrow cellularity is often increased but production of red blood cells is decreased, a condition called "ineffective erythropoiesis."

Therefore, Jean Ann had a megaloblastic anemia, characteristic of a folate or B12 deficiency.

Jean Ann's serum folic acid level was 3.1 ng/mL (reference range = 6–15), and her serum B12 level was 154 pg/mL (reference range = 150–750). Her serum iron level was normal. It was clear, therefore, that Jean Ann's megaloblastic anemia was caused by a folate deficiency (although her B12 levels were in the low range of normal). The management of a pure folate deficiency in an alcoholic patient includes cessation of alcohol intake and a diet rich in folate.

The abbreviation fL stands for *femtoliters*. One fL is 10^{-12} milliliters (mL).

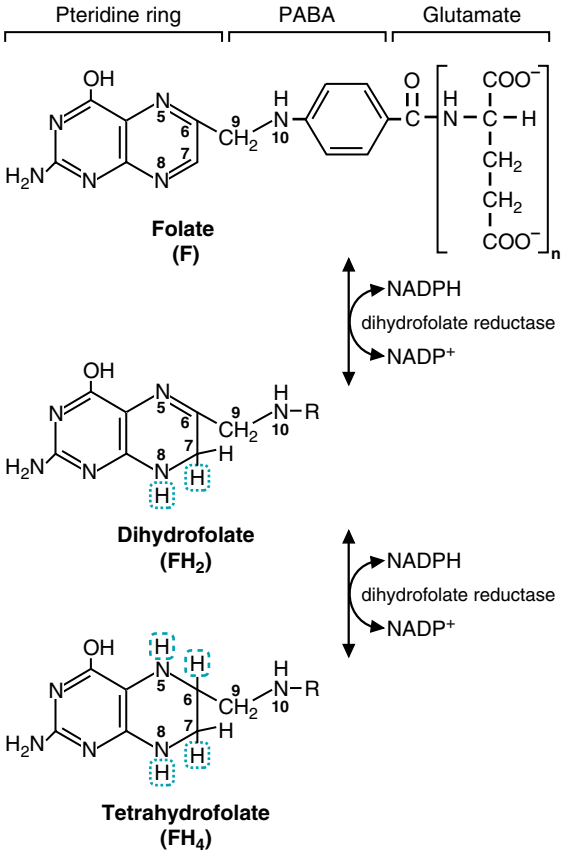


Fig. 40.2. Reduction of folate to tetrahydrofolate (FH_4). The same enzyme, dihydrofolate reductase, catalyzes both reactions. Multiple glutamate residues are added within cells (n ~ 5). Plants can synthesize folate, but humans cannot. Therefore, folate is a dietary requirement. R is the portion of the folate molecule shown to the right of N^{10}. The different precursors of FH_4 are indicated in the figure. PABA = para-aminobenzoic acid.

Sulfa drugs, which are used to treat certain bacterial infections, are analogs of para-aminobenzoic acid. They prevent growth and cell division in bacteria by interfering with the synthesis of folate. Because we cannot synthesize folate, sulfa drugs do not affect human cells in this way.

The current recommended dietary allowance (RDA) for folate equivalents is approximately 400 μg for adult men and women. In addition to being prevalent in green leafy vegetables, other good sources of this vitamin are liver, yeast, legumes, and some fruits. Protracted cooking of these foods, however, can destroy up to 90% of their folate content. A standard US diet provides 50 to 500 μg absorbable folate each day. Folate deficiency in pregnant women, especially during the month before conception and the month after, increases the risk of neural tube defects, such as spina bifida, in the fetus. To reduce the potential risk of neural tube defects for women capable of becoming pregnant, the recommendation is to take 400 μg of folic acid daily from a multivitamin pill. If the women has a history of having a child with a neural tube defect, this amount is increased to 4000 μg/day for the month before and the month after conception. Flour-containing products in the United States are now supplemented with folate to reduce the risk of neural tube defects in newborns.

of several glutamate residues joined in amide linkage. The one-carbon group that is accepted by the coenzyme and then transferred to another compound is bound to N^5, to N^{10}, or to both.

Different forms of folate may differ in the oxidation state of the one-carbon group, in the number of glutamate residues attached, or in the degree of oxidation of the pteridine ring. When the term *folate* or *folic acid* is applied to a specific chemical form, it is the most oxidized form of the pteridine ring (see Fig. 40.2). Folate is reduced to dihydrofolate and then to tetrahydrofolate by dihydrofolate reductase present in cells. Reduction is the favored direction of the reaction; therefore, most of the folate present in the body is present as the reduced coenzyme form, FH_4.

B. The Vitamin Folate

Folates are synthesized in bacteria and higher plants and ingested in green leafy vegetables, fruits, and legumes in our diet. The vitamin was named for its presence in green, leafy vegetables (foliage). Most of the dietary folate derived from natural food sources is present in the reduced coenzyme form. However, vitamin supplements and fortified foods contain principally the oxidized form of the pteridine ring.

As dietary folates pass into the proximal third of the small intestine, folate conjugases in the brush border of the lumen cleave off glutamate residues to produce the monoglutamate form of folate, which is then absorbed (see Fig. 40.2, upper

structure, when n = 1). Within the intestinal cells, folate is converted principally to N^5-methyl FH_4, which enters the portal vein and goes to the liver. Smaller amounts of other forms of folate also follow this route.

The liver, which stores half of the body's folate, takes up much of the folate from the portal circulation; uptake may be through active transport or receptor-mediated endocytosis. Within the liver, FH_4 is reconjugated to the polyglutamate form before being used in reactions. A small amount of the folate is partially degraded, and the components enter the urine. A relatively large portion of the folate enters the bile and is subsequently reabsorbed (very similar to the fate of bile salts in the enterohepatic circulation).

N^5-Methyl-FH_4, the major form of folate in the blood, is loosely bound to plasma proteins, particularly serum albumin.

C. Oxidation and Reduction of the One-Carbon Groups of Tetrahydrofolate

One-carbon groups transferred by FH_4 are attached either to nitrogen N^5 or N^{10} or they form a bridge between N^5 and N^{10}. The collection of one-carbon groups attached to FH_4 is known as the one-carbon pool. While attached to FH_4, these one-carbon units can be oxidized and reduced (Fig. 40.3). Thus, reactions requiring a carbon at a particular oxidation state may use carbon from the one-carbon pool that was donated at a different oxidation state.

