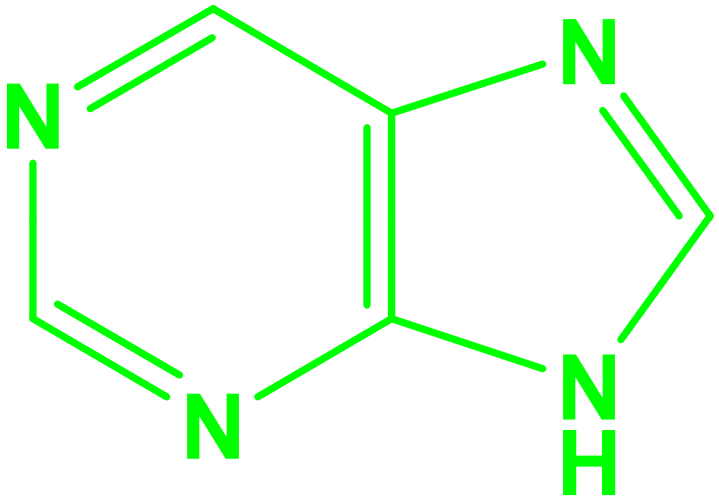
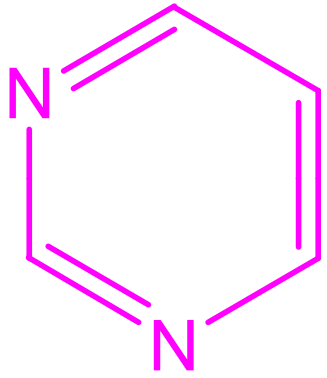
Metabolism of Nucleotides





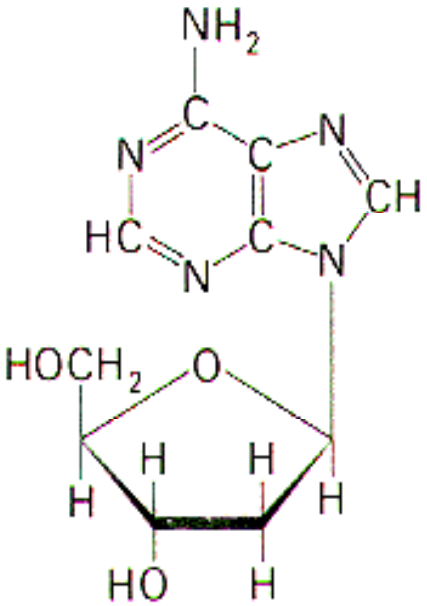
- Nucleic acid **metabolism** is the process by which nucleic acids (DNA and RNA) are synthesized and degraded. Nucleic acids are polymers of **nucleotides**.

- **Nucleotide** synthesis is an anabolic mechanism involving the chemical reaction of phosphate, pentose sugar, and a nitrogenous base.

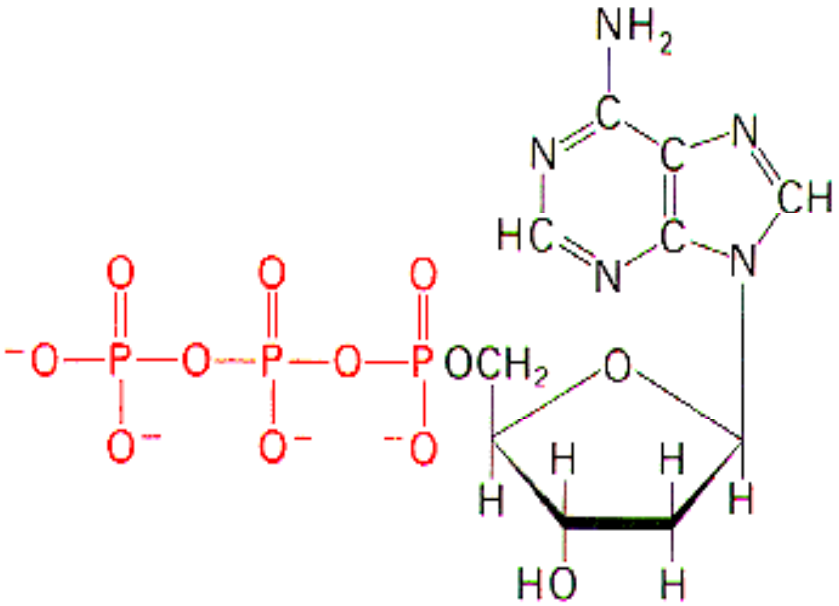
Nucleoside and Nucleotide

Nucleoside = Nitrogenous base – ribose

Nucleotide = Nitrogenous base – ribose – phosphate

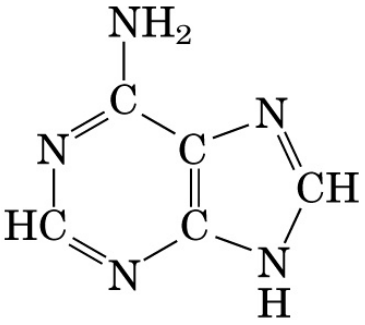


Deoxyadenosine
(A nucleoside)

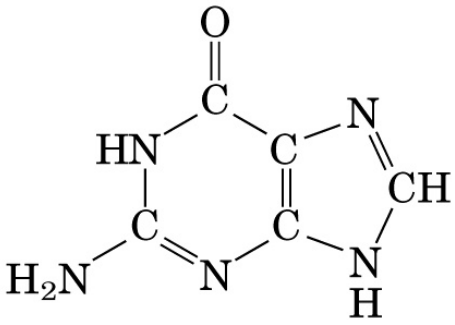


Deoxyadenosine 5′-triphosphate
(dATP)
(A nucleotide)

Purines vs Pyrimidines

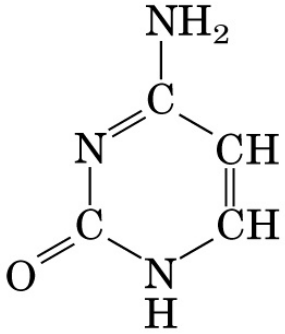


Adenine

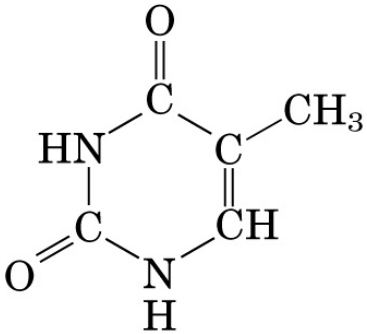


Guanine

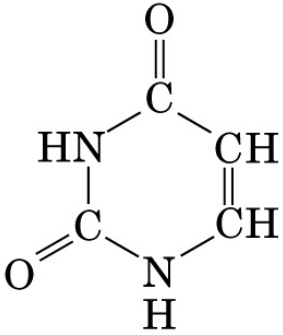
Purines



Cytosine



Thymine
(DNA)



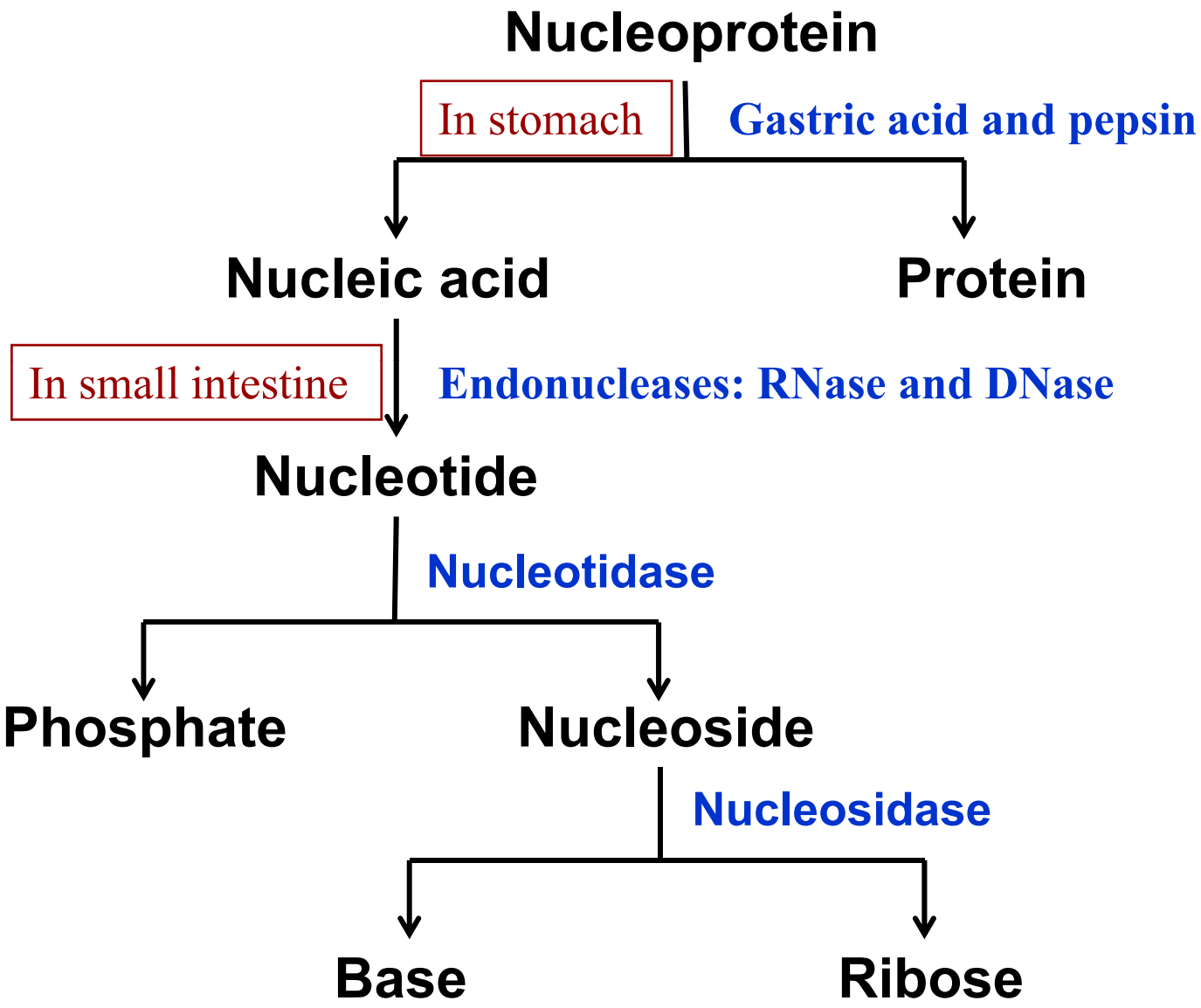
Uracil
(RNA)

