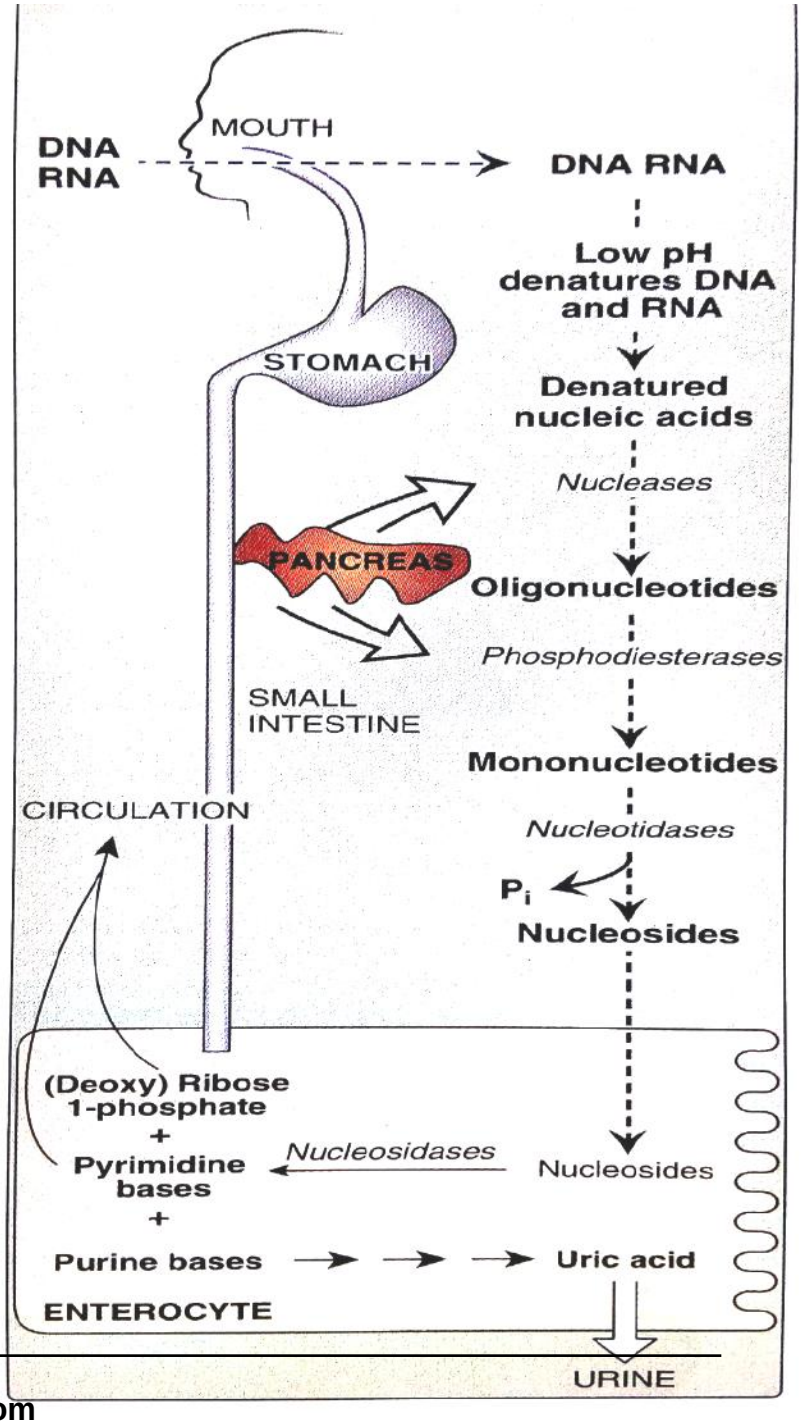
# PURINE METABOLISM

- The process of synthesis of complex end product(s) in a metabolic pathway from simple precursors molecule is called as de novo synthesis (de novo = 'anew', i.e. starting 'from scratch')
- The three processes that contribute to purine nucleotide biosynthesis are, in order of decreasing importance.

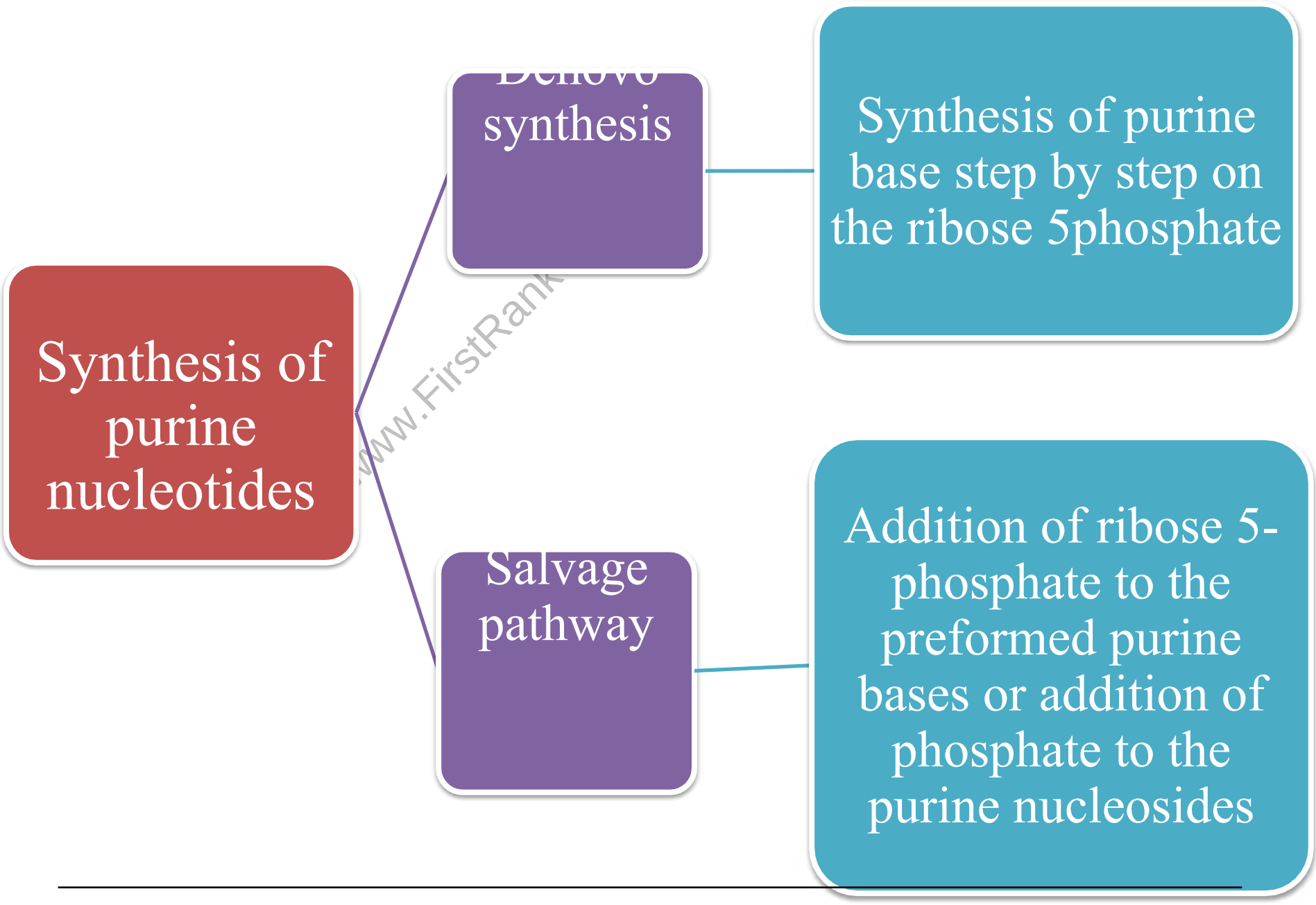
1. Synthesis from amphibolic intermediates (synthesis de novo).
2. Phosphoribosylation of purines.
3. Phosphorylation of purine nucleosides.

# DIGESTION OF NUCLEIC ACIDS

- The nucleic acids in the diet are hydrolyzed to a mixture of nucleotides by ribonuclease and deoxy ribonuclease present in pancreatic and intestinal secretions.
- Then nucleotidases liberate the phosphate from nucleotides.
- The resulting nucleosides are hydrolyzed by nucleosidases forming free bases and pentose sugars.



- Dietary purine bases are not used for synthesis of tissue nucleic acids.
- Instead they are degraded to uric acid in the enterocytes.
- Most of the uric acid enters the blood and is eventually excreted in the urine.
- Humans synthesize the nucleic acids and their derivatives ATP, NAD+, coenzyme A, etc, from amphibolic intermediates.
- However, injected purine or pyrimidine analogs, including potential anticancer drugs, may nevertheless be incorporated into DNA.
- The incorporation of injected [$^3$H]thymidine into newly synthesized DNA thus can be used to measure the rate of DNA synthesis



- Denovo purine biosynthesis occurs from basic precursors and a new purine ring is synthesized using various metabolic intermediates as sources of carbon, nitrogen etc.
- This is then used to produce nucleosides and nucleotides

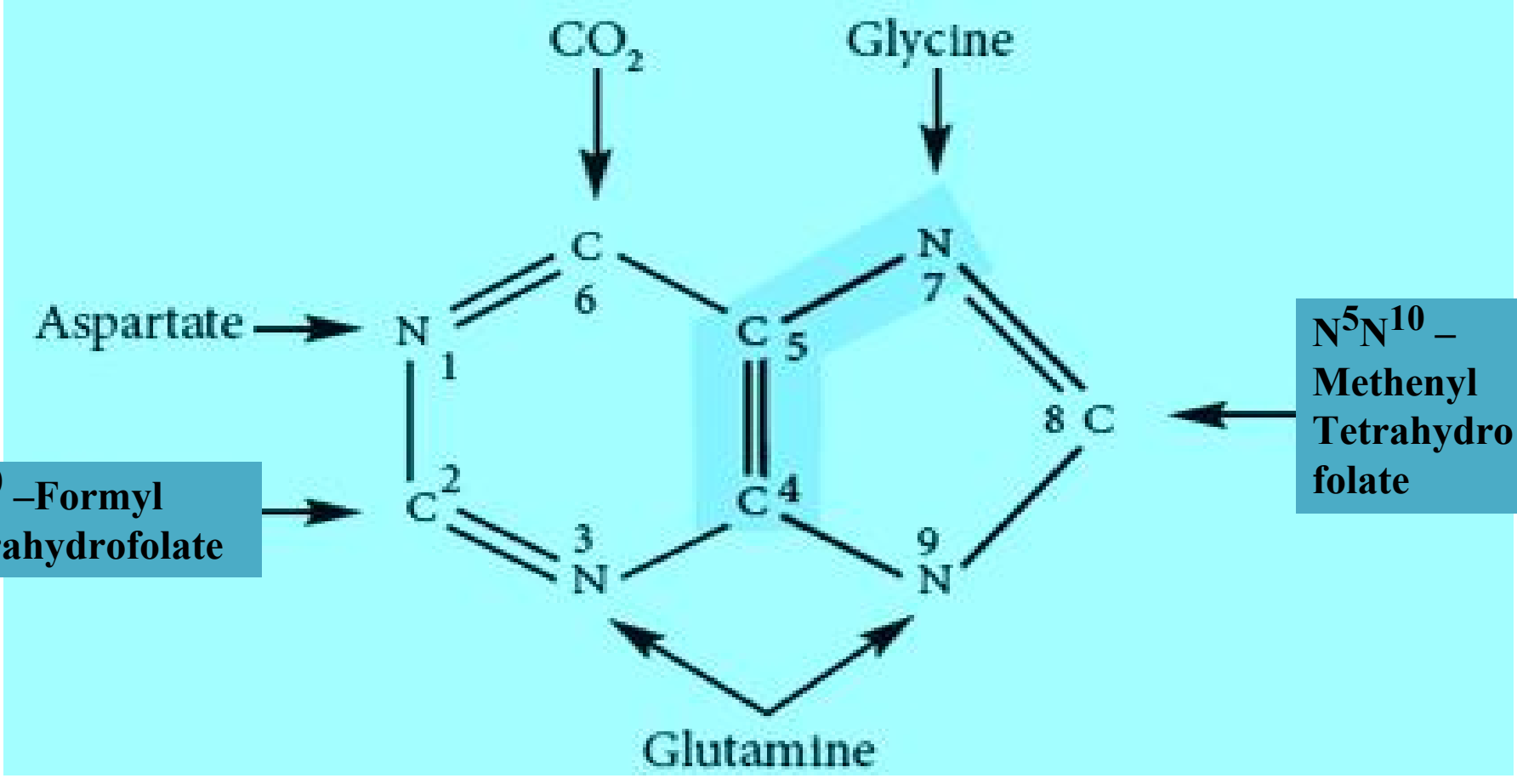
- In de novo purine biosynthesis pathway α- D-ribose 5 -phosphate is used to synthesize a nucleotide inosine monophosphate ( IMP).

- This IMP is then converted into AMP and GMP which are the end products of this pathway.

- There is regulation both at the level of synthesis of IMP and then its conversion into AMP and GMP.

- Denovo purine biosynthesis is an expensive process for the cell and uses many metabolic intermediate in the synthesis of purine ring.

# Sources of different atoms of purine ring




Sources of nitrogen and carbon atoms of the purine ring

# Denovo synthesis of purine nucleotides

- Purines are synthesized by most of the tissues
- The major site is-- liver .
- Erythrocytes, polymorphonuclear leukocytes & brain cannot produce purines.
- Subcellular site--- cytoplasm

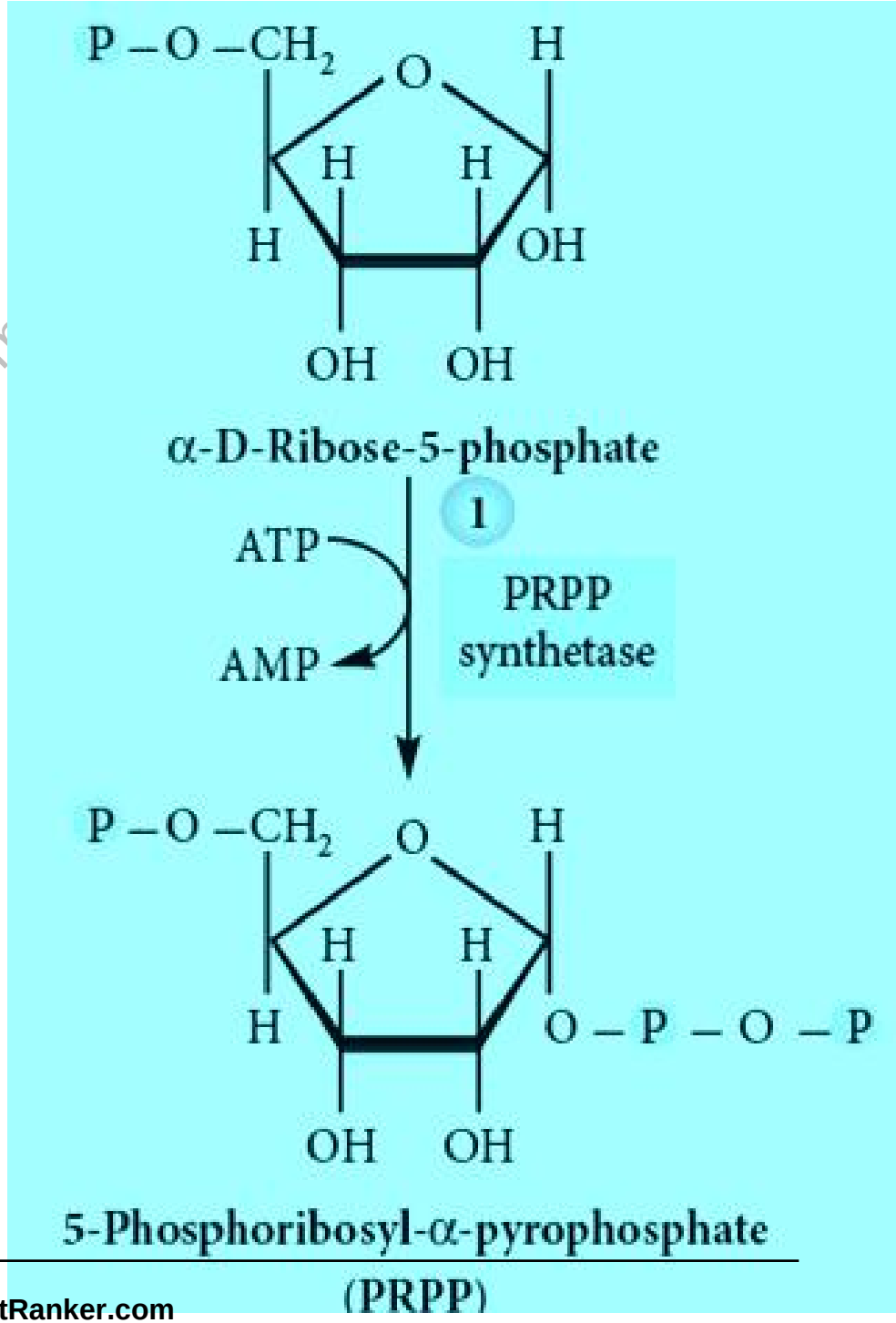
.

