

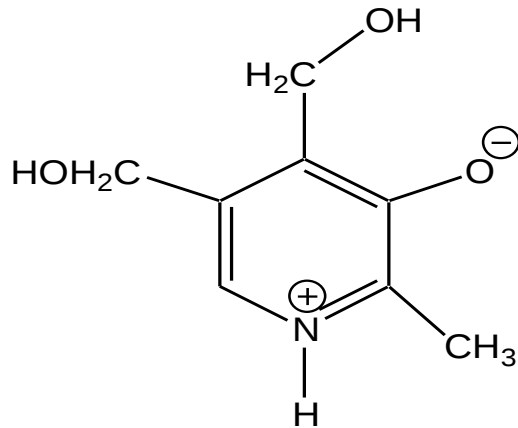
Water Soluble Vitamins

DR. PARINEETA SAMANT

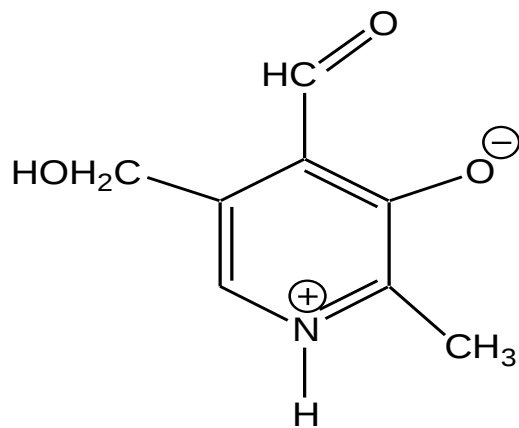
Vitamin B6 (Pyridoxime)

- Vitamin B₆ is found in: Cereals, Beans, Meat, Liver, Fish, Yeast, Nuts and some fruits as Banana and Potatoes.
- It is also produced by bacterial flora in the colon.

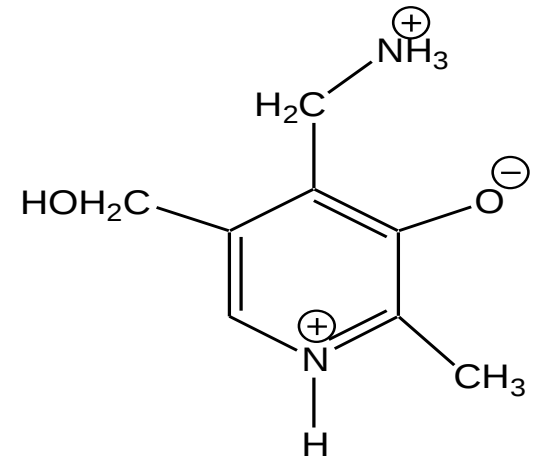
Pyridoxal



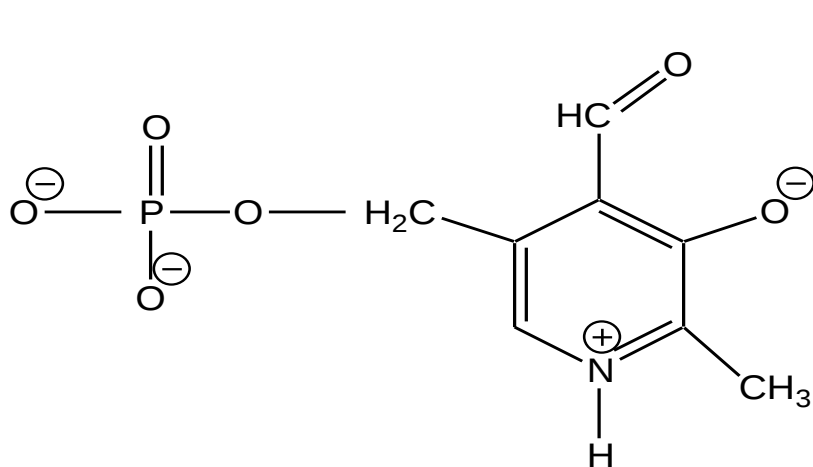
PYRIDOXINE



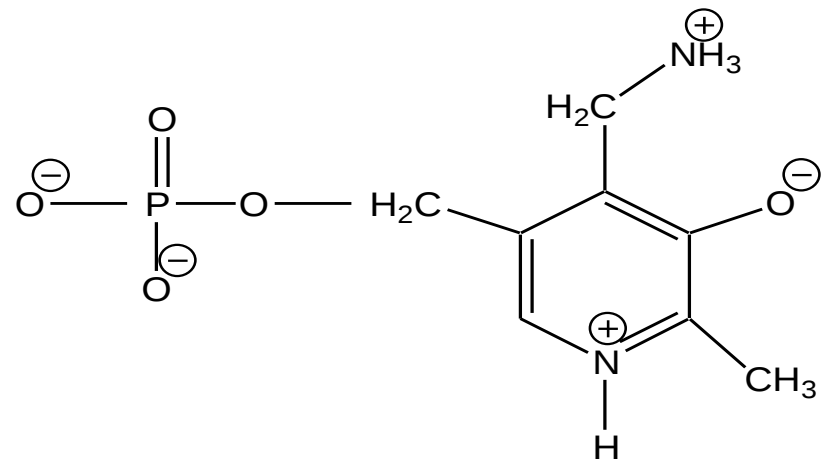
PYRIDOXAL



PRYIDOXAMINE



PYRIDOXAL 5' PHOSPHATE



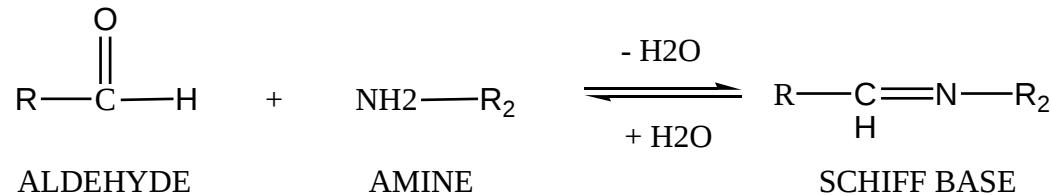
PYRIDOXAMINE 5' PHOSPHATE

RDA

- Children 1- 3 years old 1.0 mg
- 4- 6 years old 1.1 mg
- 7- 10 years old 1.4 mg
- **Adults 1.4- 2.2 mg**
- Pregnancy 2.2 mg
- Lactation (2.1 mg).

