FOLATE METABOLISM

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OUTLINE:

I. Overview of folate coenzymes

II. Daily requirement and sources of folate

III. Folate metabolic pathway
- A. Conversion of folic acid to tetrahydrofolate
- B. Tetrahydrofolate derivatives
- C. Interconversion of folate derivatives

IV. Functional roles of folate coenzymes
- A. Folate coenzymes in amino acid metabolism
- B. Purine nucleotide biosynthesis
- C. Thymidylate biosynthesis

V. Health benefits of folate

VI. Disorders associated with defects in folate metabolism
- A. Methionine synthase deficiency
- B. Methylene tetrahydrofolate reductase deficiency

VII. Folate antimetabolites in cancer treatment

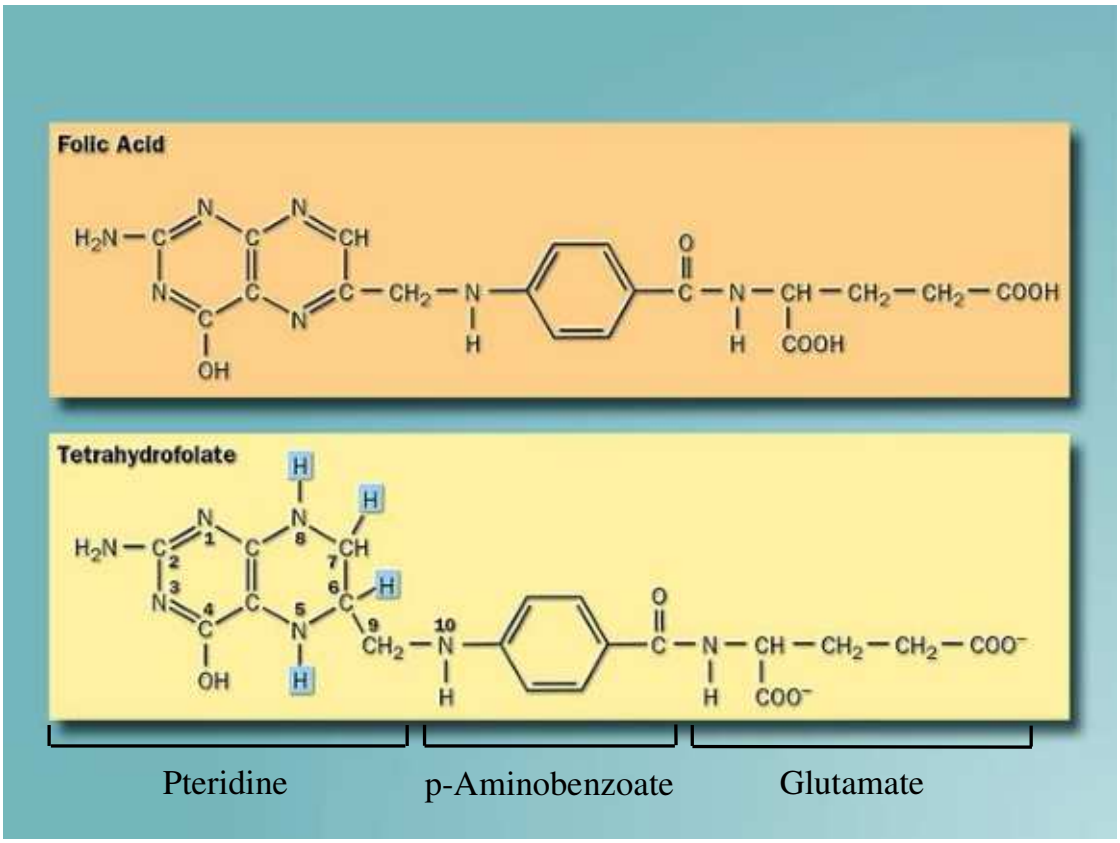
VIII. Summary

OBJECTIVES: After studying this unit you should be able to:

1. Specify the structural components of folic acid.
2. Differentiate between folic acid, dihydrofolate, and tetrahydrofolate.
3. State 5 tetrahydrofolate derivatives.
4. Identify sources that donate one-carbon groups into the folate pool.
5. Explain the importance of folate coenzyme in the methionine cycle.
6. Describe the involvement of folate coenzymes in purine and thymidylate biosynthesis.
7. Explain why it is important for pregnant women to have an adequate folate intake.
8. State some clinical manifestations associated with folate deficiency.
9. List two defects involving folate metabolic enzymes and specify their clinical outcomes.
10. Explain why methionine synthase deficiency would cause megaloblastic anemia.
11. Describe the mechanisms by which antifolate drugs inhibit cancer cell growth.