# Denovo synthesis of purine nucleotides

## SYNTHESIS OF IMP

# STEP 1

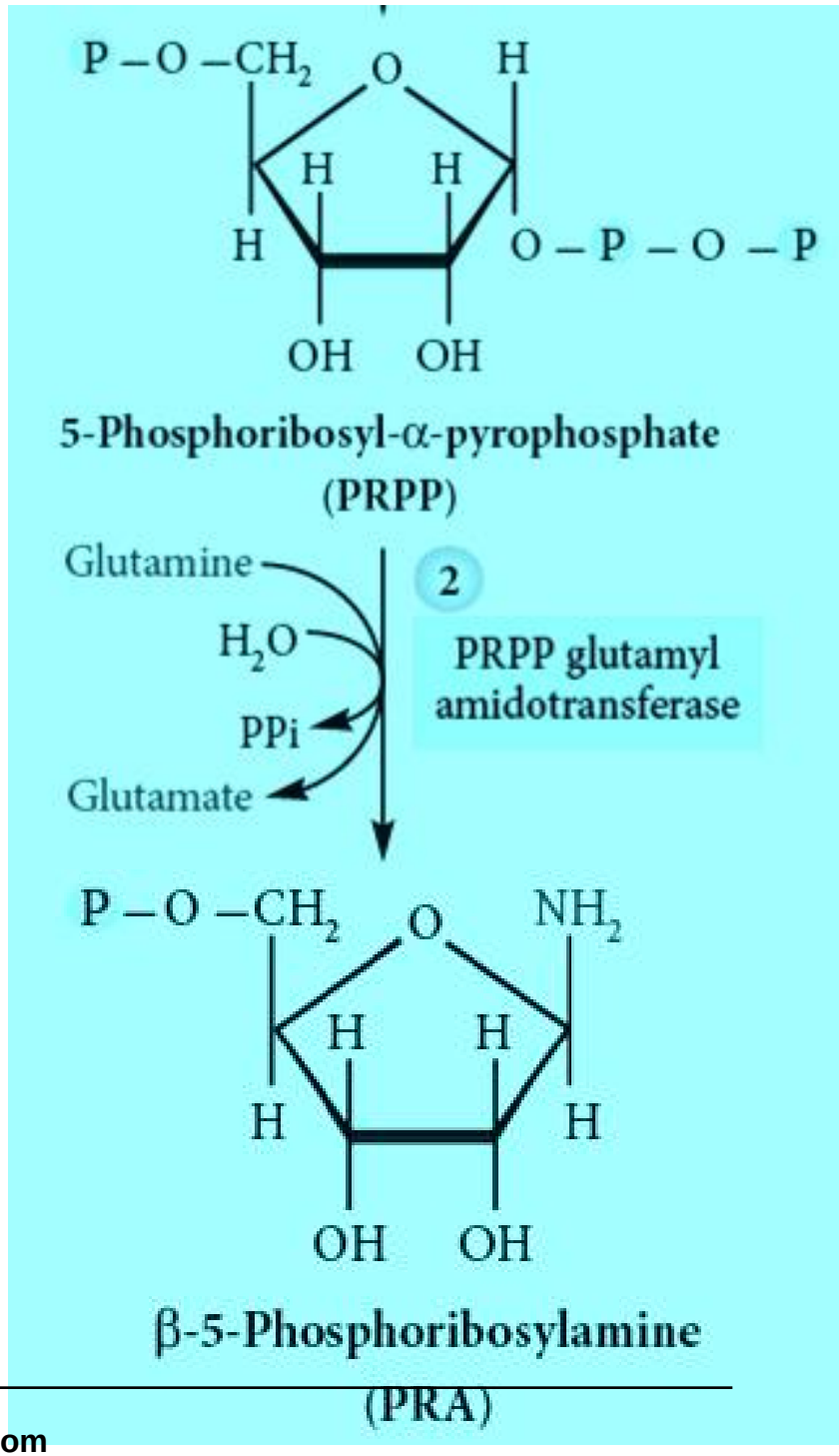
- Ribose 5-phosphate, produced in the hexose monophosphate shunt of carbohydrate metabolism is the starting material for purine nucleotide synthesis.
- It reacts with ATP to form phsophoribosyl pyrophosphate (PRPP).
- PRPP Synthetase is inhibited by PRPP



# STEP 2

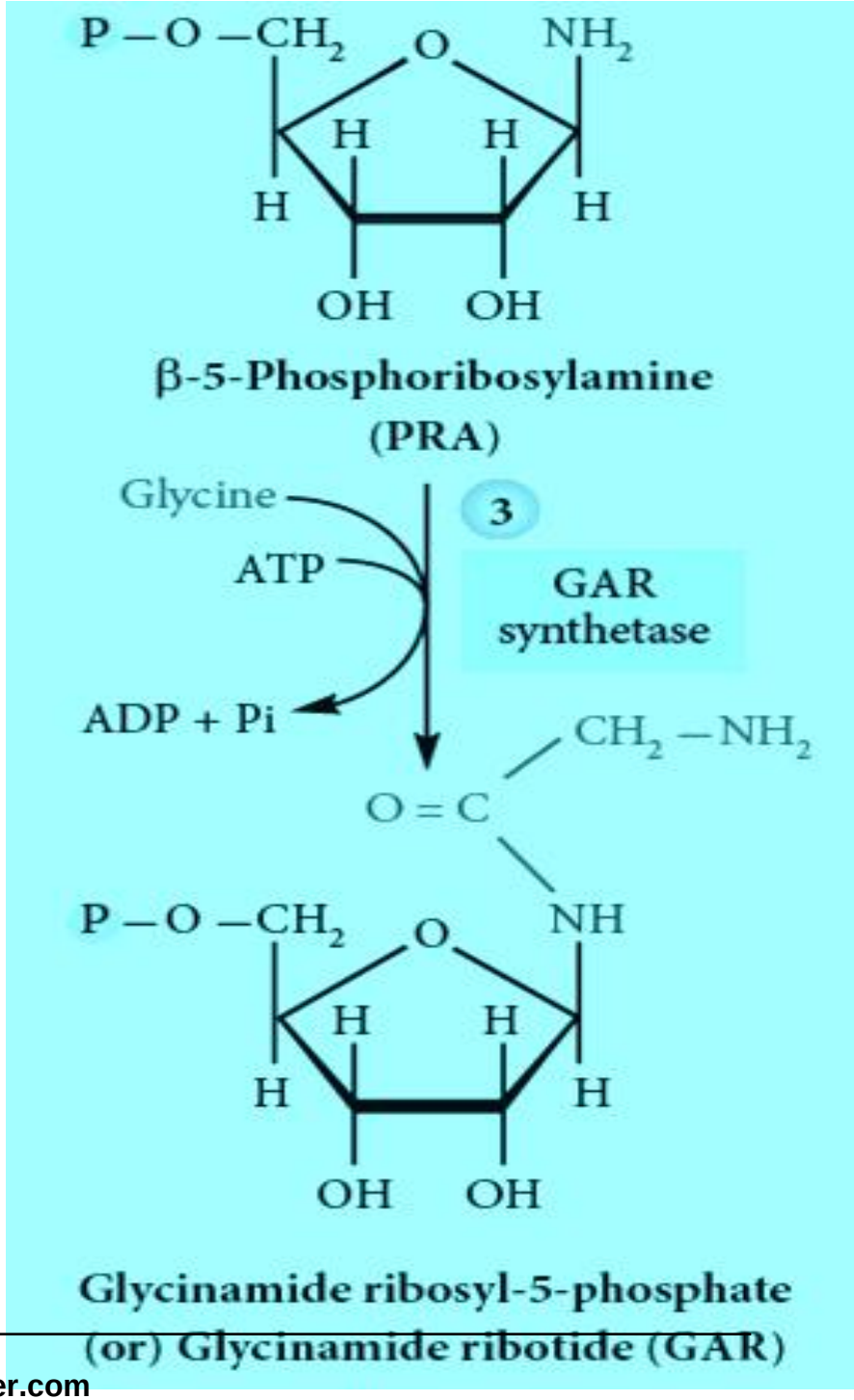
## Rate limiting step

- Glutamine transfers it's amide nitrogen to PRPP to replace pyrophosphate and produce 5phosphoribosylamine
- The enzyme PRPP glutamyl amidotransferase is controlled by feedback inhibition of nucleoltides (IMP, AMP and GMP,).
- This reaction is the 'committed.



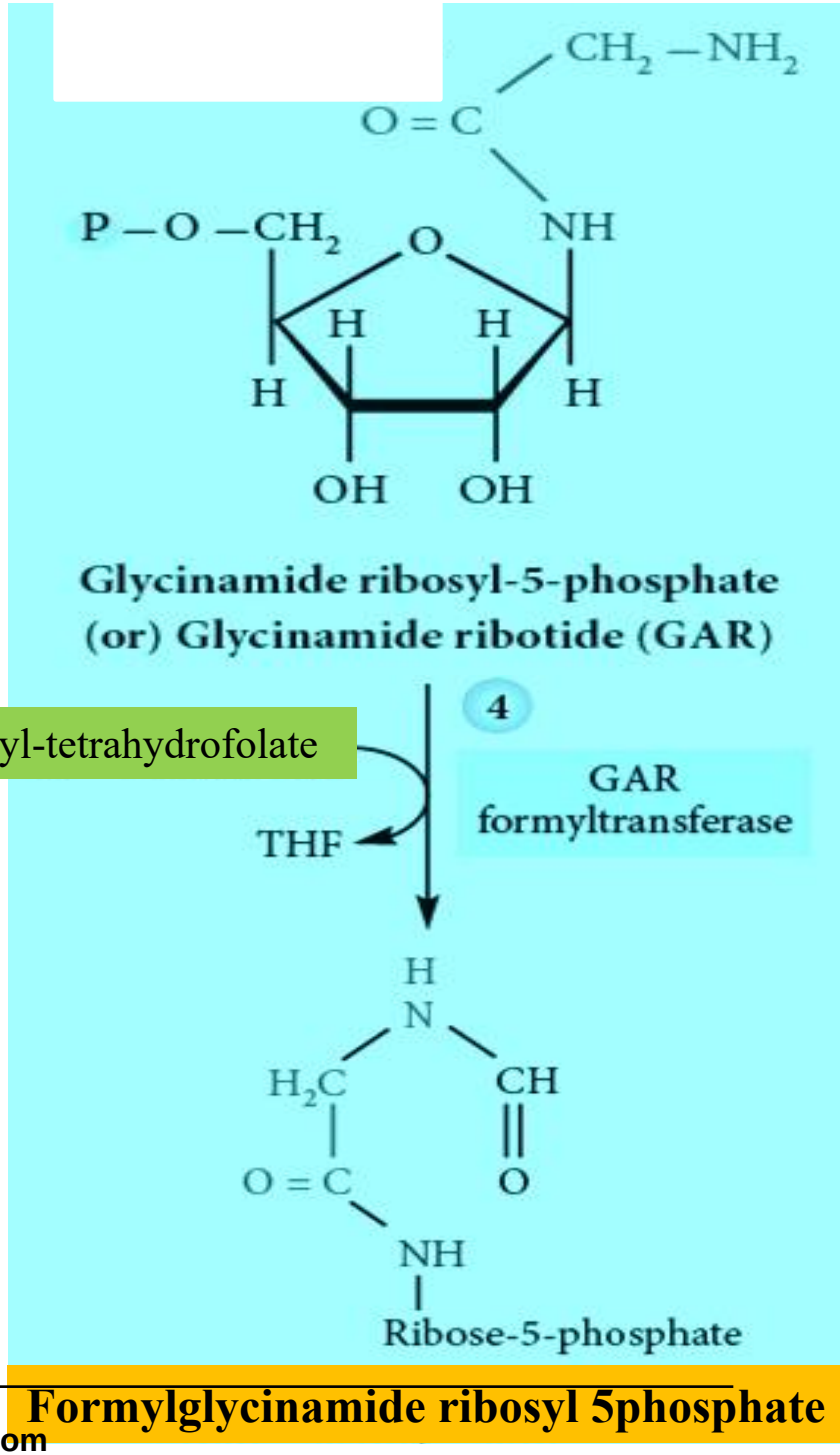
# STEP 3

- Phosphoribosylamine reacts with glycine in the presence of ATP to form glycinamide ribosyl-5-phosphate or glycinamide ribotide (GAR)