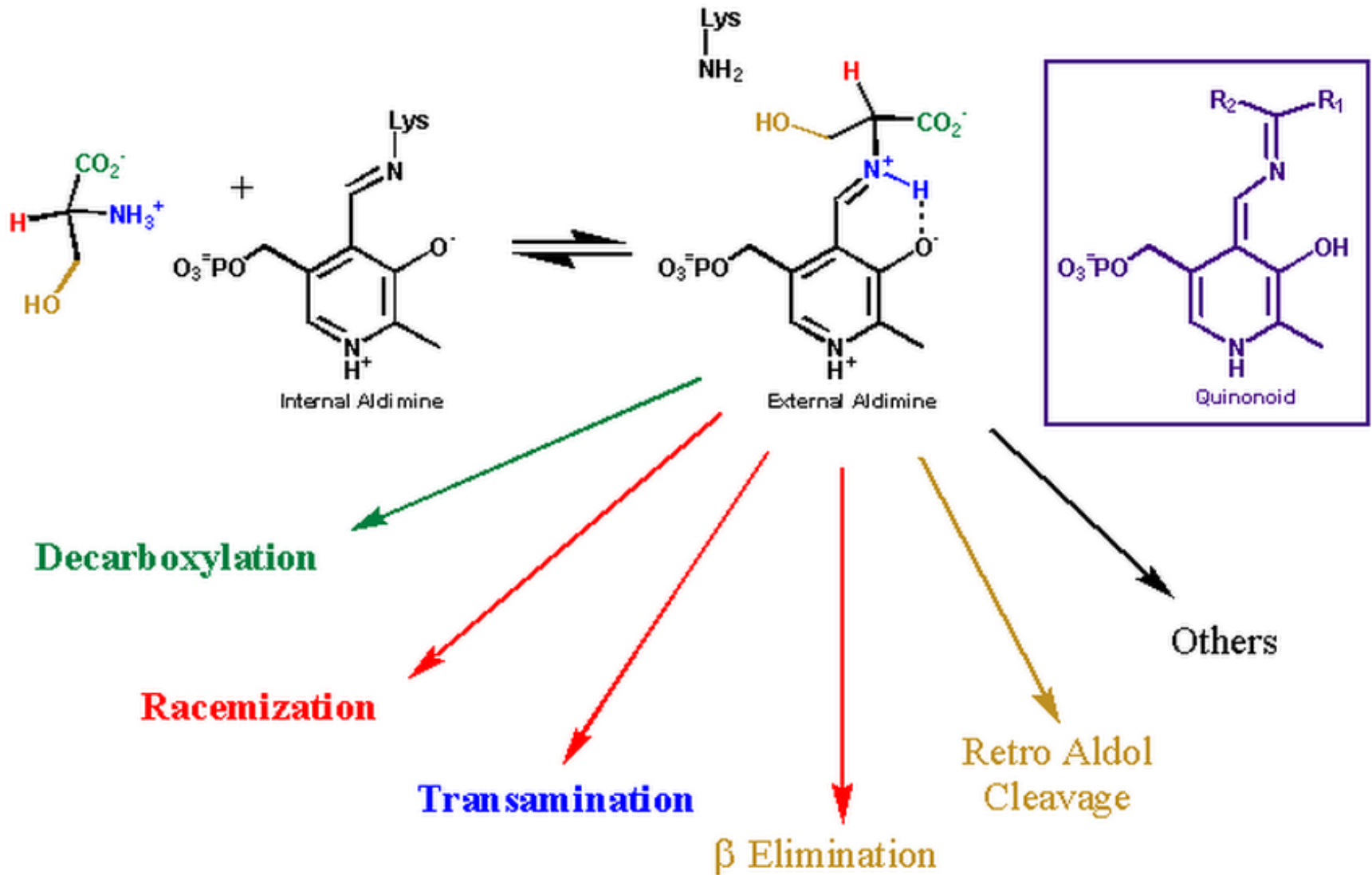
PYRIDOXAL-PHOSPHATE

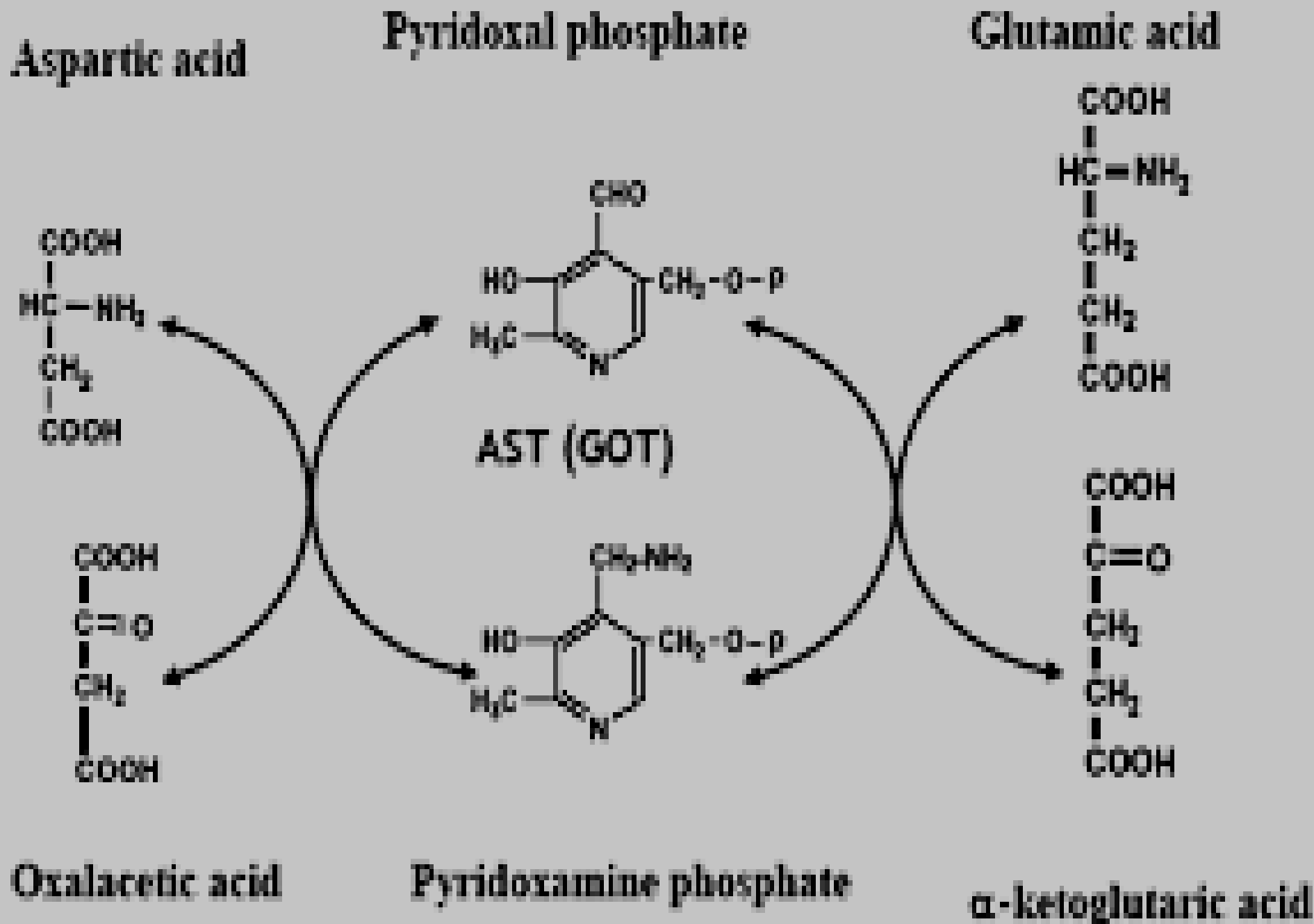
- Important in amino acid metabolism
- Bound to enzyme as a Schiff base thru rxn with lysine



- PLP functions in transamination, decarboxylation, racemization, isomerization, side-chain elimination rxns involving amino acids

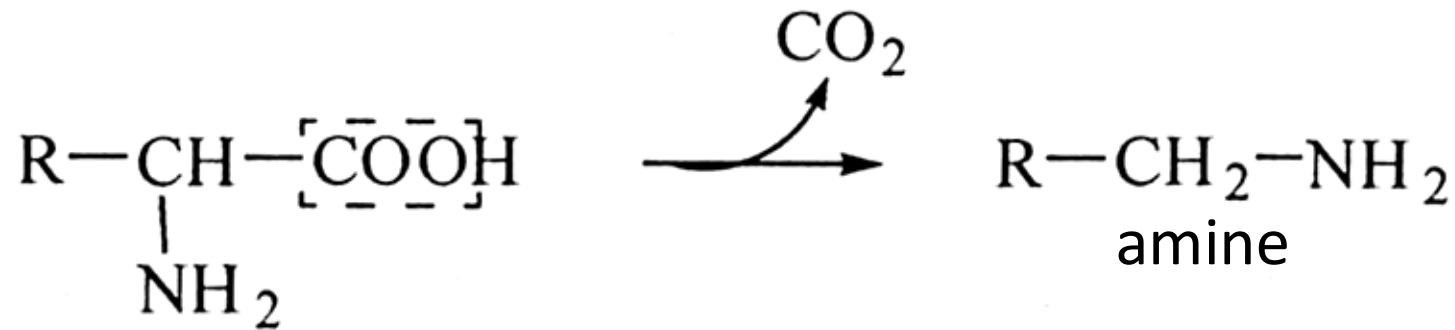
PLP Reactions





Decarboxylation of amino acids

Decarboxylation – removal of *carbon dioxide* from amino acid with formation of *amines*.



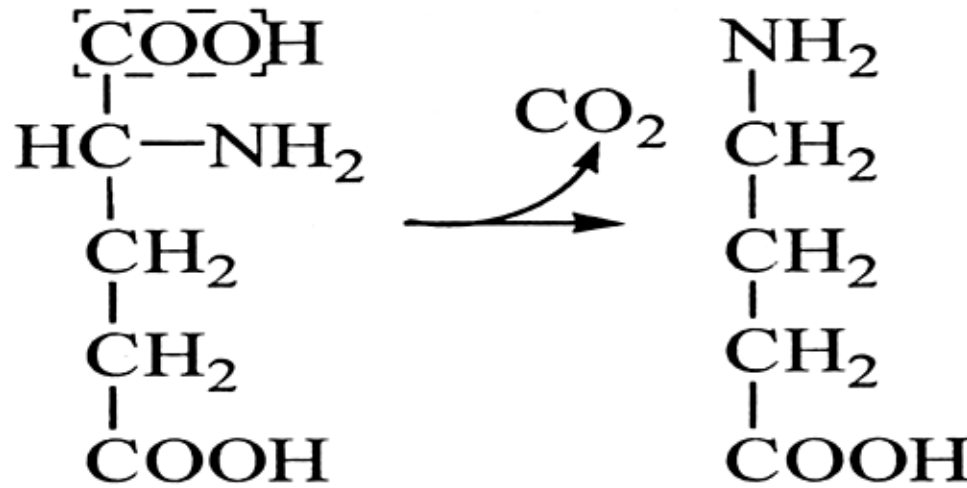
Usually amines have high physiological activity (hormones, neurotransmitters etc).

Enzyme: *decarboxylases*

Coenzyme – *pyridoxal phosphate*

Significance of amino acid decarboxylation

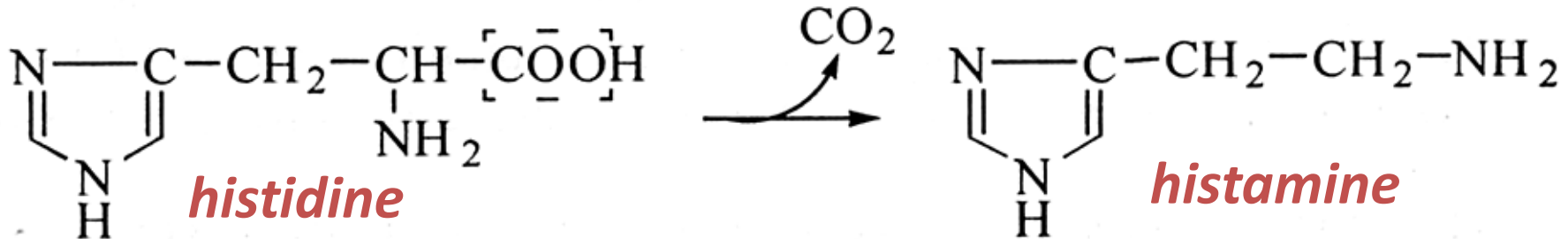
1. Formation of physiologically active compounds



glutamate

gamma-aminobutyric acid (GABA)

GABA –
mediator of
nervous
system



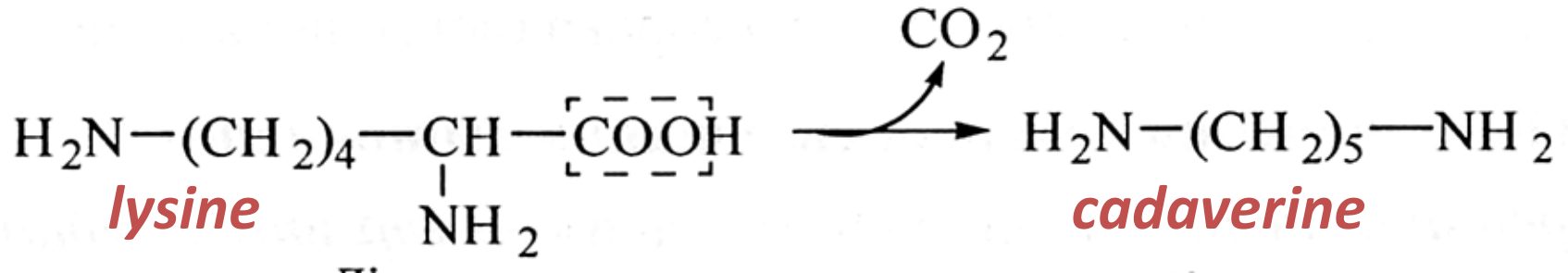
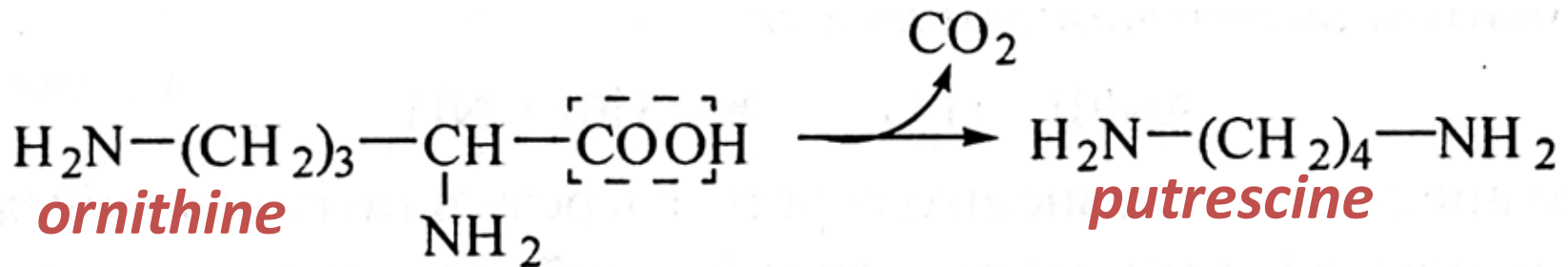
histidine

histamine

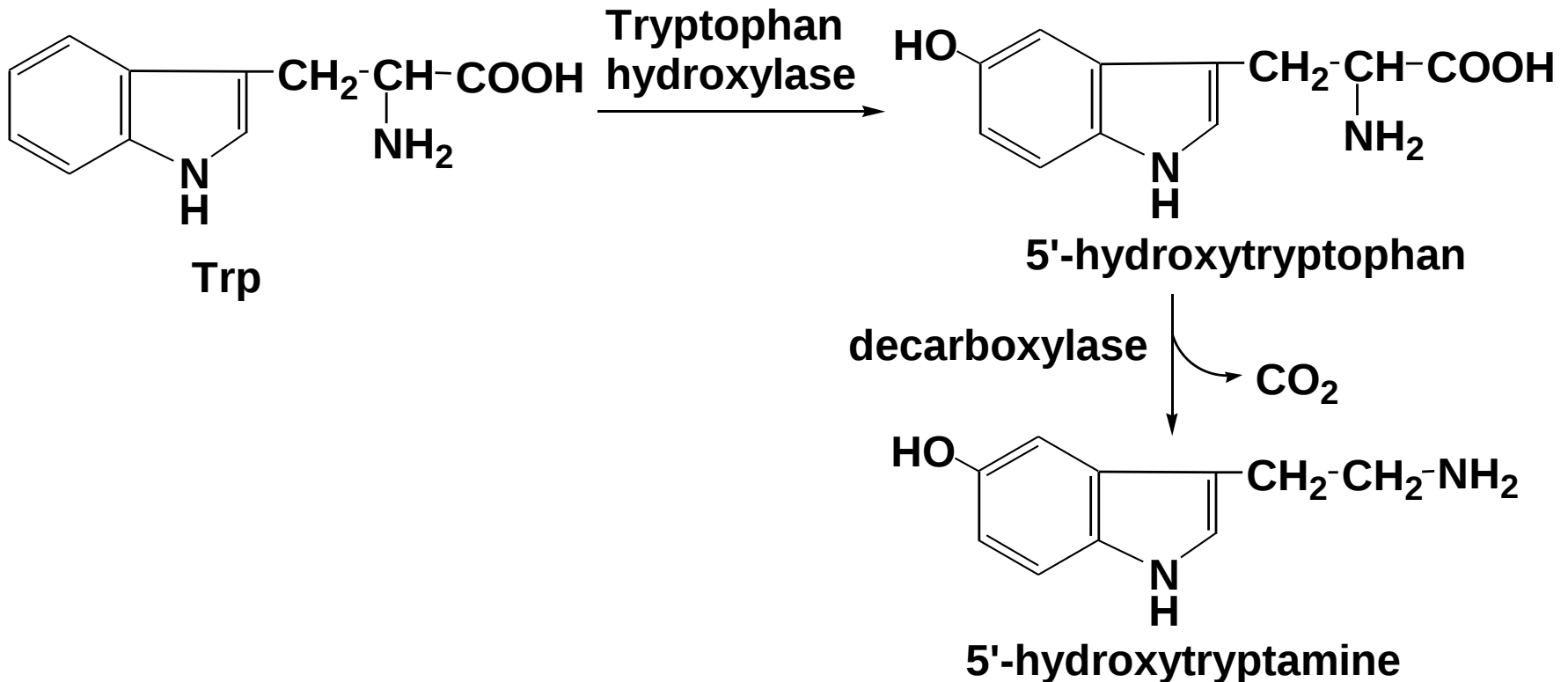
Histamine – mediator of inflammation, allergic reaction.