I. Overview of folate coenzymes

Folic acid and folate coenzymes

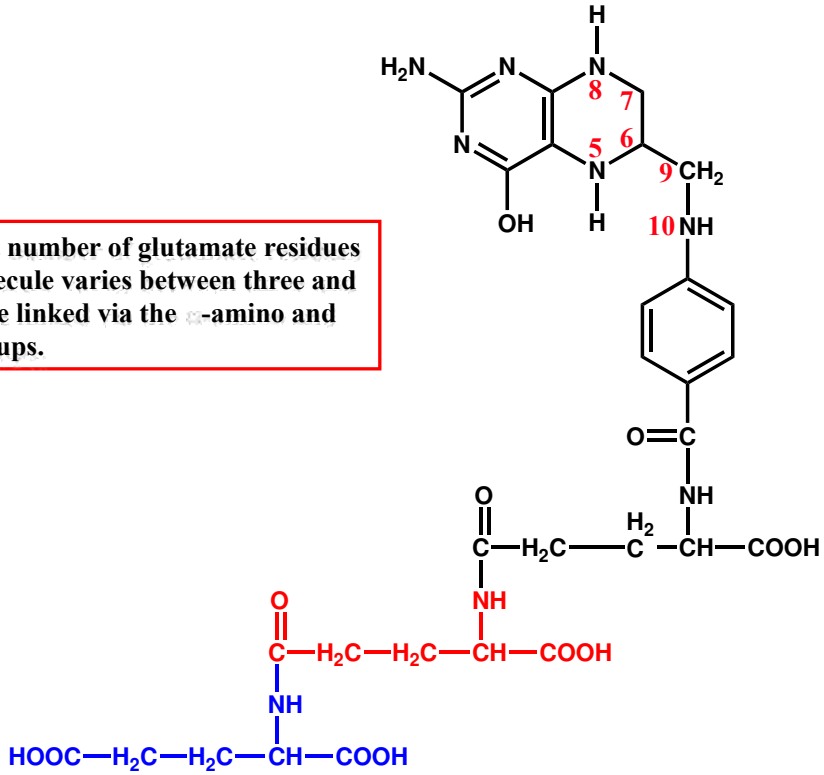


Folic acid is a water-soluble B vitamin (B_9). All forms of life require folate coenzymes to perform single-carbon transfer. Folic acid and folate represent the oxidized and reduced forms of the vitamin respectively. Folic acid or folate is made up of a pteridine ring, para-aminobenzoic acid (PABA), and glutamate. In our body, we can synthesize these components. However, we are unable to conjugate the pteridine ring to PABA. As a result, we must obtain this important nutrient from the diet or from multivitamin supplements.

The reactive form of folate is tetrahydrofolate, which is often abbreviated as THF or FH_4. The reactive groups of THF are located at the N5 and N10 positions, which can be linked to different one-carbon units. The reduced form, tetrahydrofolate, has four additional hydrogen atoms in the pteridine ring compared to folic acid. Dihydrofolate, on the other hand, has two additional hydrogen atoms. It must be reduced further in order to form the active tetrahydrofolate.

Intracellular folates are normally linked to multiple glutamate residues

In the cell, the number of glutamate residues in a folate molecule varies between three and eight. They are linked via the α-amino and γ-carboxyl groups.



Folate derivatives usually contain more than one glutamate residue. They vary between three to eight glutamate residues. These residues are linked together via the α-amino and γ-carboxyl groups, rather than the usual peptide linkage of between α-amino and α-carboxyl groups. Folate monoglutamate can be transported through membranes into the cell. Once inside, additional glutamate residues will be added. The resulting folate polyglutamate derivatives are therefore trapped inside the cell. Natural folates from foods are normally polyglutaminated and they must be converted to the monoglutamyl form before they can be absorbed in the intestine. Folic acid found in multi-vitamin supplements, in its monoglutamyl form, can be easily absorbed.

II. Daily requirement and sources of folate

The recommended dietary allowances (RDA) of folate from the food and supplements are shown in the next page. For pregnant and breastfeeding women, they need more daily folate intake. Good food sources of folate include green vegetables, legumes, lentils, fruits, liver, and fortified foods.

Recommended dietary allowance of folic acid

1-3 years	150 g
4-8 years	200 g
9-13 years	300 g
14 years and up	400 g
Pregnant women	up to 800 g
Breastfeeding women	500 g

WebMD.com

Foods containing folate

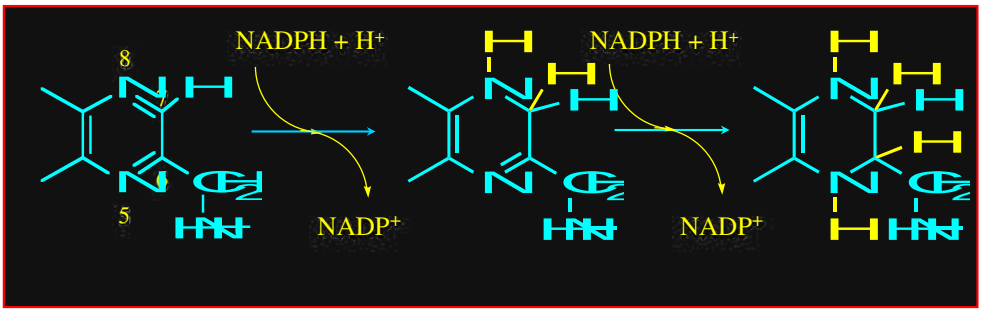
Food sources of folate	Amount of folate
100 ml breast milk	8-14 micrograms
1⁄2 cup raw chopped broccoli	31 micrograms
1⁄2 cup boiled broccoli	39 micrograms
1 medium orange	40 micrograms
1⁄2 cup raw chopped spinach	54 micrograms
1⁄4 cup wheat germ	80 micrograms
1 cup orange juice	110 micrograms
1⁄2 cup boiled kidney beans	115 micrograms
1⁄2 cup cooked dark green leafy vegetables such as spinach	130 micrograms
1⁄2 cup boiled black beans	130 micrograms
1⁄2 cup boiled chickpeas	140 micrograms
1⁄2 cup cooked lentils or legumes	180 micrograms
3 1⁄2 ounces cooked chicken liver	770 micrograms
1 cup fortified breakfast cereals	100-400 micrograms

http://ods.od.nih.gov/factsheets/folate.asp

III. Folate metabolic pathway

A. Conversion of folic acid to tetrahydrofolate

Dihydrofolate reductase converts folic acid to tetrahydrofolate



Folic acid Dihydrofolate Tetrahydrofolate (THF)

Once folic acid is taken into a cell as a monoglutamate form, it is reduced to tetrahydrofolate (glutamyl residues are also added). This requires two sequential steps involving the enzyme **dihydrofolate reductase (DHFR)**. Both reactions require NADPH.