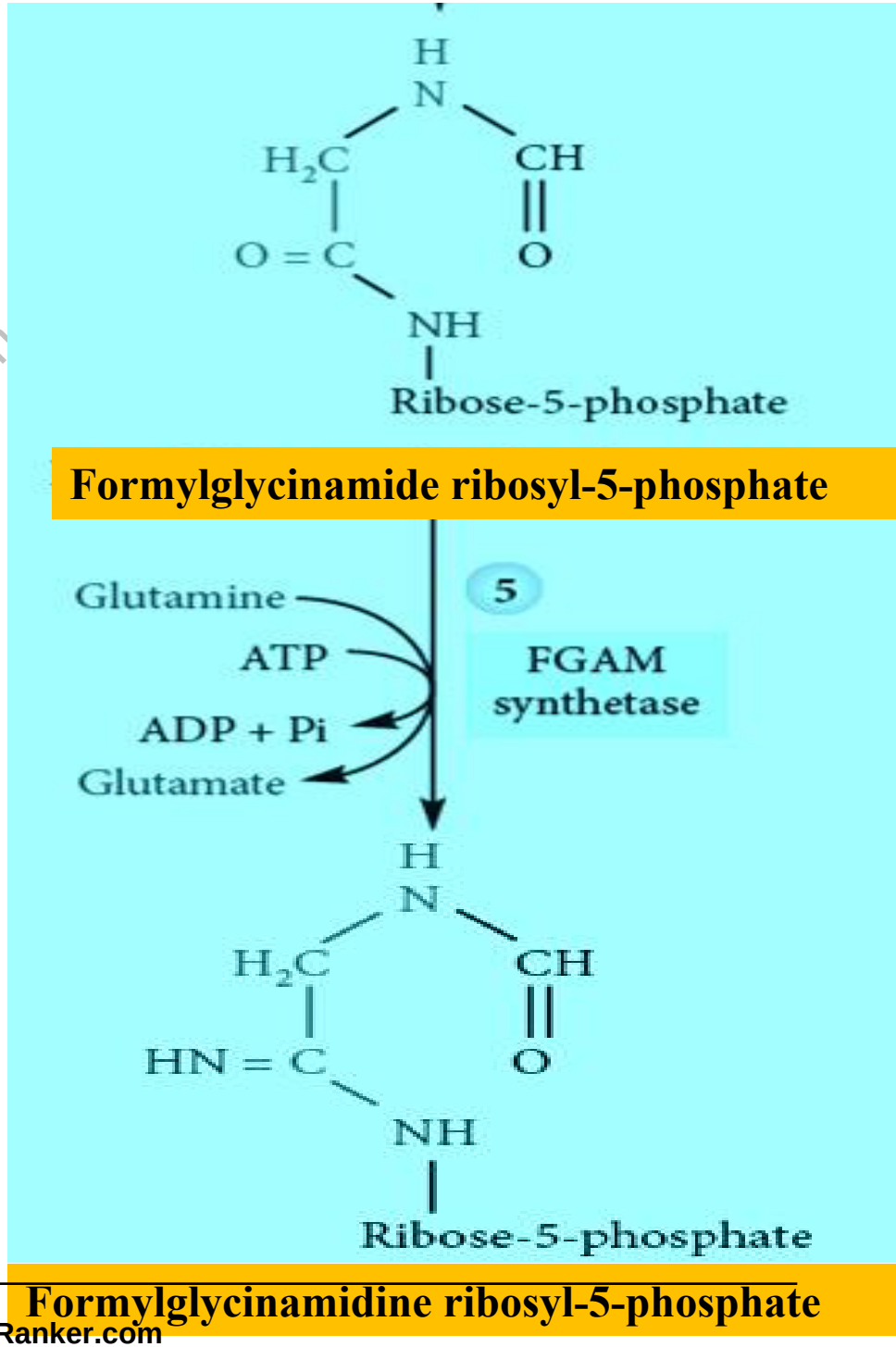
# STEP 4

- $N^5$,$N^{10}$-formyl-tetrahydrofolate donates the formyl group and the product formed is formylglycinamide ribosyl 5phosphate.



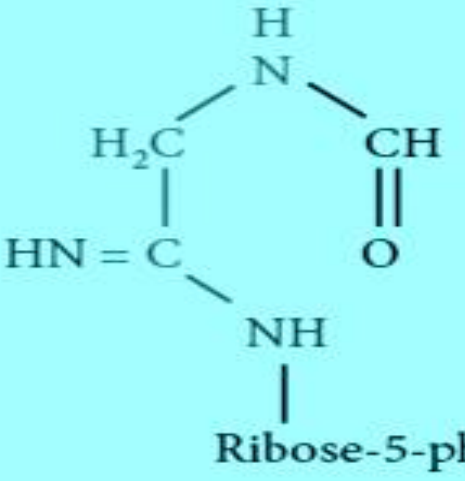
# STEP 5

- Glutamine transfers the second amido amino group to produce formylglycinamidine ribosyl 5phosphate

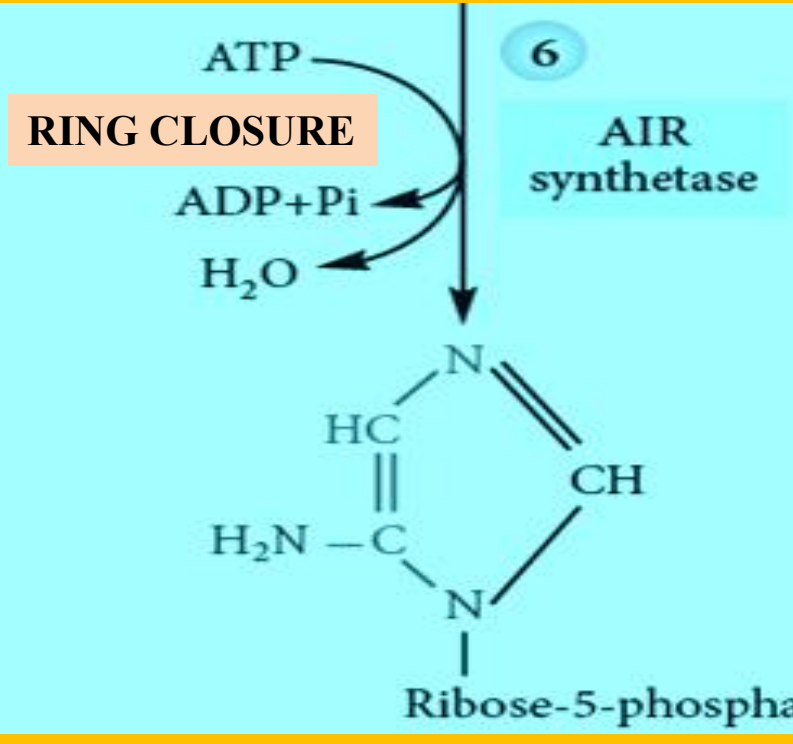


# STEP 6

- The imidazole ring of the purine is closed in an ATP dependent reaction to yield 5-aminoimidazoleribosyl 5-phosphate




**Formylglycinamidine ribosyl-5phosphate**

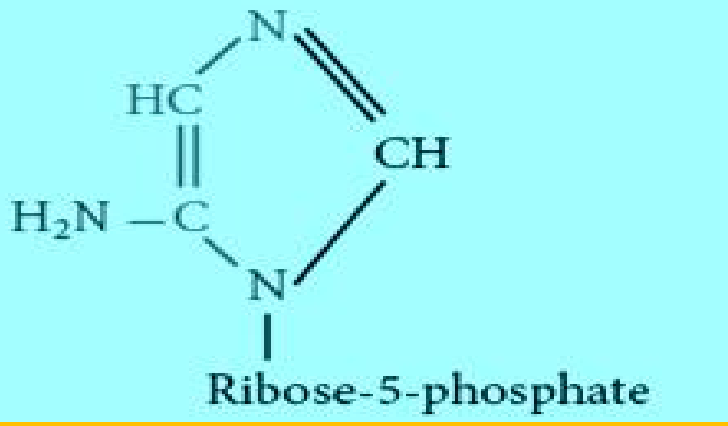



**Aminoimidazole ribosyl-5-phosphate**

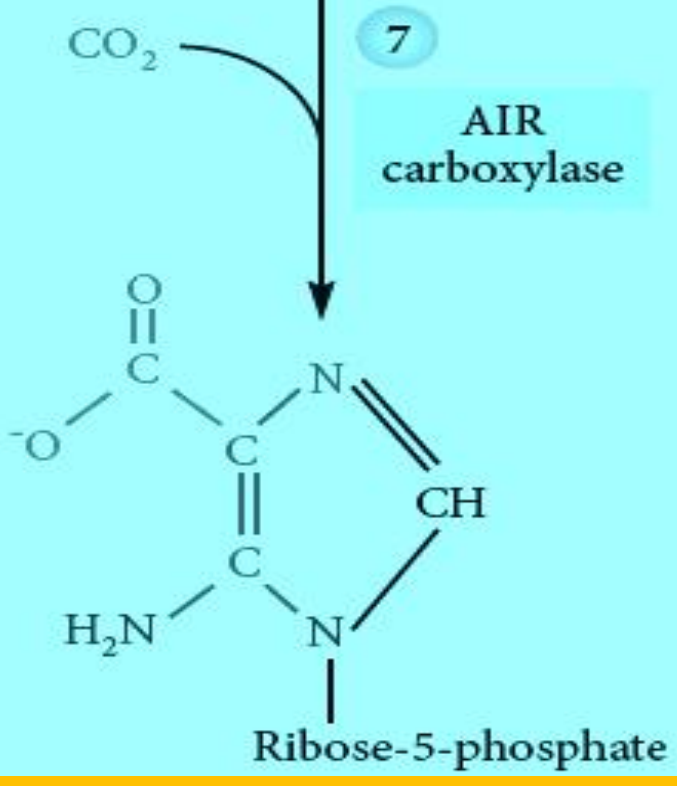
# STEP 7

- Incorporation of CO2 (carboxylation) occurs to yield aminoimidazole carboxylate ribosyl 5-phosphate.

- This carboxylation reaction does not require the vitamin biotin and /or ATP which is the case with most of the carboxylation reaction.



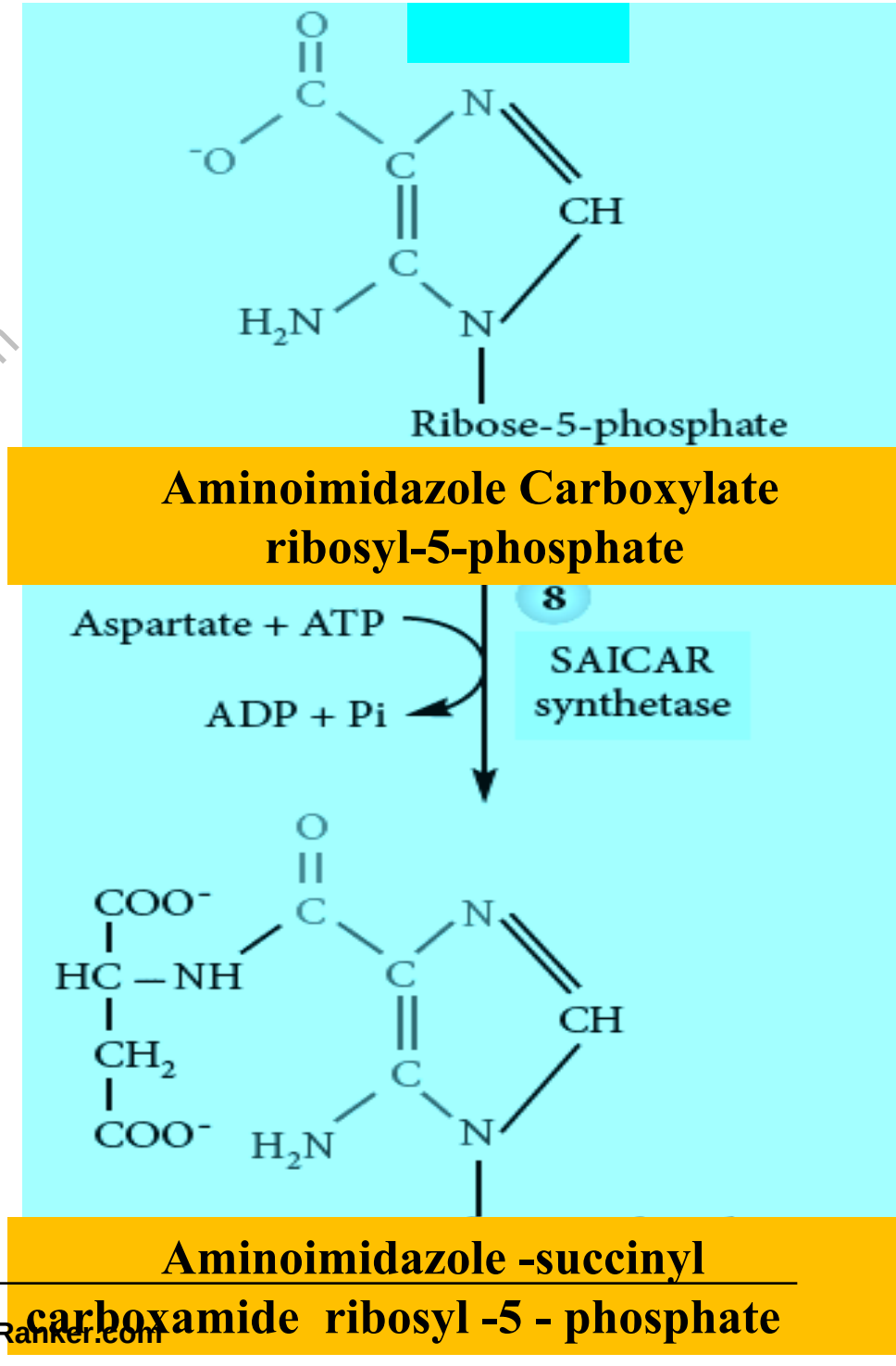

**Aminoimidazole ribosyl-5-phosphate**




**Aminoimidazole Carboxylate ribosyl-5-phosphate**

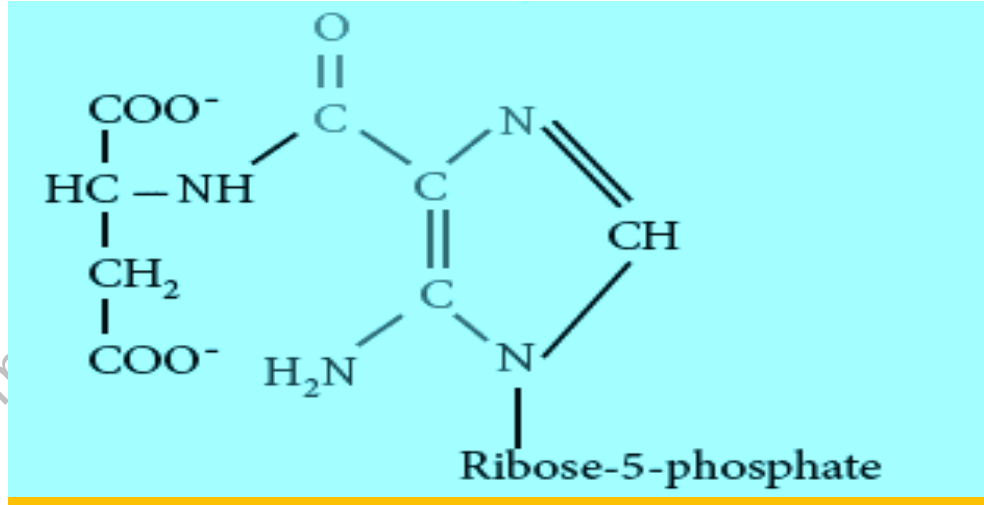
# STEP 8

- Aspartate condenses with aminoimidazole carboxylate ribosyl 5-phosphate. to form aminoimidazole 4-succinyl carboxamide ribosyl 5phosphate

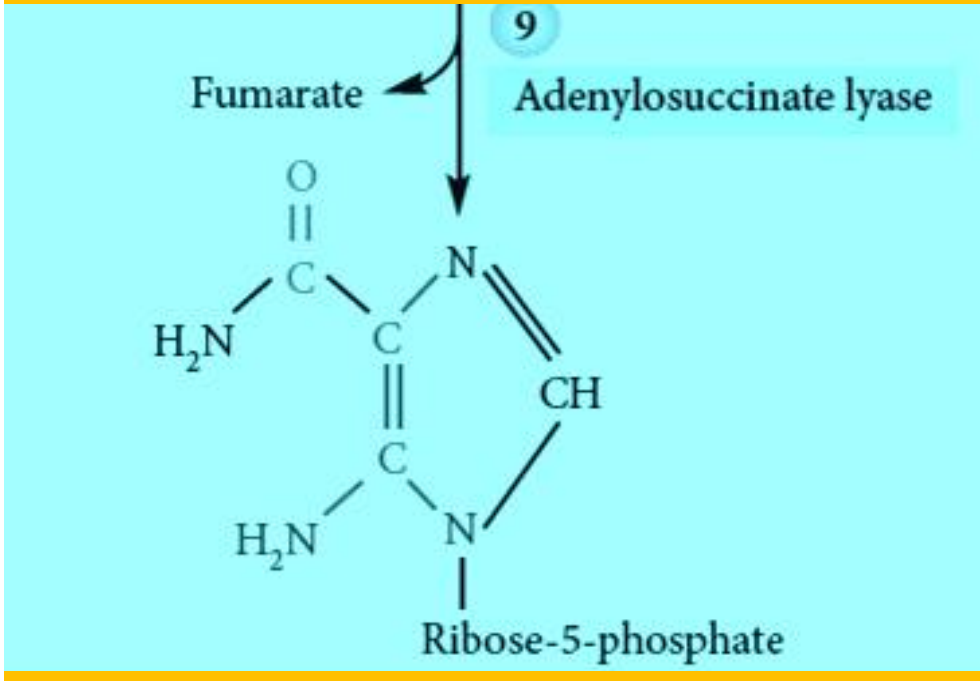


# STEP 9

- Adenylosuccinase or Adenylosuccinate lyase cleaves off fumarate and only the amino group of aspartate is retained to yield aminoimidazole carboxamide ribosyl 5-phosphate.




