UNIT OVERVIEW: HYPOMETHYLATION, FOLIC ACID METABOLISM AND VITAMIN B12

15. Folic acid and deficiency
16. Synthesis and metabolism of tetrahydrofolate (THF)
17. Biochemical actions and physiologic roles of THF (dNTPs)
18. Folic acid and hyperhomocysteinemia
19. Vitamin B_{12} metabolism and deficiency (pernicious anemia)

For further reference on folic acid, see Stryer (5th ed) pp.216-7.

Folic Acid (Folate, Folacin):

- Latin: folin – "leaf" Yellow-orange, slightly soluble in water

- Animals can't synthesize folic acid

- Dietary requirements:
  RDI: 400 ug/day (preg. 800 ug/day)
  (>1000 ug/day may cause toxicity)

-Folic acid is widely distributed in leafy green vegetables, mushrooms, asparagus or liver, kidney, steak, yeast but is destroyed by cooking and exposure to reducing agents.

Folate deficiency:

Folic acid is the most common form of vitamin deficiency. On a world-wide basis, deficiency of folic acid is believed to be the most common form of vitamin under-nutrition.

Particularly prevalent in underdeveloped countries in the tropics, where most of the population is believed to have at least marginal folic acid deficiency.

In the U.S. many indigents and elderly people suffer from deficiency of folic acid, manifested as anemia, weight loss, and weakness.

Pregnant women and infants are particularly vulnerable.

Folic acid deficiency is a major feature of tropical sprue, in which there is a general deficiency in absorption of many nutrients from the small intestine. Folate deficiency is also frequently involved in Megaloblastic anemia (B12).

Folate deficiency, effects:

- Neural tube defects in fetus (e.g. spinobifida)

- Premature atherosclerosis and thromboembolism - due to ↑ plasma homocysteine (increased plasma homocysteine found in 40% of all atherosclerosis patients)

- Suppression of DNA synthesis (GI, mucosa, b.m.)

- Malabsorption can lead to megaloblastic (macrocytic) anemia (immature erythrocytes).

- Depression, schizoid psychosis - 10-50% of psychiatric patients are folic acid deficient (due to treatment regimes).

- Increased risk of colorectal cancer (especially in drinkers) - due to ↓ DNA methylation

- Neurological - peripheral neuropathy, myelopathy, spinal cord syndromes, restless legs

Ref: Oncol. 24 (5 Suppl 18): S18-39 (1997)
Eur. J. Ped. 157 (Suppl 2): S60-6 (1998)

Folate deficiency, induced:

Low serum RBC folate has been shown to be associated with the following conditions:

Drug induced:

-Antiepileptic drugs

- Oral contraceptives, NSAIDS

- Smoking

- Alcohol

- Methotrexate (antifolate chemotherapy)

-Nitrous oxide anesthesia

- Methionine therapy

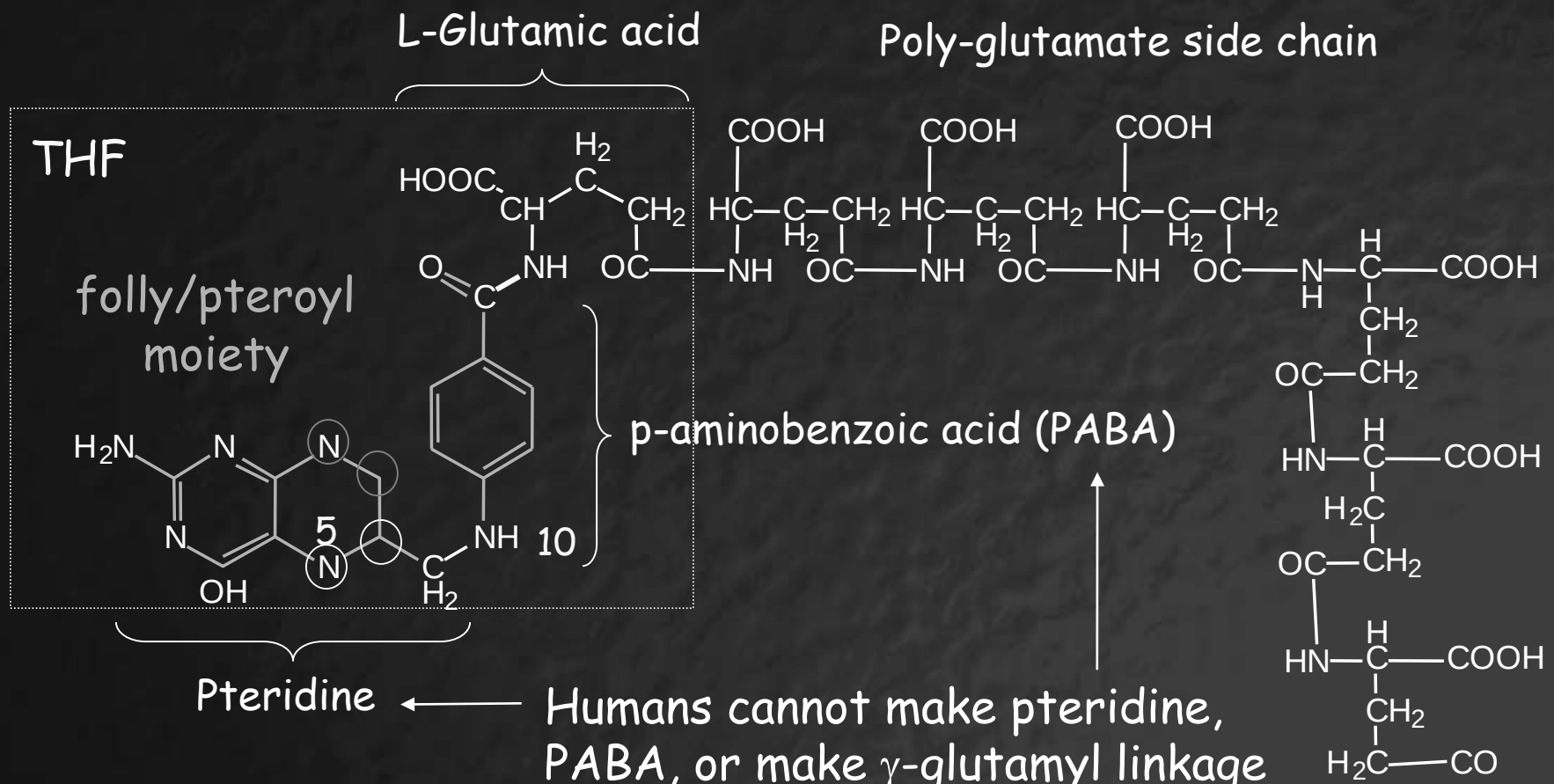
Genetic:

- Hyperhomocysteinemia patients

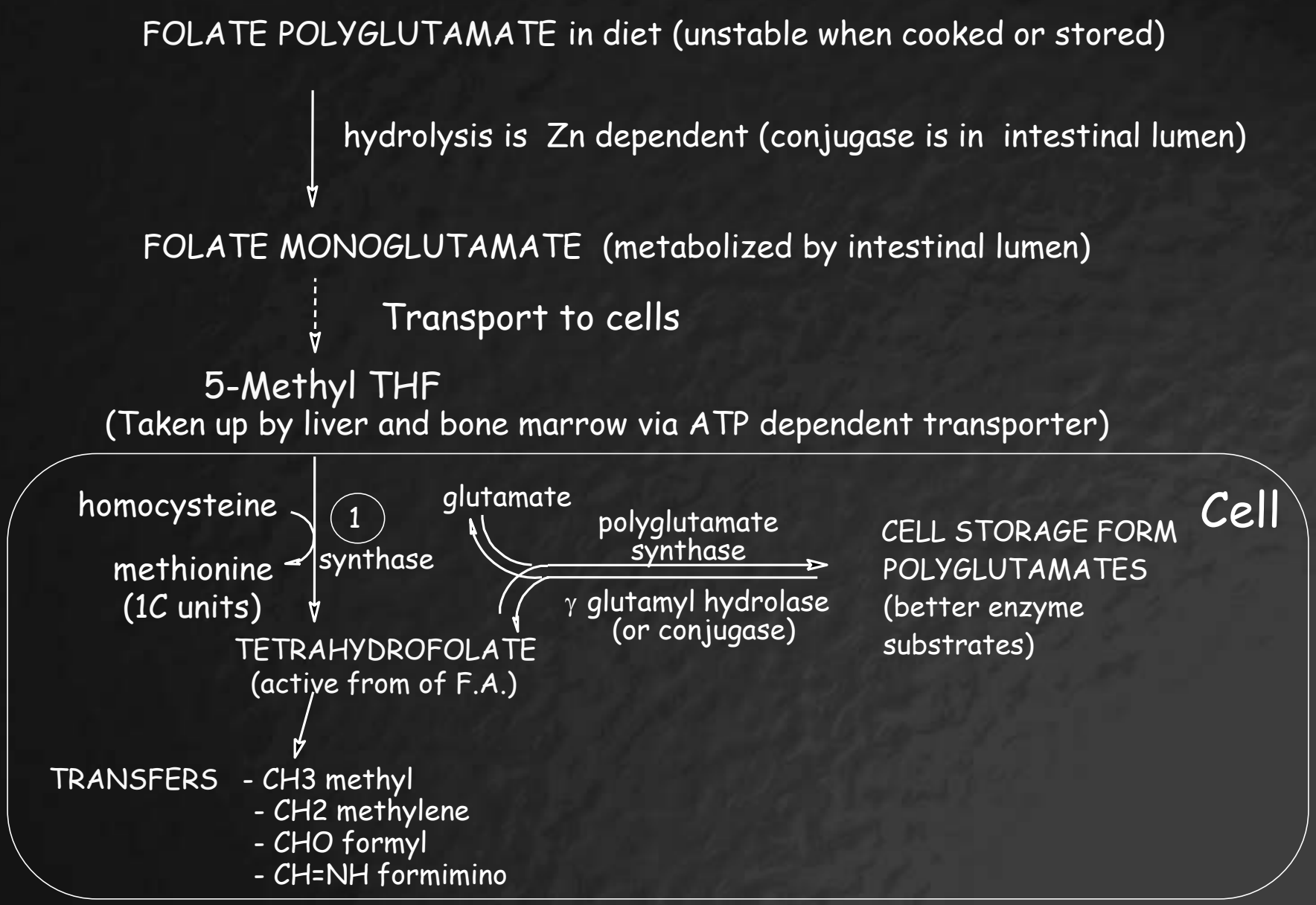
- Ulcerative cholitis (↑ Colon cancer)

Biological folate structures:

The structure of dietary folyl / pteroyl glutamic ascid is indicated below, illustrating the unique γ-glutamyl-linkage between PABA and glutamate residues.



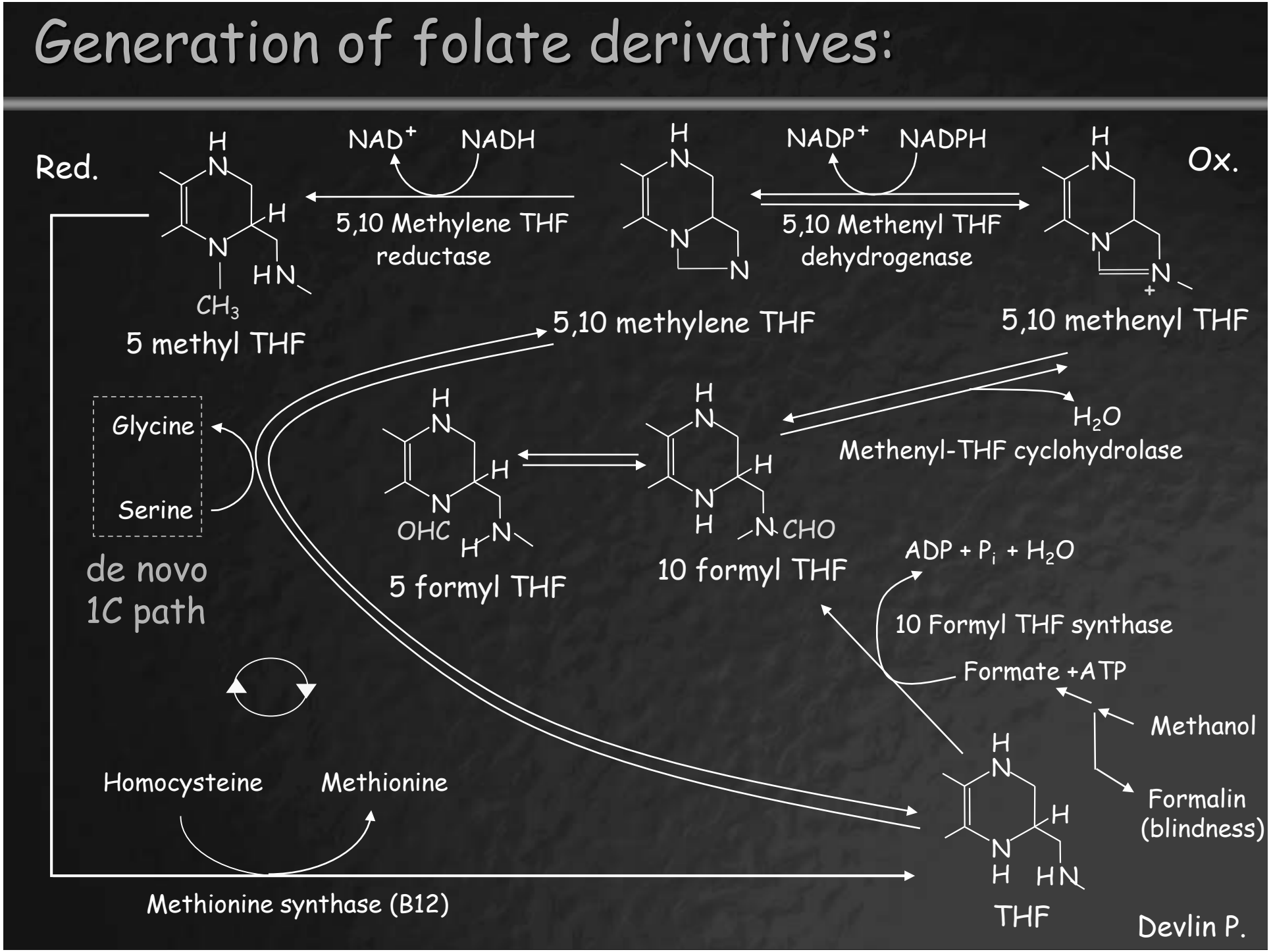
Tetrahydrafolate absorption:



Oxidation state of 1C groups carried by THF:

Oxidation Level:	Group Carried:	THF Derivative(s):
		THF (F4)
Formate	Formyl (-CH=O) (folinic acid, leucovorin)	N^5-Formyl-THF N^{10}-formyl-THF
	Methenyl (-CH=)	N^5,N^{10}-Methenyl-THF
	Formimino (-CH=NH)	N^5-Formimino-THF
Formaldehyde	Methylene (-CH_2-)	N^5,N^{10}-Methylene-THF
Methanol	Methyl (-CH_3)	N^5-Methyl-THF

Generation of folate derivatives:



Utilization of 1C units: Purine synthesis

Because of requirement for 1C fragments, conditions which enhance GMP/AMP synthesis also reduce the toxicity of methanol.