B. Tetrahydrofolate derivatives

One-carbon groups carried by tetrahydrofolate

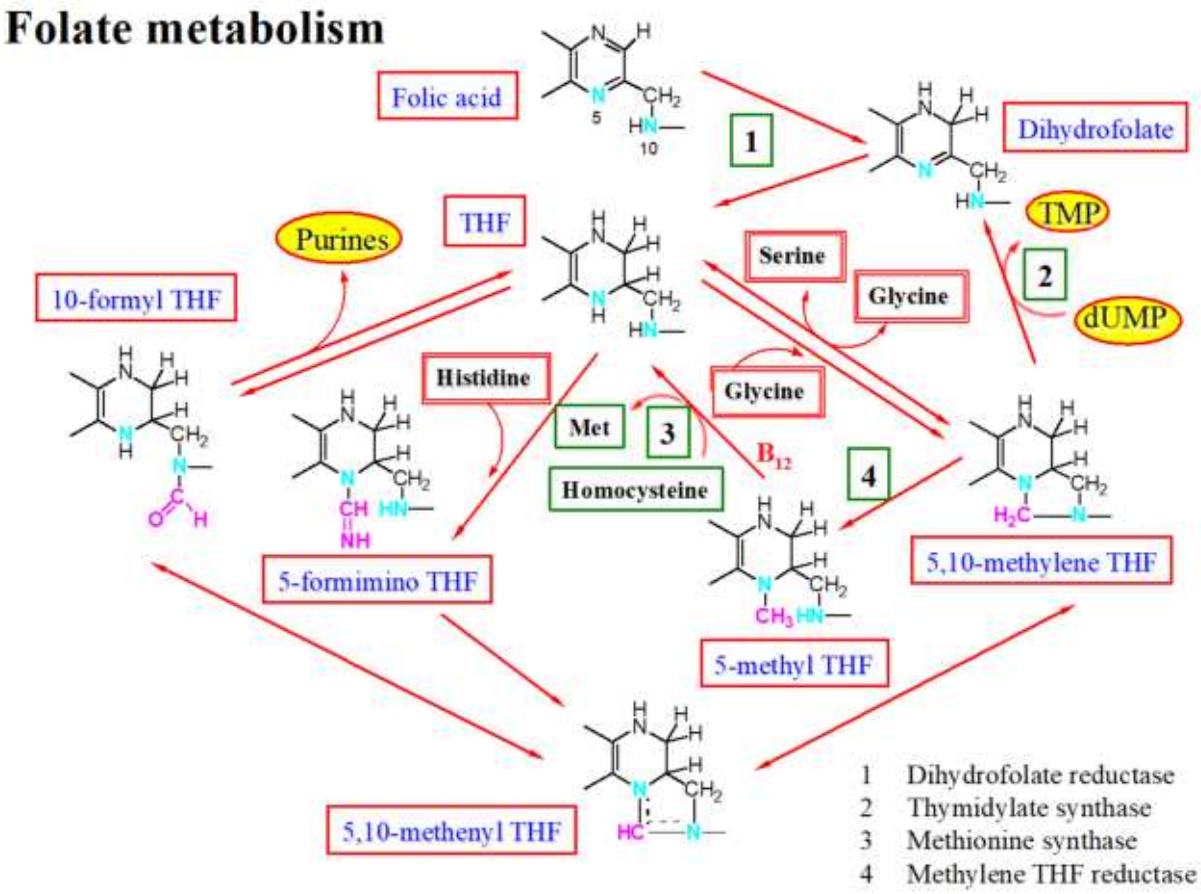
Oxidation state	Group	
Most reduced (= methanol)	$-CH_3$	Methyl
Intermediate (= formaldehyde)	$-CH_2-$	Methylene
Most oxidized (= formic acid)	–CHO	Formyl
	–CHNH	Formimino
	–CH=	Methenyl

5-Methyl THF *
5,10-Methylene THF *
10-Formyl THF *
5-Formimino THF
5,10-Methenyl THF

* Participates in metabolic pathways

Tetrahydrofolate can carry one-carbon groups, including methyl, methylene, formyl, formimino, and methenyl. These groups are linked to the N-5 or N-10 nitrogen atom of THF, or to both. Only 5-methyl, 5,10-methylene, and 10-formyl groups are known to participate in various metabolic pathways.

C. Interconversion of folate derivatives

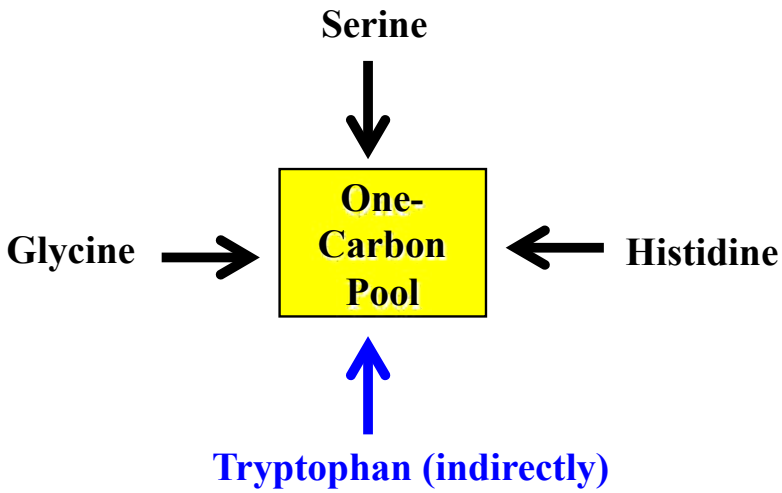


Folate metabolic pathway is rather complicated. The one-carbon units carried by THF are interconvertible. 5,10-Methylene THF can be reduced to 5-methyl THF or oxidized to 5,10-methenyl THF. Deamination of 5-formimino THF will yield 5,10-methenyl THF and hydration of 5,10-methenyl THF will produce 10-formyl THF. The key enzymes relevant to this lecture are dihydrofolate reductase, thymidylate synthase, methionine synthase, and methylene THF reductase.