**Aminoimidazole -succinyl carboxamide ribosyl -5 - phosphate**

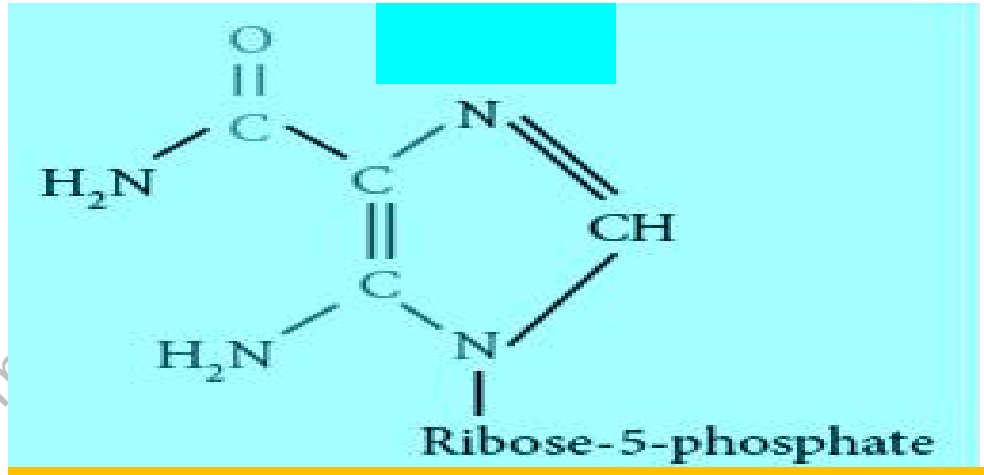



**Aminoimidazole Carboxamide ribosyl-5-phosphate**

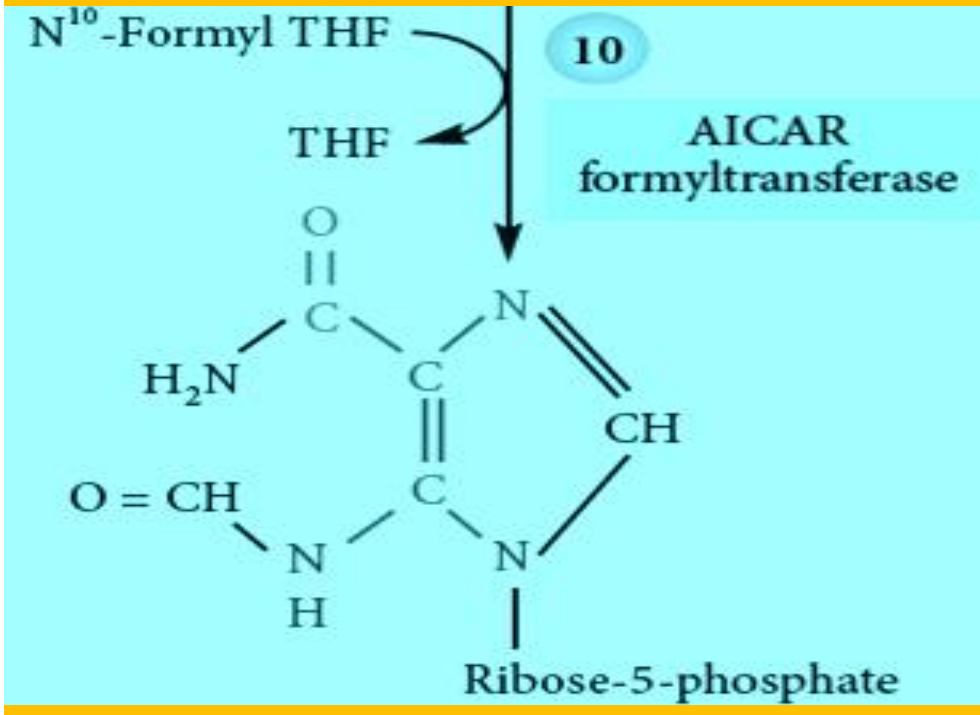
# STEP 10

- $N^{10}$-formyl-THF donates a one-carbon moiety to produce formimidoimidazole 4-carboxamide ribosyl 5-phosphate.

- With this reaction, all the carbon and nitrogen atoms of purine ring are contributed by the respective sources.

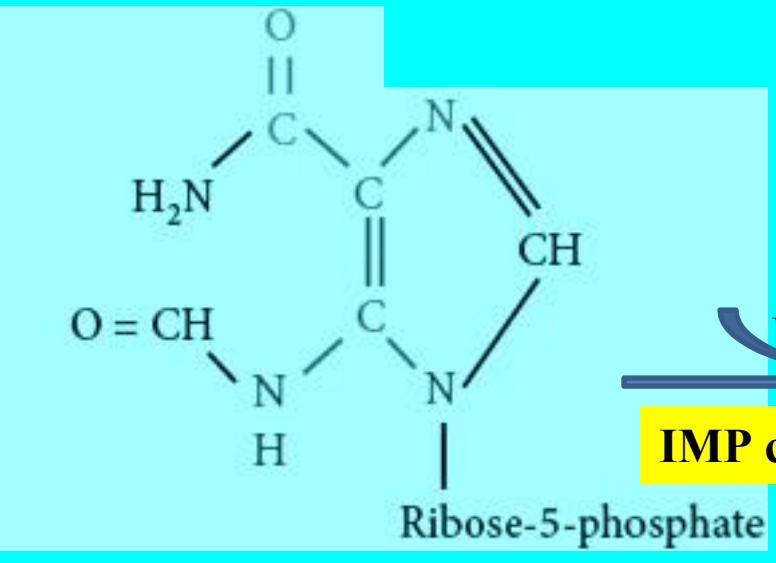



**Aminoimidazole Carboxamide ribosyl-5-phosphate**




**Formimidoimidazole Carboxamide ribosyl-5-phosphate**

# STEP 11

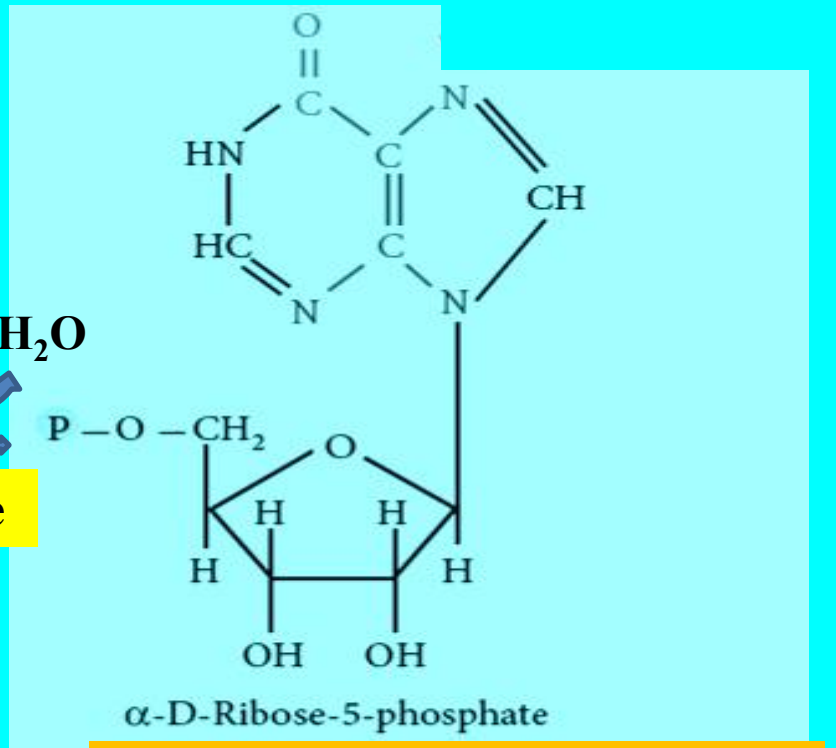



**Formimidoimidazole Carboxamide ribosyl-5-phosphate**

$H_2O$

**Ring closure**

**IMP cyclohydrolase**

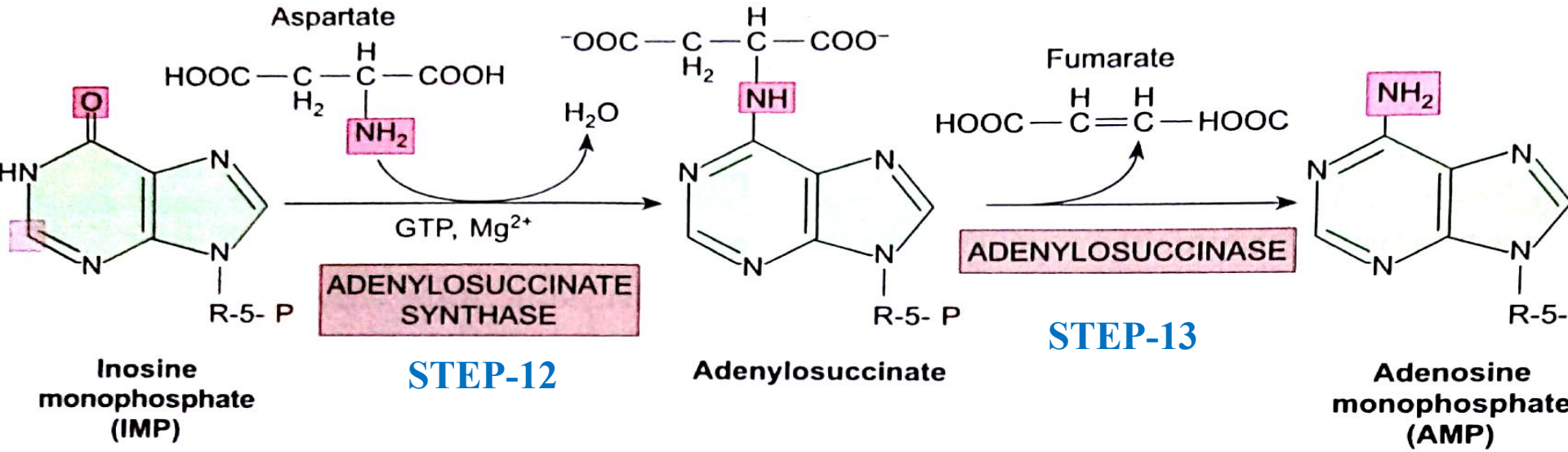


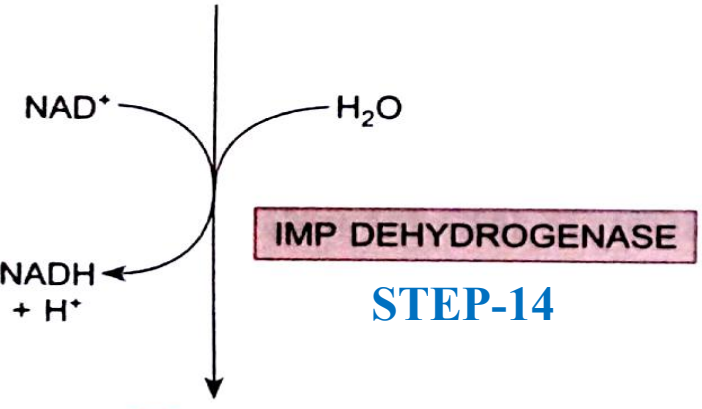

**Inosine monophosphate (IMP)**

The final reaction catalysed by cyclohydrolase leads to ring closure with an elimination of water molecule from formimidoimidazole ribosyl-5-P by Inosine monophosphate (IMP) cyclohydrolase forms IMP.

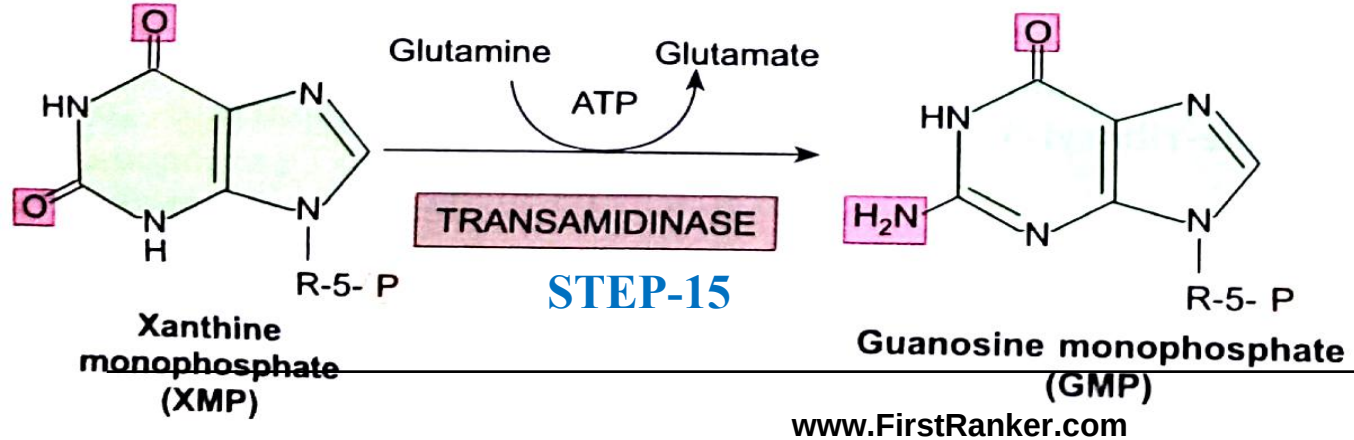
# Synthesis of AMP and GMP from IMP

- Inosine monophosphate is the immediate precursor for the formation of AMP & GMP
- Aspartate condences with IMP in the presence of GTP to produce Adenylosuccinate which on cleavage forms AMP.
- For the synthesis of GMP, IMP undergoes $NAD^+$ dependent dehydrogenation to form Xanthosine monophosphate ( XMP).
- Glutamine then transfers amide nitrogen to XMP to produce GMP. This requires ATP.