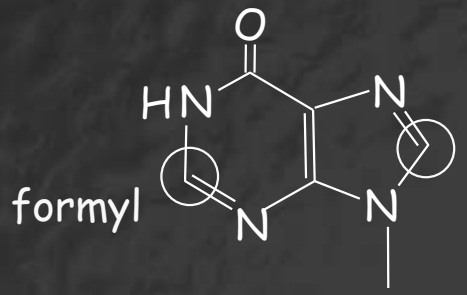
Ribose 5 phosphate

↓ Ribose phosphate pyrophosphokinase ⊣ IMP, AMP, GMP

5 phosphoribosyl-1-phosphate (PRPP)

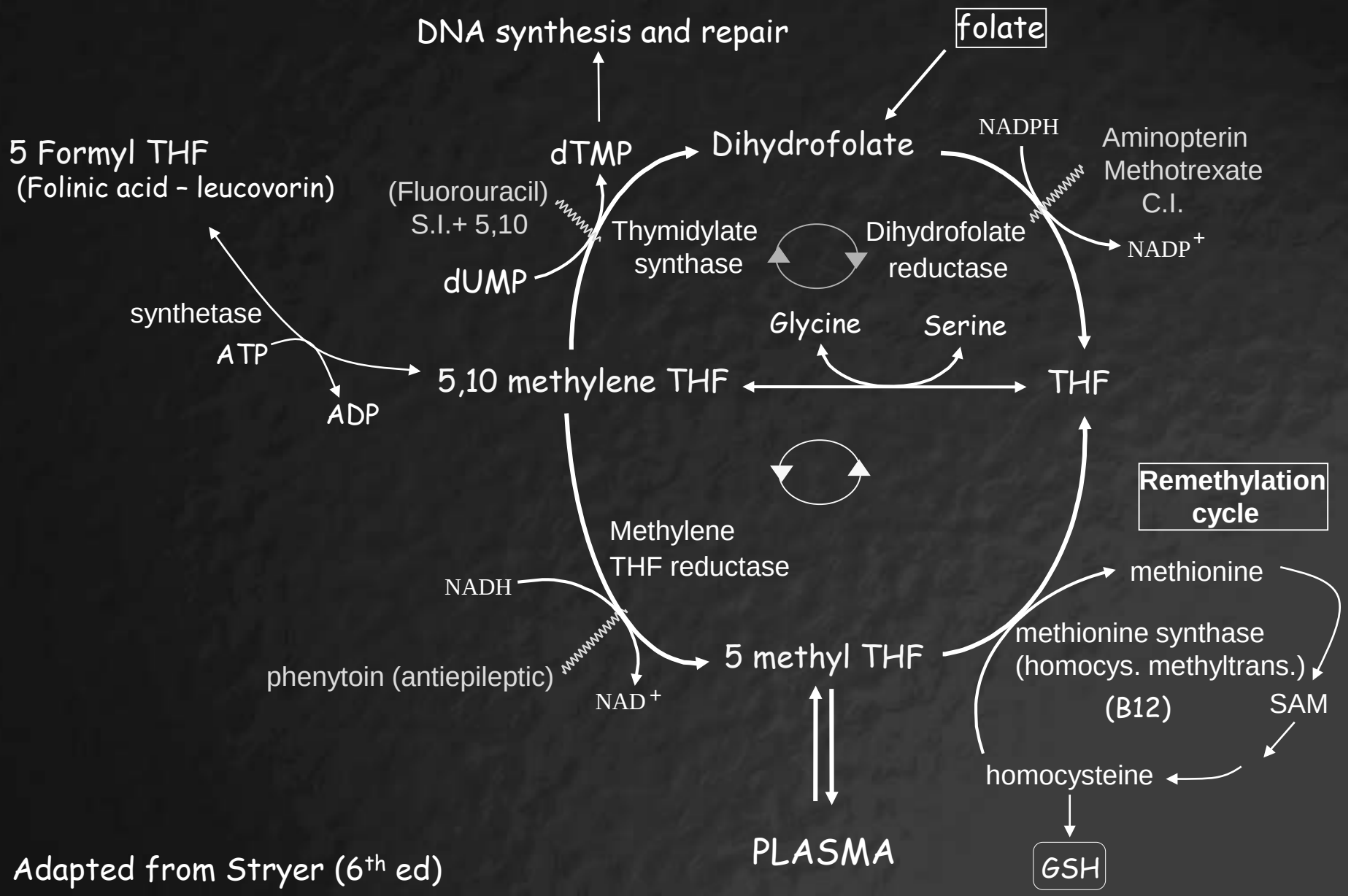
Glutamine → Glutamate (Amidophosphoribosyl transferase)

5 phosphoribosyl -1-amine (PRPP + NH2) → (9 steps) → (Inosine) → GMP, AMP



Ribose 5 phosphate

One carbon pathway / drug interactions:

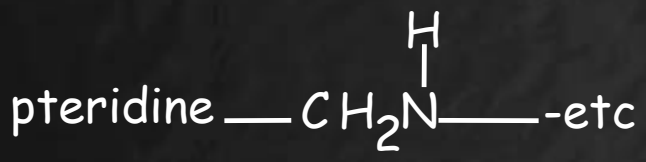


Inhibition of folate, chemotherapy:

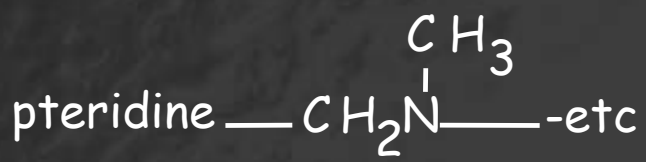
Methotrexate is a (10 methyl) THF analog which inhibits dihydrofolate reductase competitively. Systemic treatment with methotrexate has been used for leukemia, lymphoma, bladder and breast cancer.

Side effects of methotrexate therapy include myelosuppression and mucositis. These effects result from the fall in THF levels and can be ameliorated using folinic acid (leucovorin) if given promptly.

Methotrexate prevents one-carbon transfer reactions which are required for DNA, RNA and purine biosynthesis, in particular thymidylate synthase.