2. Catabolism of amino acids during the decay of proteins

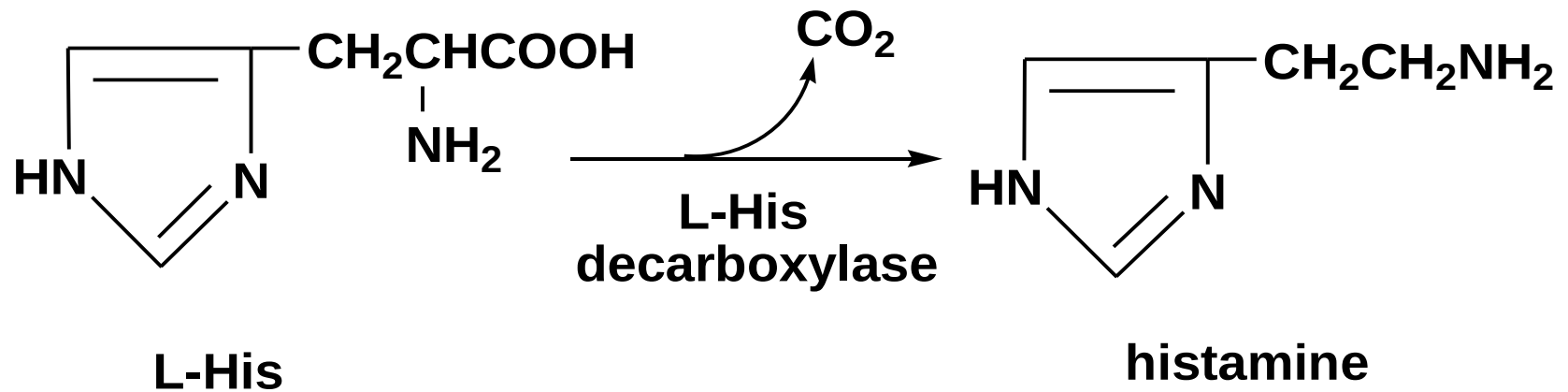
Enzymes of microorganisms (in colon; dead organisms) decarboxylate amino acids with the formation of **diamines**.



4. Trp → 5-hydroxytryptamine (5-HT) (serotonin)



3. His→histamine



Histamine acts as a neurotransmitter, particularly in the hypothalamus. It acts as an anaphylactic and inflammatory agent on being released from mast cells in response to antigens.

Causes of Deficiency

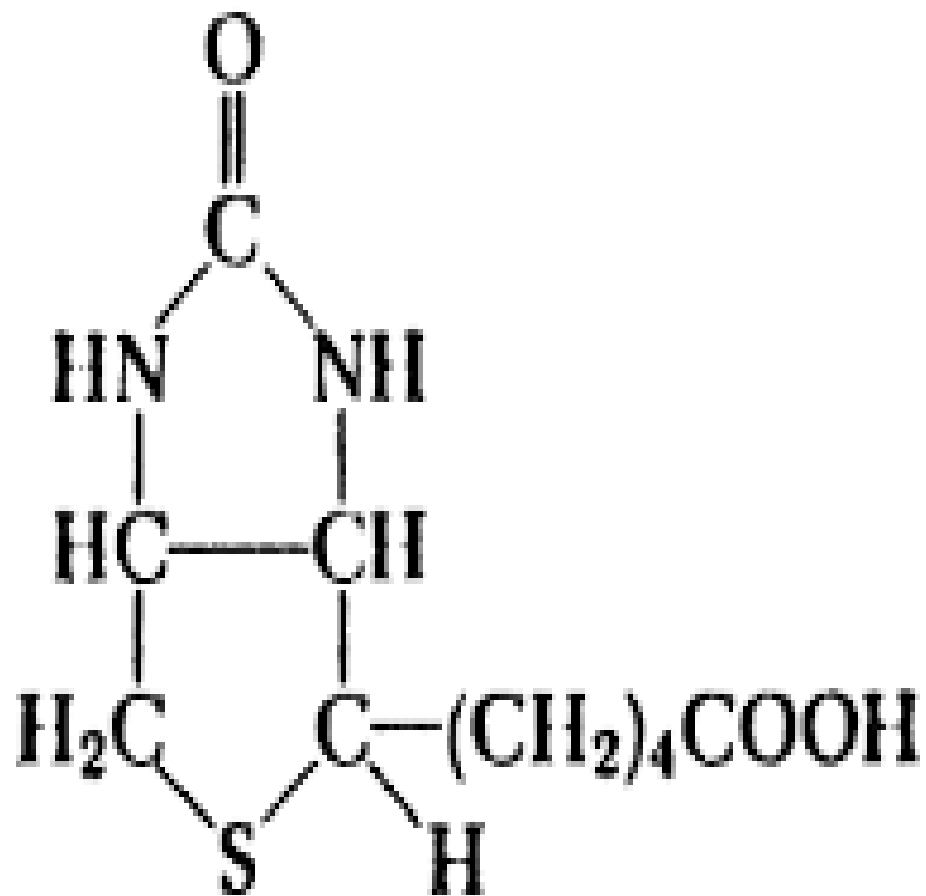
- Individuals with a poor quality diet or an inadequate B₆ intake.
- Alcoholics because alcohol also promotes the destruction and loss of vitamin B₆ from the body.
- Older people.
- Asthmatic children treated with theophylline because it decreases body stores of vitamin B6.

Deficiency

Signs of vitamin B₆ deficiency include:

- Skin: Dermatitis (skin inflammation), Stomatitis (inflammation of the mucous lining of any of the structures in the mouth), Glossitis (is inflammation or infection of the tongue).
- Neurological abnormalities: Depression, confusion, and convulsions.
- Vitamin B₆ deficiency also can cause anemia.

Biotin



Biotin

Chemistry of Biotin

- Biotin molecule consist of an imidazole ring fused to a thiophene ring.
- The chemical name is hexahydro-2-oxo-1-thieno-3,4-imidazole-4-valeric acid.
- It is also called as anti egg white injury factor.
- Raw egg white contains a basic protein, avidin which is an antagonist of biotin.
- It is heterocyclic monocarboxylic acid.

RDA

- Infants=10-15 $\mu\text{g}/\text{dl}$
- Children= 20-40 $\mu\text{g}/\text{dl}$
- Adult =25-50 $\mu\text{g}/\text{dl}$

Active form of biotin

- Biotin exist in two active forms
- α -form
- β -form

Metabolic role

- It act as a coenzyme for carboxylation reactions
- Biotin is converted to carboxy biotin complex by fixing CO₂ and utilizing ATP.
- This complex provides active CO₂ for being transferred to the substrate.

Enzyme requiring Biotin

- Pyruvate carboxylase
- Acetyl CoA carboxylase
- Propionyl CoA carboxylase

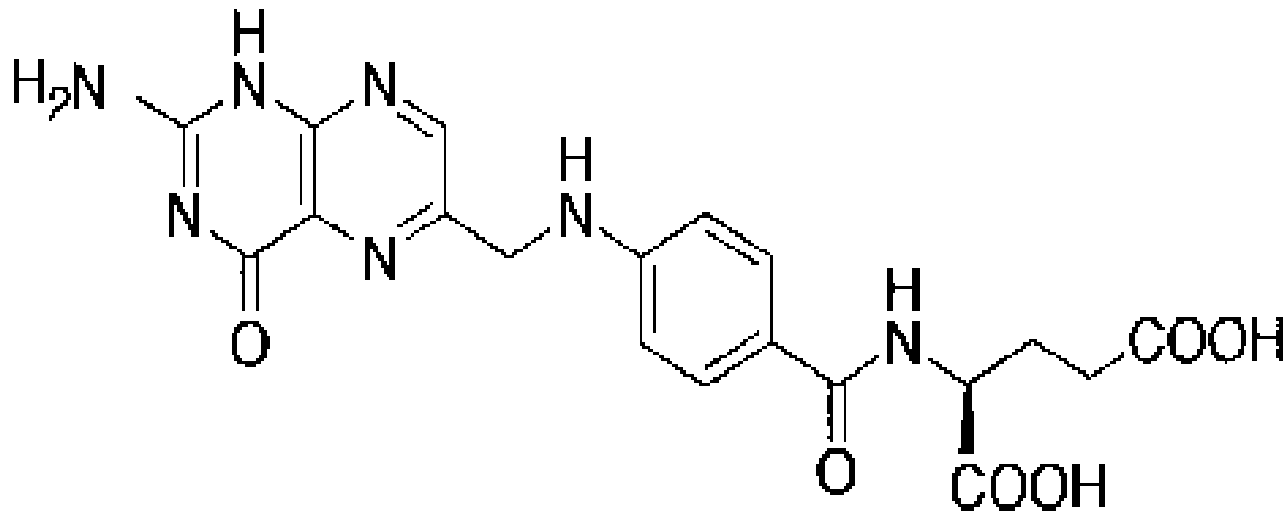
- Exception
- Carbamoyl phosphate synthetase
- Malic enzyme

Deficiency of Biotin

- No known deficiency diseases are reported as this vitamin can be synthesised by the gut microflora.
- Prolonged use of antibiotic may lead to destruction of intestinal microflora, causing deficiency.
- Symptoms are: Muscle cramp, anorexia, dermatitis, pallor, depression, sleepiness, anemia, neurological symptoms.

Folates

- The parent of folate family compounds is folic acid which has the following basic structure. Humans are incapable to synthesize it so the only source is diet.