# Conversion Of IMP to AMP and GMP



# MUST REMEMBER

## ANTIFOLATE DRUGS & GLUTAMINE ANALOGS BLOCK PURINE NUCLEOTIDE BIOSYNTHESIS



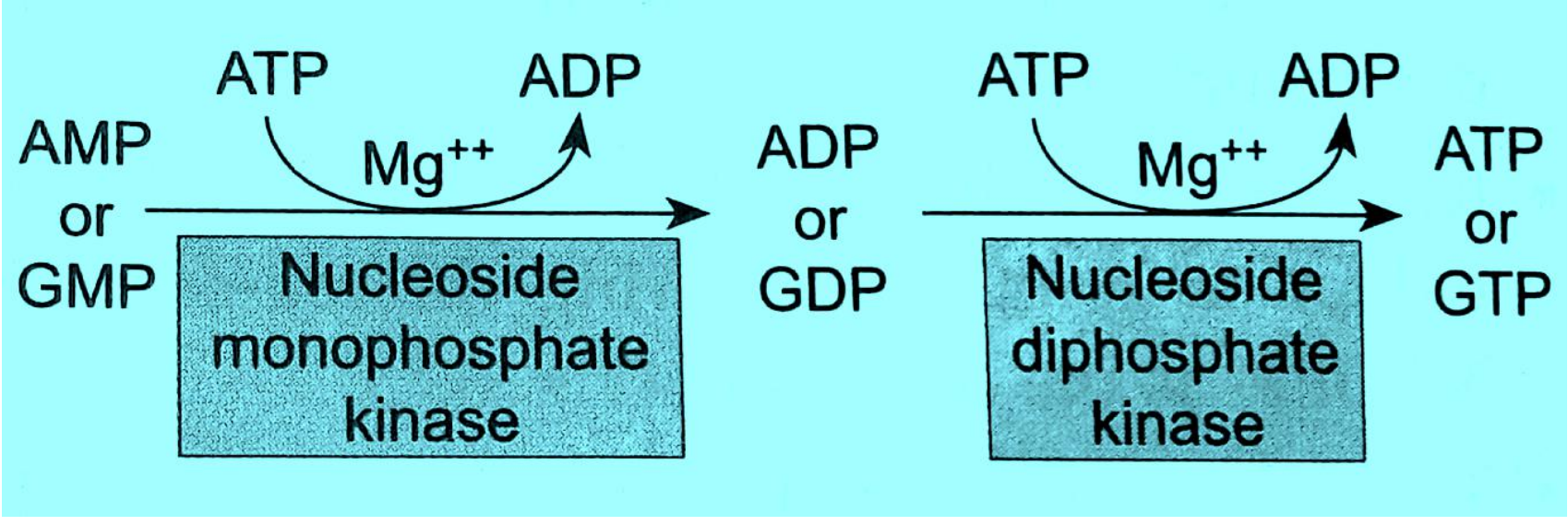
Compounds that inhibit formation of tetrahydrofolates and therefore block purine synthesis have been used in cancer chemotherapy.

Inhibitory compounds and the reactions they inhibit include

- Azaserine ----------reaction ⑤
- Diazanorleucine ---------reaction ②
- 6-mercaptopurine -------reactions ⑬ and ⑭
- mycophenolic acid -------reaction ⑭

# FORMATION OF DIPHOSPHATE AND TRIPHOSPHATE NUCLEOTIDES

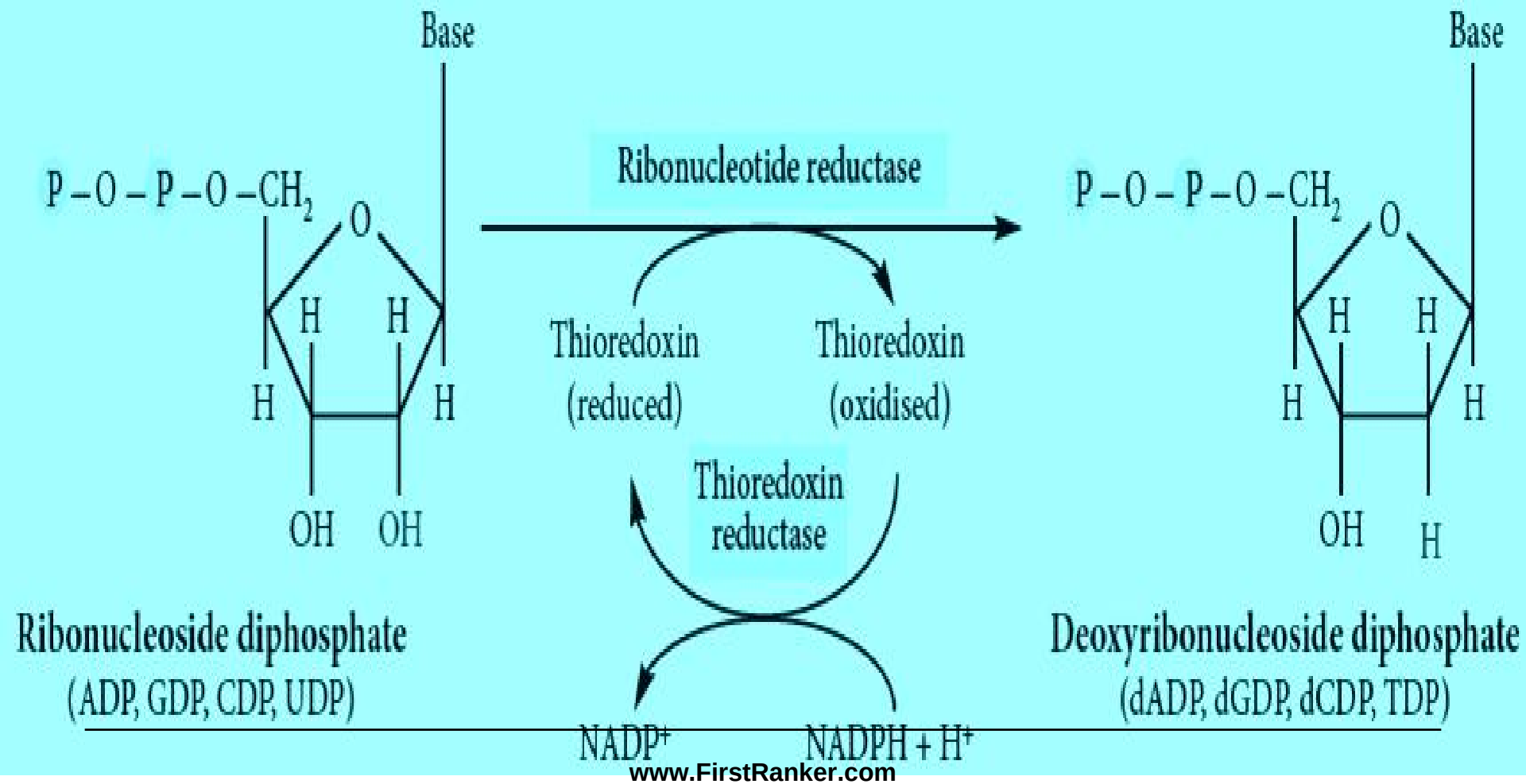
- AMP and GMP are phosphorylated using ATP as the source of phosphate to first make nucleoside di phosphate and then nucleoside triphosphate i.e. ADP, ATP, GDP and GTP



# CONVERSION OF RIBONUCLEOTIDES TO DEOXY RIBONUCLEOTIDES

- The synthesis of purine & pyrimidine deoxy ribonucleotides occur from ribonucleotides by a reduction at the C2 of ribose moity.
- This reaction is catalyzed by enzyme RIBONUCLEOTIDE REDUCTASE.
- The enzyme ribonucleotide reductase itself provides the hydrogen atoms needed for reduction from its sulfhydryl groups.
- The reducing equivalents, in turn, are supplied by Thioredoxin, a monomeric protein with two cysteine residues.
- NADPH-dependent thioredoxin reductase converts the oxidised thioredoxin to reduced form

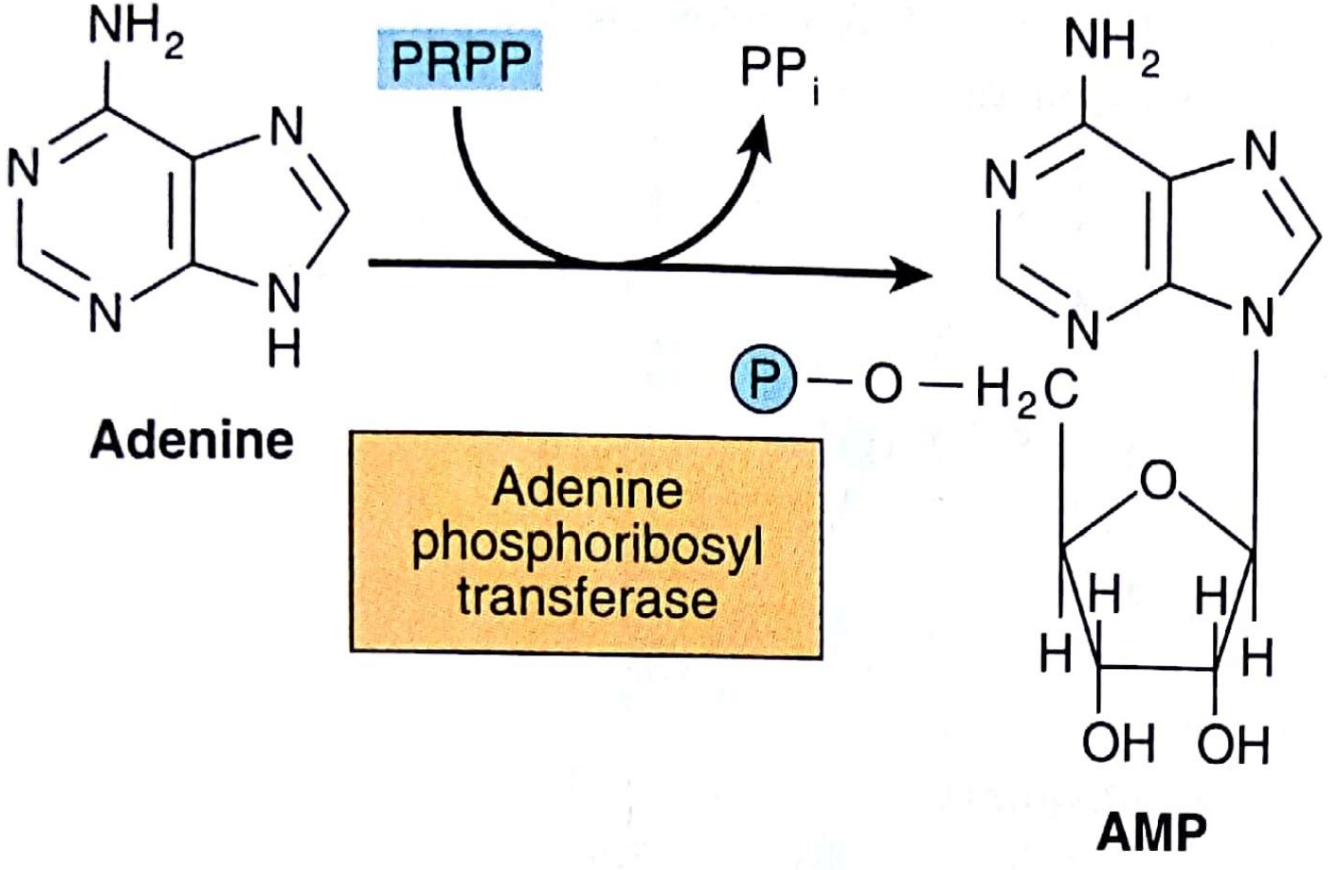
- Deoxy ribonucleotides are formed from reduction of ribo-nucleoside diphosphates.
- Monophosphate and triphosphate are not reduced to corresponding deoxy ribonucleotides

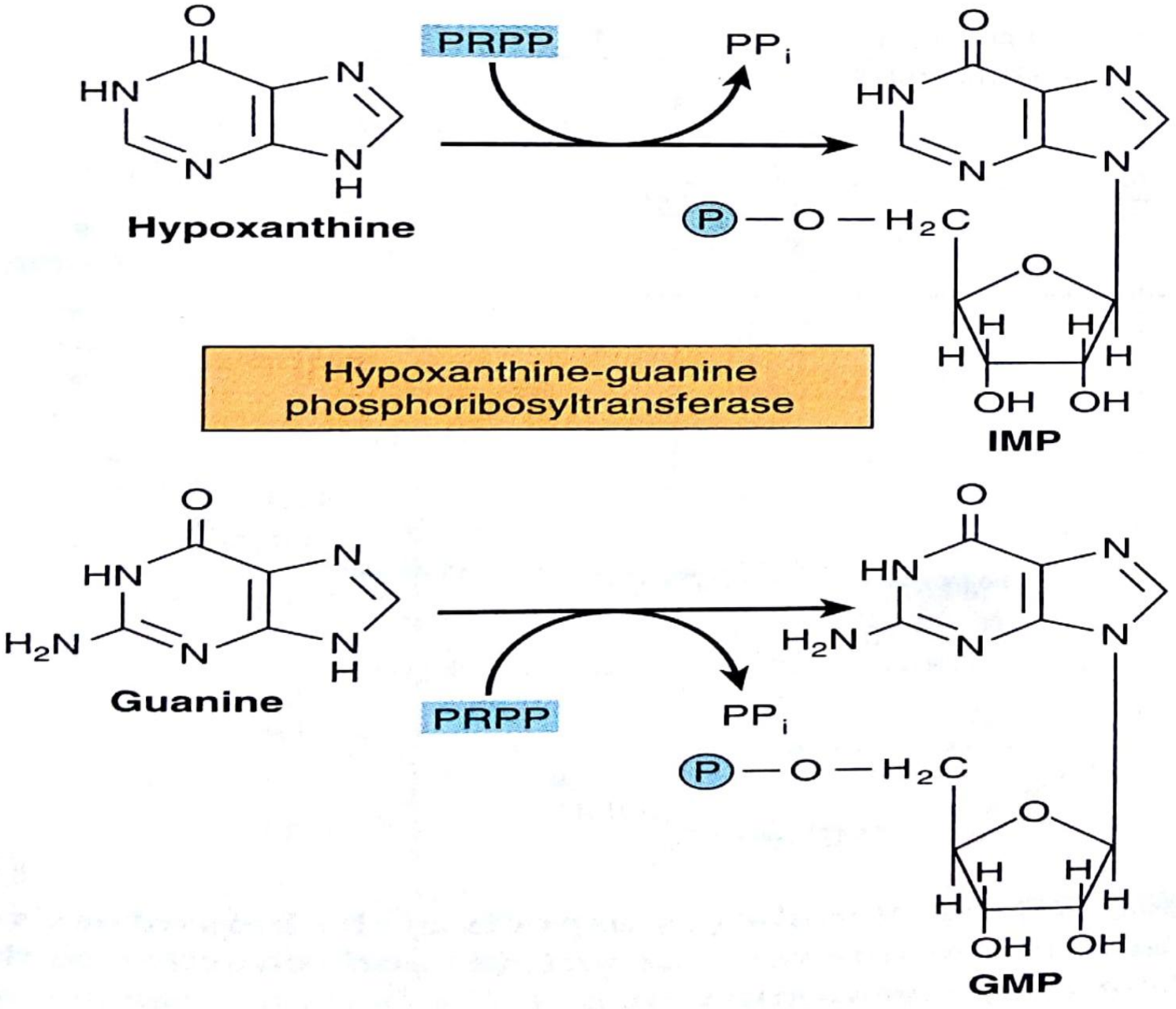


# PURINE SALVAGE PATHWAY

- The free purines ( adenine, guanine & hypoxanthine ) are formed in the normal turnover of nucleic acids & also obtained from the dietary sources.
- The free purines are converted to corresponding nucleotides, & this process is known as 'salvage pathway'.
- Adenine phosphoribosyl transferase catalyses the formation of AMP from adenine.
- Hypoxanthine-guanine phosphoribosyl transferase (HGPRT) converts guanine & hypoxanthine respectively, to GMP & IMP.
- Phosphoribosyl pyrophosphate (PRPP) is the donor of ribose 5 phosphate in the salvage pathway.
- The salvage pathway is particularly important in certain tissues such as erythrocytes & brain where denovo synthesis of purine nucleotides is not operative.