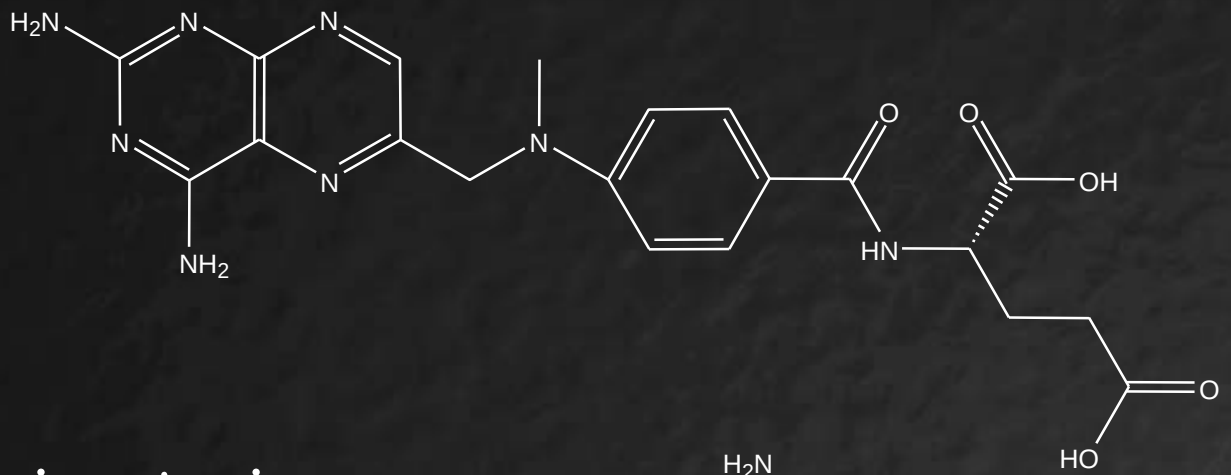
Folic Acid



Methotrexate

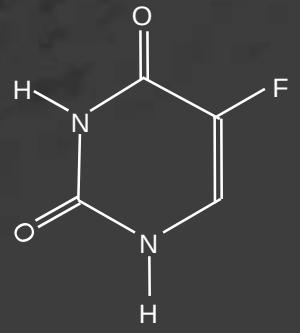
Antimetabolite therapy:

Methotrexate

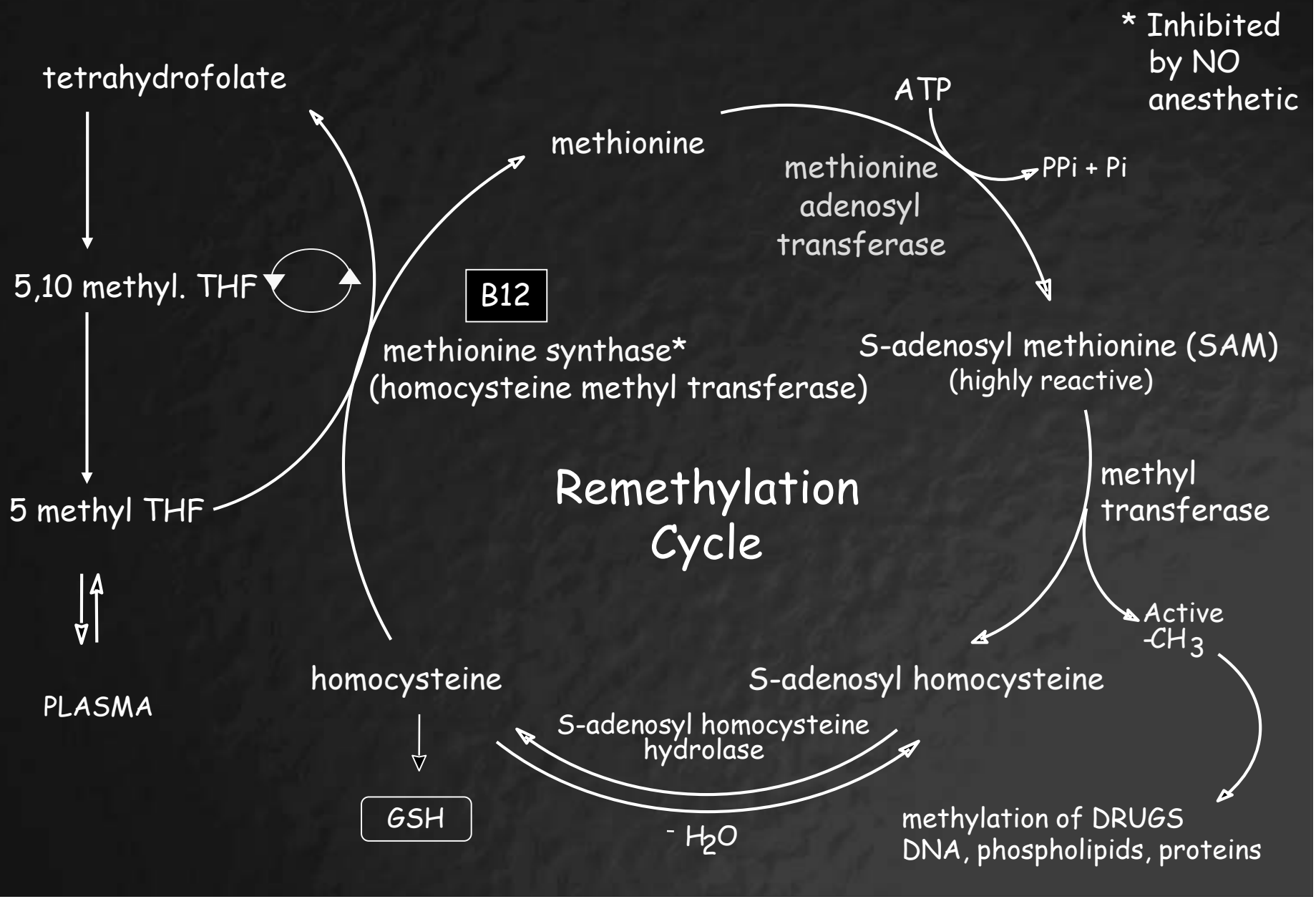


Aminopterin

5-Fluorouracil



Remethylation cycle, details:



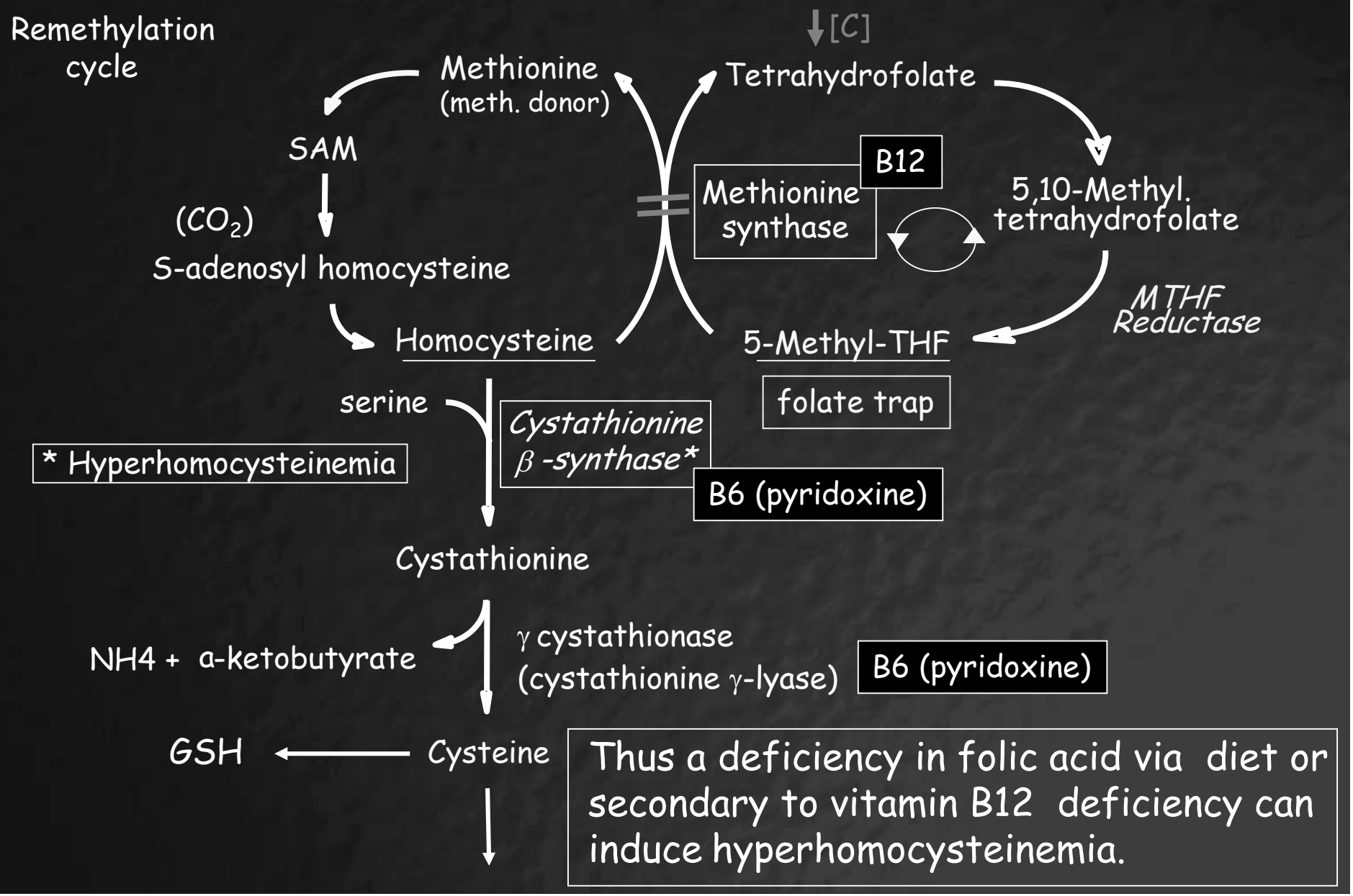
Methionine adenosyl transferase:

Chronic alcoholism causes inactivation of methionine adenosyltransferase so that methionine is not converted to SAM. This causes methylation deficiency as well as cysteine deficiency, resulting in GSH deficiency.

Hypoxia, viral liver cirrhosis, septic shock also form oxygen radicals which inactivate methionine adenosyltransferase

Gastroenterol. 114, 364-71 (1998)
Pathol. Biol 49, 738-52 (2000)

B12, Folate, and Hyperhomocysteinemia:



Thus a deficiency in folic acid via diet or secondary to vitamin B12 deficiency can induce hyperhomocysteinemia.

Hyperhomocysteinemia:

- Individuals with hyperhomocysteinemia (5-7% of general population) can develop atherosclerosis (premature coronary artery disease) and/or atherothrombosis by 30-40 years of age.

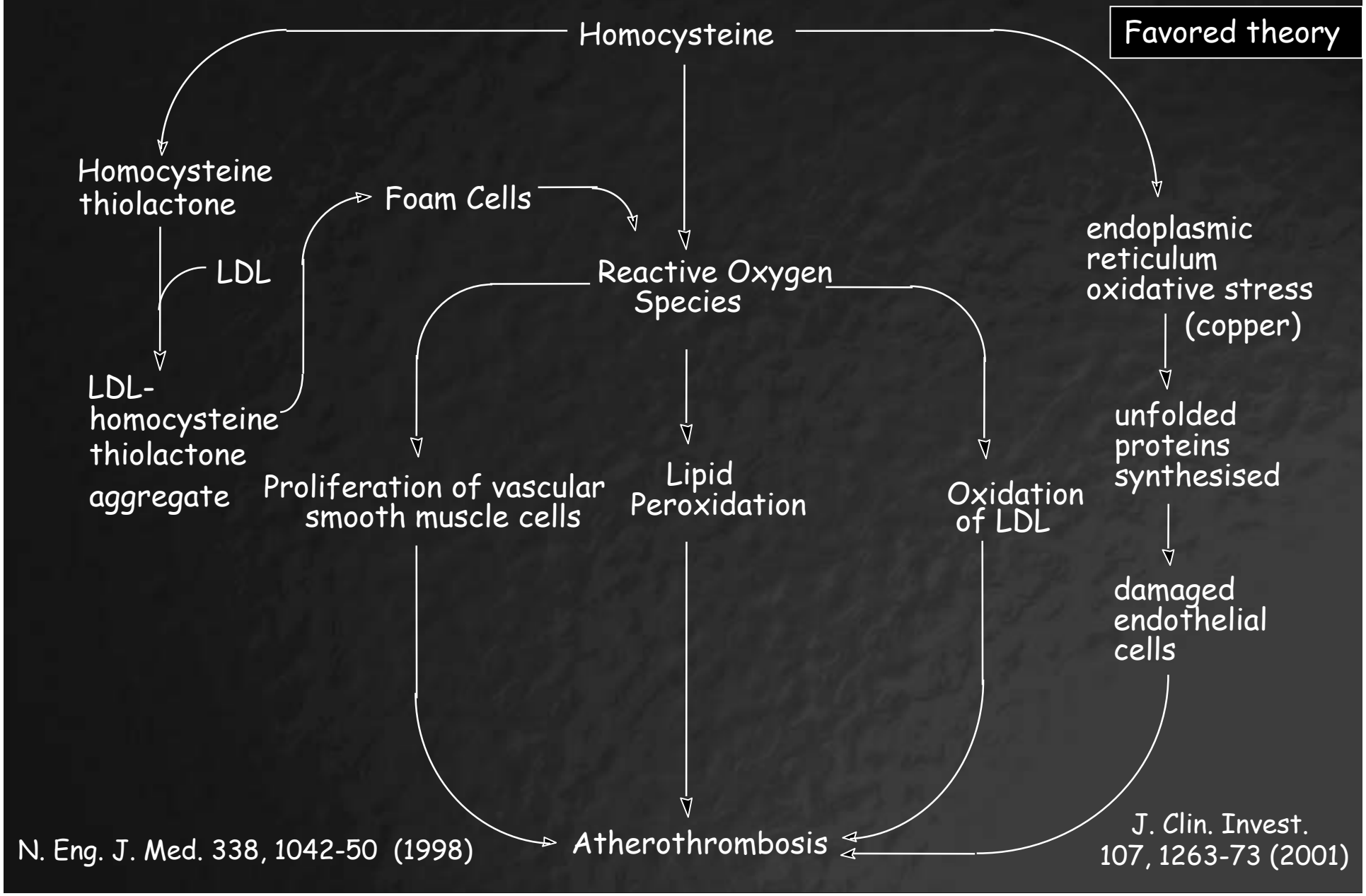
- Causes: vitamin deficiencies of pyridoxine (B6), folic acid (B9), or B12 can result in elevated homocysteine levels. Elevated homocysteine may also occur as a result of genetic abnormalities (MTHFR, methionine synthase, cystathione beta-synthase).