The individual steps for reduction of the one-carbon group are shown in Fig. 40.4. The most oxidized form is N^{10}-formyl FH_4. The most reduced form is N^5-methyl-FH_4. Once the methyl group is formed, it is *not readily reoxidized back to N^5, N^{10} methylene FH_4, and thus N^5-methyl-FH_4 will tend to accumulate in the cell.*

D. Sources of One-Carbon Groups Carried by FH_4

Carbon sources for the one-carbon pool include serine, glycine, formaldehyde, histidine, and formate (see Fig. 40.4). These donors transfer the carbons to folate at different oxidation states. Serine is the major carbon source of one-carbon groups in the human. Its hydroxymethyl group is transferred to FH_4 in a reversible reaction, catalyzed by the enzyme serine hydroxymethyltransferase. This reaction produces glycine and N^5, N^{10}-methylene-FH_4. Because serine can be synthesized from 3-phosphoglycerate, an intermediate of glycolysis, dietary carbohydrate can serve as a source of carbon for the one-carbon pool. The glycine that is produced may be further degraded by donation of a carbon to folate. Additional donors that form N^5, N^{10} methylene-FH_4 are listed in Table 40.1.

Table 40.1. One-Carbon Pool: Sources and Recipients of Carbon

Source[a]	Form of One-Carbon Donor Produced[b]	Recipient	Final Product
Formate	N^{10}-formyl-FH_4	Purine precursor	Purine (C2 and C8)
Serine Glycine Formaldehyde	N^5, N^{10} methylene FH_4	dUMP Glycine	dTMP Serine
N^5, N^{10} methylene FH_4	N^5-methyl FH_4	Vitamin B12	Methylcobalamin
Histidine	N^5-formimino FH_4 is converted to N^5, N^{10} methylene FH_4		
Choline	Betaine	Homocysteine	Methionine and Dimethylglycine
Methionine	S-adenosylmethionine (SAM)	Glycine (there are many others; see figure 40.9B)	N-methylglycine (sarcosine)

[a]The major source of carbon is serine.
[b]The carbon unit attached to FH_4 can be oxidized and reduced (see Fig. 40.3). At the methyl level, reoxidation does not occur.

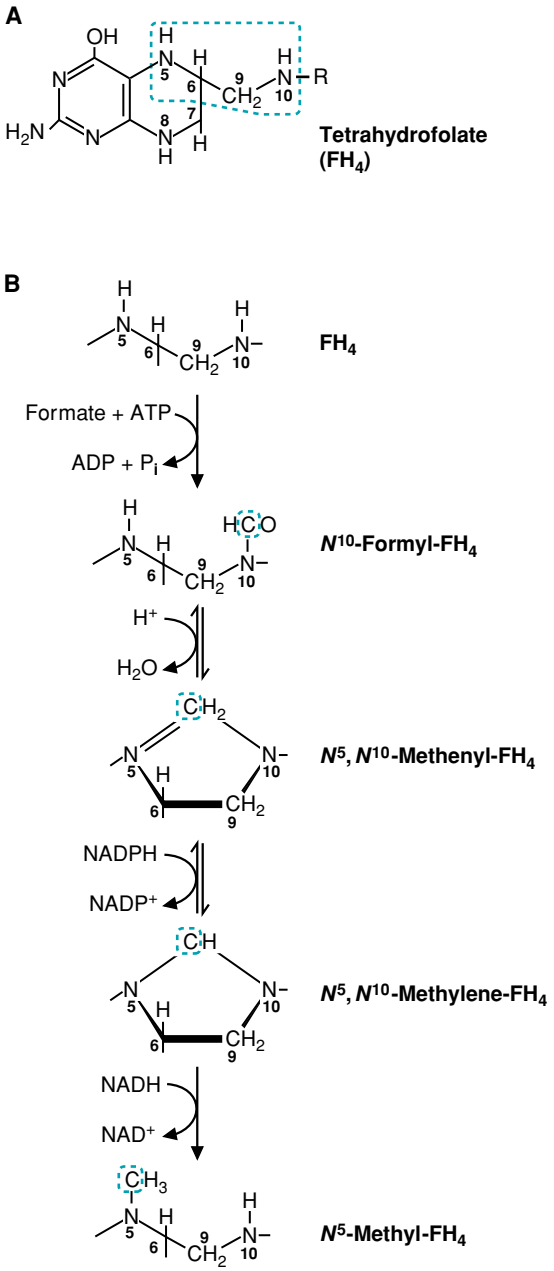


Fig. 40.3. One-carbon units attached to FH_4. A. The active form of FH_4. For definition of R, see Figure 40.2. B. Interconversions of one-carbon units of FH_4. Only the portion of FH_4 from N^5 to N^{10} is shown, which is indicated by the dashed line in Part A. After a formyl group forms a bridge between N^5 and N^{10}, two reductions can occur. Note that N^5-methyl-FH_4 cannot be reoxidized. The most oxidized form of FH_4 is at the top of the figure, whereas the most reduced form is at the bottom.

A deficiency of folate results in the accumulation of FIGLU, which is excreted in the urine. A histidine load test can be used for detecting folate deficiencies. Patients were given a test dose of histidine (a histidine load), and the amount of FIGLU that appeared in the urine was measured.

Histidine and formate provide examples of compounds that donate carbon at different oxidation levels (see Fig. 40.4). Degradation of histidine produces formiminoglutamate (FIGLU), which reacts with FH_4 to donate a carbon and nitrogen (generating N^5-formimino-FH_4), thereby releasing glutamate. Formate, produced from tryptophan oxidation, can react with FH_4 and generate N^{10}-formyl-FH_4, the most oxidized folate derivative.