Pyrimidines

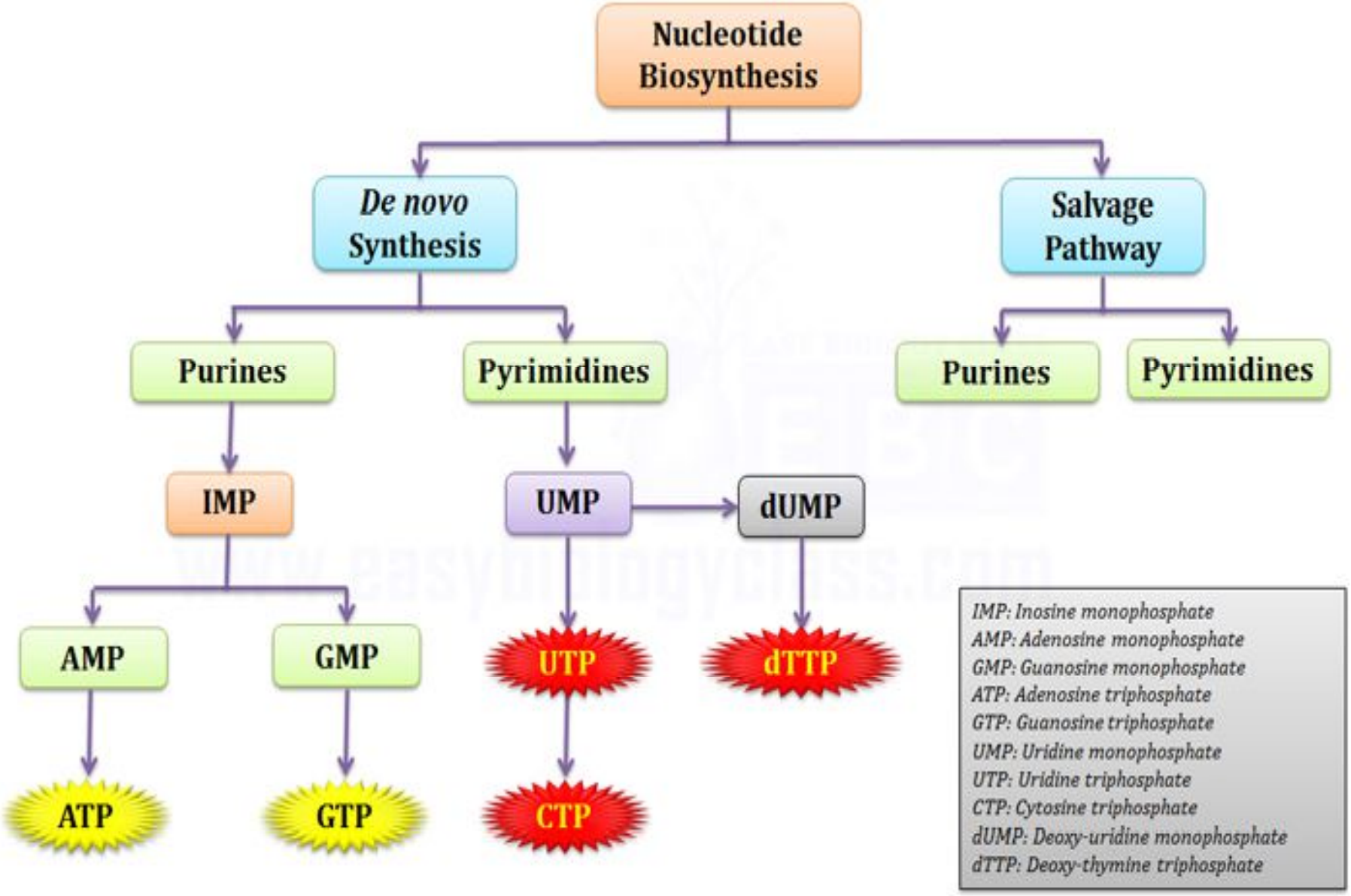
Functions of Nucleotides

- Activated precursors of DNA & RNA.
- ATP – Universal currency of energy.
- Required for activation of intermediates in many biosynthetic pathway.
- Carrier of methyl group in the form of SAM
- GTP-involved in protein biosynthesis as source of energy.
- Components of coenzymes: NAD, FAD & CoA.
- Metabolic regulators, e.g. cAMP, cGMP.

Degradation of nucleic acid



- Synthesis of purine nucleotides


Nucleotide Biosynthesis
De novo Synthesis
Salvage Pathway
Purines
Pyrimidines
Purines
Pyrimidines
IMP
UMP
dUMP
AMP
GMP
UTP
dTTP
ATP
GTP
CTP
IMP: Inosine monophosphate
AMP: Adenosine monophosphate
GMP: Guanosine monophosphate
ATP: Adenosine triphosphate
GTP: Guanosine triphosphate
UMP: Uridine monophosphate
UTP: Uridine triphosphate
CTP: Cytosine triphosphate
dUMP: Deoxy-uridine monophosphate
dTTP: Deoxy-thymine triphosphate

There are two pathways leading to nucleotides

- **De novo synthesis:** **The synthesis of nucleotides begins with their metabolic precursors: *amino acids, ribose-5-phosphate, CO_2, and one-carbon units*.**
- **Salvage pathways:** **The synthesis of nucleotide by recycle the free bases or nucleosides released from nucleic acid breakdown.**

Biosynthetic Routes: De novo and salvage pathways

- Most organisms can synthesize purine and pyrimidrne nucleotides from low-molecular-weight precursors in amounts sufficient for their needs. These so-called de novo pathways are essentially identical throughout the biological world.
- Salvage pathways involve the utilization of preformed purme and pyrimidine compounds that would be otherwise lost to biodegradation. Salvage pathways represent important sites for manipulation of biological systems.

De novo synthesis

- **Site:**
  - **in cytosol of liver, small intestine and thymus**
- **Characteristics:**

  a. Purines are synthesized using 5-phosphoribose(R-5-P) as the starting material step by step.

  b. PRPP(5-phosphoribosyl-1-pyrophosphate) is active donor of R-5-P.

  c. AMP and GMP are synthesized further at the base of IMP(Inosine-5'-Monophosphate).

Contd.

- The pathway can be divided into two stages.
- Stage one : formation of inosine monophosphate (IMP)
- Stage two : conversion of IMP to either AMP or GMP

- Stage One

- PRPP synthetase
- R5P + ATP----------→PRPP + AMP

- amidotransferase
- PRPP + Gln----------→PRA + Glu

Contnd.

Stage Two

- The conversion of IMP either to AMP or GMP requires two reactions.

GTP,Mg++,adenylosuccinate synthase

- IMP + Asp----------→adenylosuccinate

adenylosuccinate lyase

- Adenylosuccinate----------→AMP + fumarate

Contd.

IMP dehydrogenase

- IMP + H2O + NAD+----------→XMP + NADH + H+

ATP, Mg++, GMP synthase

- XMP + Gln----------→GMP + Glu

- Nucleoside triphosphates are the most common nucleotide used in metabolism.
- ATP is synthesized from ADP and Pi via oxidative. phosphorylation or substrate level phosphorylation.