- Homocystinuria due to CBS deficiency is a special sub-type of hyperhomocysteinemia. Major organ systems typically affected in this autosomal recessive disorder include ocular (dislocation of lens a common presenting symptom), CNS and skeletal abnormalities.

- The minimum required folic acid intake to prevent defects in the fetus is 200 ug/day (400). Recommended folic acid intake to prevent homocysteine induced vascular disease is 350 ug/day.

- In 1998 cereal flours in the US were required to be enriched with folic acid to a higher level. Multivitamin dose can be 800 ug/day.

Homocystinuria, suggested mechanisms:



Cystathionine beta-synthase overexpression:

- Downs syndrome represents most common genetic cause of mental retardation. Associated with trisomic duplication on chromosome 21.

- CBS gene located in trisomic region of 21. Analysis of individuals with Downs syndrome demonstrate significant elevation of CBS activity, resulting in lowered levels of homocysteine and elevated cystathionine levels in blood plasma. Leukocyte DNA samples exhibit hypermethylation in DS patients compared to normal siblings.

Vitamin B12, general properties:

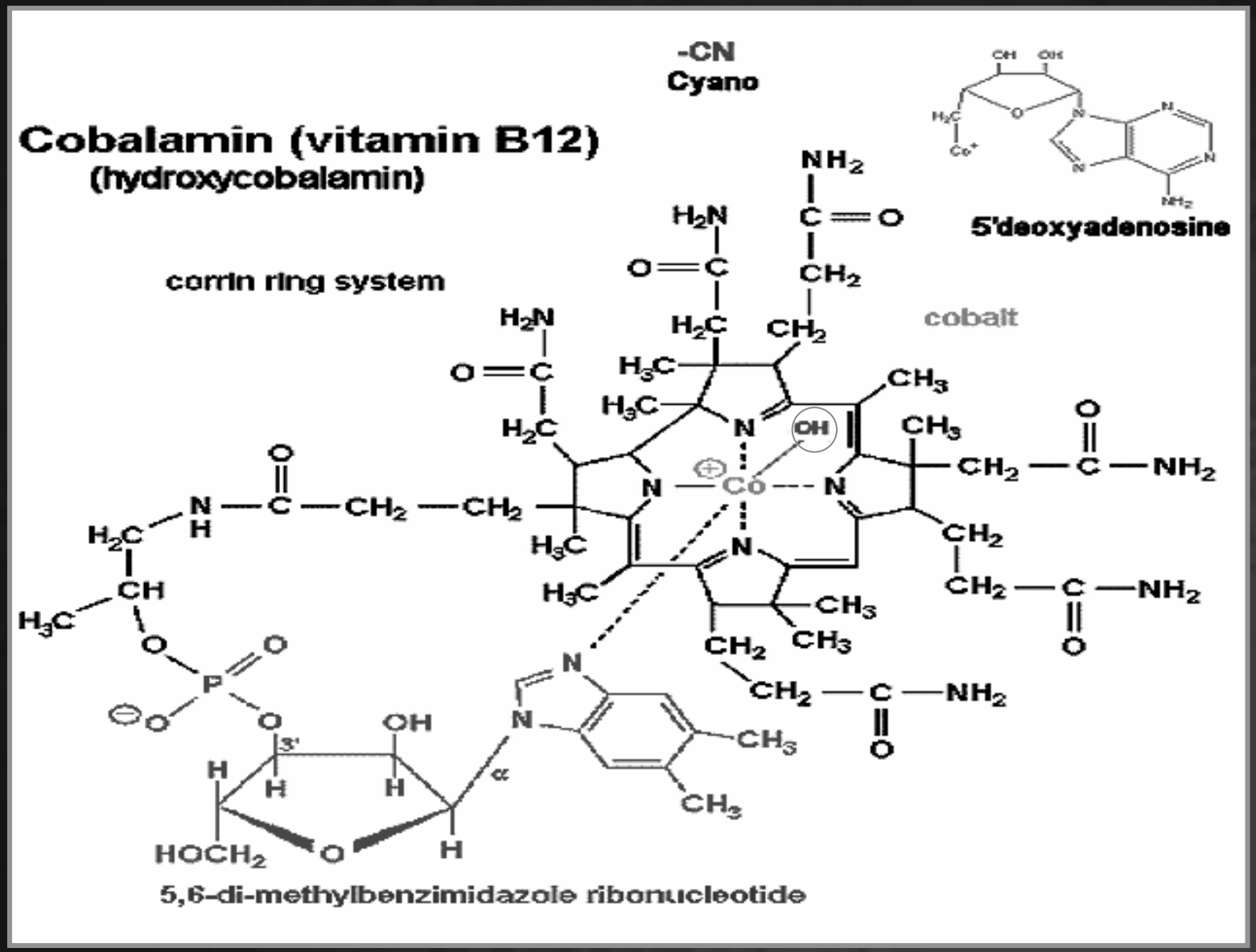
- Name given to cobalt-containing compounds possessing a corrin ring

- B12 is a water soluble vitamin produced in bacteria, and is obtained from foods of animal origin (i.e. meat 2.7- liver 95, kidney, and dairy products). Vitamin B12 may be present, but is not available to humans from plant sources (lacto-ovo vegetarians usually OK).

- Vitamin B12 (cyanocobalamin) USDA requirements are 5 ug per day (8 ug per day for pregnancy, elderly) (RNI is actually 1.5 ug/day. B12 is required for maintenance of the hematopoietic and nervous systems.

- Three enzymatic reactions depend upon B12 : methylmalonyl CoA mutase, leucine aminomutase, and methionine synthase.

Cobalamins, Vitamin B12:



Vitamin B12, ligands:

Derivative:	Application:
Co(III)-CN cyano*cobalamin (B12)*	Commercial product
Co(III)-OH *hydroxocobalamin*	Bacterial form, Cyanide & hydrogen sulfide antidote
Co(III)-Ado 5' deoxy*adenosylcobalamin*	Body reserves, a.a. metabol.
Co(III)-CH3 *methylcobalamin*	Methylation reactions homocysteine →methionine

Vitamin B12, uptake and distribution:

Protein digestion in the stomach makes vitamin B12 available to bind to cobalophilin (saliva).

The cobalophilin/B12 complex is hydrolyzed in the duodenum, releasing B12 for binding to intrinsic factor (a glycoprotein made by parietal cells of the gastric mucosa).