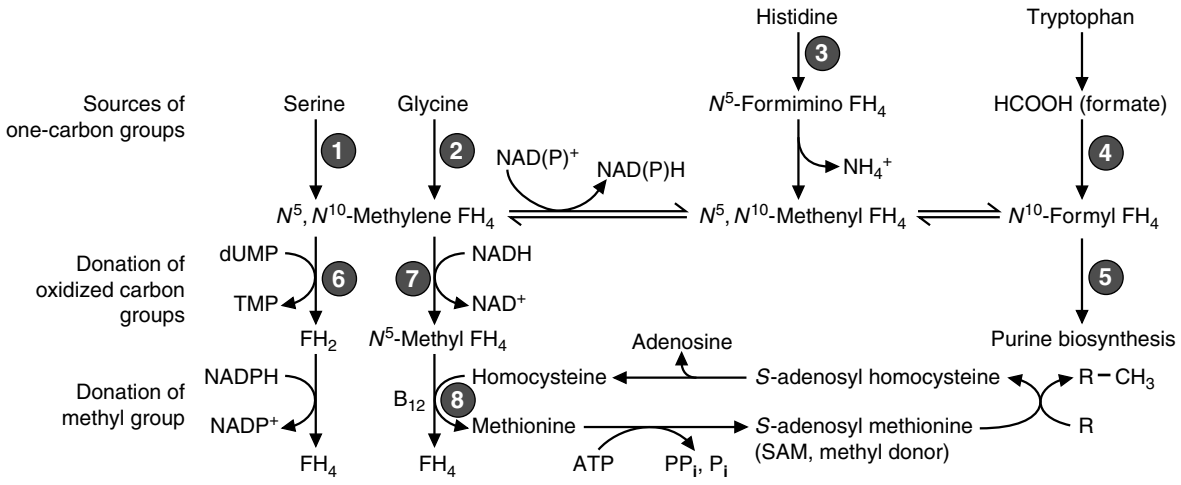


Fig. 40.4. Sources of carbon (reactions 1–4) for the FH_4 pool and the recipients of carbon (reactions 5–8) from the pool. See Figure 40.3 to view the FH_4 derivatives involved in each reaction.

E. Recipients of One-Carbon Groups

The one-carbon groups on FH_4 may be oxidized or reduced (see Fig. 40.3) and then transferred to other compounds (see Fig. 40.4 and Table 40.1). Transfers of this sort are involved in the synthesis of glycine from serine, the synthesis of the base thymine required for DNA synthesis, the purine bases required for both DNA and RNA synthesis, and the transfer of methyl groups to vitamin B12.

Because the conversion of serine to glycine is readily reversible, glycine can be converted to serine by drawing carbon from the one-carbon pool.

The nucleotide deoxythymidine monophosphate (dTMP) is produced from deoxyuridine monophosphate (dUMP) by a reaction in which dUMP is methylated to form dTMP (Fig. 40.5). The source of carbon is N^5, N^{10}-methylene FH_4. Two hydrogen atoms from FH_4 are used to reduce the donated carbon to the methyl level. Consequently, dihydrofolate (FH_2) is produced. Reduction of FH_2 by NADPH in a

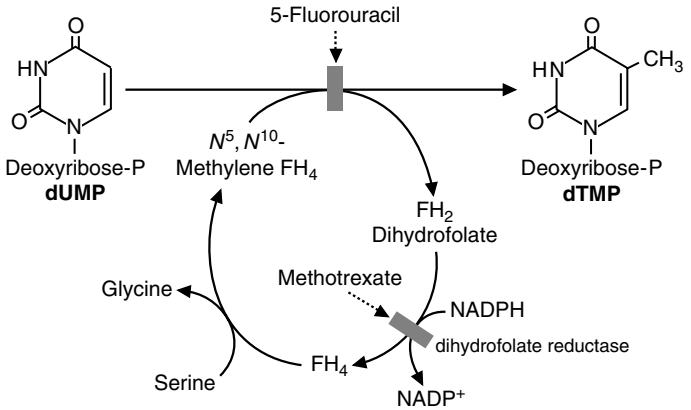


Fig. 40.5. Transfer of a one-carbon unit from N^5, N^{10} methhylene FH_4 to dUMP to form dTMP. FH_4 is oxidized to FH_2 (dihydrofolate) in this reaction. FH_2 is reduced to FH_4 by dihydrofolate reductase and FH_4 is converted to N^5, N^{10} methylene FH_4 using serine as a carbon donor. Shaded bars indicate the steps at which the antimetabolites 5-fluorouracil (5-FU) and methotrexate act. 5-FU inhibits thymidylate synthase. Methotrexate inhibits dihydrofolate reductase.

FH_4 is required for the synthesis of deoxythymidine monophosphate and the purine bases used to produce the precursors for DNA replication. Therefore, FH_4 is required for cell division. Blockage of the synthesis of thymine and the purine bases either by a dietary deficiency of folate or by drugs that interfere with folate metabolism results in a decreased rate of cell division and growth.

A better understanding of the structure and function of the purine and pyrimidine bases and of folate metabolism led to the development of compounds having antimetabolic and antifolate action useful for treatment of neoplastic disease. For example, **Colin Tuma** was successfully treated for colon cancer with 5-fluorouracil (5-FU) (see Chapter 12 and Fig. 40.5). 5-FU is a pyrimidine analog, which is converted in cells to the nucleotide fluorodeoxyuridylate (FdUMP). FdUMP causes a "thymineless death," especially for tumor cells having a rapid turnover rate. It prevents the growth of cancer cells by blocking the thymidylate synthase reaction, i.e., the conversion of dUMP to dTMP.

Jean Ann Tonich's megaloblastic anemia was treated, in part, with folate supplements (see Clinical Comments). Within 48 hours of the initiation of folate therapy, megaloblastic or "ineffective" erythropoiesis usually subsides, and effective erythropoiesis begins.

A megaloblastic anemia is caused by a decrease in the synthesis of thymine and the purine bases. These deficiencies lead to an inability of hematopoietic (and other) cells to synthesize DNA and, therefore, to divide. Their persistently thwarted attempts at normal DNA replication, DNA repair, and cell division produce abnormally large cells (called megaloblasts) with abundant cytoplasm capable of RNA and protein synthesis, but with clumping and fragmentation of nuclear chromatin (see Chapter 44). Some of these large cells, although immature, are released early from the marrow in an attempt to compensate for the anemia. Thus, peripheral blood smears will also contain megaloblasts. Many of the large immature cells, however, are destroyed in the marrow and never reach the circulation.

The average daily diet in Western countries contains 5–30 μg vitamin B12, of which 1–5 μg is absorbed into the blood. (The RDA is 2.4 μg/day.) Total body content of this vitamin in an adult is approximately 2–5 mg, of which 1 mg is present in the liver. As a result, a dietary deficiency of B12 is uncommon and is only observed after a number of years on a diet deficient in this vitamin.