Contd.

- ADP is synthesized from AMP in a reaction catalyzed by adenylate kinase.

AMP + ATP----------→ 2ADP

- Other NTPs are also synthesized in ATP-requiring reactions catalyzed by corresponding NMP kinases.

NMP + ATP----------→NDP + ADP

- NDP kinase catalyzes the formation of NTP.

NDP + ATP----------→NTP + ADP

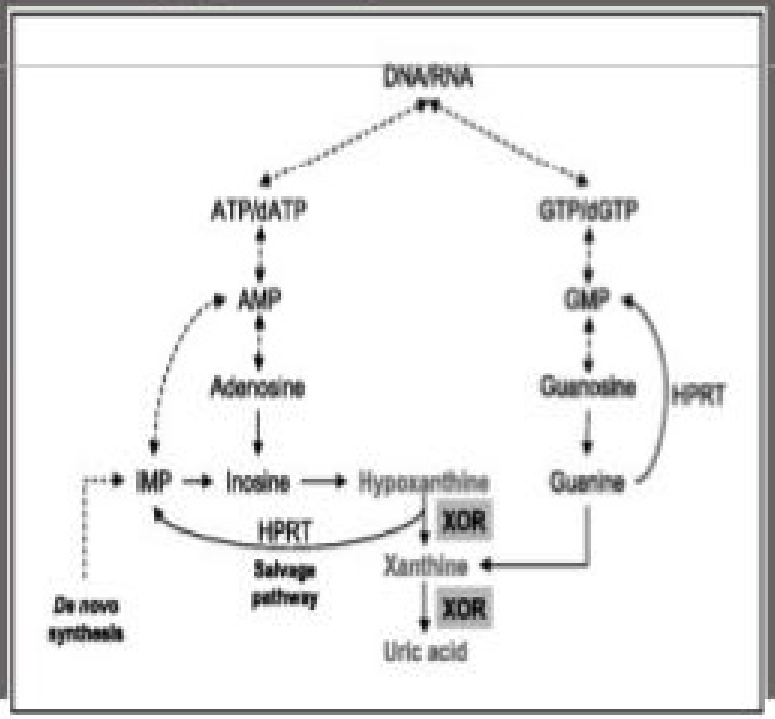
Regulation of de novo Pathway

- PRPP activates amidotransferase.
- IMP , AMP and GMP inhibit PRPP synthetase.
- AMP inhibits conversion of IMP to GMP and GMP inhibits conversion of IMP to AMP.
- ATP stimulates conversion of IMP to GMP and GTP stimulates conversion of IMP to AMP.
- That ensures a balanced synthesis of both families of purine nucleotides.

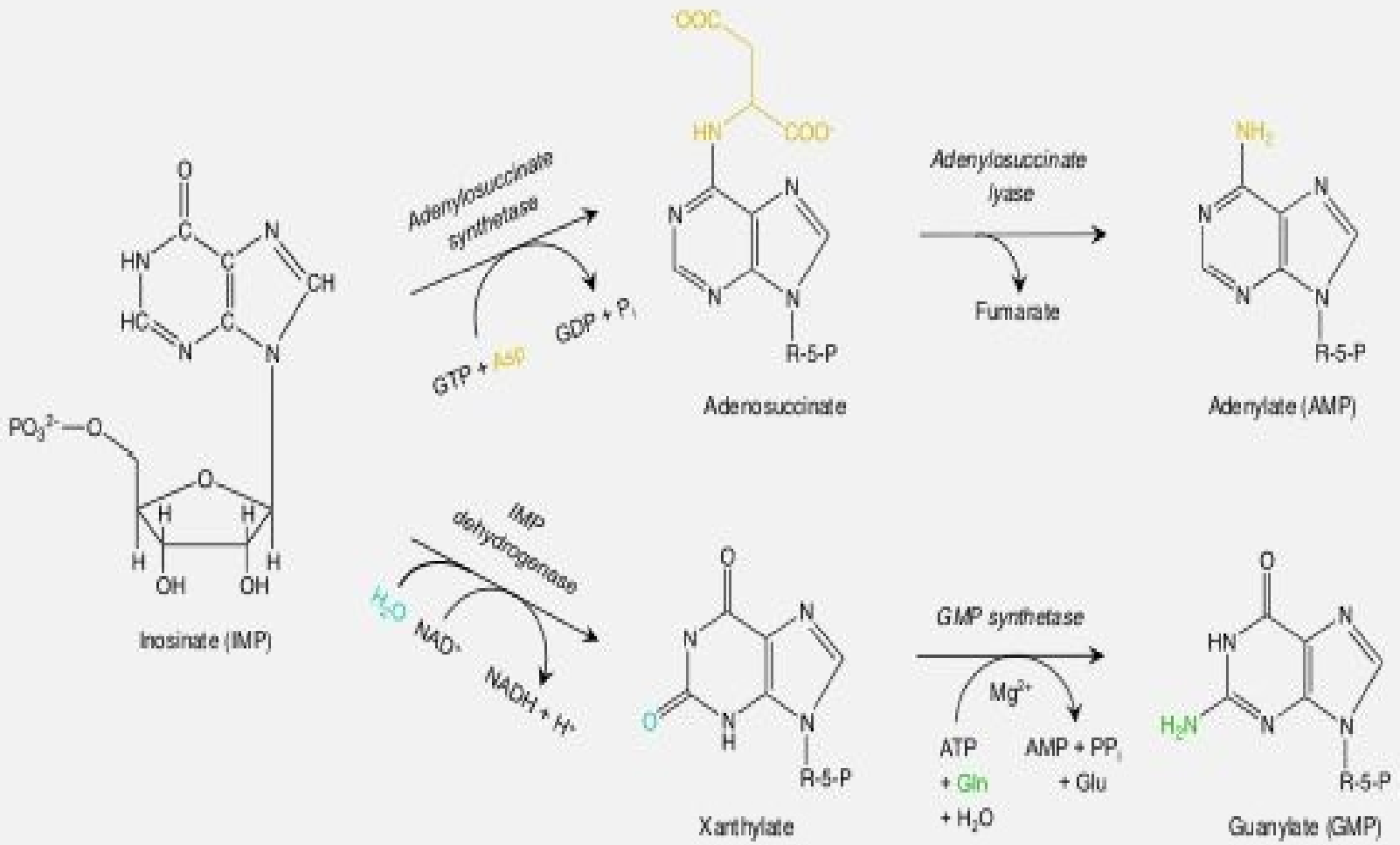
Salvage Pathway of Purine Nucleotides

- Many cells have mechanisms to retrieve purine bases and purine nucleosides. They are used to synthesize purine nucleotides.

  This is the salvage pathway.



❖ Formation of AMP and GMP from IMP branch point



Contd.

- From Base to Nucleotides

APRT

- A + PRPP----------→AMP + ppi

HGPRT

- H + PRPP----------→ IMP + ppi

HGPRT

- G + PRPP----------→GMP + ppi

Contnd.

- From Nucleoside to Nucleotide

AR kinase

- AR + ATP----------→AMP + ADP

- In comparison to de novo pathway, salvage pathway is energy-saving.

- In brain and bone marrow tissues salvage pathway is the only pathway of nucleotide synthesis.

- Deficiency of HGPRT causes Lesch Nyhan syndrome

AR – andrenergic receptors.

Antimetabolites of purine nucleotides

- Antimetabolites of purine nucleotides are structural analogs of purine, amino acids and folic acid.
- They can interfere, inhibit or block synthesis pathway of purine nucleotides and further block synthesis of DNA, RNA, and proteins.
- Widely used to control cancer.

Antimetabolites of Purine Nucleotides

- Antimetabolites of purine nucleotides are analogues of purine, amino acids or folic acid.
- They either act as competitive inhibitors of enzymes in purine nucleotides synthesis or can be incorporated into purine nucleotides.
- Thus they block purine nucleotides synthesis or interfere in nucleic acids synthesis.

Contd.

- 6-MP and 6-MG are purine analogues

.

- 6-MP nucleotide is structurally similar to IMP and inhibits conversion of IMP to AMP and GMP.

- It also blocks synthesis of PRA from PRPP ,, synthesis of GMP and IMP from G and H respectively.

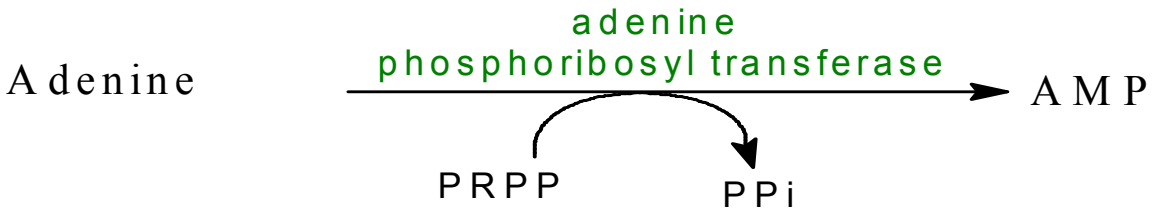
Contd.