Folic Acid

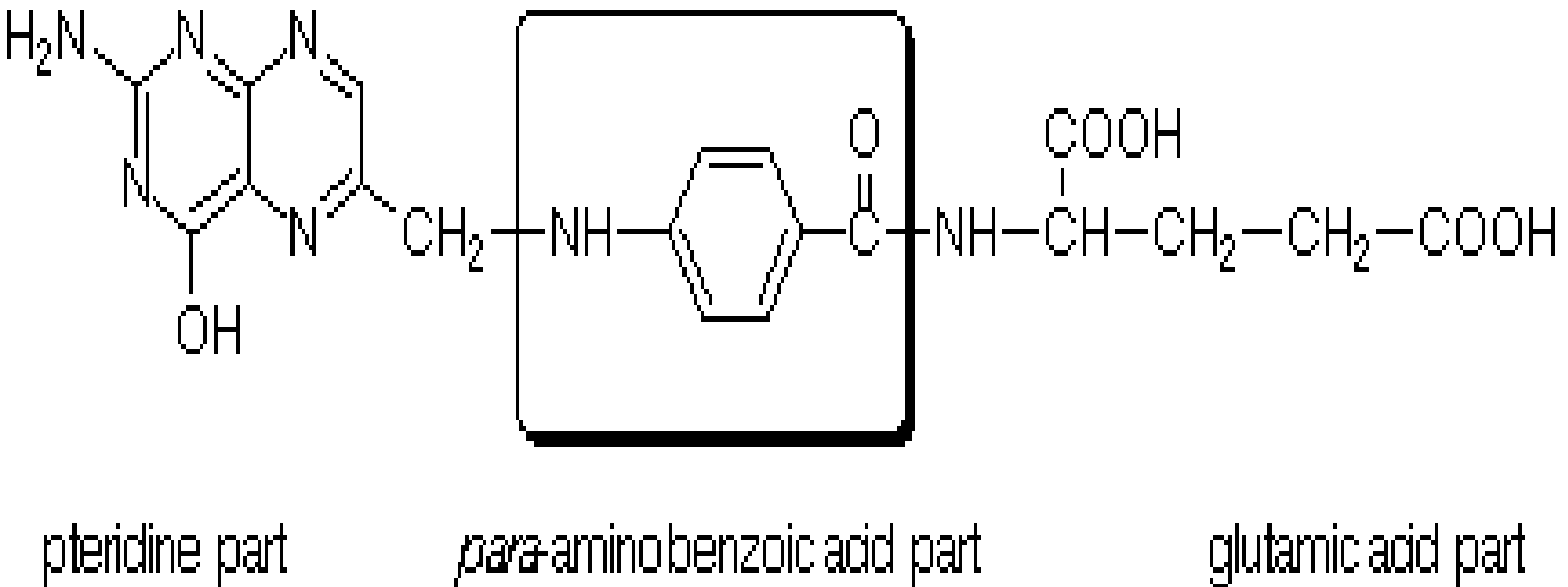
RDA

- Folate Recommendations (1998 RDA)
 - RDA Adults: 400 µg/day
 - **There are higher recommendations for pregnant women.**

Folate

- Conjugate of Pteridine, *p*-Aminobenzoic acid and Glutamic acid.

Folic acid



Folate

- Folate Food Sources
 - Fortified grains
 - **Leafy green vegetables**
 - Legumes and seeds
 - Liver
- Other Information
 - Easily destroyed by heat and oxygen

Daily folate requirement and storage

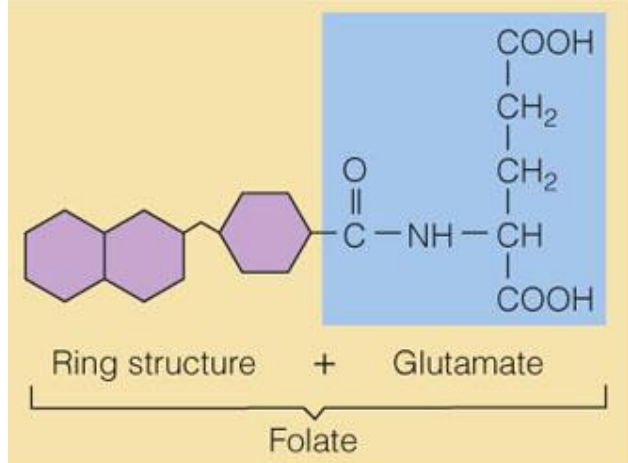
- Daily folate losses are about 100 μg per day, mainly in the faeces, urine, and sweat and skin cells.
- Faeces contain large amount of vitamin B12 and folic acid, but these are due to the microbial flora activities rather than losses from body stores.
- Folate is extremely sensitive to heat. Cooking involves prolonged boiling result in sever loss.

Daily folate requirement and storage

- Normal mixed diet may contain as much as 700 μg of folate per day but improper food preparation can reduce this amount close to the minimum daily requirement.
- Typical body stores of folate in a normal, healthy adult are about 10mg and are located in liver. Thus, if dietary folate intake or intestinal absorption ceased, the body stores would become exhausted in about 3-4 months.

Folate transport and retention in the body

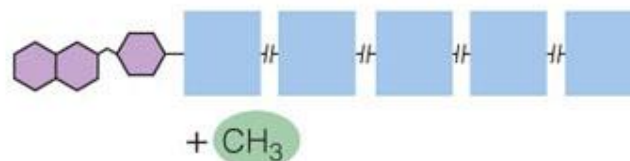
- Dietary folates, primarily polyglutamates, are hydrolyzed by a brush border hydrolase, prior to absorption in the gut.
- Monoglutamate forms circulate in plasma and are transported into tissues via a membrane carrier and/or by a receptor-mediated process.
- Folate retention by tissues requires their conversion to long chain length polyglutamate forms. Incomplete conversion to polyglutamates by tissues such as intestinal mucosa and liver leads to release of folate, mainly as methylfolate, into plasma.
- Tissue folates turn over primarily by catabolism to pterins and aminobenzoylpolyglutamates. The half life of whole body folate is approximately 100-200 days, depending on folate intake.



In foods, folate naturally occurs as polyglutamate. (Folate occurs as mono-glutamate in fortified foods and supplements.)



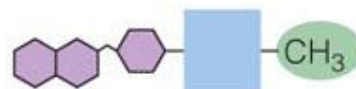
Spinach



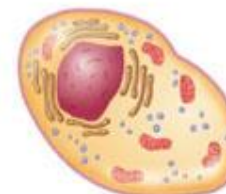
In the intestine, digestion breaks glutamates off . . . and adds a methyl group. Folate is absorbed and delivered to cells.



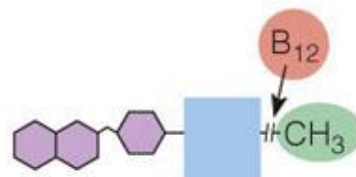
Intestine



In the cells, folate is trapped in its inactive form.



Cell



To activate folate, vitamin B₁₂ removes and keeps the methyl group, which activates vitamin B₁₂.

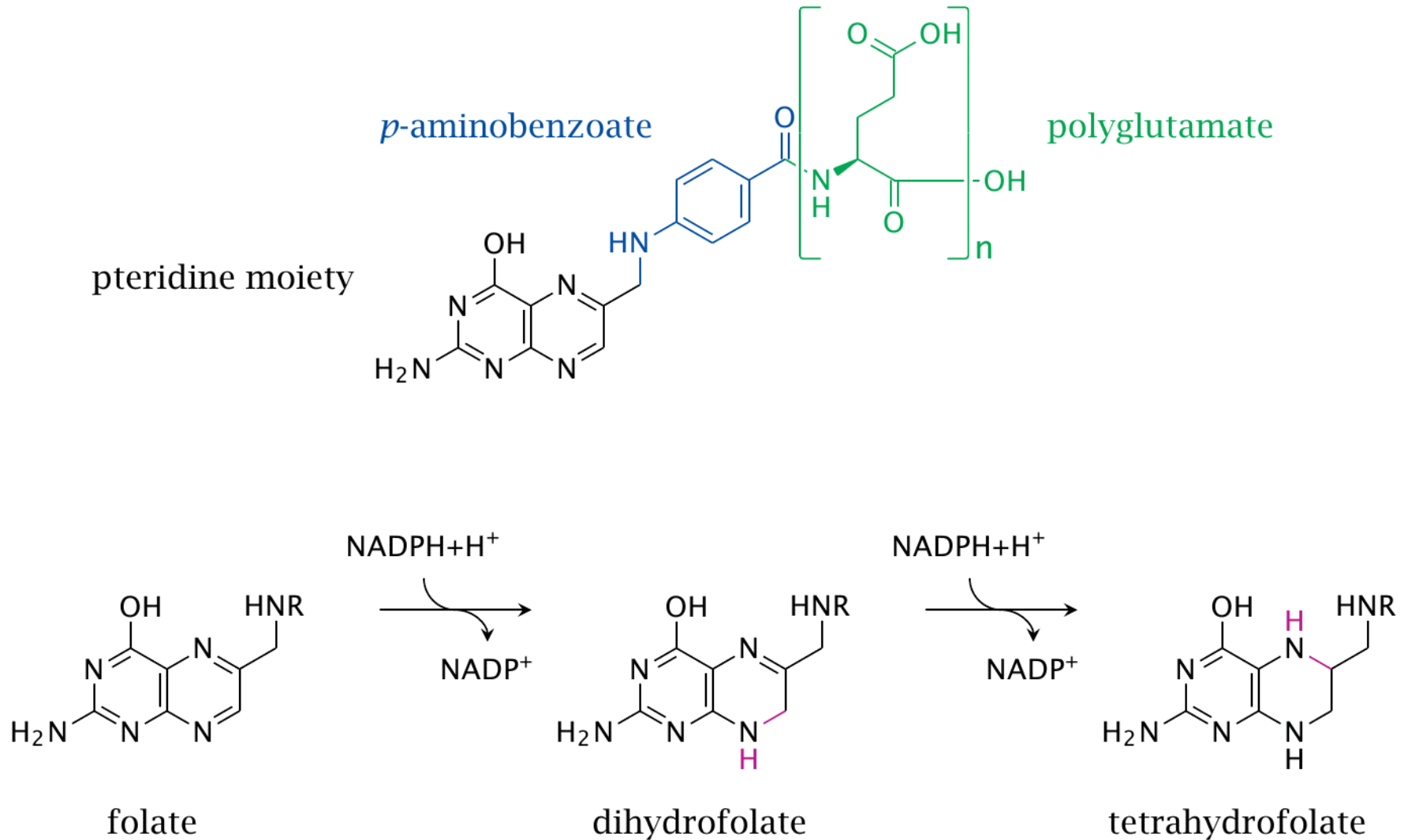


Both the folate coenzyme and the vitamin B₁₂ coenzyme are now active and available for DNA synthesis.