In spite of **Jean Ann Tonich's** relatively malnourished state because of chronic alcoholism, her serum cobalamin level was still within the low-to-normal range. If her undernourished state had continued, a cobalamin deficiency would eventually have developed.

reaction catalyzed by dihydrofolate reductase (DHFR) regenerates FH_4. This is the only reaction involving FH_4 in which the folate group is oxidized as the one-carbon group is donated to the recipient. Recall that DHFR is also required to reduce the oxidized form of the vitamin, which is obtained from the diet (see Fig. 40.2). Thus, DHFR is essential for both regenerating FH_2 in the tissues and from the diet. These reactions contribute to the effect of folate deficiency on DNA synthesis because dTMP is only required for the synthesis of DNA.

During the synthesis of the purine bases, carbons 2 and 8 are obtained from the one-carbon pool (see Chapter 41). N^{10}-Formyl-FH_4 provides both carbons. Folate deficiency would also hinder these reactions, contributing to an inability to replicate DNA because of the lack of precursors.

After the carbon group carried by FH_4 is reduced to the methyl level, it is transferred to vitamin B12. This is the only reaction through which the methyl group can leave FH_4 (recall that the reaction creating N^5-methyl FH_4 is not reversible).

II. VITAMIN B12

A. Structure and Forms of Vitamin B12

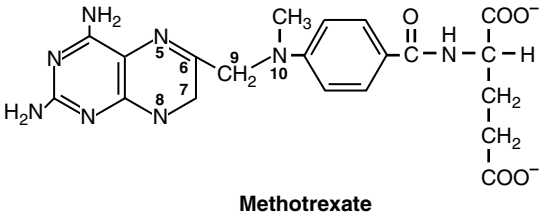
The structure of vitamin B12 (also known as cobalamin) is complex (Fig. 40.6). It contains a corrin ring, which is similar to the porphyrin ring found in heme. The corrin ring differs from heme, however, in that two of the four pyrrole rings are joined directly rather than by a methylene bridge. Its most unusual feature is the presence of cobalt, coordinated with the corrin ring (similar to the iron coordinated with the porphyrin ring). This cobalt can form a bond with a carbon atom. In the body, it reacts with the carbon of a methyl group, forming methylcobalamin, or with the 5′-carbon of 5′-deoxyadenosine, forming 5′-deoxyadenosylcobalamin (note that in this case the deoxy designation refers to the 5′ carbon, not the 2′ carbon as is the case in the sugar found in DNA). The form of B12 found in vitamin supplements is cyanocobalamin, in which a CN group is linked to the cobalt.

B. Absorption and Transport of Vitamin B12

Although vitamin B12 is produced by bacteria, it cannot be synthesized by higher plants or animals. The major source of vitamin B12 is dietary meat, eggs, dairy products, fish, poultry, and seafood. The animals that serve as the source of these foods obtain B12 mainly from the bacteria in their food supply. The absorption of B12 from the diet is a complex process (Fig. 40.7).

Ingested B12 can exist in two forms, either free or bound to dietary proteins. If free, the B12 binds to proteins known as R-binders (haptocorrins, also known as transcobalamin I), which are secreted by the salivary glands and the gastric mucosa, in either the saliva or the stomach. If the ingested B12 is bound to proteins, it must be released from the proteins by the action of digestive proteases both in the stomach

Q: Individuals with non-Hodgkin's lymphoma receive a number of drugs to treat the tumor, including the use of methotrexate. The structure of methotrexate is shown below.



Methotrexate

What compound does methotrexate resemble?

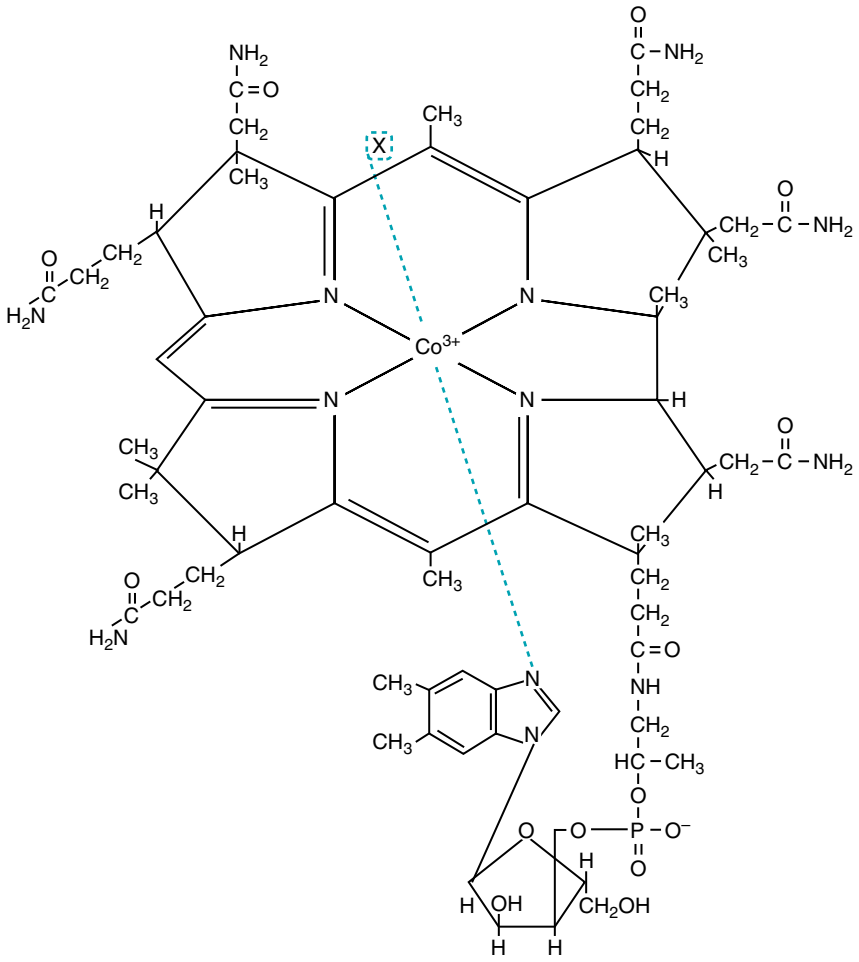


Fig. 40.6. Vitamin B12. X = 5′-deoxyadenosine in deoxyadenosylcobalamin; X = CH_3 in methylcobalamin; X = CN in cyanocobalamin (the commercial form found in vitamin tablets).

and small intestine. Once the B12 is released from its bound protein, it will bind to the haptocorrins. In the small intestine, the pancreatic proteases digest the haptocorrins, and the released B12 then binds to intrinsic factor, a glycoprotein secreted by the parietal cells of the stomach when food enters the stomach. The intrinsic factor–B12 complex attaches to specific receptors in the terminal segment of the small intestine known as the ileum, after which the complex is internalized.