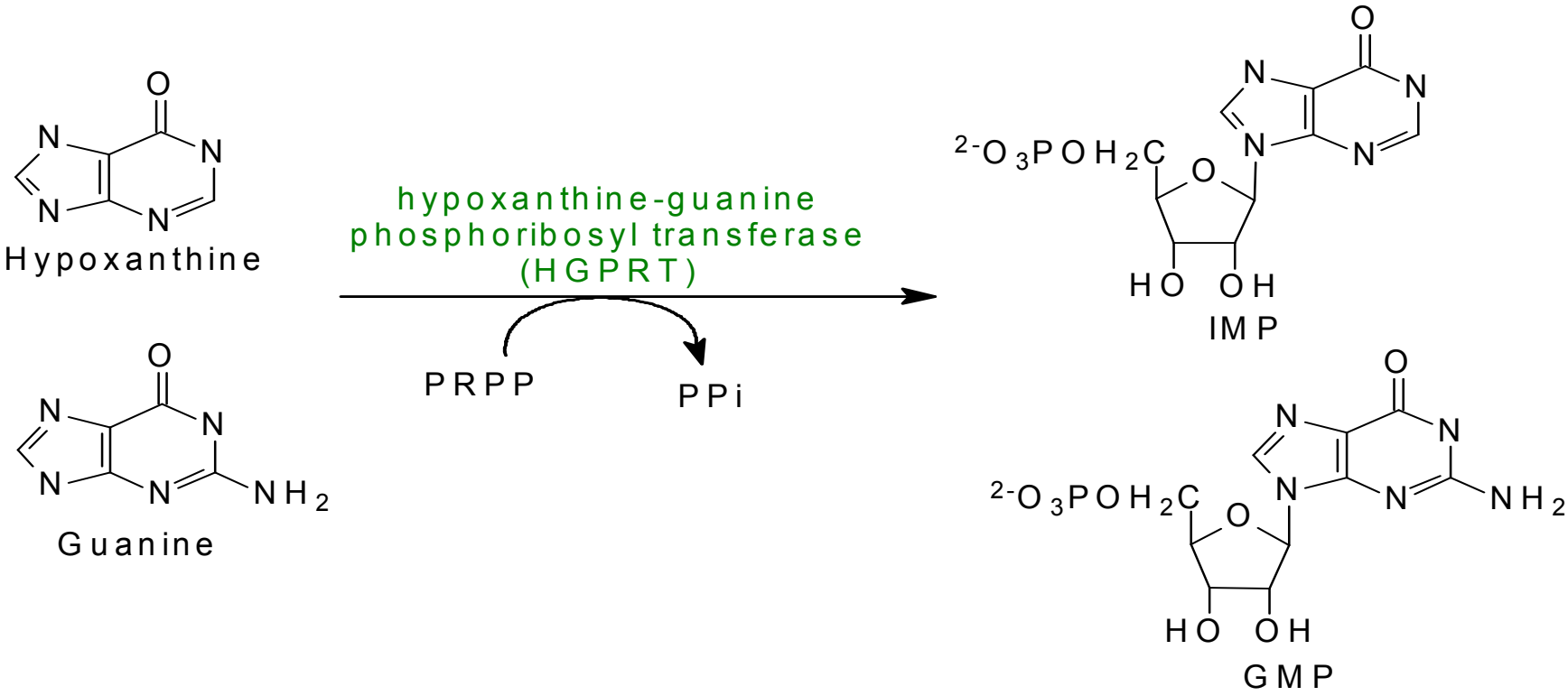
- Azaserine and diazonorleucine are amino acid analogues.
- They are analogues of Gln and interfere with Gln in purine nucleotide de novo synthesis.
-

2. Salvage pathway

- **Purine bases created by degradation of RNA or DNA and intermediate of purine synthesis directly converted to the corresponding nucleotides.**
-
- **The significance of salvage pathway :**
  - **Save the fuel.**
  - **Some tissues and organs such as brain and bone marrow are only capable of synthesizing nucleotides by salvage pathway.**
- **Two phosphoribosyl transferases are involved:**
  - **APRT (adenine phosphoribosyl transferase) for adenine.**
  - **HGPRT (hypoxanthine guanine phosphoribosyl transferase) for guanine or hypoxanthine.**

Purine Salvage Pathway



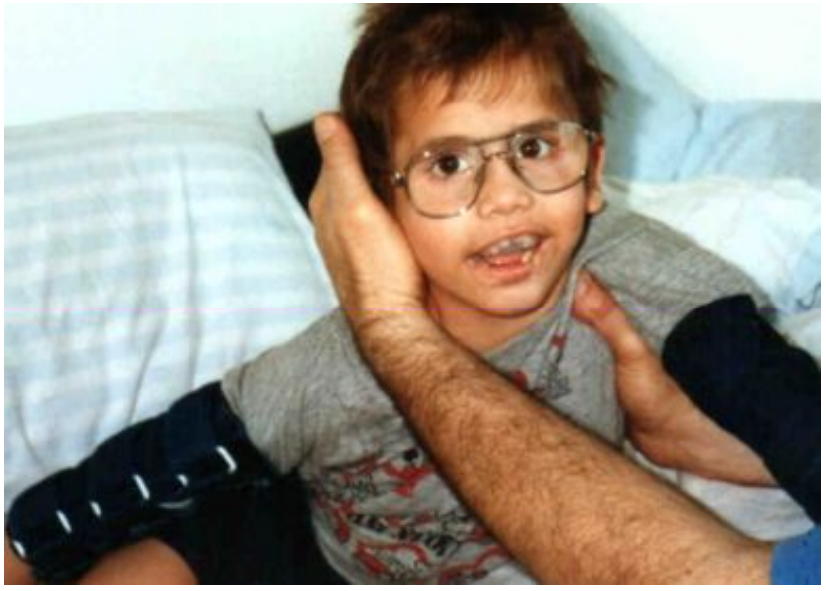


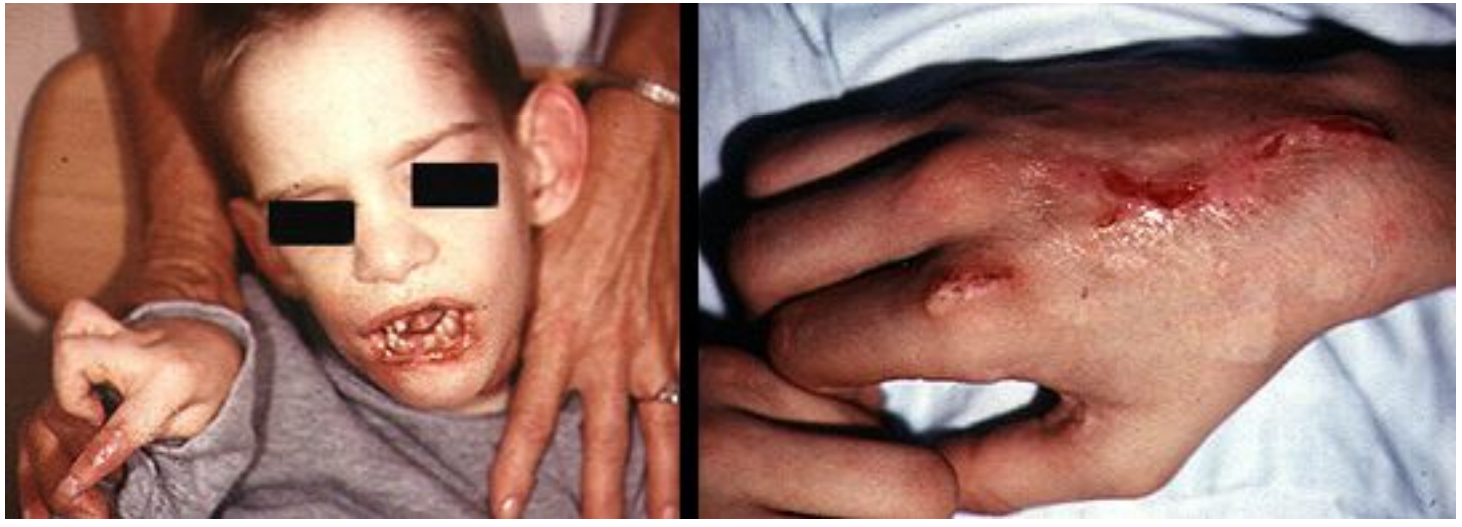
Absence of activity of HGPRT leads to Lesch-Nyhan syndrome.