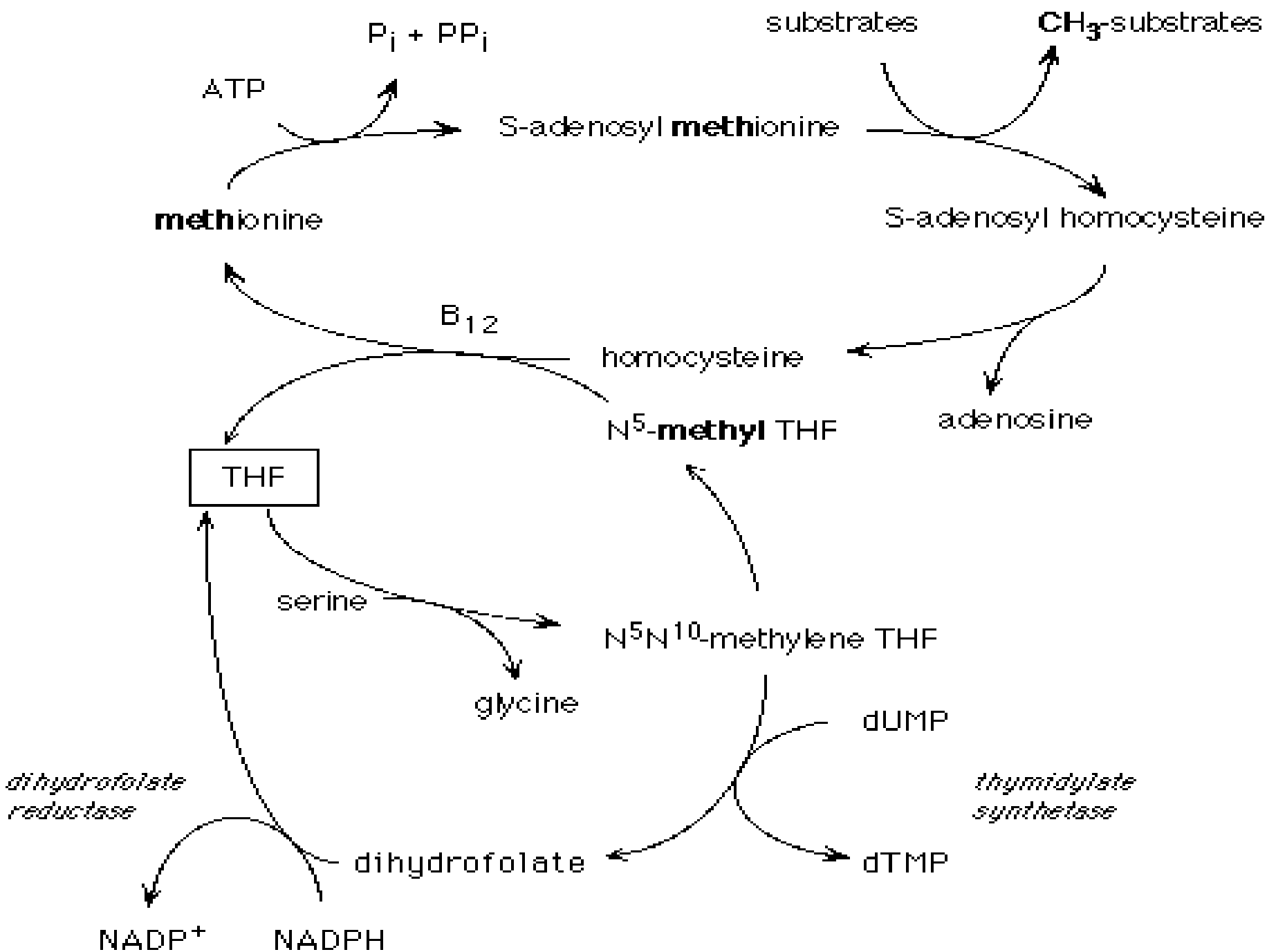
DNA

Folic acid is reduced by dihydrofolate reductase (DHFR)



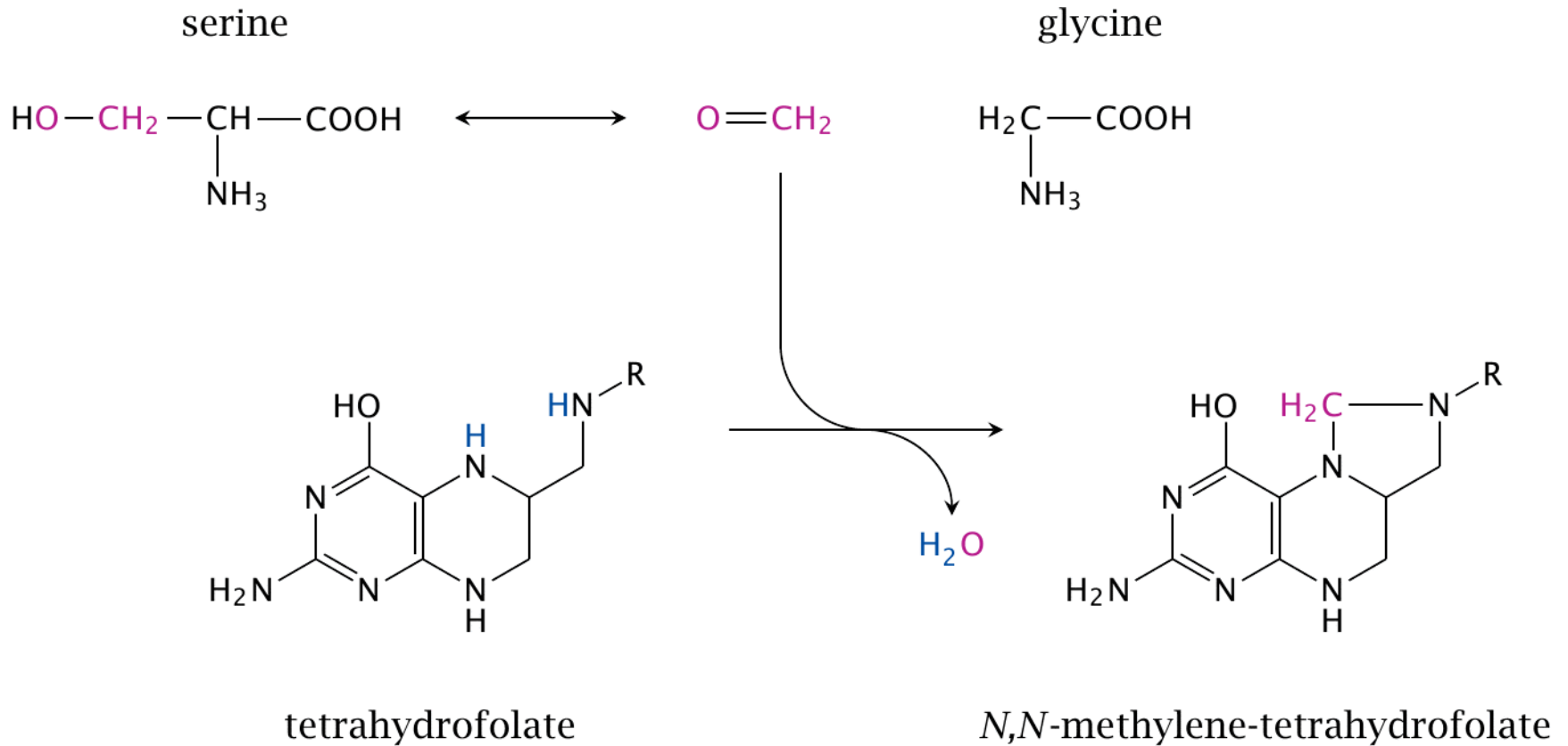
The role of folate within the body

- The various form of folate function as a single-carbon donor-acceptors in a variety of biosynthetic reactions as shown below:
 - (1) Synthesis of methionine. By donation of methyle group from N-5-methyl-tetrahydrofolate and requires vitamin B₁₂ as a coenzyme.
 - (2) Pyrimidine synthesis which is a rate limiting step in DNA synthesis.
 - (3) Purine synthesis.
 - (4) Conversion of serine into glycine.
 - (5) Histidine catabolism.

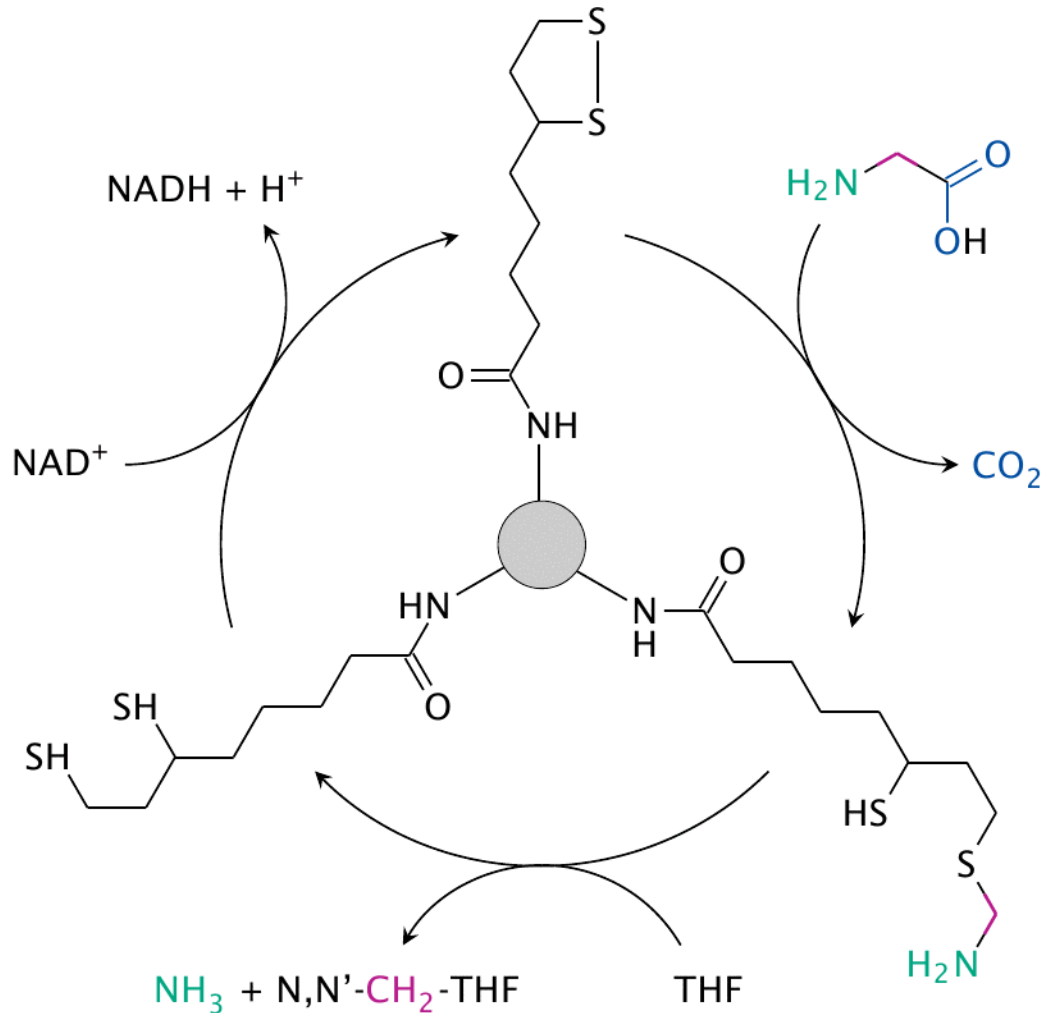


- **Folate** is involved in the synthesis of DNA and the **formation of new cells**.
- The coenzymes THF (tetrahydrofolate) and DHF (dihydrofolate) require **vitamin B₁₂** to function correctly.