The B12 within the enterocyte complexes with transcobalamin II and then is released into circulation. The transcobalamin II–B12 complex delivers B12 to the tissues, which contain specific receptors for this complex. The liver takes up approximately 50% of the vitamin B12, and the remainder is transported to other tissues. The amount of the vitamin stored in the liver is large enough that 3 to 6 years pass before symptoms of a dietary deficiency occur.

C. Functions of Vitamin B12

Vitamin B12 is involved in two reactions in the body: the transfer of a methyl group from N^5-methyl FH_4 to homocysteine to form methionine and the rearrangement of the methyl group of L-methylmalonyl CoA to form succinyl CoA (Fig. 40.8).

Tetrahydrofolate receives a one-carbon group from serine or from other sources. This carbon is reduced to the methyl level and transferred to vitamin B12, forming

A: Methotrexate has the same structure as folate except that it has an amino group on C4 and a methyl group on N10. Anticancer drugs such as methotrexate are folate analogs that act by inhibiting dihydrofolate reductase, thereby preventing the conversion of FH_2 to FH_4 (see Fig. 40.5). Thus, the cellular pools of FH_4 are not replenished, and reactions requiring FH_4 cannot proceed.

Pernicious anemia, a deficiency of intrinsic factor, is a relatively common problem caused by malabsorption of dietary cobalamin. It may result from an inherited defect that leads to a decreased ability of gastric parietal cells to synthesize intrinsic factor or from partial resection of the stomach or of the ileum. Production of intrinsic factor often declines with age and may be low in elderly individuals. An alternative circumstance that leads to the development of a B12 deficiency is pancreatic insufficiency or a high intestinal pH, which would result from too little acid being produced by the stomach. Both of these conditions prevent the degradation of the R-binder-B12 complex; as a result, B12 will not be released from the R-binder protein and, therefore, cannot bind to intrinsic factor.

Q: How should vitamin B12 be administered to a patient with pernicious anemia?

A: Because the problem in pernicious anemia is a lack of intrinsic factor, which results in an inability to absorb vitamin B12 from the gastrointestinal tract, B12 cannot be administered orally to treat this condition. In the past, it was usually given by injection. An effective nasal spray containing B12 has recently been marketed, however, and its use precludes the need for lifelong injections of this vitamin.

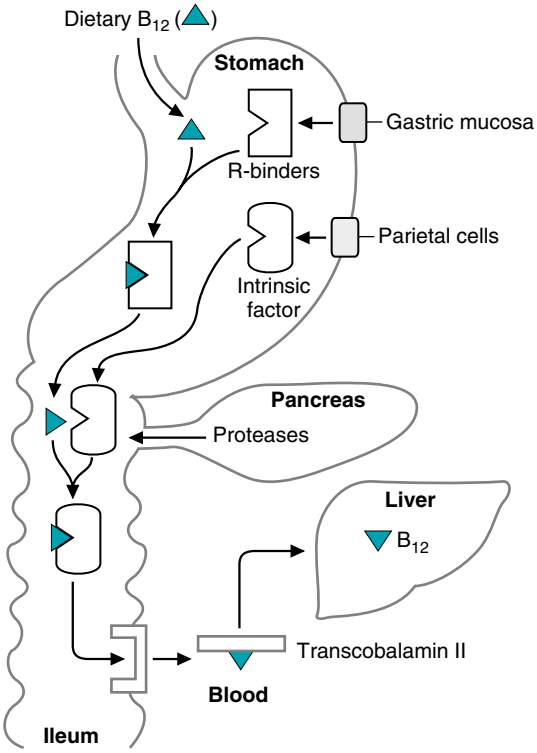


Fig. 40.7. Absorption, transport, and storage of vitamin B12. Dietary B12 binds to R-binders (haptocorrins) in the stomach and travels to the intestine, where the R-binders are destroyed by pancreatic proteases. The freed B12 then binds to intrinsic factor (IF). B12 is absorbed in the ileum and carried by called transcobalamins (TC) to the liver, where B12 is stored.

methyl-B12 (or methylcobalamin). Methylcobalamin transfers the methyl group to homocysteine, which is converted to methionine by the enzyme methionine synthase. Methionine can then be activated to SAM to transfer the methyl group to other compounds (Fig. 40.9).

Vitamin B12 also participates in the conversion of L-methylmalonyl CoA to succinyl CoA. In this case, the active form of the coenzyme is 5′-deoxyadenosylcobalamin. This reaction is part of the metabolic route for the conversion of carbons from valine, isoleucine, threonine, thymine, and the last three carbons of odd-chain fatty acids, all of which form propionyl CoA, to the TCA cycle intermediate succinyl CoA (see Chapter 39).

There are two major clinical manifestations of cobalamin (B12) deficiency. One such presentation is hematopoietic (caused by the adverse effects of a B12 deficiency on folate metabolism), and the other is neurologic (caused by hypomethylation in the nervous system).

The hemopoietic problems associated with a B12 deficiency are identical to those observed in a folate deficiency and, in fact, result from a folate deficiency secondary to (i.e., caused by) the B12 deficiency (i.e., the methyl trap hypothesis). As the FH_4 pool is exhausted, deficiencies of the tetrahydrofolate derivatives needed for purine and dTMP biosynthesis develop, leading to the characteristic megaloblastic anemia.

The classical clinical presentation of the neurologic dysfunction associated with a B12 deficiency includes symmetric numbness and tingling of the hands and feet, diminishing vibratory and position sense, and progression to a spastic gait disturbance. The patient may become somnolent or may become extremely irritable ("megaloblastic madness"). Eventually, blind spots in the central portions of the visual fields develop, accompanied by alterations in gustatory (taste) and olfactory (smell) function. This is believed to be caused by hypomethylation within the nervous system, brought about by an inability to recycle homocysteine to methionine and from there to S-adenosylmethionine. The latter is the required methyl donor in these reactions. The nervous system lacks the betaine pathway of methionine regeneration and is dependent on the B12 system. With a B12 deficiency, this pathway is inoperable in the nervous system.