IV. Functional roles of folate coenzymes

A. Folate coenzymes in amino acid metabolism

Amino acids contribute one-carbon units into the folate pool



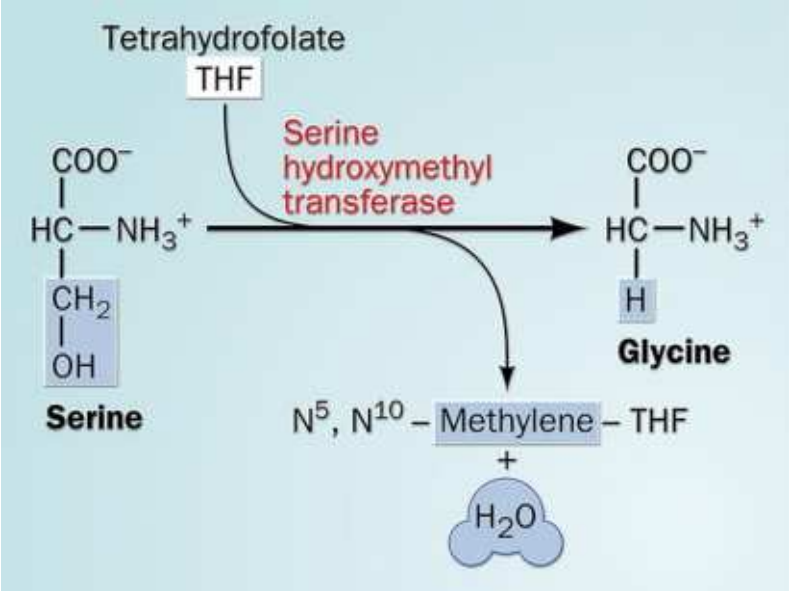
Serine and glycine - Methylene group
Histidine - Formimino group
Tryptophan - Formyl group (indirectly)

Amino acid degradation represents the major source of one-carbon units entering the folate pool. Serine and glycine degradation can lead to the production of 5,10-methylene THF, while degradation of histidine will yield 5-formimino THF. The formyl group produced from the degradation of tryptophan can be linked to THF to form 10-formyl THF.

Folate coenzymes are necessary for:

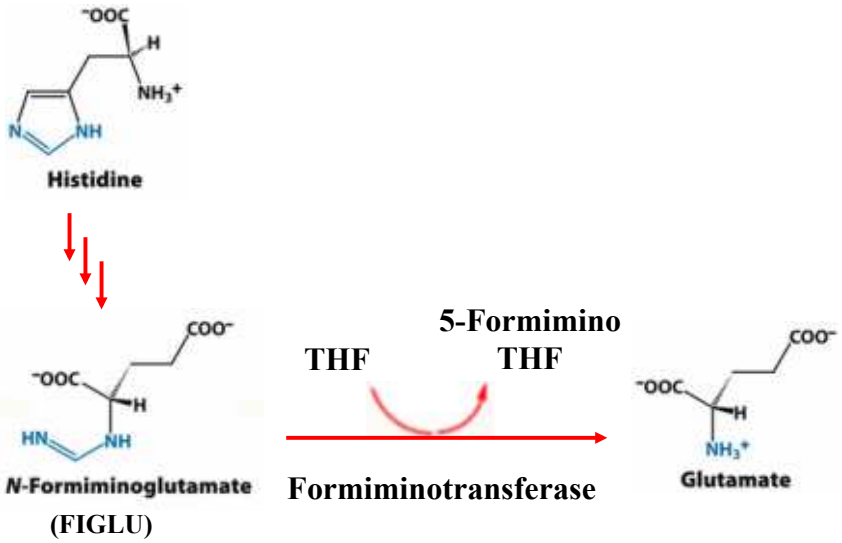
- Serine, glycine, and histidine metabolism
- Methionine regeneration
- Purine nucleotide biosynthesis
- Thymidylate synthesis