Lesch-Nyhan syndrome

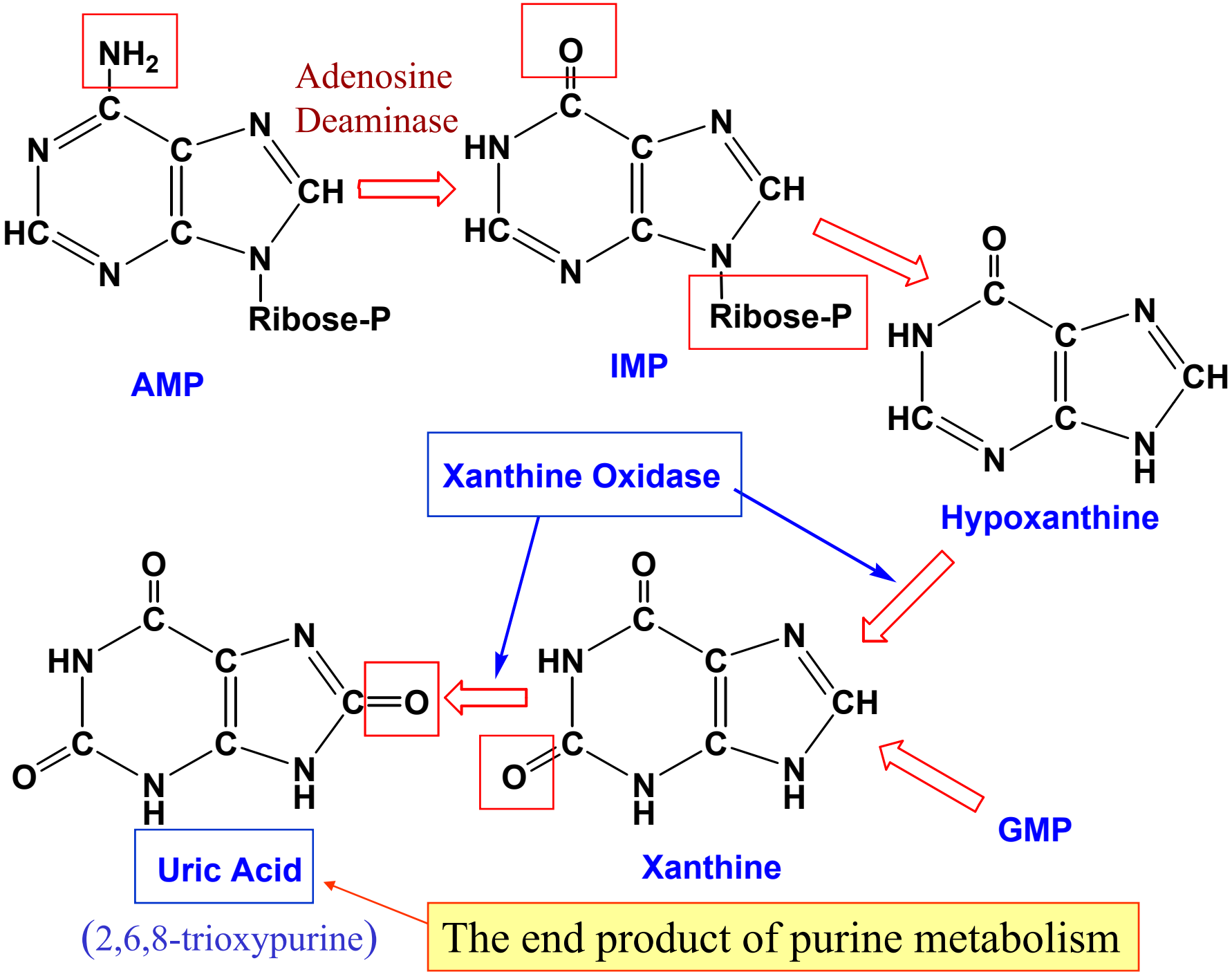
- first described in 1964 by Michael Lesch and William L. Nyhan.
- there is a defect or lack in the HGPRT enzyme
- Sex-linked metabolic disorder: only males
- the rate of purine synthesis is increased about 200-fold
  - Loss of HGPRT leads to elevated PRPP levels and stimulation of de novo purine synthesis.
- uric acid level rises
- in addition there are mental aberrations
- patients will self-mutilate by biting lips and fingers off

Lesch-Nyhan syndrome





DEGRADATION OF PURINE NUCLEOTIDES


NH2
Adenosine
Deaminase
O
Ribose-P
Ribose-P
AMP
IMP
Hypoxanthine
Xanthine Oxidase
GMP
Uric Acid
Xanthine
(2,6,8-trioxypurine)
The end product of purine metabolism

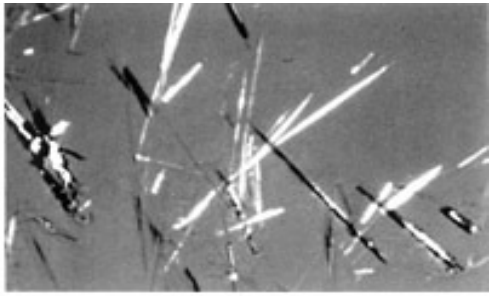
Uric acid

- Uric acid is the excreted end product of purine catabolism in primates, birds, and some other animals.

- The rate of uric acid excretion by the normal adult human is about 0.6 g/24 h, arising in part from ingested purines and in part from the turnover of the purine nucleotides of nucleic acids.

- The normal concentration of uric acid in the serum of adults is in the range of 3-7 mg/dl.

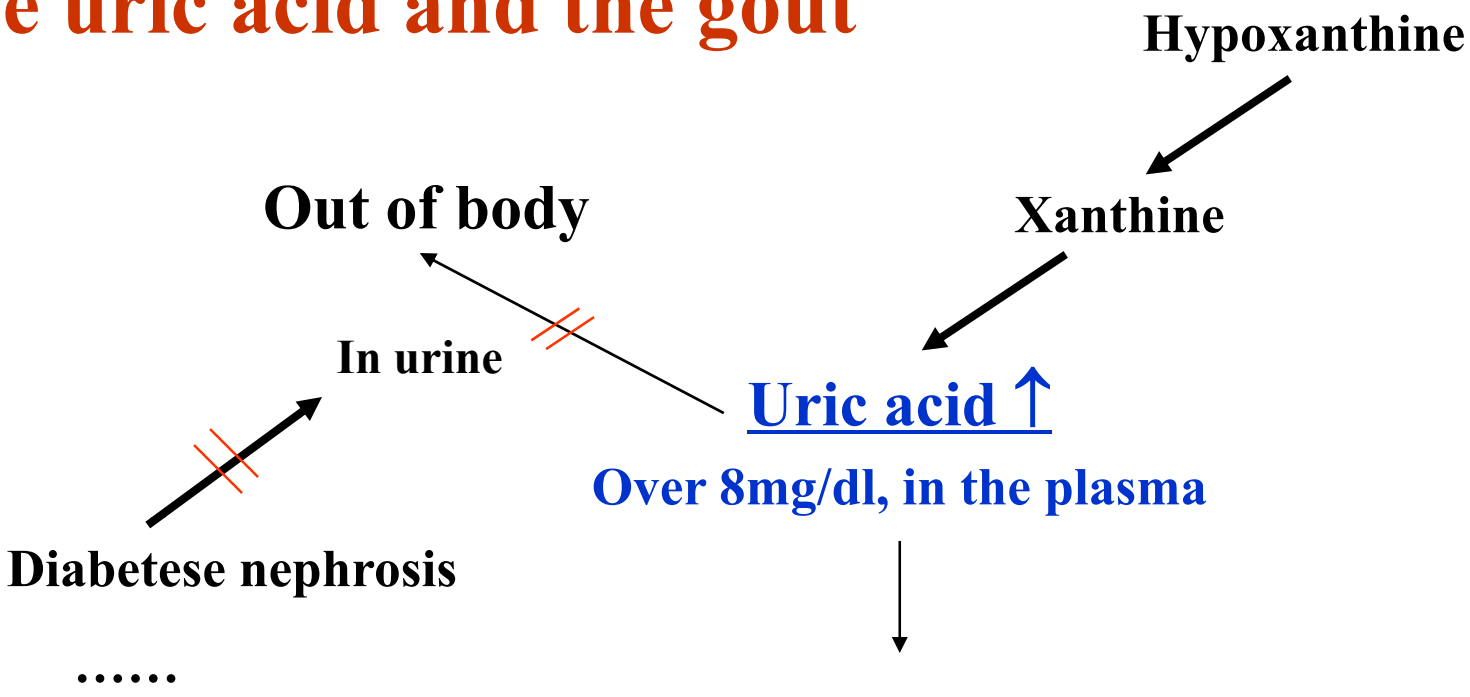
GOUT



Sodium Urate Crystals

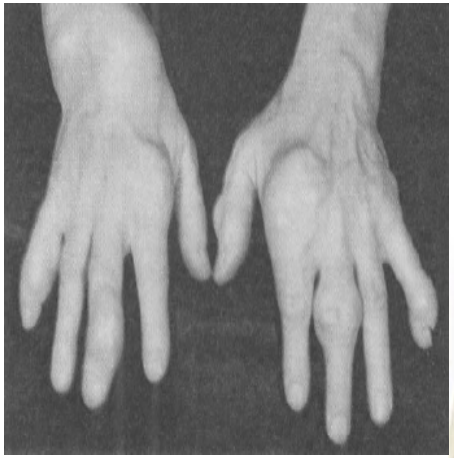
- The disease gout, is a disease of the joints, usually in males, caused by an elevated concentration of uric acid in the blood and tissues.
- The joints become inflamed, painful, and arthritic, owing to the abnormal deposition of crystals of sodium urate.
- The kidneys are also affected, because excess uric acid is deposited in the kidney tubules.