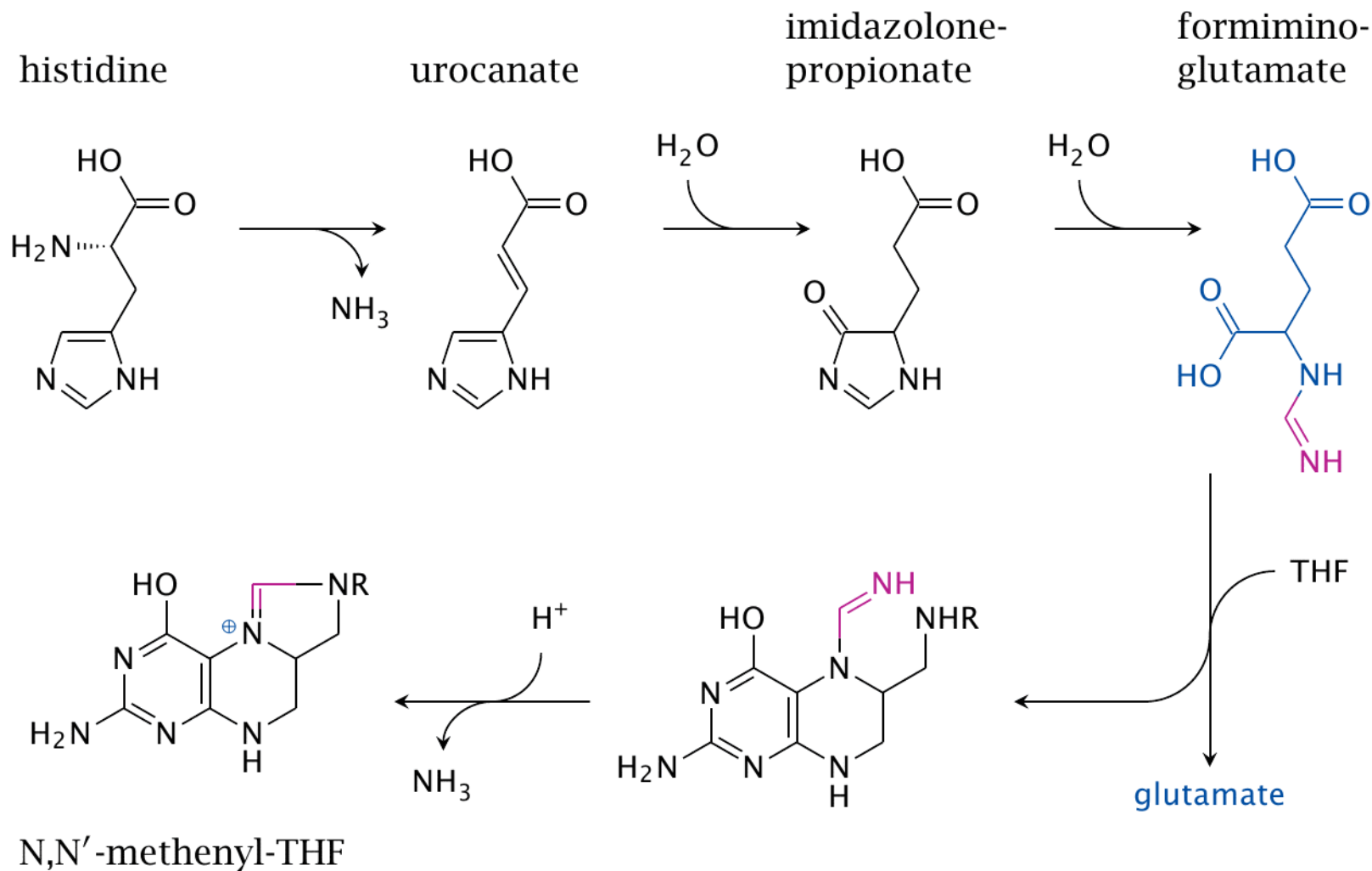
Capture of formaldehyde by THF yields N,N'-methylene-THF



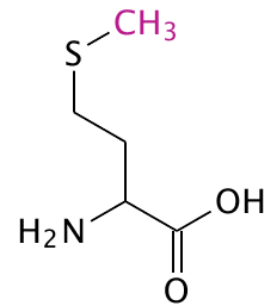
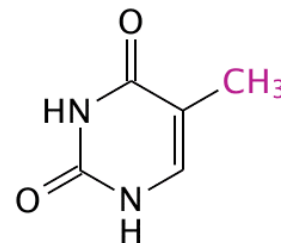
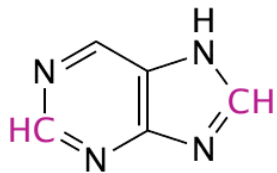
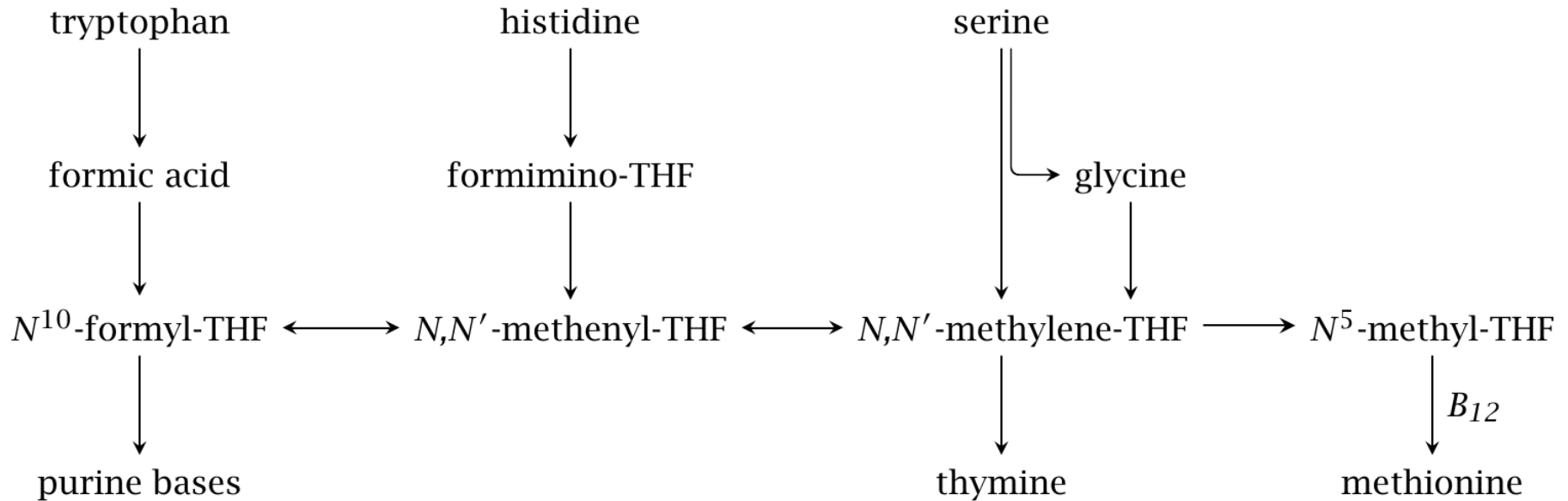
N,N'-methylene-THF production by the glycine cleavage system



Histidine degradation produces *N,N'*-methenyl-THF



Overview of flux through the C₁-THF pool



Why Take Folic Acid?

- Low amounts of folic acid in the expecting mother has also been linked to several other factors:
 - Premature delivery
 - Poor prenatal development
 - Placental abruption
 - Trouble with blood vessels within the placenta

Folate and Neural Tube Defects

- Neural tube defects include spina bifida and anencephaly.
- Women of childbearing age should eat folate-rich foods and folate-fortified foods and take folate supplements containing 0.4 mg (400 microgram) of folate daily.
- Pregnant women should take folate supplements.

NTD

- neural tube defects (NTDs) are birth defects that occur early in pregnancy
 - often before a woman knows she is pregnant
- neural tube grows to become baby's spinal cord, spine, brain and skull

- Spina Bifida
 - a condition that results when the lower part of the neural tube fails to develop properly
- Anencephaly
 - a fatal condition in which the upper end of the neural tube fails to close

Spina Bifida

- Can Lead to:
 - Muscle weakness
 - Paralysis
 - loss of sensation in legs
 - poor bladder and bowel control
- Baby will need surgery 24-48 hours after birth.



Spina Bifida

- The location on the spine and the severity of the defect will determine the level of impairment.
- May cause paralysis which will affect walking and bowel/bladder control.
- Treatment throughout the child's lifetime will be needed to monitor kidney functions.

Anencephaly (an-in-SEF-uh-lee)

- A neural tube defect at the top part of the skull.
- Usually results in miscarriage, stillbirth, or death shortly after birth.
- There is no surgical correction available.



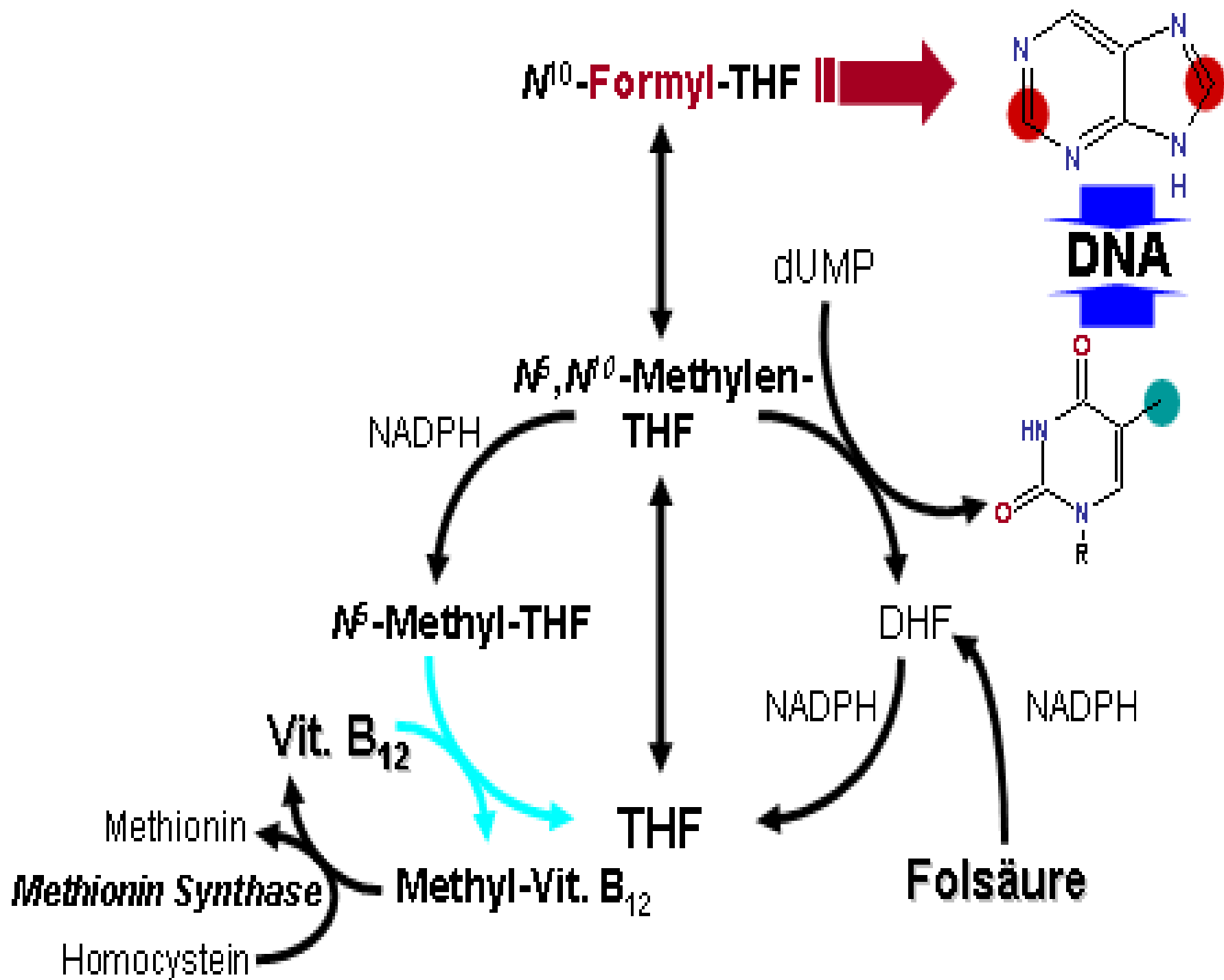
Encephalocele (in-SEF-uh-low-seel)

- Defect of the skull (smaller than anencephaly) that causes the brain tissue to herniate and form a sac protruding from the skull.
- Rarer than other types of neural tube defects.
- Surgical correction may be possible.
- Functioning levels of baby depend on and severity of encephalocele.



Consequences of impaired folate status or metabolism

Metabolic Disruption	Biochemical Markers	Clinical Associations
S-adenosylmethionine (MTHFR, MS, B ₁₂ deficiency) →	Hypomethylated DNA Elevated homocysteine Reduced methylation →	Cancer CVD demyelination NTDs
Thymidylate →	Increased uracil in DNA Decreased DNA synthesis & reduced cell division →	Cancer NTDs Anemia
Purines (A,G) →	Decreased DNA synthesis & reduced cell division →	Anemia



Folate and Heart Disease

- High levels of **homocysteine** and low levels of folate increase risk of heart disease.
- Folate breaks down homocysteine.
- Folate may help to prevent cancer.