Conversion of serine to glycine forms 5,10-methylene THF



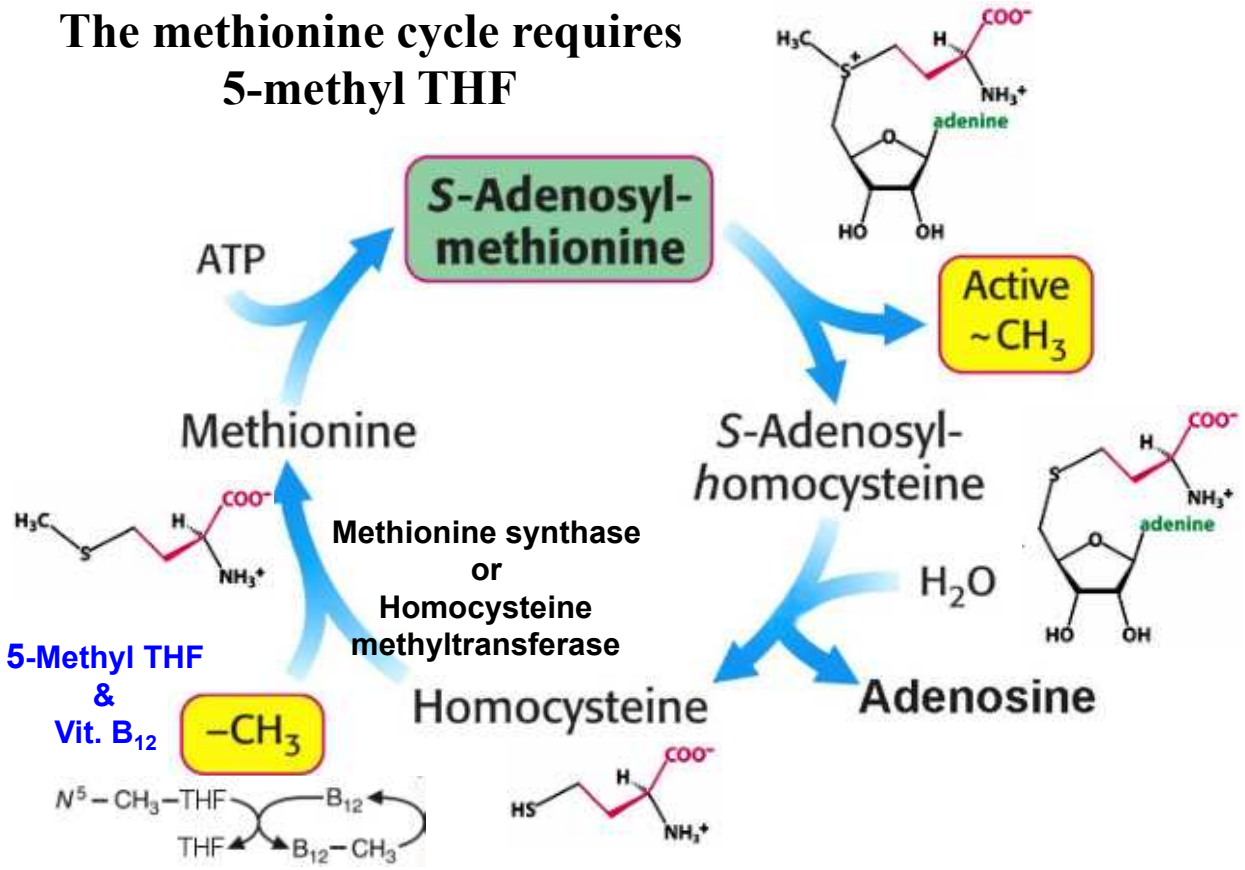
Serine hydroxymethyltransferase catalyzes a 1-step reaction that converts serine to glycine. This reaction involves the transfer of the hydroxymethyl group from serine to the cofactor tetrahydrofolate (THF), producing glycine, 5,10-methylene THF, and water. Glycine produced from serine, or obtained from the diet, can also be oxidized by the glycine cleavage complex (GCC). This will yield a second equivalent of 5,10-methylene-tetrahydrofolate, as well as ammonia and CO_2 (not shown). Glycine is involved in many anabolic reactions other than protein synthesis, including the synthesis of purine nucleotides, heme, glutathione, and creatine.

Histidine degradation yields 5-formimino THF



A histidine loading test is commonly used to determine if a person is deficient in folate.

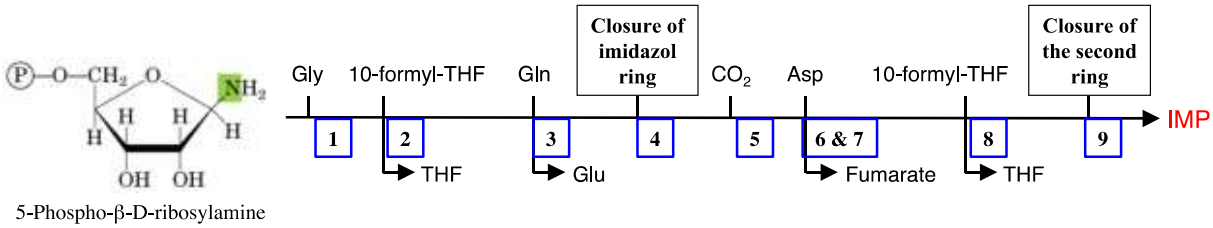
The final reaction in the breakdown of histidine involves the transfer of a formimino group from formiminoglutamate (FIGLU) to THF via formiminotransferase. In the process, 5-formimino-THF is formed. This reaction can be used as a basis to determine if a person has folate deficiency. In the diagnostic assay, the patients are given an oral dose of histidine. The lack of folate will be indicated by the accumulation of FIGLU in the blood and a higher than normal amount of this compound in the urine. 5-Formimino THF can go on to form other folate derivatives such as 5,10-methylene THF, 5-methyl THF, and 10-formyl THF.



Regeneration of methionine can be performed by the transfer of a methyl group from 5-methyl THF to homocysteine. This reaction is catalyzed by methionine synthase and requires cobalamin (vitamin B_{12}). 5-Methyl THF first transfers its methyl group to cobalamin to form methyl cobalamin. In the process, THF is regenerated and it can go on to become other THF derivatives. Methyl cobalamin, on the other hand, then transfers its newly acquired methyl group to homocysteine to form methionine. The methionine cycle is essential for the synthesis of S-adenosyl methione (SAM), an important methyl donor in over a hundred biological reactions.