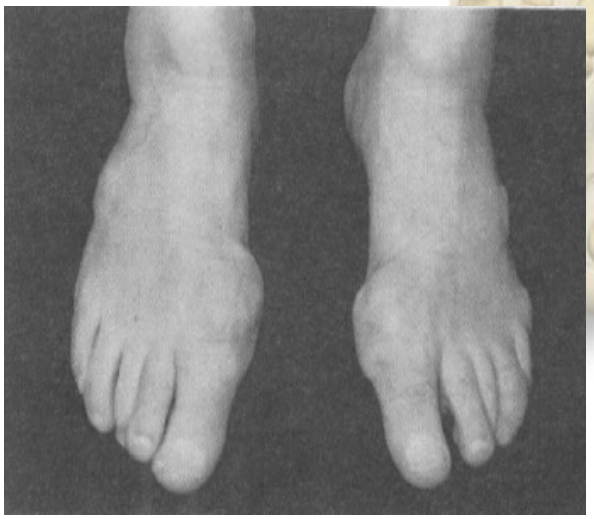
The uric acid and the gout

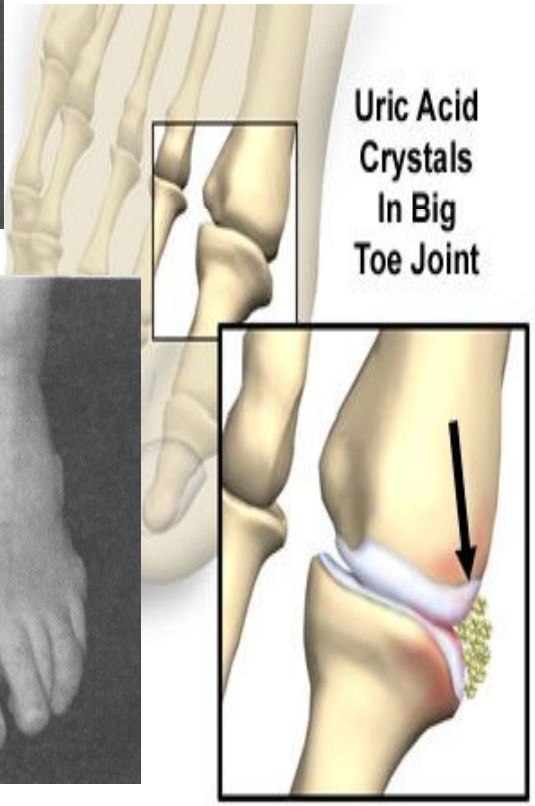


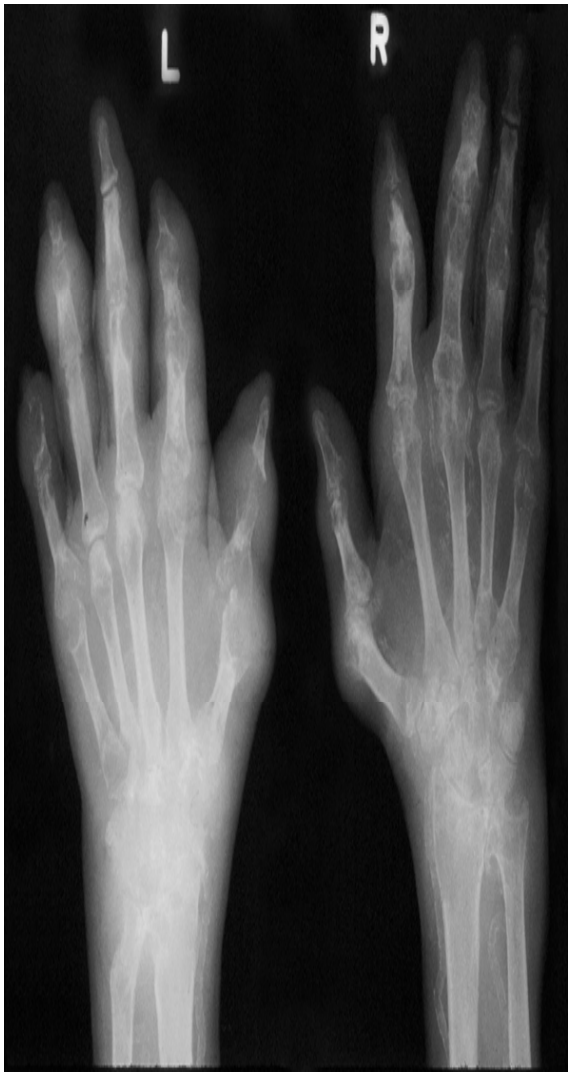
Gout, Urate crystallization

in joints, soft tissue, cartilage and kidney

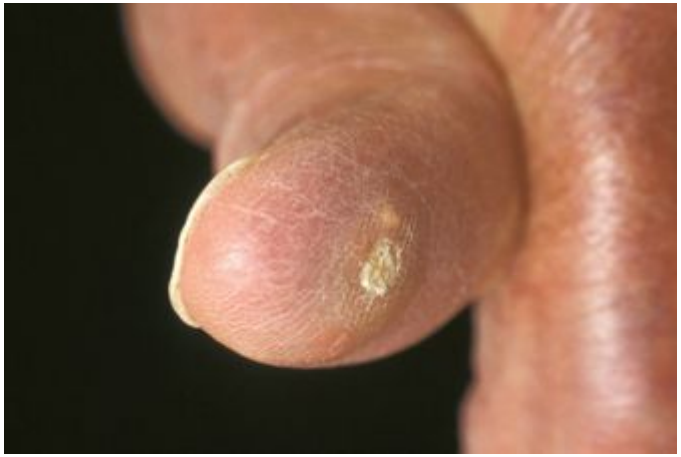


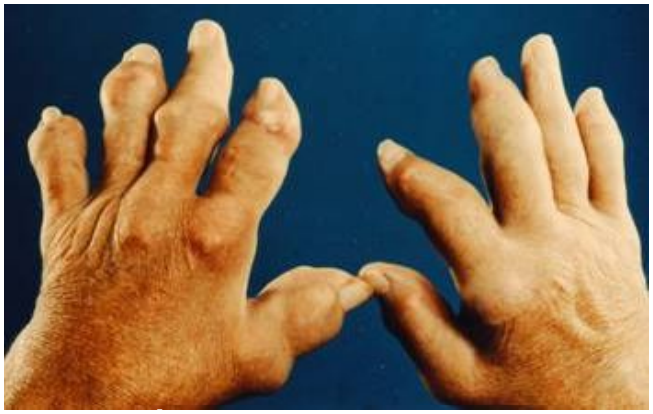


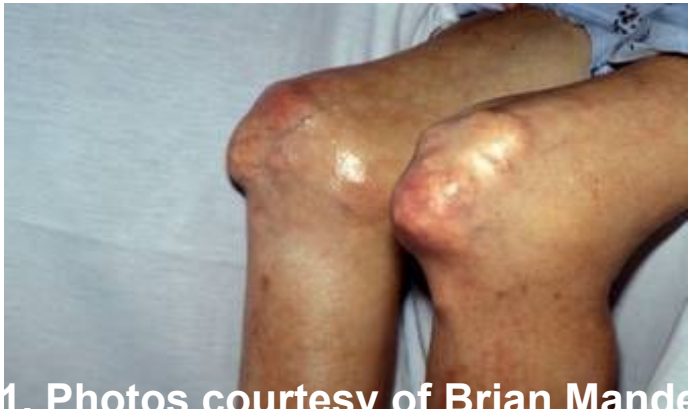

Uric Acid
Crystals
In Big
Toe Joint

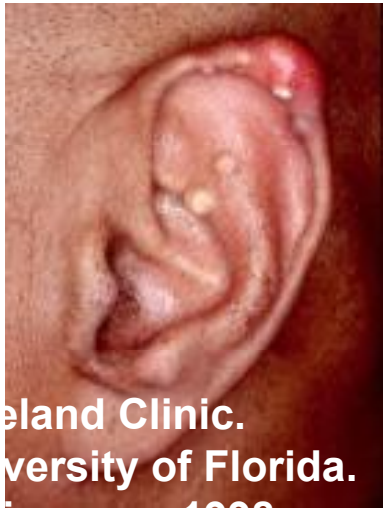

L
R

Advanced Gout
Clinically Apparent Tophi







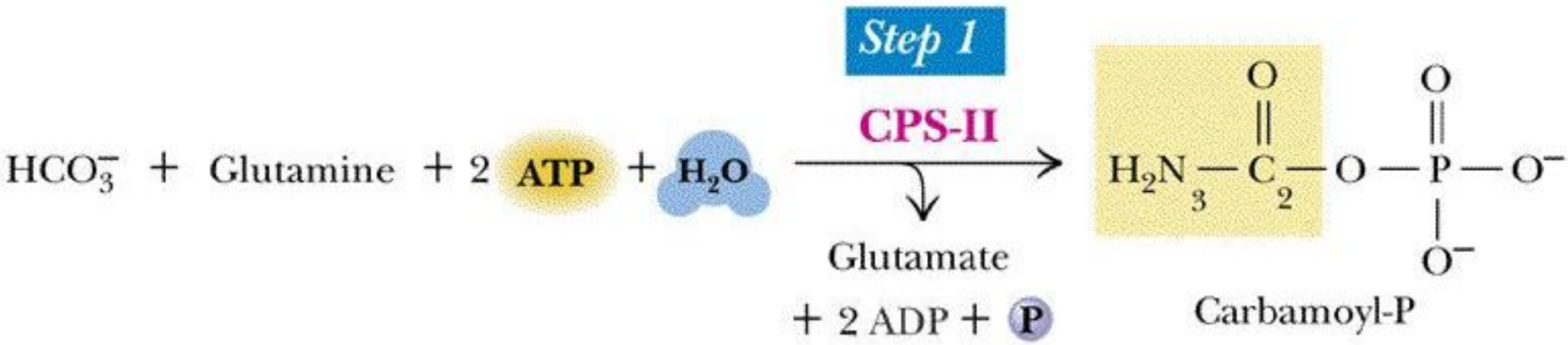


- SYNTHESIS OF PYRIMIDINE NUCLEOTIDES

De novo synthesis

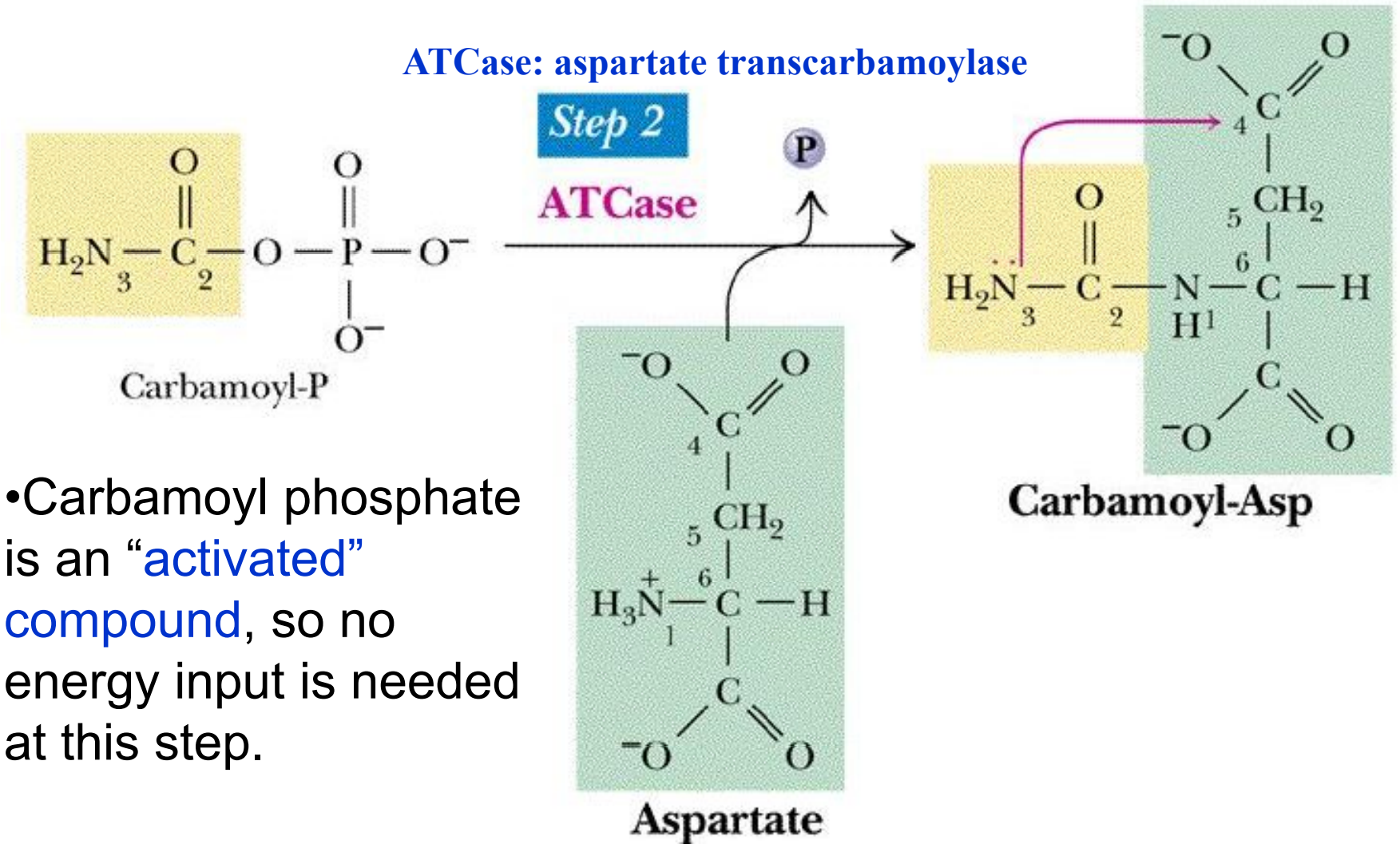