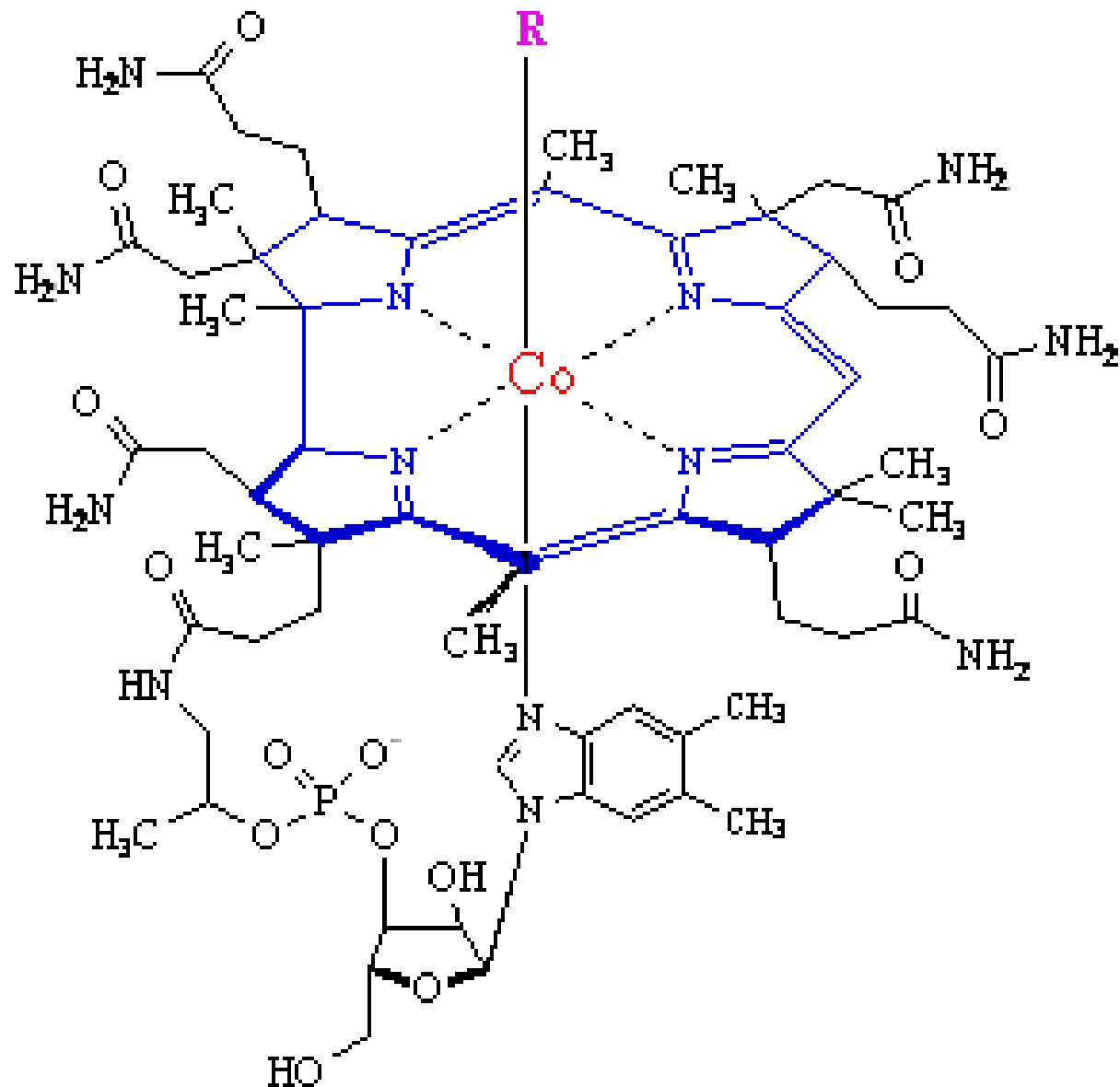
Megaloblastic Anaemia

- Megaloblastic anemia is referred to a group of panhypoplastic disorders, which are characterized by retardation of DNA synthesis but RNA synthesis proceeds at a normal rate.
- The resulting asynchrony between nuclear and cytoplasm maturation in developing cells is responsible for the distinctive morphological and biochemical features of the megaloblastic anaemias.

Megaloblastic Anaemias Causes

- Deficiency of either vitamin B12 or folic acid or sometimes both.

Vitamin B₁₂



Structure of Vitamin B12

- Vitamin B₁₂ is relatively stable and little is lost during cooking.
- It contains cobalt with co ordinate linkage with four pyrrole rings. The ring system is called as corrin.
- Cobalt forms fifth covalent linkage with substituted benzimidazole ring. The resultant molecule is called cobalamine.
- The sixth cordinate position of cobalt is occupied by a chemical group which may be hydroxyl, cyanide, adenosyl or methyl grp.

RDA

- 2.4 micrograms

Daily vitamin B₁₂ requirement

- The only source available to man is dietary.
 - The main dietary source is liver, kidney, red meat, eggs, shellfish and dairy products.
 - Normal mixed diet contains 5-30 µg /day.
-
- Typical daily losses of vitamin B₁₂ are between 1-4 µg.
 - The vitamin is lost mainly in urine and faeces.
 - Since normally there is no consumption of vitamin B₁₂ within the body, the daily requirement matches daily losses.

Vitamin B12 Stores

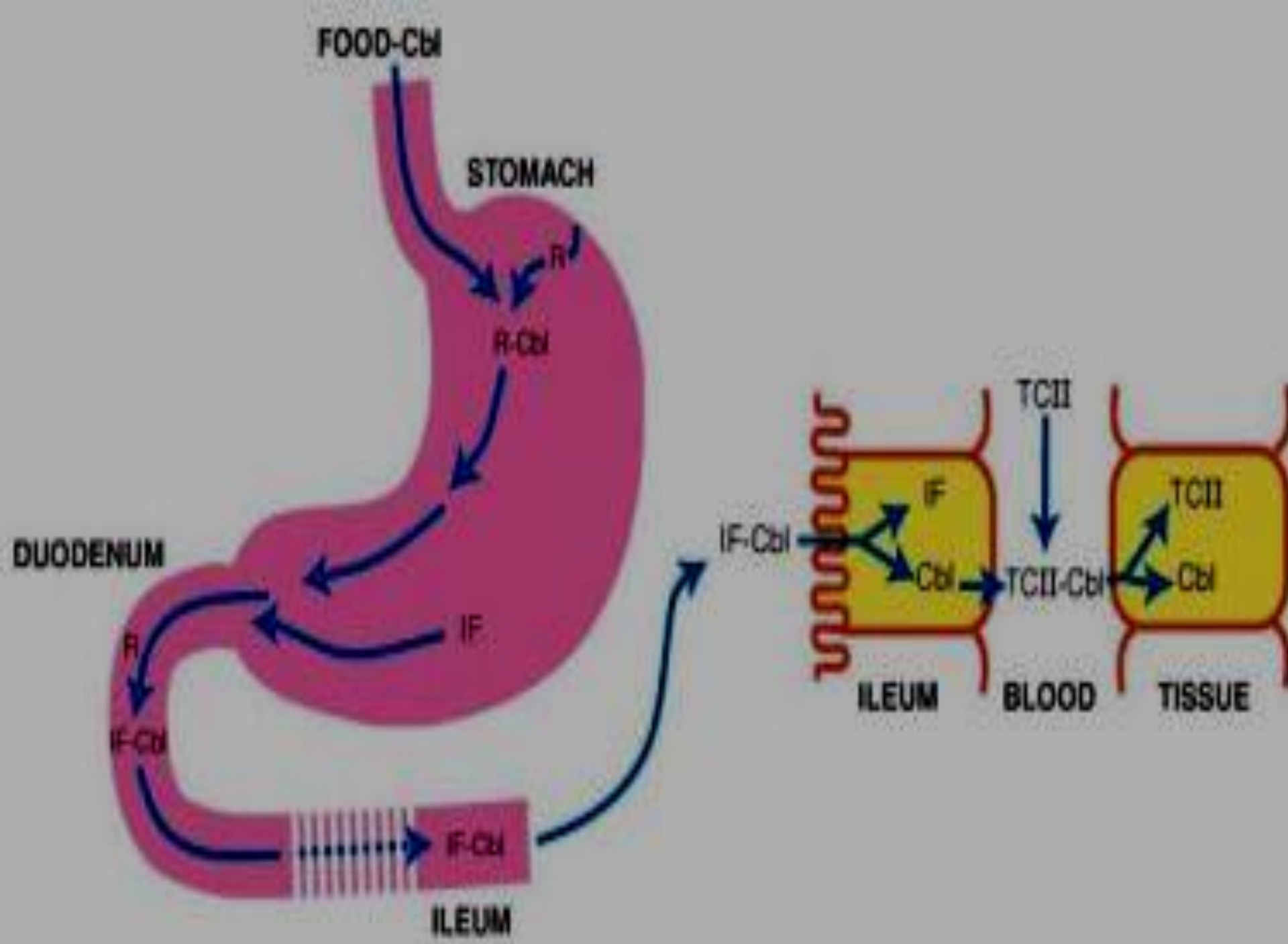
- Normal body stores of vitamin B12 about 3-4 mg, primarily in liver. This would be sufficient for 3 years if dietary intake ceased or if the ability to absorb the vitamin was lost.

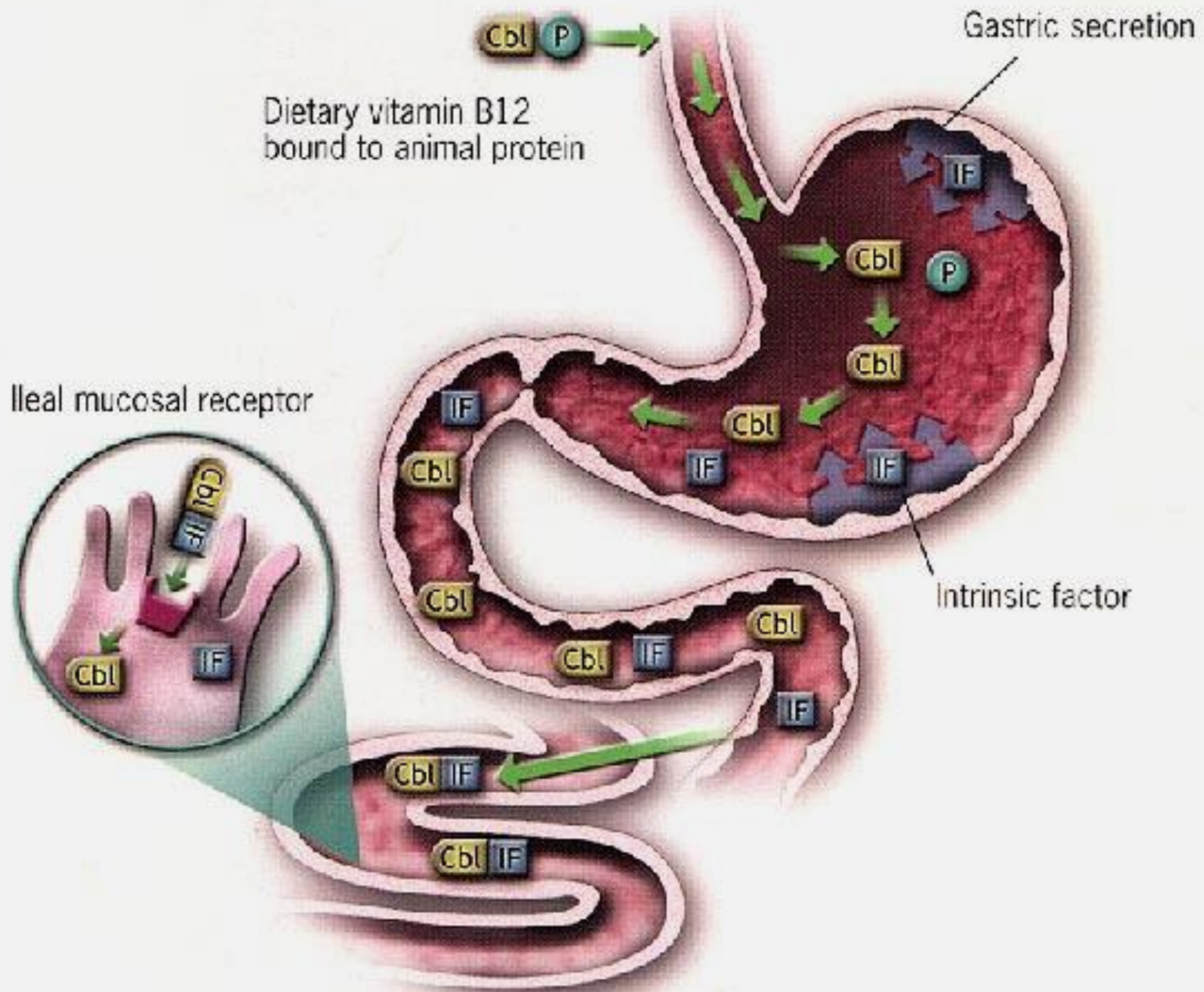
Vitamin B12 absorption

- VitaminB12 absorption is an active process, which occurs in the ileum.
- Vitamin B12 is liberated from food by gastric and duodenal enzymes and complexes in a 1:1 ratio with the **intrinsic factor**.
-
- IF is a glycoprotein, MW 45,000 which is synthesized and secreted by gastric parietal cells.
- IF: B12 complex then progresses to the ileum where it attaches to specific receptors on the ileal mucosal cells.
- This process requires the presence of calcium ions and neutral pH.
- The vitamin is internalized from the complex and released into the portal circulation after 6 hours.