B. Purine nucleotide biosynthesis

De novo purine biosynthesis requires 10-formyl THF



5-Phospho-β-D-ribosylamine

Table 25.2 The enzymes of de novo purine synthesis

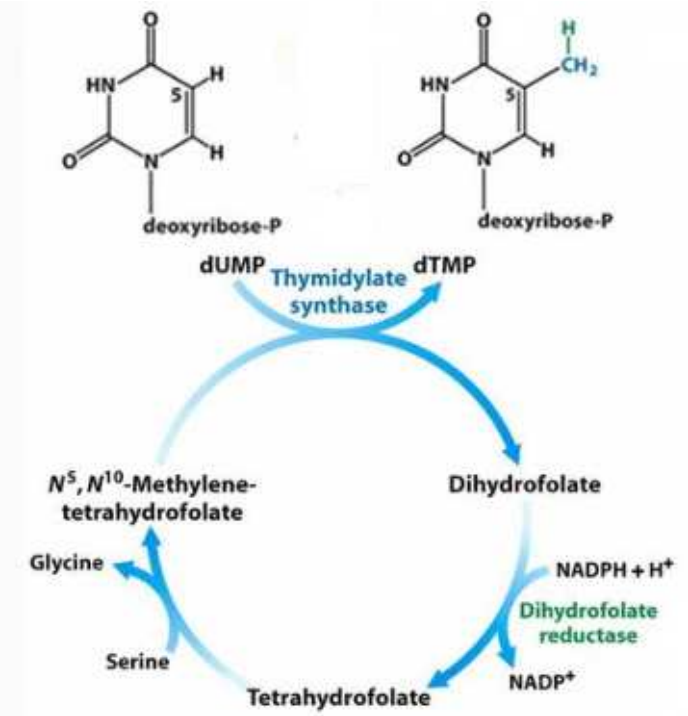
Step	Enzyme
1	Glycinamide ribonucleotide (GAR) synthetase
2	GAR transformylase
3	Formylglycinamidine synthase
4	Aminoimidazole ribonucleotide synthetase
5	Carboxyaminoimidazole ribonucleotide synthase
6	Succinylaminoimidazole carboxamide ribonucleotide synthetase
7	Adenylosuccinate lyase
8	Aminoimidazole carboxamide ribonucleotide transformylase
9	Inosine monophosphate cyclohydrolyase

The synthetic pathway of purine nucleotides is rather complicated. The nucleotide base is built on 5-phosphoribosylamine. Two of the steps in this pathway require 10-formyl-THF. Thus, there is potential of using antifolate drugs that specifically target the two folate-dependent enzymes in this pathway to inhibit the proliferation of unwanted cells. IMP or inosine monophosphate is the first purine nucleotide made in the pathway. AMP and GMP can subsequently be synthesized from IMP.

C. Thymidylate biosynthesis

Uridylate (UMP), produced by the pyrimidine nucleotide biosynthetic pathway, is not incorporated into DNA. DNA contains thymine deoxyribonucleotide, a methylated analog of uracil deoxyribonucleotide. dUMP is the immediate precursor of thymidylate (TMP). Conversion of dUMP to TMP is catalyzed by thymidylate synthase. A one-carbon unit is transferred from 5,10-methylene THF to dUMP to form thymidylate. In the process, dihydrofolate is generated. Dihydrofolate is reduced to tetrahydrofolate by dihydrofolate reductase. This reaction is essential for many processes that depend on the availability of tetrahydrofolate.

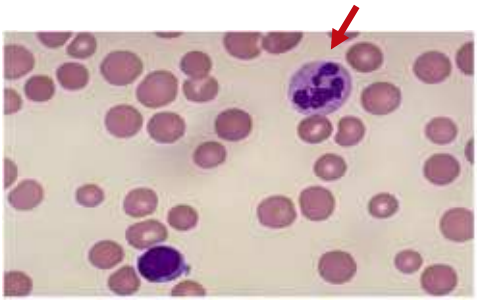
Folate-dependent thymidylate synthesis



V. Health benefits of folate

Health benefits of folate

- Adequate intake of folate during the periconception period helps to protect against pregnancy complications, cleft lip, and congenital malformations including neural tube (spina bifida), heart, limb, and urinary tract defects.
- Necessary for fertility
- Reduces the risk of stroke, colon cancer, and age-related macula degeneration
- Folate deficiency is associated with megaloblastic anemia, weakness, and behavioral disorders.



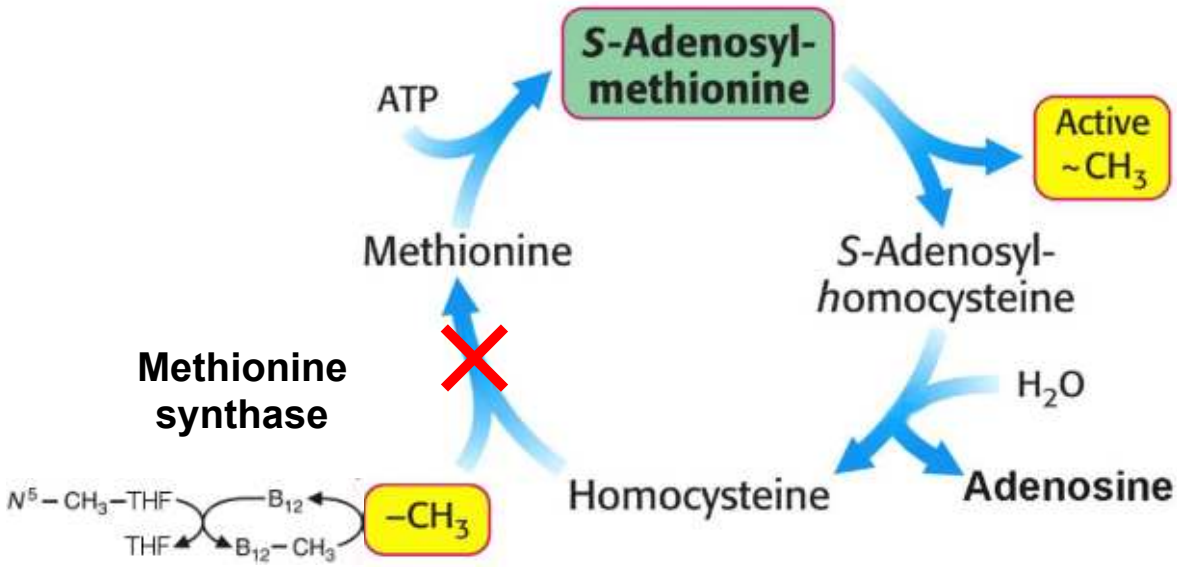
Folate is known to be vital for the prevention of neural tube defects (NTDs). Spina bifida is an example of NTDs. All women of reproductive age are advised to take multivitamin supplements containing 0.4 mg folic acid daily, because the nervous system becomes evident on day 18 and neural tube closure occurs during days 22-28, long before most women are aware of pregnancy. The recommended dietary allowance (RDA) for folic acid in normal adults is 0.4 mg. For pregnant women, it is up to 0.8 mg. Folate is also important for the prevention of other congenital malformations.