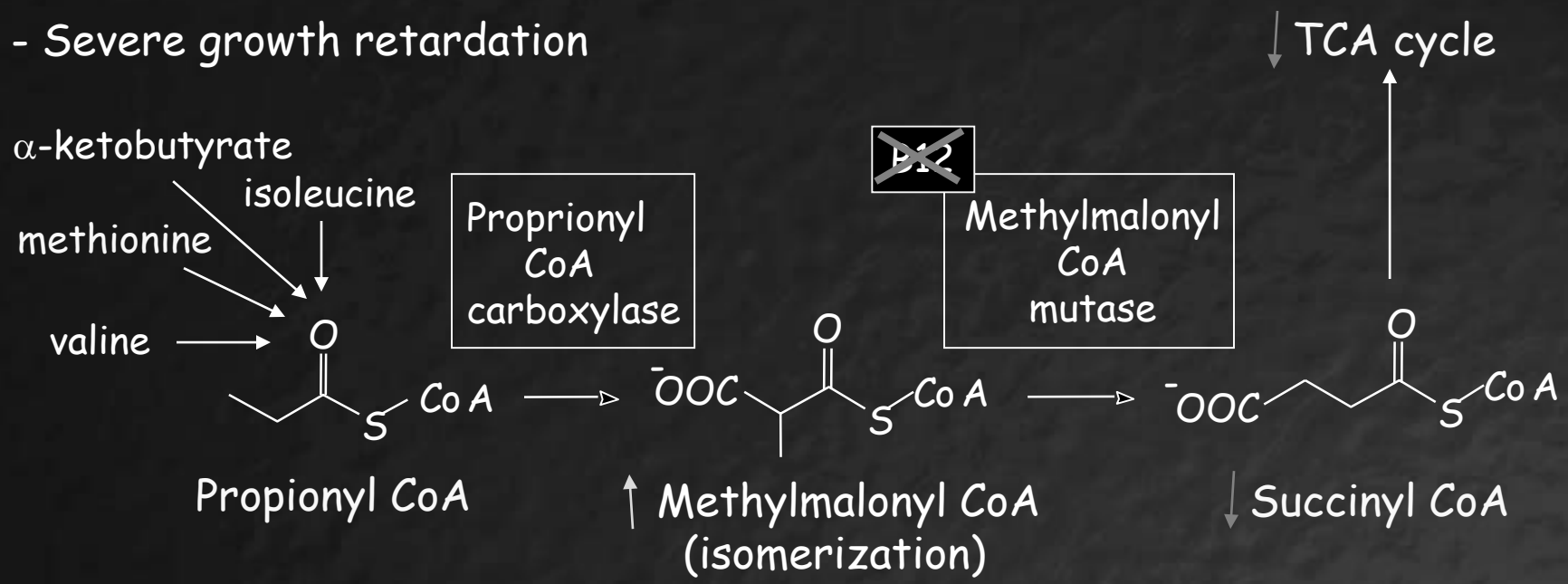
The B12/ IF complex (but neither alone), can then be absorbed from the distal third of the ileum.

In the blood, vitamin B12 is bound to transcobalamin II.
In cells, vitamin B12 is stored bound to transcobalamin I and III.

Frequently, the defect in cases of vitamin B12 deficiency is that binding to intrinsic factor is impaired. Such is the case in pernicious anemia (both genetic and autoimmune forms are known) and in Crohn's disease.

Vitamin B12, methylmalonyl CoA:

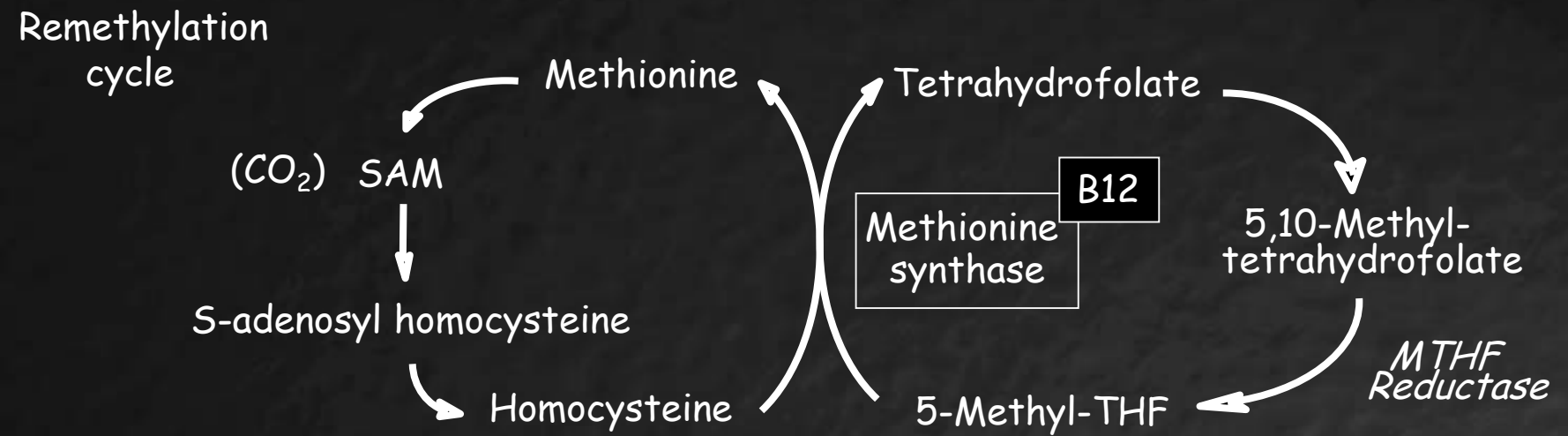
- Decreased dicarboxylate carrier cap. of mitochondria
- Severe growth retardation