Vitamin B12 Transport

- There are three vitamin B12 transport proteins normally present in the plasma, which are known as **Transcobalamines** (TCI-TCII-TCIII).
- The physiologically active is TCII which complex in a 1:1 ratio with vitamin B12.
- The complex is then binds to specific surface receptors on developing blood cells in the bone marrow. Vitamin B12 is then released by hydrolysis. The TCII is not reutilized.
- The plasma half-life of TCII is 12 hours and congenital absence of it causes megaloblastic anaemia within weeks of birth.
- Transcobalamines I and III are α -globulins synthesized by granulocytes and known as R-binders that are found in a wide range of body fluids. TCI&III do not readily release vitamin B12 to the developing tissues. The plasma half-life is 9-12 days and congenital absence of them causes no physiological impairment.





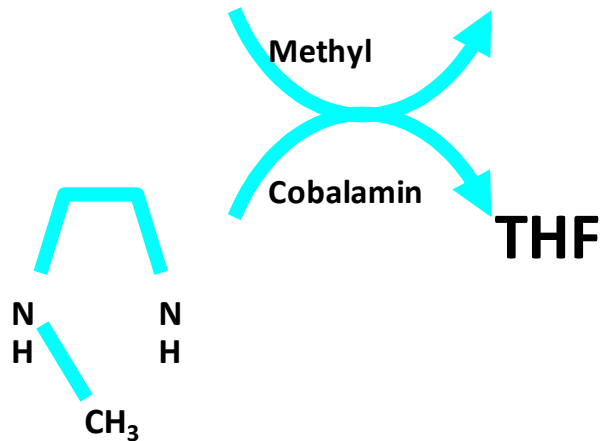
The role of vitamin B₁₂ within the body

Vitamin B₁₂ is required as coenzyme for two metabolic reaction:

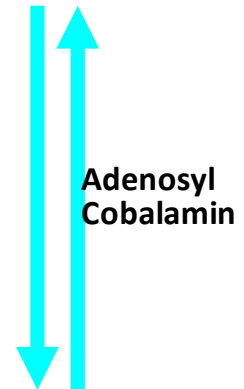
- (1) Isomerization of L-methylmalonyl CoA to succinyl CoA. This is important substrate in Hb synthesis.
- (2) Methylation of homocystine to methionine. This step is important in intracellular synthesis of folate coenzyme.

COBALAMIN REACTIONS

Homocysteine Methionine



Methylmalonyl
CoA



Succinyl CoA

Causes of Vitamin B12 Deficiency

- 1- Inadequate dietary intake.
- 2- Intestinal malabsorption.
- 3- Increased requirements, which cannot be met from the diet.
- 4- Failure of utilization of absorbed vitamin.

Inadequate Dietary Intake

This is uncommon for three main reasons:

- 1- Vitamin B12 is present in a wide range of readily available foodstuffs.
- 2- Vitamin B12 is relatively heat-stable.
- 3- Body stores of vitamin B12 are sufficient to meet the requirements for at least three years following complete cessation of dietary intake or intestinal absorption.

Malabsorption of Vitamin B12

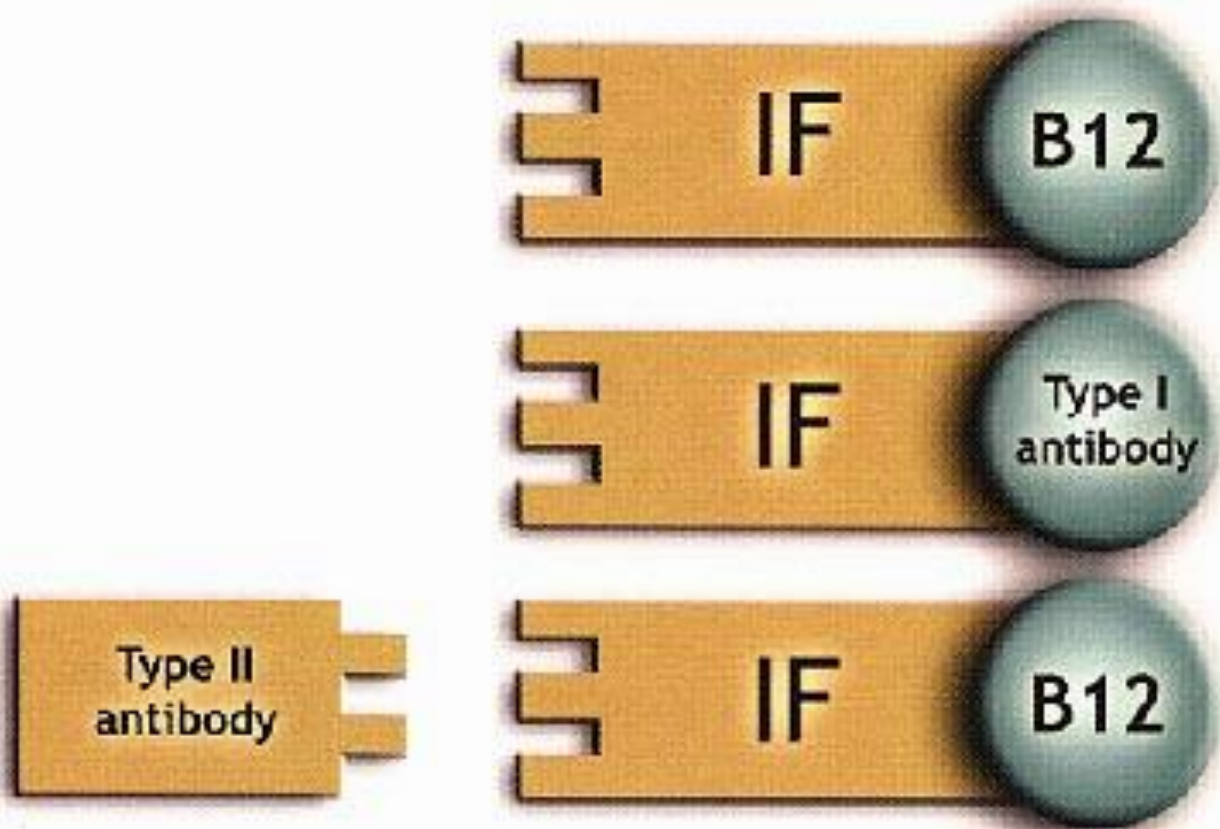
- The most common cause of the deficiency, which could be due to:
 - Lack of intrinsic factor
 - Gastrointestinal disease.
 - Drug-induced Malabsorption .

1- Lack of intrinsic factor (pernicious anaemia)

- Pernicious anemia is by far the most common cause of B12 deficiency.
- This condition is especially common among the elderly, with an observed prevalence of up to 1.9%.
- The disease is more common in women than in men and is associated with blood group A.

Pernicious anaemia

- Pernicious anemia is caused by intestinal malabsorption due to atrophy of the gastric mucosa and decreased secretion of intrinsic factor.
- One recent hypothesis suggests an autoimmune mechanism.
- The question of a relationship between pernicious anemia and *Helicobacter pylori* has also been investigated, but evidence for this theory has not been conclusive.



Pernicious anaemia

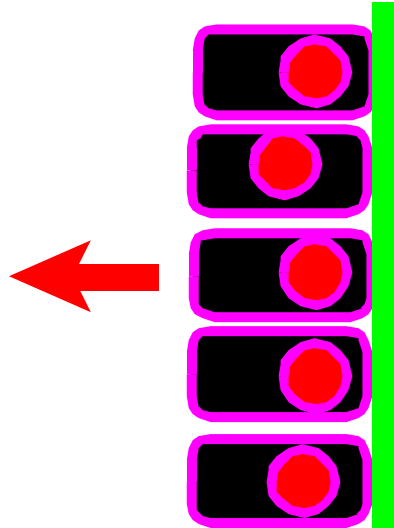
- About 90% of patients have cytotoxic IgG directed against gastric parietal cells or intrinsic factor demonstrated in serum.
- In about 75% of these the antibody is demonstrated in gastric juice. Polyclonal IgG or IgA are demonstrated in serum and gastric juice in 50% of patients with pernicious anaemia, this acts in one of two ways:
 - Either prevents the binding of vitamin B12 to intrinsic factor. (Type 1 Ab)
 - OR inhibit the absorption of VitB12-IF complex. (Type 2 Ab).

Pernicious anaemia

- Pernicious anaemia is associated with an increased incidence with congenital deficiency of autoimmune thyroid disease, rheumatoid arthritis and gastric carcinoma.
- A rare type of pernicious anaemia is associated with congenital deficiency of intrinsic factor or the synthesis of dysfunctional variant of intrinsic factor.

Stomach

Acid +
IF



Normal
gastric parietal
cells

Stomach



Atrophic gastritis
Achlorhydria
No IF

2- Gastrointestinal Disease

The most obvious follows surgical removal of the source of intrinsic factor, or the site of absorption of the vitamin.

- a)** Total gastrectomy: the anaemia is developed after depletion of the body stores, which is usually, occur within 5 years. This is severe when accompanied with iron deficiency anaemia.
- b)** Partial gastrectomy: (stagnant or blind loop syndrome)
Partial removal of the stomach, and refashioning the junction with the gut. The sterile duodenal part will be colonized with bacteria, which will consume huge amount of the vitamin.
- c)** Ileal resection or ileostomy: Involve removal of the vitB₁₂ receptor.

Gastrointestinal Disease

- d) Crohn's disease: Granulomatous disease, which most commonly affects the terminal ileum and the ascending colon. It's manifested by generalized Malabsorption of nutrients from the diet.

- e) Infestation of the gut with the fish tapeworm *Diphyllobothrium latum*, which is capable of extracting substantial quantities of vitamin B12 both complexed with intrinsic factor and free.

3- Drug-induced Malabsorption

A number of drugs have been reported to impair vitamin B12 absorption such as:

- Anticonvulsant, phenytoin
- Antimicrobial, neomycin
- Anticancer, colchicine
- Alcohol.

Increased Requirements

The requirements are increased during pregnancy. The increase is not sufficient to cause deficiency unless the pregnant was previously borderline body stores of the vitamin.