- shorter pathway than for purines
- Pyrimidine ring is made first, then attached to ribose-P (unlike purine biosynthesis)
- only 2 precursors (aspartate and glutamine, plus HCO_3^-) contribute to the 6-membered ring
- requires 6 steps
- the product is UMP (uridine monophosphate)

Step 1: synthesis of carbamoyl phosphate



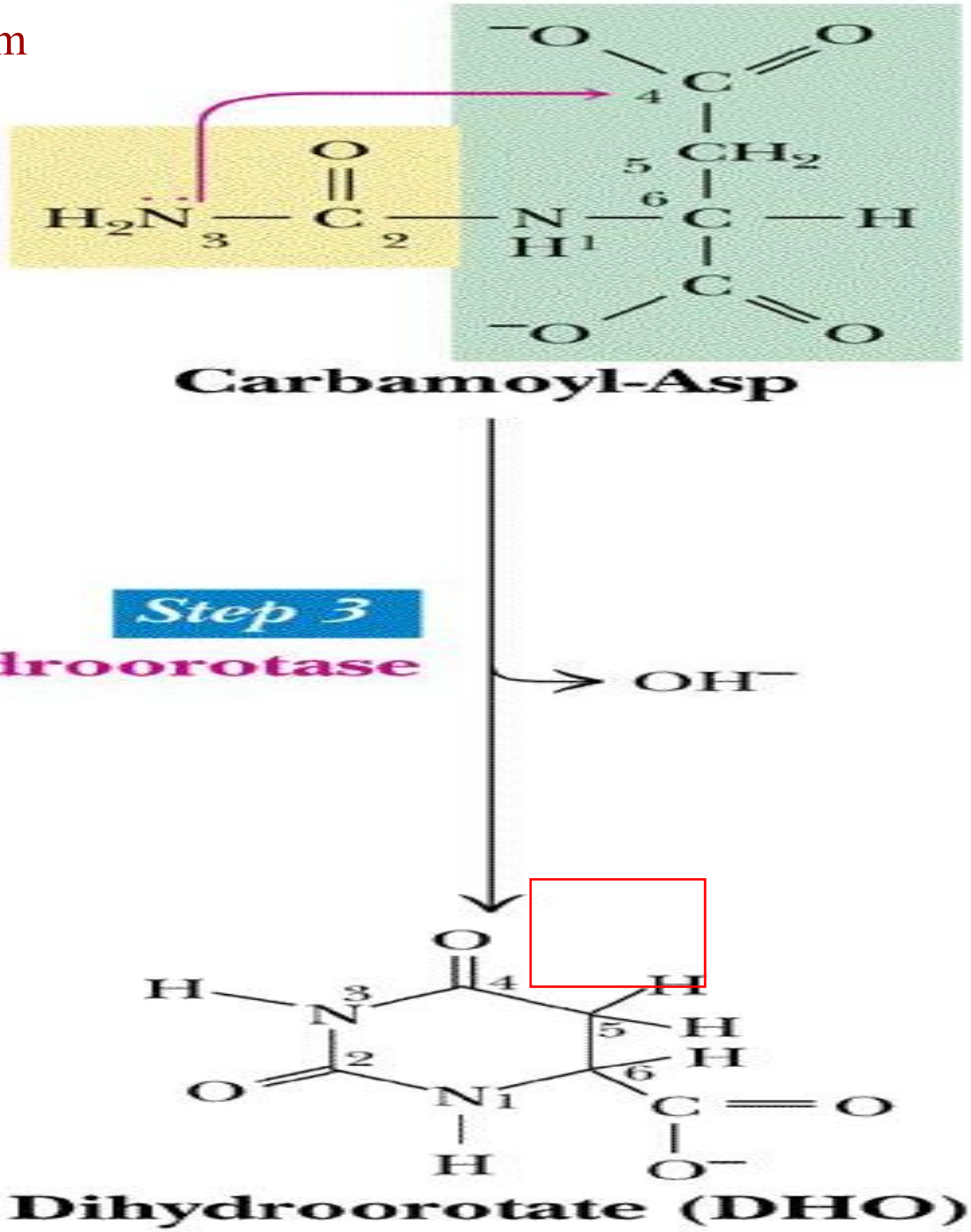
- Carbamoyl phosphate synthetase(CPS) exists in 2 types:
  - CPS-I, a mitochondrial enzyme, is dedicated to the urea cycle and arginine biosynthesis.
  - CPS-II, a cytosolic enzyme, used here. It is the committed step in animals.