Folate is important in spermatogenesis and thus it is necessary for fertility. Because folate reduces homocyteine levels in the body by promoting methionine synthesis, it is believed to play a role in reducing the risk of stroke. Adequate intake of folate has also been shown to associate with decreased risk of developing colorectal cancer and age-related macular degeneration. Folate deficiency in adults leads to loss of appetite, weakness, heart palpitations, and behavioral disorders. Megaloblastic anemia represents a sign of advanced folate deficiency.

VI. Disorders associated with defects in folate metabolism

A. Methionine synthase deficiency

Methionine synthase deficiency and folate trap

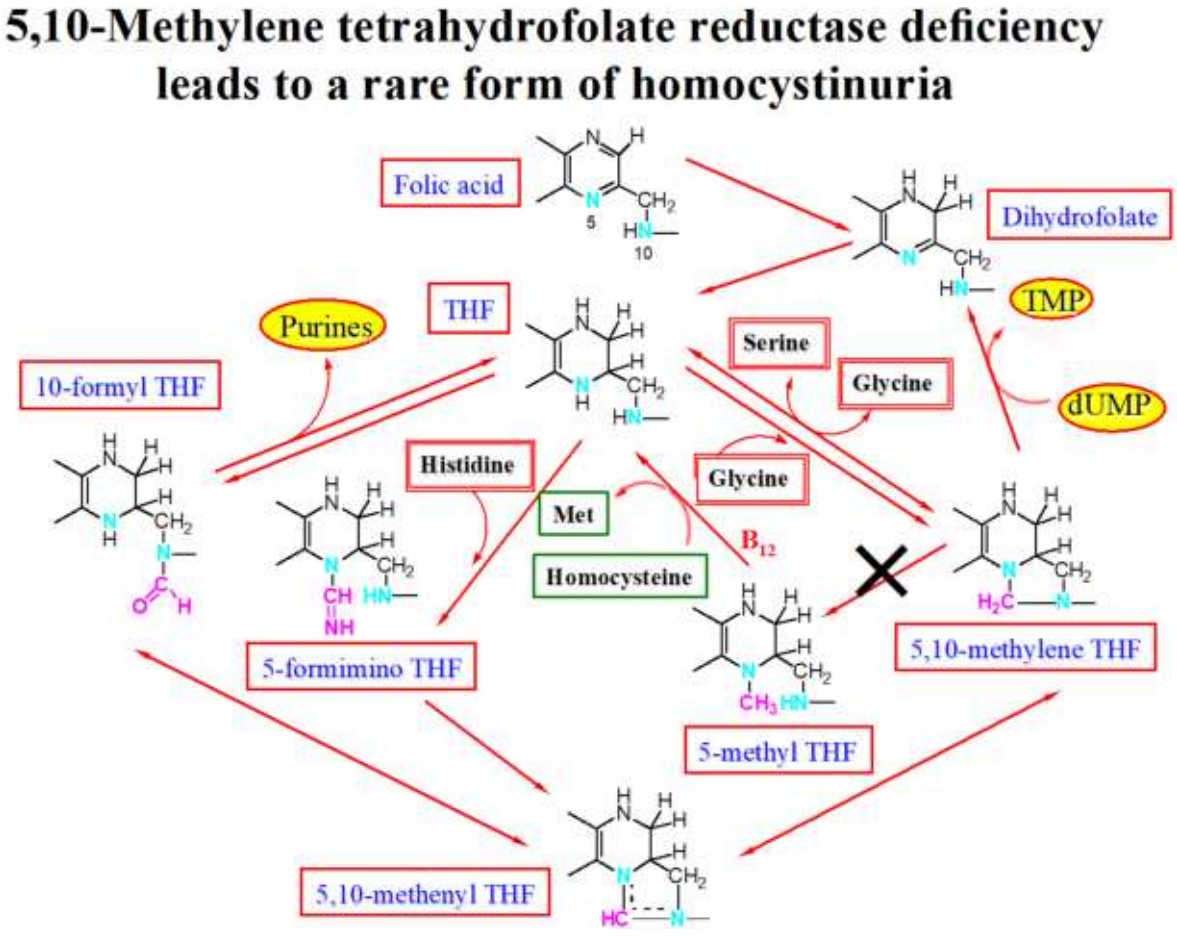


- Methionine synthase deficiency leads to the accumulation of 5-methyl THF and depletion of other folate coenzymes.
- Individuals with methionine synthase deficiency would develop both homocystinuria and megaloblastic anemia.