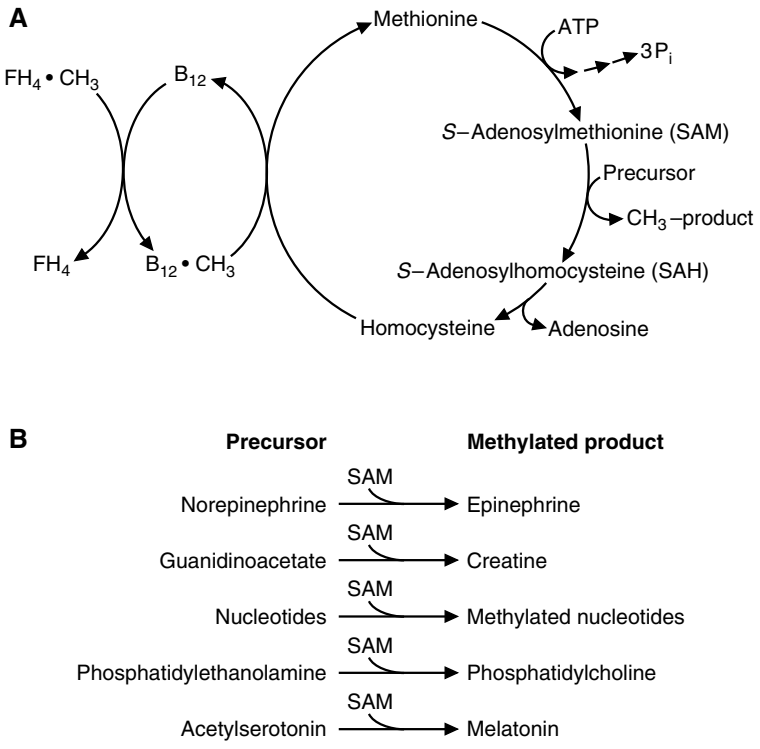


Fig. 40.9. Relationship between FH_4, B12, and SAM. A. Overall scheme. B. Some specific reactions requiring SAM.

III. *S*-ADENOSYLMETHIONINE

S-Adenosylmethionine (SAM) participates in the synthesis of many compounds that contain methyl groups. It is used in reactions that add methyl groups to either oxygen or nitrogen atoms in the acceptor (contrast that to folate derivatives, which can add one-carbon groups to sulfur or to carbon). As examples, SAM is required for the conversion of phosphatidylethanolamine to phosphatidylcholine, guanidinoacetate to creatine, norepinephrine to epinephrine, acetylserotonin to melatonin, and nucleotides to methylated nucleotides (see Fig. 40.9B). It is also required for the inactivation of catecholamines and serotonin (see Chapter 48). More than 35 reactions in humans require methyl donation from SAM.

SAM is synthesized from methionine and ATP. As with the activation of vitamin B12, ATP donates the adenosine. With the transfer of its methyl group, SAM forms *S*-adenosylhomocysteine, which is subsequently hydrolyzed to form homocysteine and adenosine.

Methionine, required for the synthesis of SAM, is obtained from the diet or produced from homocysteine, which accepts a methyl group from vitamin B12

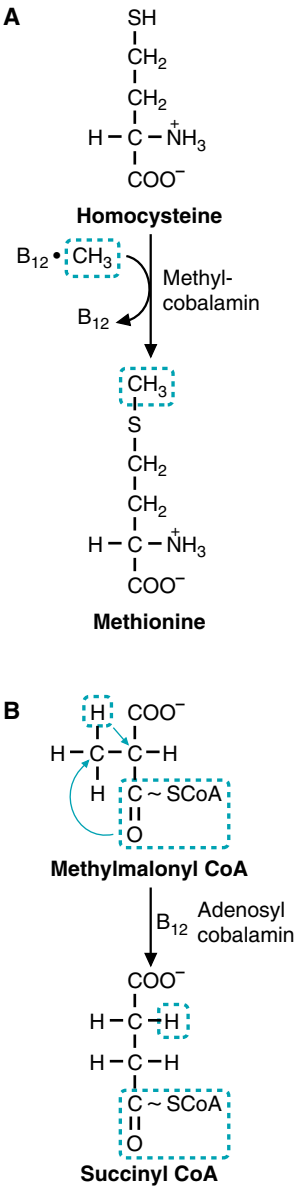


Fig. 40.8. The two reactions involving vitamin B12 in humans.

Many health food stores now sell SAMe, a stabilized version of S-adenosylmethionine. SAMe has been hypothesized to relieve depression because the synthesis of certain neurotransmitters requires methylation by SAM (see Chapter 47). This has led to the hypothesis that by increasing SAM levels within the nervous system, the biosynthesis of these neurotransmitters will be accelerated. This in turn might alleviate the feelings of depression. There have been reports in the literature indicating that this may occur, but its efficacy as an antidepressant must be confirmed. The major questions that must be addressed include the stability of SAMe in the digestive system and the level of uptake of SAMe by cells of the nervous system.

(see Fig. 40.9A). Thus, the methyl group of methionine is regenerated. The portion of methionine that is essential in the diet is the homocysteine moiety. If we had an adequate dietary source of homocysteine, methionine would not be required in the diet. However, there is no good dietary source of homocysteine, whereas methionine is plentiful in the diet.