1. **Pu + PR-PP → Pu-RP + PPi**

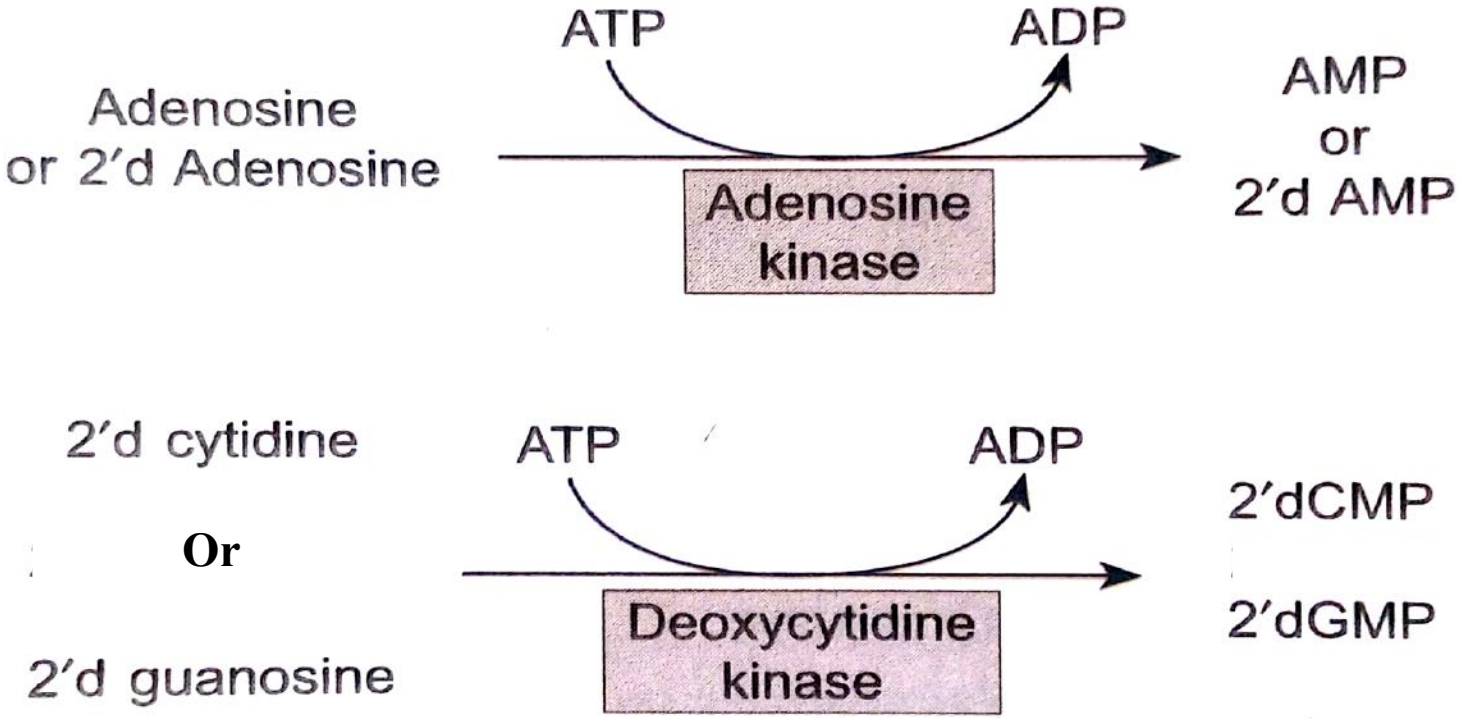




- A second salvage mechanism involves phosphoryl transfer from ATP to a purine ribonucleoside (Pu-R):

  Pu-R + ATP → PuR-P + ADP

- Phosphorylation of the purine nucleotides, catalyzed by adenosine kinase converts adenosine and deoxyadenosine to AMP and dAMP.



# REGULATION OF PURINE NUCLEOTIDE BIOSYNTHESIS



- The de novo biosynthesis is regulated in two ways

1. Regulation of IMP formation
2. Regulation of AMP and GMP formation from IMP.

# 1. Regulation of IMP formation

- Occurs at first two reaction catalyzed by
  i. PRPP synthase
  ii. Glutamine phosphoribosyl – amido-transferase.



Though both are regulatory enzymes, the second step catalyzed by amidotransferase is also a committed step for purine synthesis. Hence it is more important.

# i. REGULATION OF PRPP SYNTHASE

- The overall determinant of the rate of de novo purine nucleotide biosynthesis is the concentration of PRPP.
- This, in turn, depends on the rate of PRPP synthesis, utilization, degradation, and regulation.
- The rate of PRPP synthesis depends on the availability of ribose 5-phosphate and on the activity of PRPP synthase
- Activity of PRPP synthase is allosterically inhibited by both the adenosine and guanosine nucleotides i.e AMP, ADP, GMP and GDP.

# ii. REGULATION OF AMIDOTRANSFERASE

- Amidotransferse is feedback inhibited by products i.e. AMP, ADP, GMP and GDP.
- AMP and GMP act as competitive inhibitors.
- So, at a high PRPP (substrate ) concentration, AMP and GMP will not be able to inhibit the amidotransferase enzyme.
- Amidotransferse is stimulated by its substrate PRPP. (feed forward reaction)
- A very high PRPP concentration will lead to increased purines and their catabolism producing hyperuricemia.

# Regulation of AMP and GMP formation from IMP

- AMP feedback inhibits its own synthesis at the adenylosuccinate synthase level.
- Simultaneously ATP stimulate GMP synthesis at xanthine transaminidase step.( cross regulation)
- Similarly GMP inhibits its own synthesis at the IMP dehydrogenase step.
- GTP stimulates AMP synthesis at the adenylosuccinate synthase step. ( cross regulation)
- This cross regulation ensures that adenine and guanine nucleotide synthesis is in equal proportion. If AMP is decreased, it stimulates its own synthesis and inhibits GMP synthesis and vice versa.

# REGULATION OF PURINE NUCLEOTIDE BIOSYNTHESIS

- Regulation of IMP is shown by solid lines
- AMP and GMP synthesis are shown by dotted lines

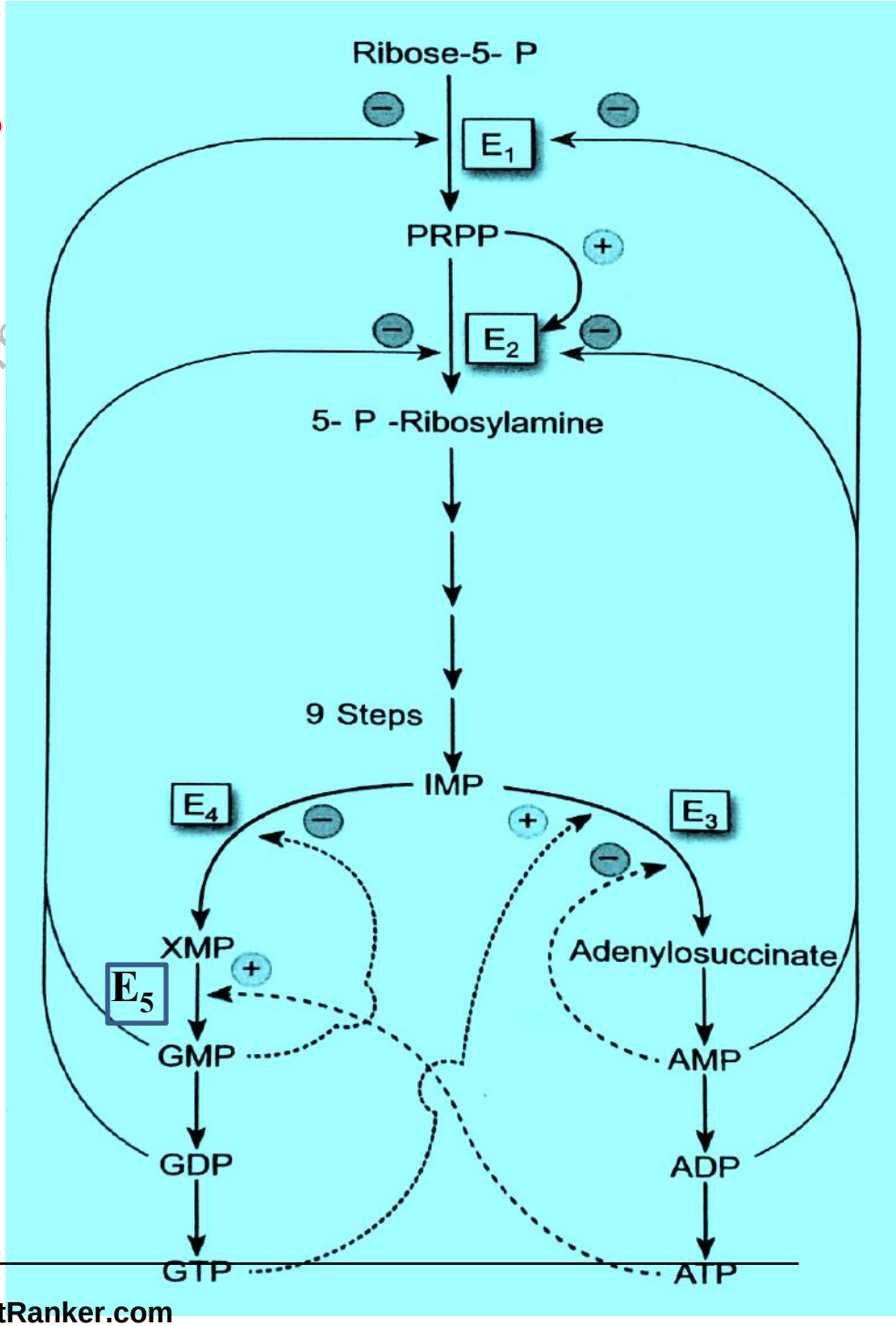
E1 = PRPP Synthetase

E2 = Amido Transferase

E3 = Adenylosuccinate synthase

E4 = IMP dehydrogenase

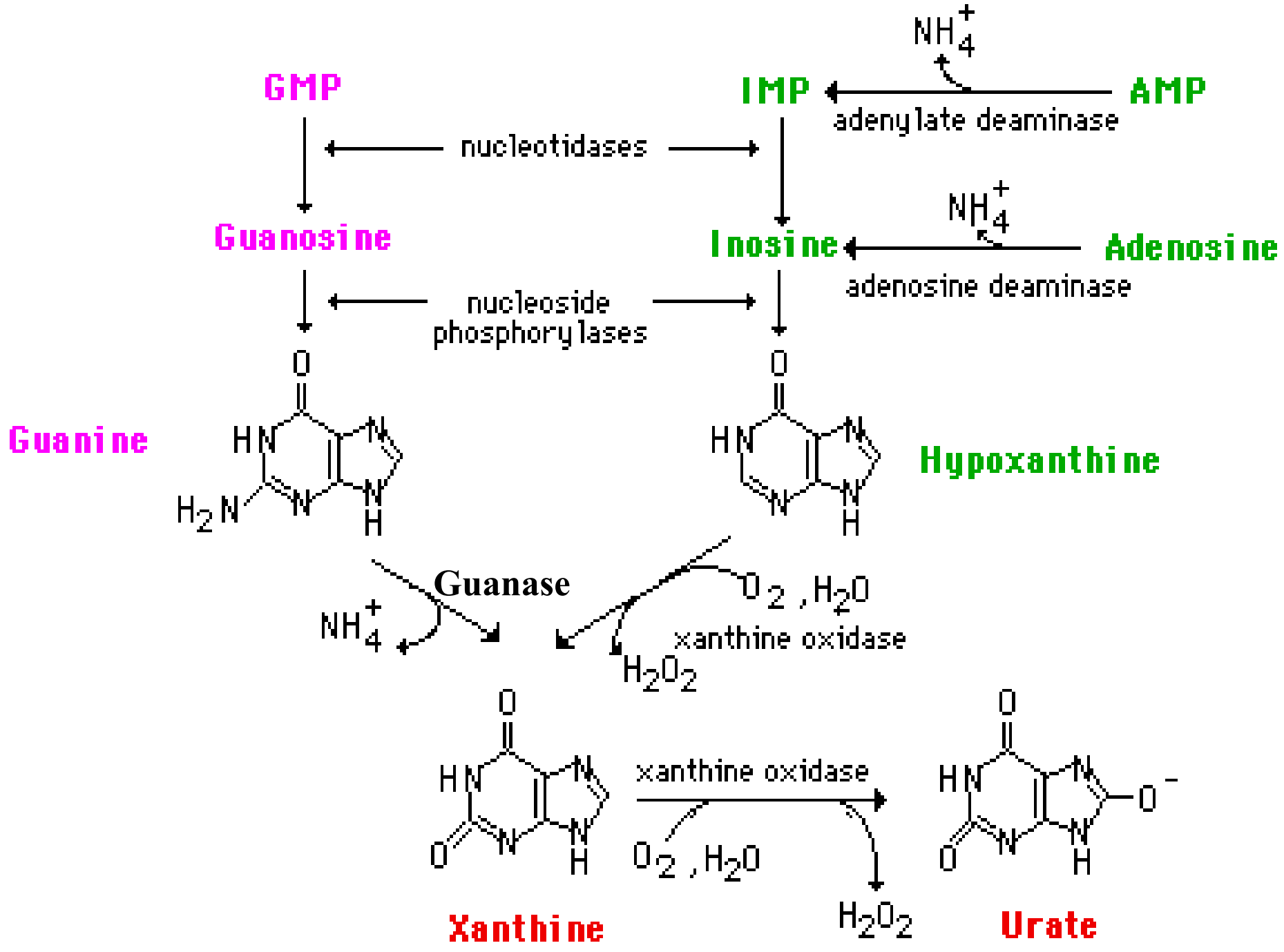
E5 = Xanthine transaminidase



# DEGREDATION OF PURINE NUCLEOTIDES

1. The end product of purine metabolism in humans is uric acid.
2. The nucleotide monophosphates (AMP , IMP & GMP) are converted to their respective nucleoside forms (adenosine,inosine & guanosine ) by the action of nucleotidase.
3. The amino group, either from AMP or adenosine, can be removed to produce IMP or inosine respectively by deaminase.
4. Inosine & guanosine are, raspectively , converted to hypoxanthine & guanine (purine bases) by purine nucleoside phosphorylase.

5. Adenosine is not degreded by this enzyme, hence it has to be converted to inosine by deaminases.
6. Guanine undergoes deamination by guanase to form xanthine.
7. Xanthine oxidase is an important enzyme that converts hypoxanthine to xanthine, & xanthine to uric acid.
8. This enzyme contains FAD, Molybdenum & Iron, & is mainly found in liver & small intestine.
9. Uric acid ( 2,6,8-trioxopurine ) is the final excretory product of purine metabolism in humans.
10. Uric acid can serve as an important antioxidant by getting itself converted non enzymatically to allantoin.



# DISORDERS OF PURINE METABOLISM

1. Hyperuricemia And Gout
2. Lesch-Nyhan syndrome
3. Severe Combined Immuno Deficiency (SCID)
4. Purine Nucleoside Phophorylase Deficiency

# URIC ACID

1. Uric acid (2,6,8-trioxopurine) is the end product of purine metabolism in humans.

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

3. In women, it is slightly lower (by about 1mg) than in men.

4. The daily excreation of uric acid is about 400-600 mg. It is filtered , reabsorbed and secreted by kidney tubules.

5. At the pH of 5.75 and above, it forms monosodium urate salt which is 10 times more soluble than uric acid.

6. In plasma (pH of 7.4), monosodium urate is predominant form present which is relatively more soluble.



7. Any condition which decreases blood pH (acidosis) ,therefore, promotes the formation of insoluble uric acid than the more soluble monosodium urate.