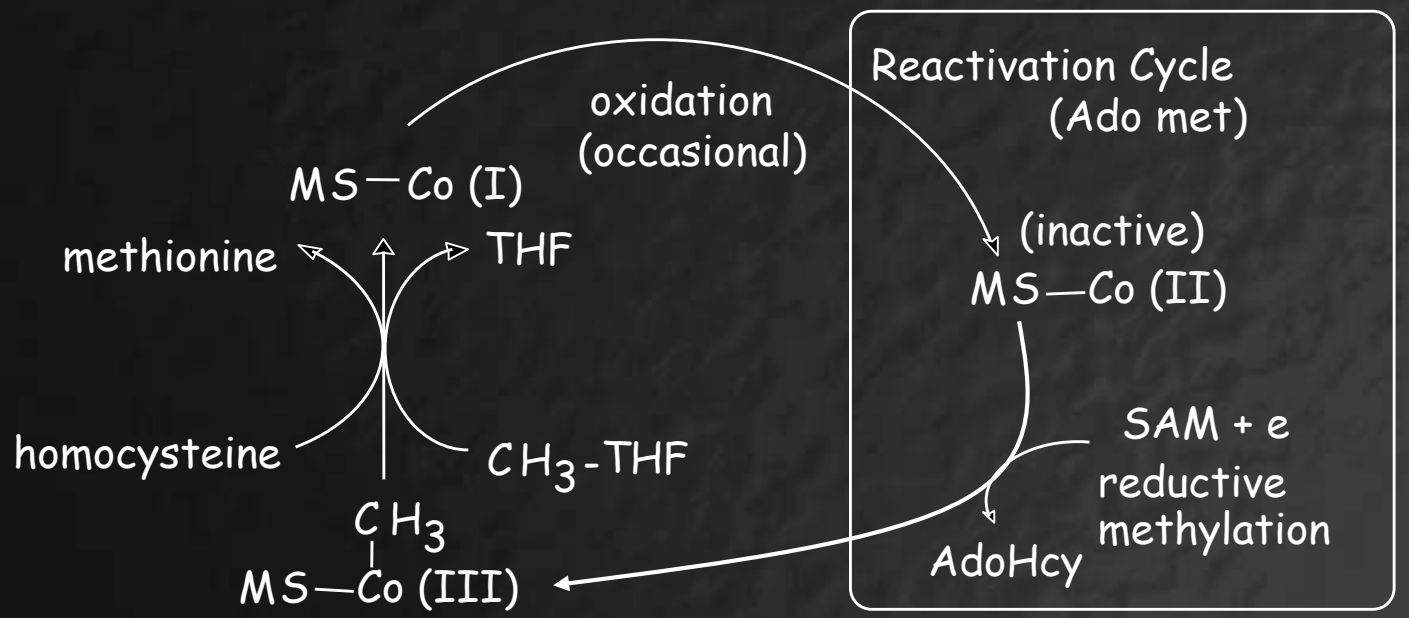


Succinyl CoA is thus the point of entry of some carbon atoms of methionine, isoleucine and valine. Propionyl CoA and then methyl-malonyl CoA are intermediates in the breakdown of these three non-polar amino acids.

REF: Stryer (5th ed) pp. 216, 611-14, 623-24, 652
also Br. J. Nut. 75, 929-38 (1996)

Vitamin B12 and methionine synthase:





Diet provides only 50% of methionine requirement (synthesis of proteins, SAM, polyamines)

Biochem. 36, 8082-91, JBC 276, 27296

Vitamin B12 deficiency:

Symptoms:

↑↑ plasma methymalony CoA and homocysteine

- pale, shiny tongue → red, sore, glossitis
- vegans, gastrectomy patients, crohns disease, 6-8% elderly
- genetic mutation, N_2O anaesthetic (oxidises Co(I) of Co(II)), oral contraceptives, hormone replacement therapy

Consequences:

- pernicious anemia
- diseases of bone marrow, intestinal tract, CNS
- increased methyl malonyl CoA causes growth retardation
- increased plasma homocysteine → premature atherosclerosis, thromboembolism
- depression and dementia in geriatrics
- cognitive impairment in the elderly, peripheral neuropathy
- multiple sclerosis (demyelination disorder)
- morbidity in transplant patients

Ref. [Nut. Rev. 54, 382-9 (1996)].

Pernicious anemia:

- Pernicious anemia arises under conditions of vitamin B12 or intrinsic factor deficiency.

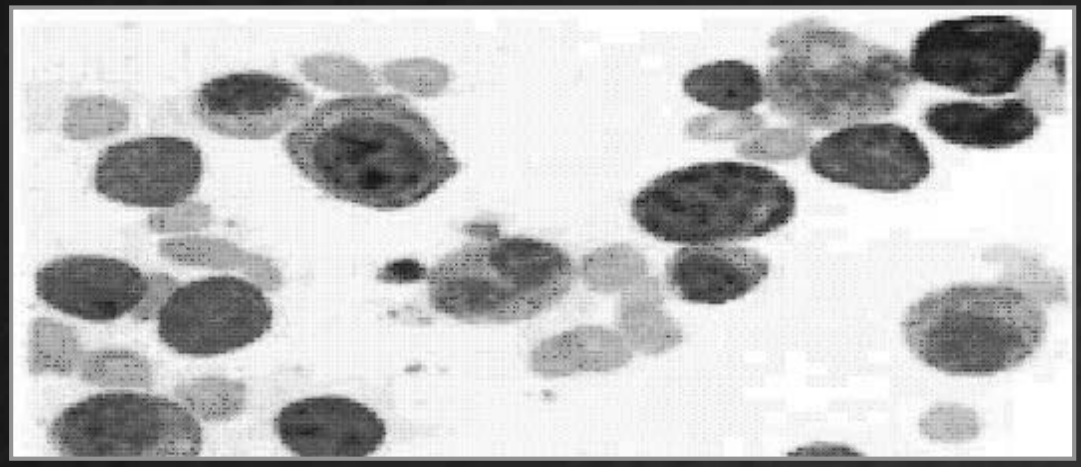
- Reduction in B12 levels blocks the metabolism of folic acid, resulting in secondary folate deficiency.

- Folate deficiency results in impaired erythropoiesis, resulting in the premature release of immature erythrocyte precursors (megaloblastic anemia)

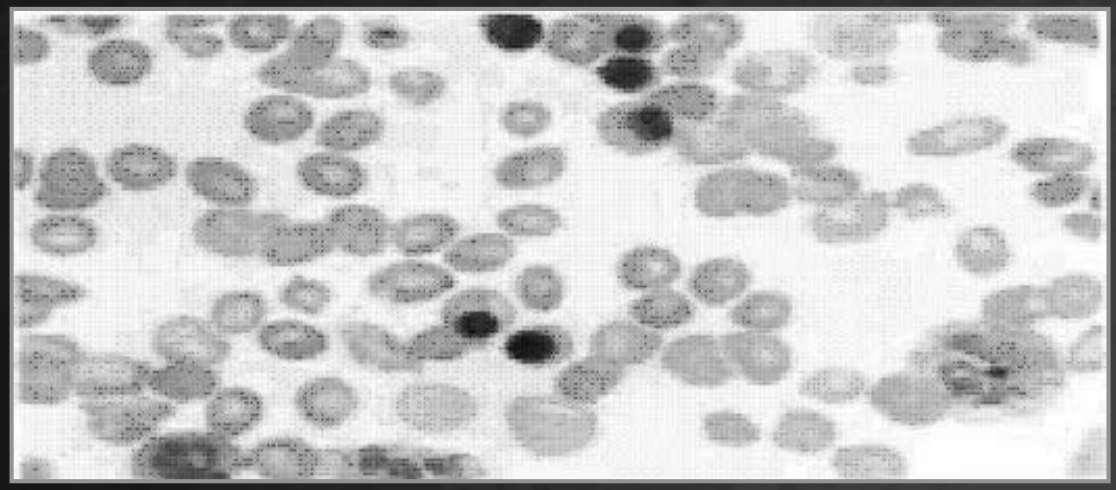
- The most common cause of this form of anemia is dietary deficiency of vitamin B12, however it can also arise from defects in "intrinsic factor" (autoimmune).

Pernicious anemia, therapy:

Blood smear from anaemic patient, showing a number of megaloblasts



Blood smear from patient 72 hours after treatment with 25 υg of vitamin B12. Note the reduction in megaloblast numbers.



Summary of material covered to date:

1. Hemoglobin, myoglobin, role of allosteric modulators in O_2 and CO_2 transport
2. Role of glycolysis in hemoglobin function
3. Drugs, toxins, mutations which affect erythrocyte function
4. Role of Glutathione
5. Pentose Phosphate Pathway
6. Genetic polymorphisms / Diseases and Malaria
7. Iron Homeostasis and treatment of anemia
8. Iron homeostasis and metabolism
   - Iron deficiency anaemia and iron therapy
   - Iron overload and Genetic disease: hemochromatosis
   - Erythropoietin Therapy
9. Folic acid metabolism, hyperhomocysteinemia and folate deficiency
10. Vitamin B12 metabolism and deficiency (pernicious anemia)