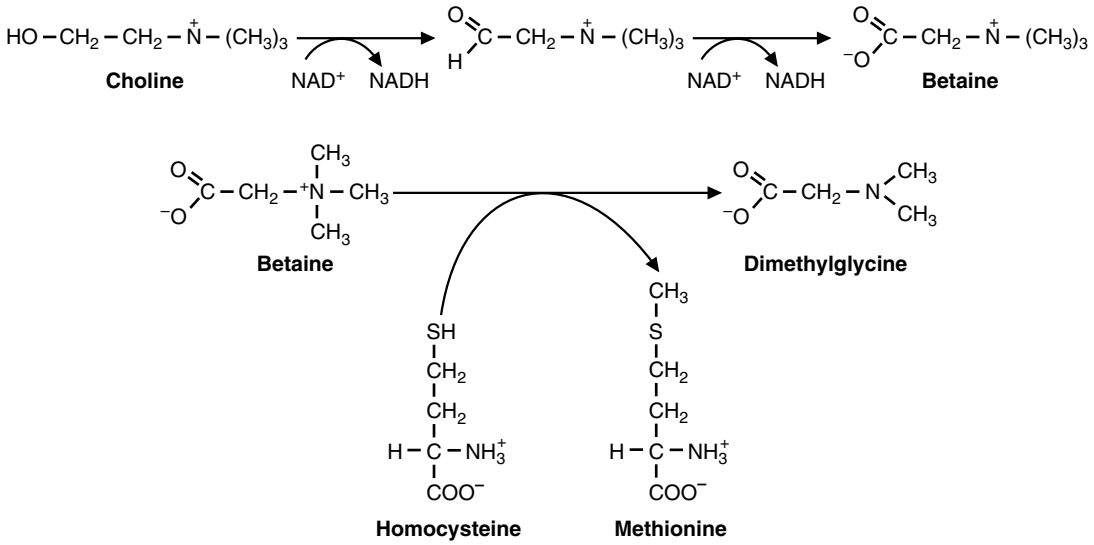
Homocysteine provides the sulfur atom for the synthesis of cysteine (see Chapter 39). In this case, homocysteine reacts with serine to form cystathionine, which is cleaved, yielding cysteine and α-ketobutyrate. The first reaction in this sequence is inhibited by cysteine. Thus, methionine, via homocysteine, is not used for cysteine synthesis unless the levels of cysteine in the body are lower than required for its metabolic functions. An adequate dietary supply of cysteine, therefore, can "spare" (or reduce) the dietary requirement for methionine.

IV. RELATIONSHIPS BETWEEN FOLATE, VITAMIN B12, AND SAM

A. The Methyl-Trap Hypothesis

If one analyzes the flow of carbon in the folate cycle, the equilibrium lies in the direction of the N^5-methyl FH_4 form. This appears to be the most stable form of carbon attached to the vitamin. However, in only one reaction can the methyl group be removed from N^5-methyl FH_4, and that is the methionine synthase reaction, which requires vitamin B12. Thus, if vitamin B12 is deficient, or if the methionine synthase enzyme is defective, N^5-methyl FH_4 will accumulate. Eventually most folate forms in the body will become "trapped" in the N^5-methyl form. A functional folate deficiency results because the carbons cannot be removed from the folate. The appearance of a functional folate deficiency caused by a lack of vitamin B12 is known as the "methyl-trap" hypothesis, and its clinical implications are discussed in following sections.

Other compounds involved in one-carbon metabolism are derived from degradation products of choline. Choline, an essential component of certain phospholipids, is oxidized to form betaine aldehyde, which is further oxidized to betaine (trimethylglycine). In the liver, betaine can donate a methyl group to homocysteine to form methionine and dimethyl glycine. This allows the liver to have two routes for homocysteine conversion to methionine. Under conditions in which SAM accumulates, glycine can be methylated to form sarcosine (N-methyl glycine). This route is used when methionine levels are high and excess methionine needs to be metabolized.



B. Hyperhomocysteinemia

Elevated homocysteine levels have been linked to cardiovascular and neurologic disease. Homocysteine levels can accumulate in a number of ways, which are related to both folic acid and vitamin B12 metabolism. Homocysteine is derived from S-adenosyl homocysteine, which arises when SAM donates a methyl group (Fig. 40.10). Because SAM is frequently donating methyl groups, there is a constant production of S-adenosyl homocysteine, which leads to a constant production of homocysteine. Recall from Chapter 39 that homocysteine has two biochemical fates. The homocysteine produced can either be remethylated to methionine or condensed with serine to form cystathionine. There are two routes to methionine production. The major one is methylation by N^5-methyl FH_4, requiring vitamin B12. The liver also contains a second pathway in which betaine (a degradation product of choline) can donate a methyl group to homocysteine to form methionine, but this is a minor pathway. The conversion of homocysteine to cystathionine requires pyridoxal phosphate. Thus, if an individual is deficient in vitamin B12, the conversion of homocysteine to methionine by the major route is inhibited. This will direct homocysteine to produce cystathionine, which eventually produces cysteine. As cysteine levels accumulate, the enzyme that makes cystathinonine undergoes feedback inhibition, and that pathway is also inhibited (see Fig. 40.10). This, overall, leads to an accumulation of homocysteine, which is released into the blood.

Homocysteine also accumulates in the blood if a mutation is present in the enzyme that converts N^5, N^{10} methylene FH_4 to N^5-methyl FH_4. When this occurs, the levels of N^5-methyl FH_4 are too low to allow homocysteine to be converted to methionine. The loss of this pathway, coupled with the feedback inhibition by cysteine on cystathionine formation, will also lead to elevated homocysteine levels in the blood.

A third way in which serum homocysteine levels can be elevated is by a mutated cystathinone-β-synthase or a deficiency in vitamin B6, the required cofactor for that enzyme. These defects block the ability of homocysteine to be converted to cystathionine, and the homocysteine that does accumulate cannot all be accommodated by conversion to methionine. Thus, an accumulation of homocysteine results.

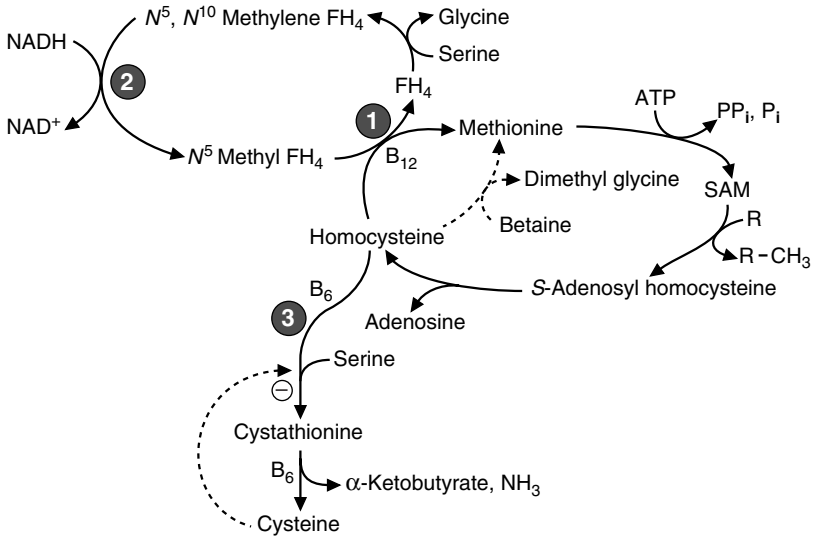


Fig. 40.10. Reaction pathways involving homocysteine. Defects in numbered enzymes (1 = methionine synthase, 2 = N^5, N^{10} methylene FH_4 reductase, 3 = cystathionine-β-synthase) lead to elevated homocysteine. Recall that as cysteine accumulates, there is feedback inhibition on cystathionine-β-synthase to stop further cysteine production.