# 1. HYPERURICEMIA AND GOUT

- Increase in blood uric acid level above the normal value of >7 mg% is called hyperuricemia.
- This is sometimes associated with increased uric acid excreation ( Uricosuria)
- In severe hyperuricemia, crystals of sodium urate get deposited in the soft tissues, particularly in the joints.
- Such deposits are commonly known as TOPHI.
- This causes inflammation in the joints resulting in a painful arthritis.
- Sodium urate &/or uric acid may also precipitate in kidneys & ureters that result in renal damage & stone formation.

**Figure 28-29** *The Gout*, a cartoon by James Gilroy (1799).

Gout is a disease characterized by elevated levels of uric acid in body fluids.
Caused by deposition of nearly insoluble crystals of sodium urate or uric acid.

# GOUT

- Hyperuricemia leading to arthritis is called gout.
- Gout is often called a disease of bones and stones due to recurrent stones formation and inflammmation of joints.
- Gouty arthritis is debilitating painful condition leading to deformity of joints.
- Typical gouty arthritis affects first metatarsophalangeal joint.(GREAT TOE)

# TYPES OF GOUT

Two types:

1. Primary –

   due to metabolic defect of uric acid where its synthesis as such is increased.

2. Secondary –

   - Due to increased nucleotide turn over wherein more uric acid is formed .
   - Uric acid metabolism is normal.
   - This occurs as a consequence of some other primary disease associated with increased tissue catabolism

# CAUSES OF GOUT

- PRIMARY GOUT

1. PRPP synthetase over activity due to defective enzyme

   varient forms of PRPP synthetase-which are not subjected to feedback regulation-have been detected. This leades to increased production of purines.



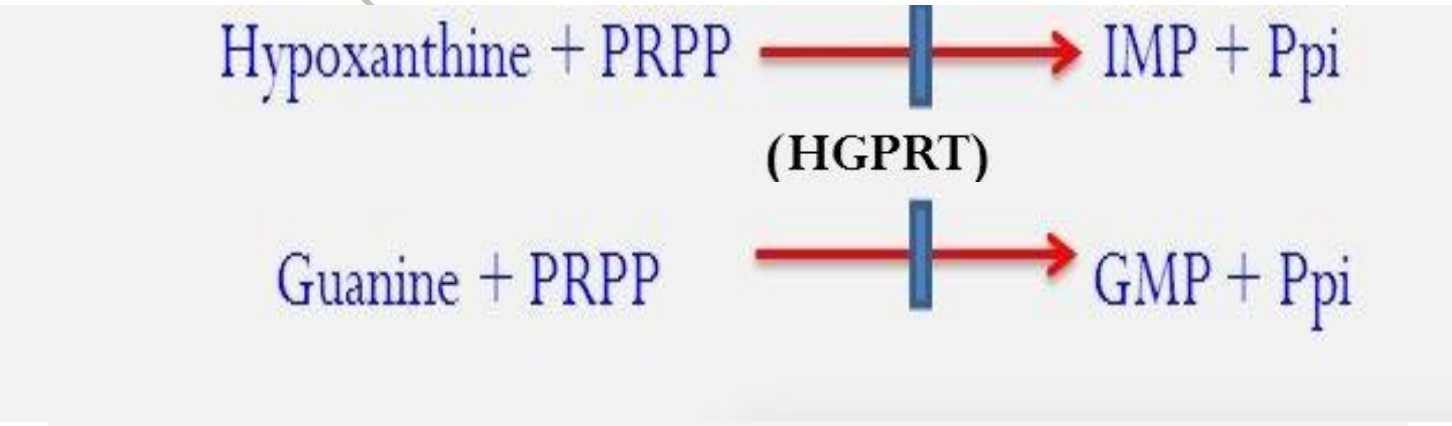
2. PRPP-glutamyl-amidotransferase - defective enzyme

   The lack of feedback control of this enzyme by purine nucleotides also leads to their elevated synthesis.

## 3.HGPRTase deficiency or Lesch-Nyhan syndrome

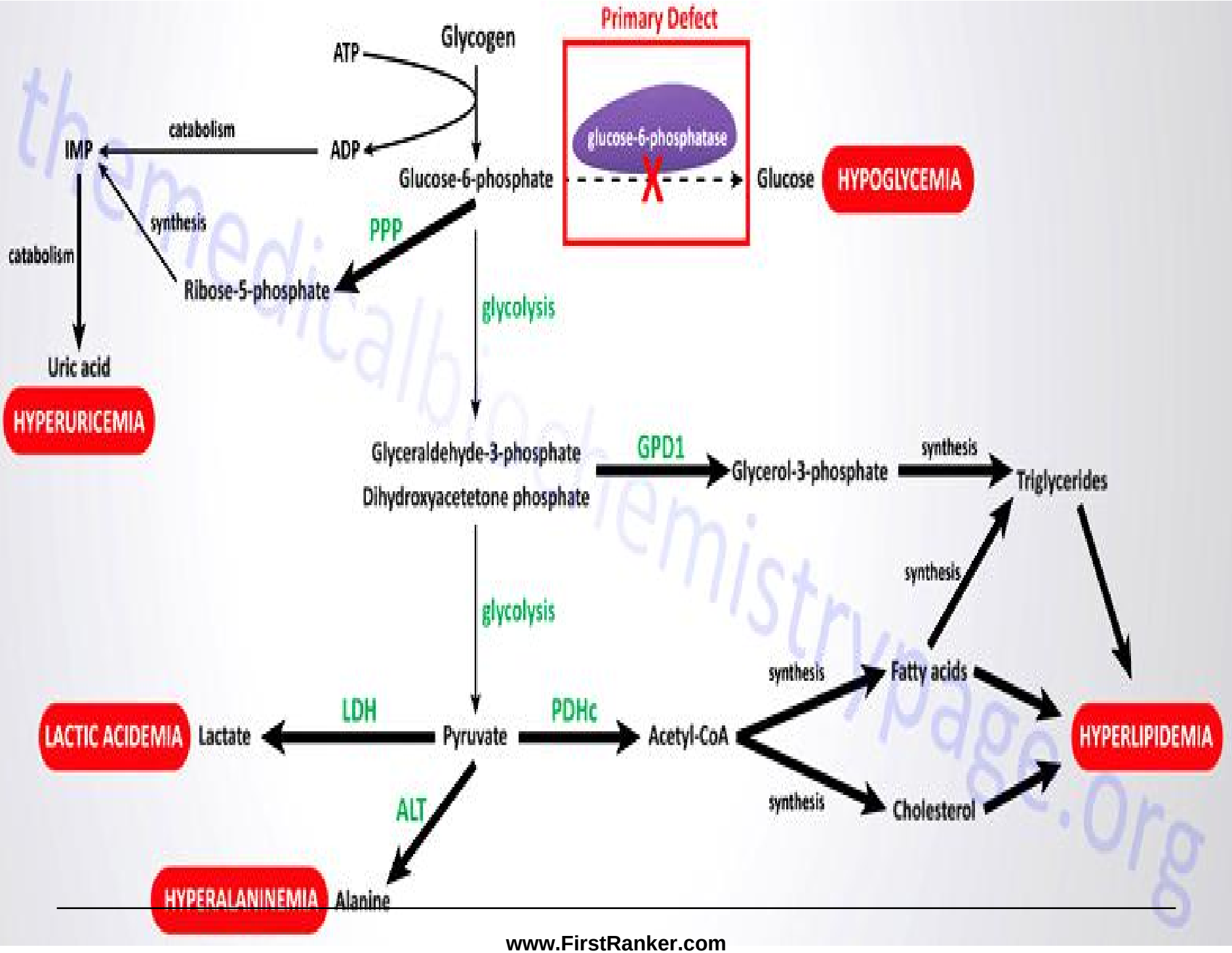
- Deficiency of HGPRTase causes a decrease in salvage pathway of hypoxanthine and guanine to reform nucleotide.
- This in turn spares PRPP and results in overproduction of purine nucleotides and their degradation to uric acid.





# 4. Glu-6-phosphatase dificiency or Von-Gierke's disease

- When this enzyme is deficient, glucose-6-phosphate cannot be converted to glucose.
- So more glucose-6-phosphate is channeled into the pentose phosphate shunt pathway, resulting in increased availability of ribose-5-phosphate.
- This would lead to increased formation of PRPP ultimately, purine over production.
- Von Gierke's disease is also associated with increased activity of glycolysis. Due to this, lactic acid accumulates in the body which interferes with the uric acid excretion through renal tubules



# SECONDARY GOUT

## A. Increased Production of Uric Acid

i. Rapidly growing malignant tissues, e.g. leukemias, lymphomas, polycythemia.

ii. Cancer patients on radiotherapy or chemotherapy (tumor lysis syndrome) due to increased cellular turnover

iii. Increased tissue damage due to trauma and raised rate of catabolism as in starvation

iv Psoriasis – skin disease

# Secondary Hyperuricemia

## B. Reduced Excretion Rate

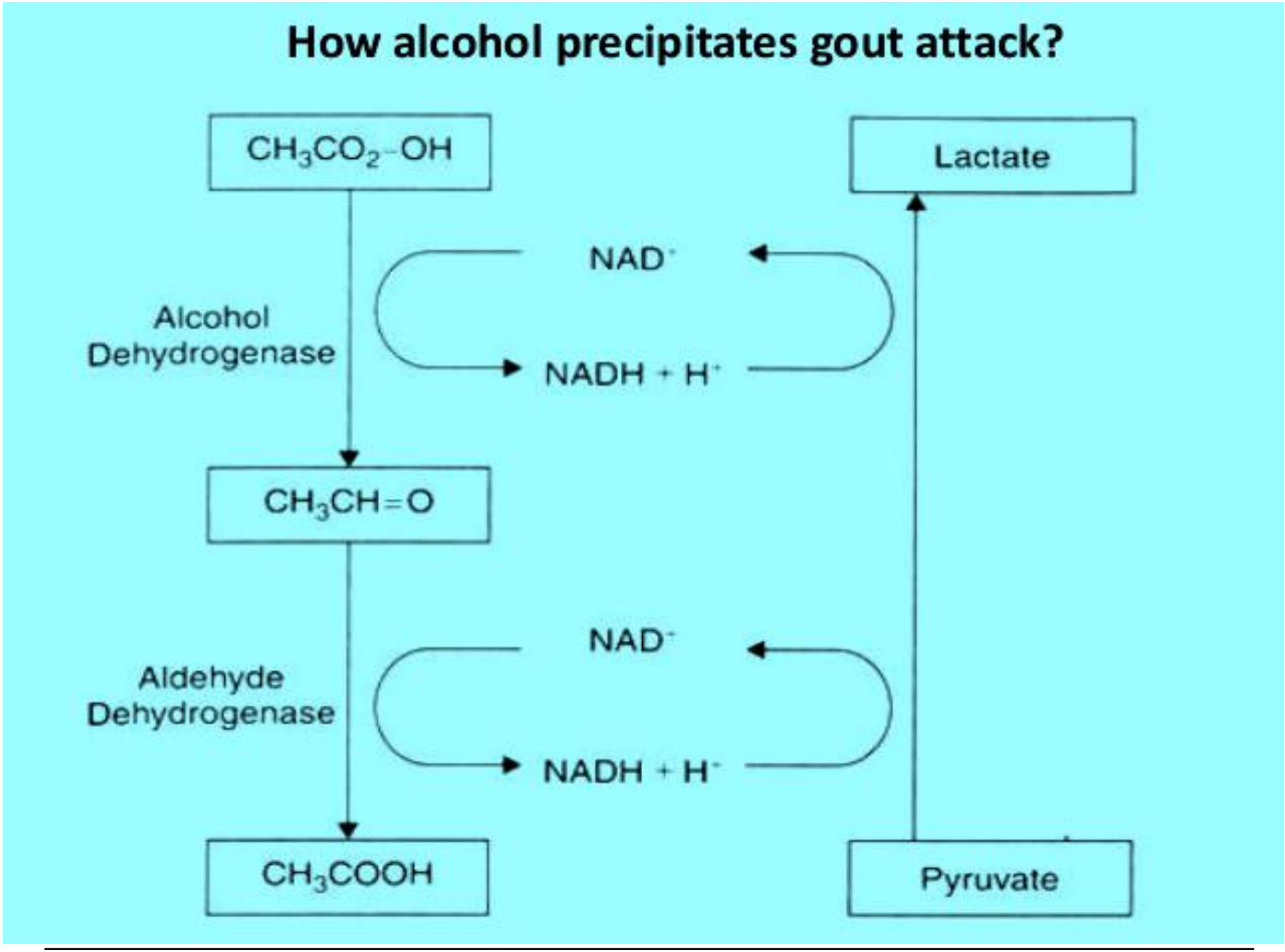
i. Renal failure

ii. Treatment with thiazide diuretics which inhibit tubular secretion of uric acid

iii. Lactic acidosis and keto-acidosis due to interference with tubular secretion.

# Clinical Findings of Gout

- The typical gouty arthritis affects the first metatarsophalangeal joint (big toe), but other joints may also be affected.
- The joints are extremely painful.
- Synovial fluid will show birefringent urate crystals.
- In chronic cases, uric acid may get deposited around joints causing swelling (tophi) composed of sodium urate
- Gouty attacks may be precipitated by high purine diet and increased intake of alcohol.
- Often the patients have a few drinks, go to sleep symptomless, but are awakened during the early hours of morning by excruciating joint pains.
- Alcohol leads to accumulation of lactic acid.