Step 2: synthesis of carbamoyl aspartate

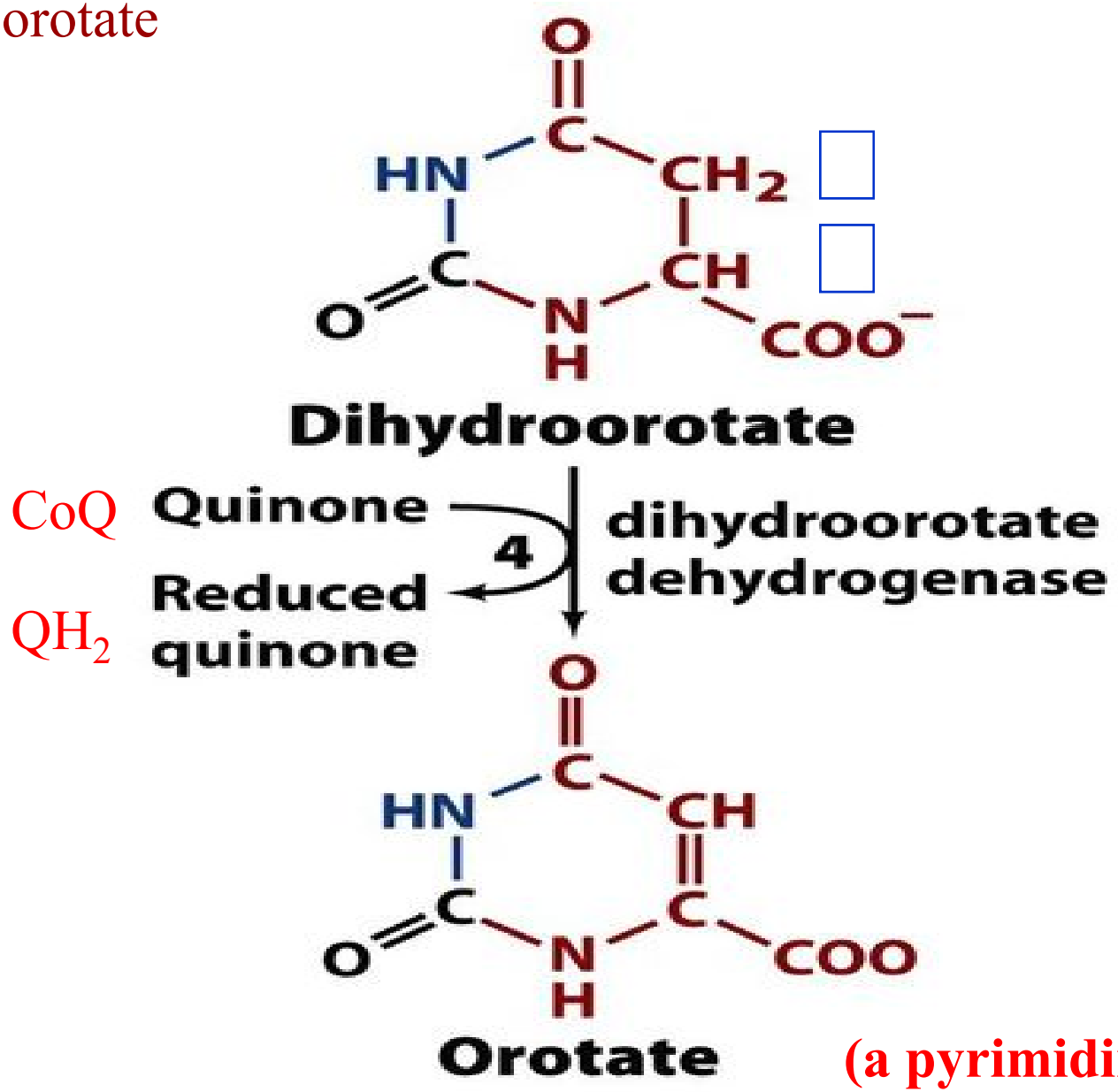


- Carbamoyl phosphate is an "activated" compound, so no energy input is needed at this step.

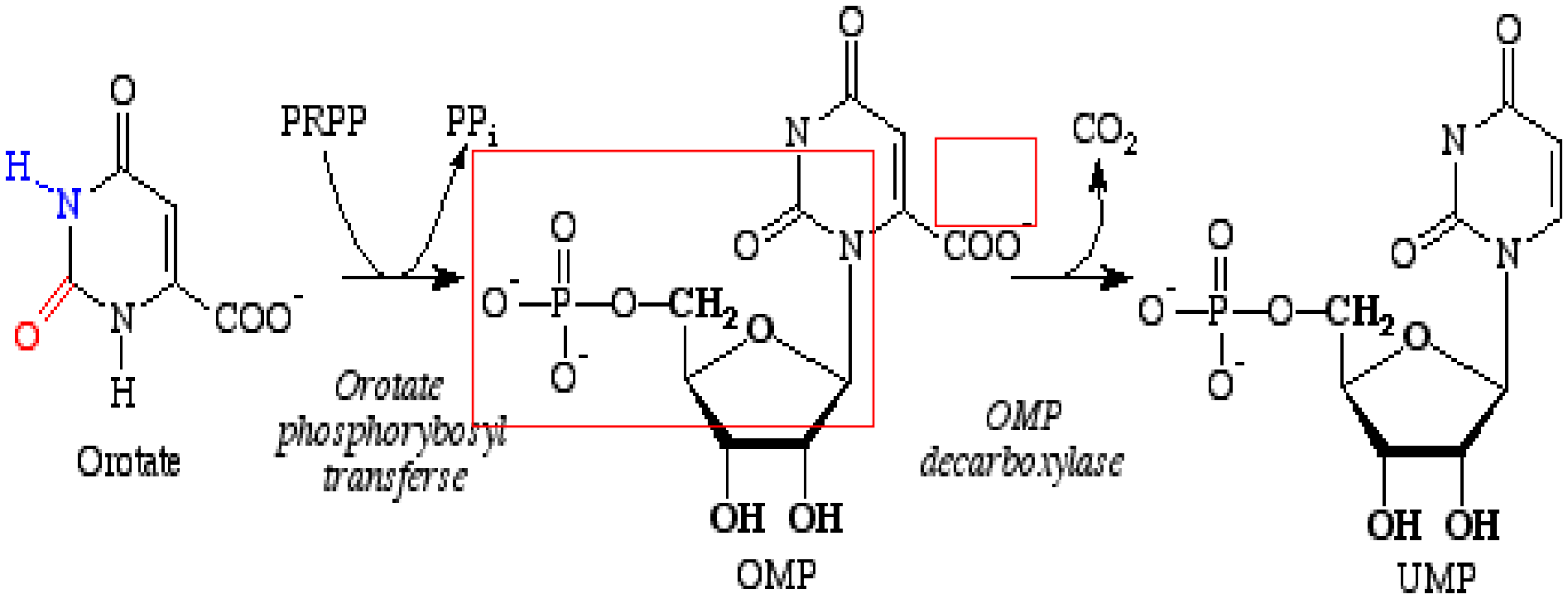
Step 3: ring closure to form dihydroorotate



Step 4: oxidation of dihydroorotate to orotate



Step 5: acquisition of ribose phosphate moiety



Step 6: decarboxylation of OMP

SYNTHESIS OF PYRIMIDINE NUCLEOTIDES

Garrett/Grisham, Biochemistry with a Human Focus
Figure 21.36