C. Neural Tube Defects

Folate deficiency during pregnancy has been associated with an increased risk for neural tube defects in the developing fetus. This risk is significantly reduced if women take folic acid supplements periconceptually. The link between folate deficiency and neural tube defects was first observed in women with hyperhomocysteinemia brought about by a thermolabile variant of N^5, N^{10} methylene tetrahydrofolate reductase. This form of the enzyme, which results from a single nucleotide change (C to T) in position 677 of the gene encoding the protein, is less active at body temperature than at lower temperatures. This results in a reduced level of N^5-methyl tetrahydrofolate being generated and, therefore, an increase in the levels of homocysteine. Along with the elevated homocysteine, the women were also folate deficient. The folate deficiency and the subsequent inhibition of DNA synthesis leads to neural tube defects. The elevated homocysteine is one indication that such a deficit is present. These findings have led to the recommendation that women considering getting pregnant begin taking folate supplements before conception occurs, and for at least 1 month after conception. The Department of Agriculture has, in fact, mandated that folate be added to flour-containing products in the United States.

CLINICAL COMMENTS

Jean Ann Tonich developed a folate deficiency and is on the verge of developing a cobalamin (vitamin B12) deficiency as a consequence of prolonged moderately severe malnutrition related to chronic alcoholism. Before folate therapy is started, the physician must ascertain that the megaloblastic anemia is not caused by a pure B12 deficiency or a combined deficiency of folate and B12.

If folate is given without cobalamin to a B12-deficient patient, the drug only partially corrects the megaloblastic anemia because it will "bypass" the methyl-folate trap and provide adequate FH_4 coenzyme for the conversion of dUMP to dTMP and for a resurgence of purine synthesis. As a result, normal DNA synthesis, DNA repair, and cell division occur. However, the neurologic syndrome, resulting from hypomethylation in nervous tissue, may progress unless the physician realizes that B12 supplementation is required. In Jean Ann's case, in which the serum B12 concentration was borderline low and in which the dietary history supported the possibility of a B12 deficiency, a combination of folate and B12 supplements is required to avoid this potential therapeutic trap.

Colin Tuma continued to do well and faithfully returned for his regular colonoscopic examinations.

Bea Twelvlow was diagnosed with an inability to absorb dietary B12 but not crystalline B12 (the Schilling test results were normal). One of the consequences of aging is a reduced acid production by the gastric mucosa (atrophic gastritis), which limits the ability of pepsin to work on dietary protein. A reduced pepsin efficiency would then reduce the amount of bound B12 released from dietary protein, as a result of which the B12 would be unavailable for absorption. Because Bea absorbs crystalline B12 without a problem, her condition can be easily treated by taking vitamin B12 supplements orally.

BIOCHEMICAL COMMENTS

Folate Deficiencies and DNA Synthesis. Folate deficiencies result in decreased availability of the deoxythymidine and purine nucleotides that serve as precursors for DNA synthesis. The decreased concentrations of these precursors affect not only the DNA synthesis that occurs during replication

before cell division, but also the DNA synthesis that occurs as a step in the processes that repair damaged DNA.

Decreased methylation of deoxyuridine monophosphate (dUMP) to form deoxythymidine monophosphate (dTMP), a reaction that requires N^5, N^{10}-methylene tetrahydrofolate as a coenzyme (see Fig. 40.5), leads to an increase in the intracellular dUTP/dTTP ratio. This change causes a significant increase in the incorporation of uracil into DNA. Although much of this uracil can be removed by DNA repair enzymes, the lack of available dTTP blocks the step of DNA repair catalyzed by DNA polymerase. The result is fragmentation of DNA as well as blockade of normal DNA replication.

These abnormal nuclear processes are responsible for the clumping and polysegmentation seen in the nuclei of neutrophilic leukocytes in the bone marrow and in the peripheral blood of patients with a megaloblastic anemia caused either by a primary folate deficiency or one that is secondary to a B12 deficiency. The abnormalities in DNA synthesis and repair lead to an irreversible loss of the capacity for cell division and eventually to cell death.

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REVIEW QUESTIONS—CHAPTER 40

1. Which of the following reactions requires N^5, N^{10} -methylene FH_4 as a carbon donor?

   (A) Homocysteine to methionine
   (B) Serine to glycine
   (C) Betaine to dimethylglycine
   (D) dUMP to dTMP
   (E) The de novo biosynthesis of the purine ring

2. Propionic acid accumulation from amino acid degradation would result from a deficiency of which of the following vitamins?

   (A) Vitamin B6
   (B) Biotin
   (C) Folic acid
   (D) Vitamin B12
   (E) Vitamin B1
   (F) Vitamin B2

3. A dietary vitamin B12 deficiency can result from which of the following?

   (A) Excessive intrinsic factor production by the gastric parietal cells
   (B) Eating a diet high in animal protein
   (C) Pancreatic insufficiency
   (D) Increased absorption of folic acid
   (E) Inability to conjugate the vitamin with glutamic acid

4. Which of the following forms of tetrahydrofolate is required for the synthesis of methionine from homocysteine?

   (A) N^5, N^{10} -methylene tetrahydrofolate
   (B) N^5 -methyl tetrahydrofolate
   (C) N^5, N^{10} -methenyl tetrahydrofolate
   (D) N^{10} -formyl tetrahydrofolate
   (E) N^5 -formimino tetrahydrofolate

5. An alternative method to methylate homocysteine to form methionine is which of the following?

   (A) Using glycine and FH_4 as the methyl donor
   (B) Using dimethylglycine as the methyl donor
   (C) Using choline as the methyl donor
   (D) Using sarcosine as the methyl donor
   (E) Using betaine as the methyl donor