If there is a deficiency in methionine synthase, 5-methyl THF cannot be effectively used to synthesize methionine from homocysteine. This leads to homocystinuria. 5-Methyl THF then accumulates, because it cannot be converted to other derivatives of THF. As a result, the

levels of other derivatives of THF will go down. This is known as the folate trap hypothesis. The lack of other derivatives of THF, such as 5,10-methylene THF and 10-formyl THF, will lead to impairment of DNA synthesis, especially in tissues that have high cell-division rates, such as bone marrow and intestinal mucosal cells. This would give rise to megaloblastic anemia.

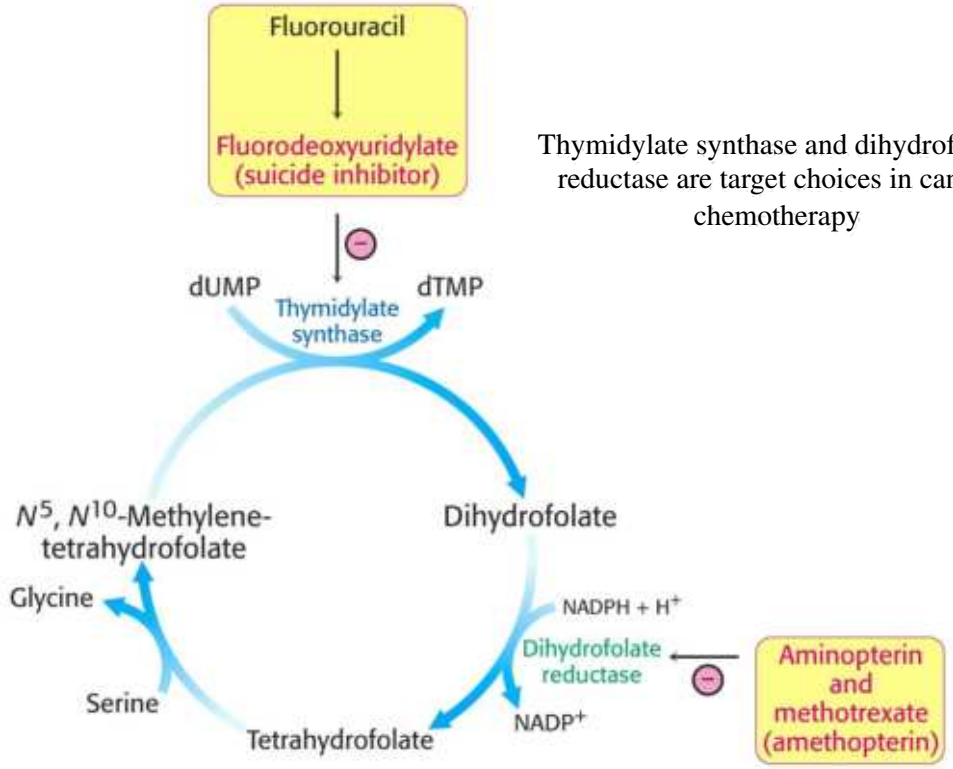
B. Methylene tetrahydrofolate reductase deficiency



Methylene tetrahydrofolate reductase is required for reducing 5,10-methylene THF to 5-methyl THF. A defect in methylene tetrahydrofolate reductase then compromises the production of 5-methyl THF. Since 5-methyl THF serves as a coenzyme for methionine synthase, its lacking would cause a rare form of homocystinuria.

VII. Folate antimetabolites in cancer treatment

Chemotherapeutic targets in folate metabolism



Thymidylate synthase and dihydrofolate reductase are target choices in cancer chemotherapy

An antimetabolite is a compound, which is usually a structural analog of a substrate, and it interferes with the utilization of the normal substrate. Two classic analogs of folate are aminopterin and methotrexate (MTX). The structures of these compounds are similar to that of folic acid and thus they are folate antimetabolites (also called antifolates). These two compounds are powerful inhibitors of dihydrofolate reductase. Tetrahydrofolate is the active form of folate coenzymes. If its regeneration from dihydrofolate is inhibited, then nucleotide synthesis will be compromised. Thus, these drugs are useful in the treatment of certain types of leukemia and cancer. MTX was developed in 1948 and gained FDA approval as an oncology drug in 1953. It is still the mainstay for the treatment of many neoplastic disorders such as acute lymphoblastic leukemia. More recently, it has come into use for the treatment of some autoimmune diseases, including rheumatoid arthritis. Newer antifolate drugs include raltitrexed (Tomudex) and pemetrexed (Alimta).

Thymidylate synthase is another folate-related target for cancer therapy. DNA contains thymine deoxyribonucleotides, so thymidylate synthesis is critical to cell division. A specific inhibitor of thymidylate synthase is 5-fluorouracil (5-FU). *In vivo*, it is converted to fluorodeoxyuridylate (F-dUMP), which can form a stable covalent complex with 5,10-methylene THF and a sulfhydryl group within the active site pocket of thymidylate synthase. This blocks the enzyme from catalyzing additional reactions. This is an example of suicide inhibition, in which an enzyme converts a pseudosubstrate into an active inhibitor.

VIII. Summary

Summary

- Folate coenzymes participate in one-carbon metabolism.
- Tetrahydrofolate is the active form of folate coenzyme.
- Tetrahydrofolate can carry various one-carbon units to form 5-methyl, 5,10-methylene, 10-formyl, 5-formimino, and 5,10-methenyl THF derivatives.
- Folate coenzymes play important roles in amino acid and nucleotide metabolism.
- Adequate folate intake during pregnancy reduces the risks of pregnancy complications and neural tube defects.
- The lack of folate can lead to megaloblastic anemia.
- Methionine synthase and methylene tetrahydrofolate reductase defects can lead to homocystinuria.
- Antifolate drugs targeting dihydrofolate reductase and thymidylate synthase are used in cancer treatment.

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