**Gout:** Increase lactic acid in chronic alcoholism

↓

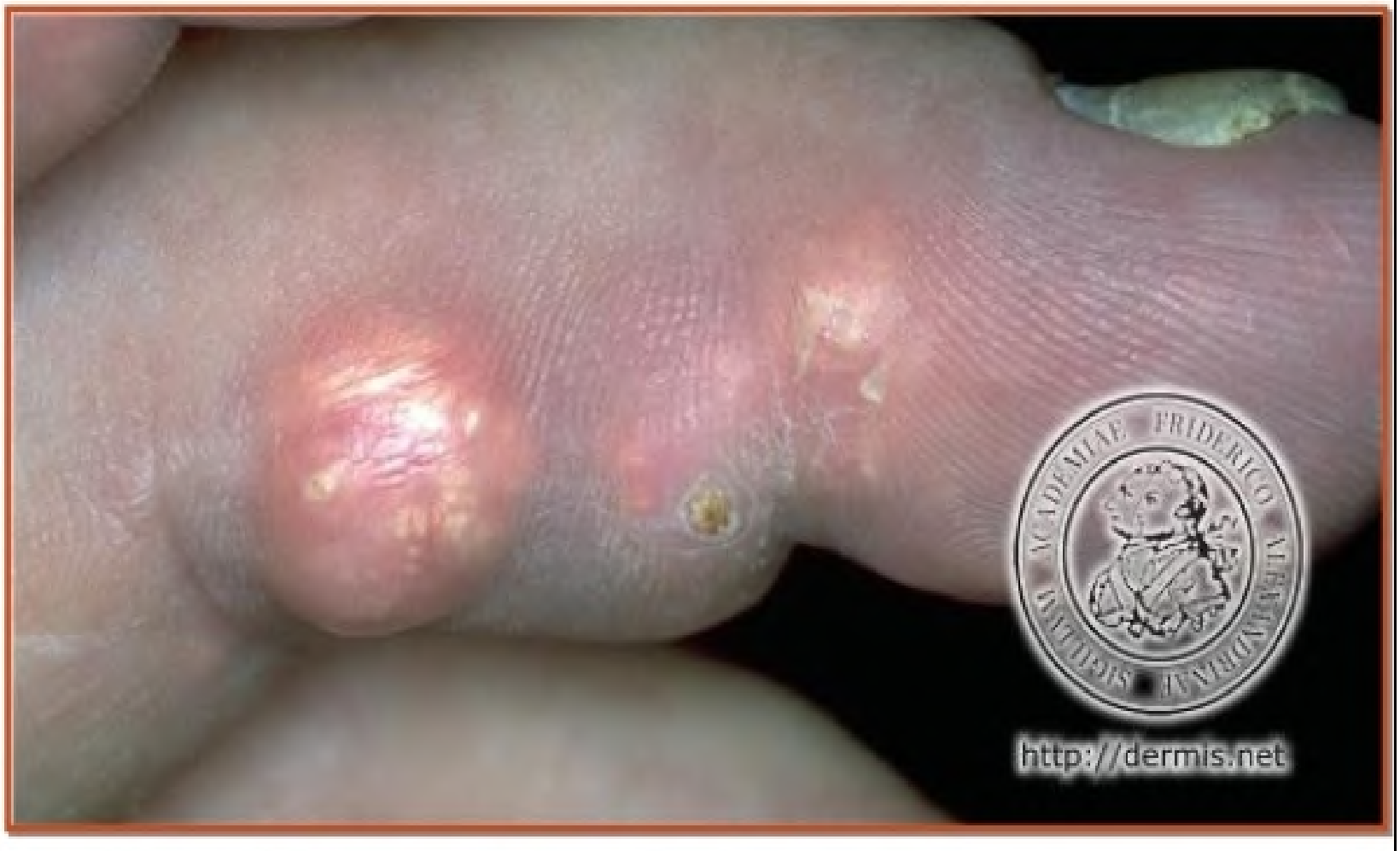
Competes with uric acid for same excretory pathway in kidney

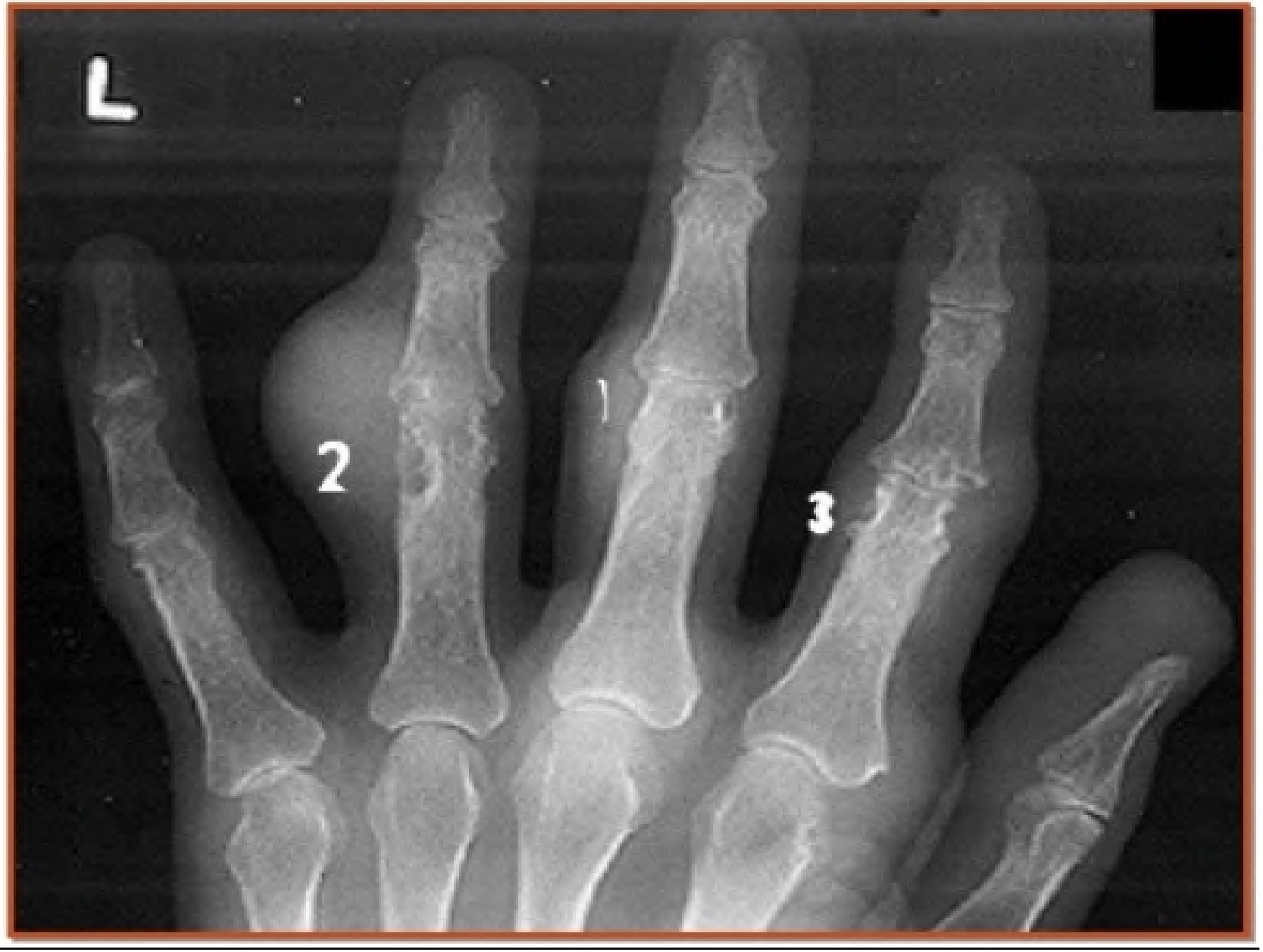
↓

Leads to accumulation of uric acid in plasma

↓

Hyperuricemia and gout



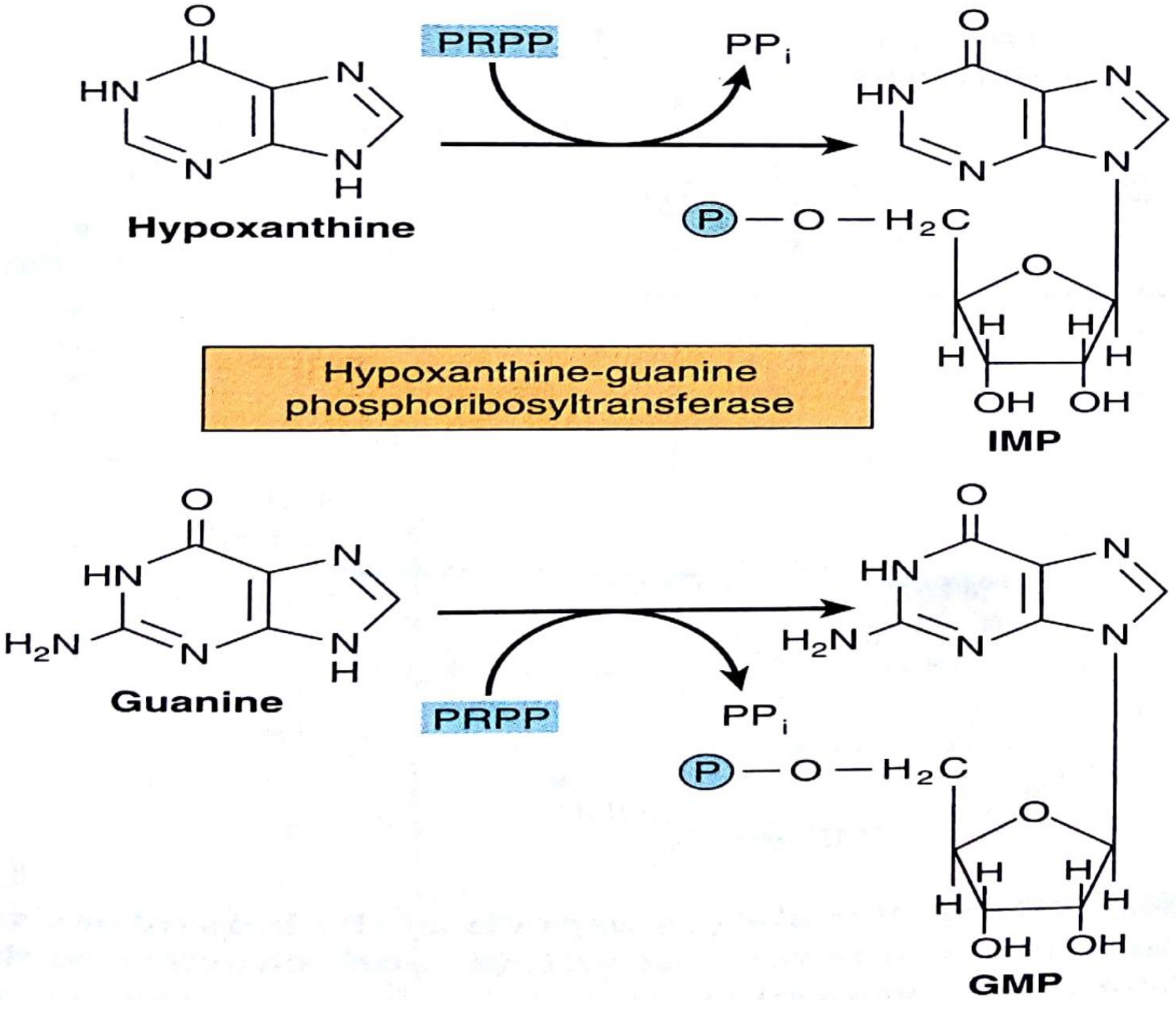


# TREATMENT

- Reduce dietary purine intake and restrict alcohol.
- Increase renal excretion of urate by uricosuric drugs, which decrease the reabsorption of uric acid from kidney tubules, e.g. probenecid.
- Reduce urate production by allopurinol, an analog of hypoxanthine and competitive inhibitor of xanthine
- Xanthine and hypoxanthine are more soluble and so are excreted more easily.
- Xanthine oxidase converts allopurinol to alloxanthine. It is a more effective inhibitor of xanthine oxidase.('suicide inhibition').
- Colchicine, an anti-inflammatory agent is very useful to arrest the arthritis in gout.

# Lesch-Nyhan Syndrome

- Inability of the body to salvage hypoxanthine and guanine due to the complete deficiency of HGPRTase (Hypoxanthine-Guanine phosphoribosyl transferase)
- It is an X-linked inherited disorder of purine metabolism, the disease is limited to males only
- Different types of mutations in HGPRTase gene have been identified in patients with Lesch Nyhan syndrome.
- Incidence is 1:10,000 males.



- HGPRT deficiency results in the accumulation of PRPP and decrease in GMP and IMP.
- Increased level of Hypoxanthine and Guanine

↓

↑ in degradation to uric acid

- Also PRPP accumulates

↓

stimulates production of Purine nucleotides

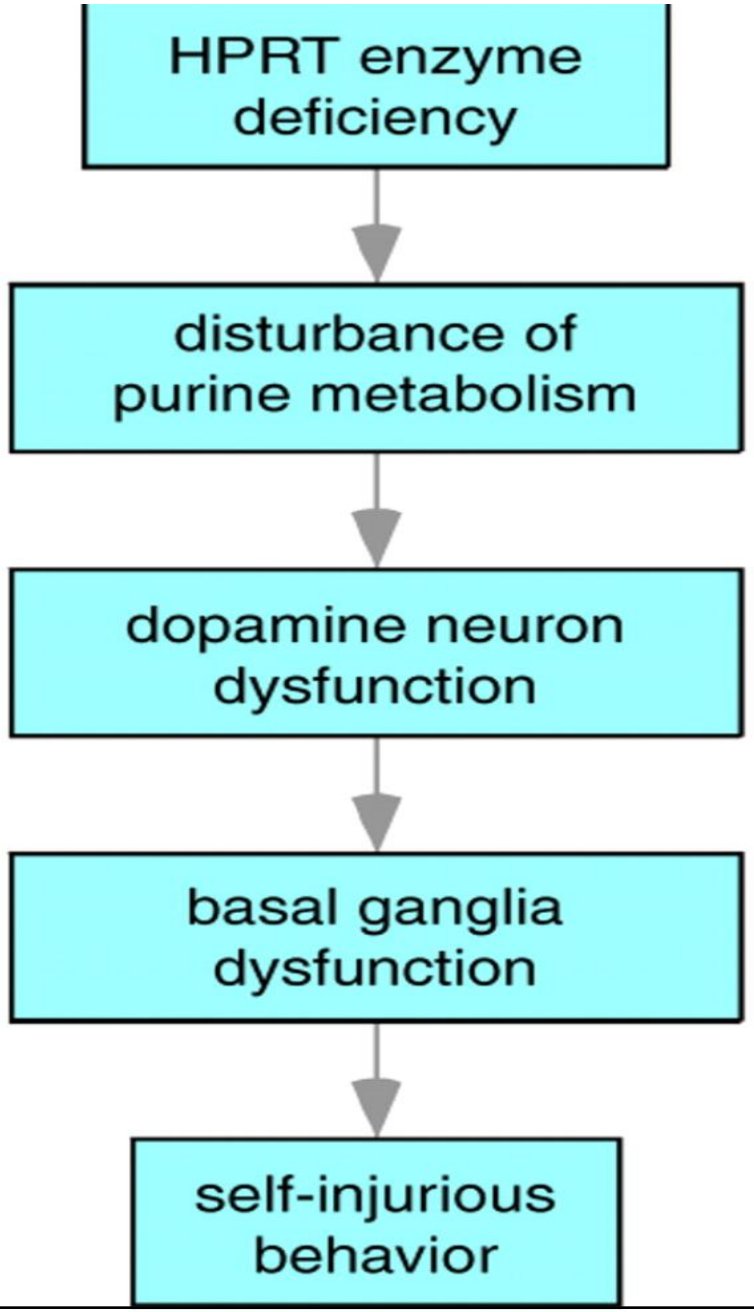
↓

increases their degradation to uric acid

- Leads to hyperuricemia---**Gout-like symptoms**
  **Nephrolithiasis** ( Renal stones)

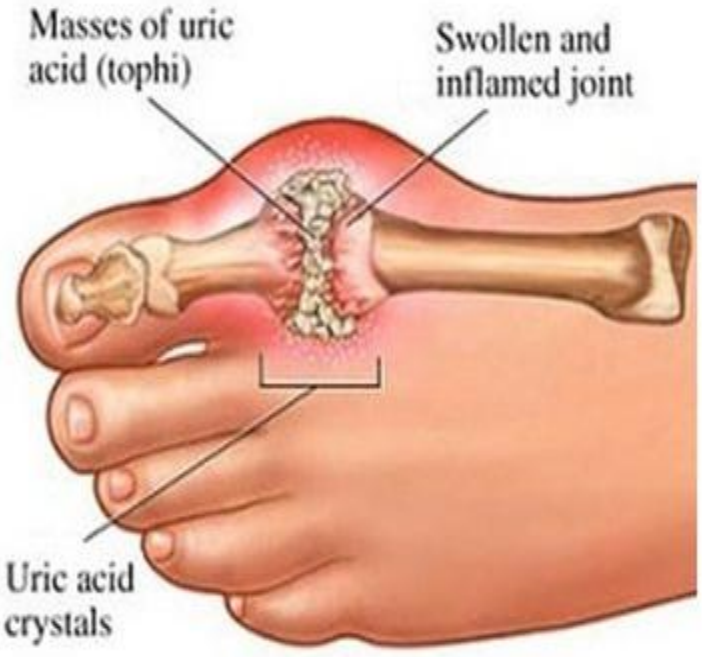
Neurological symptoms

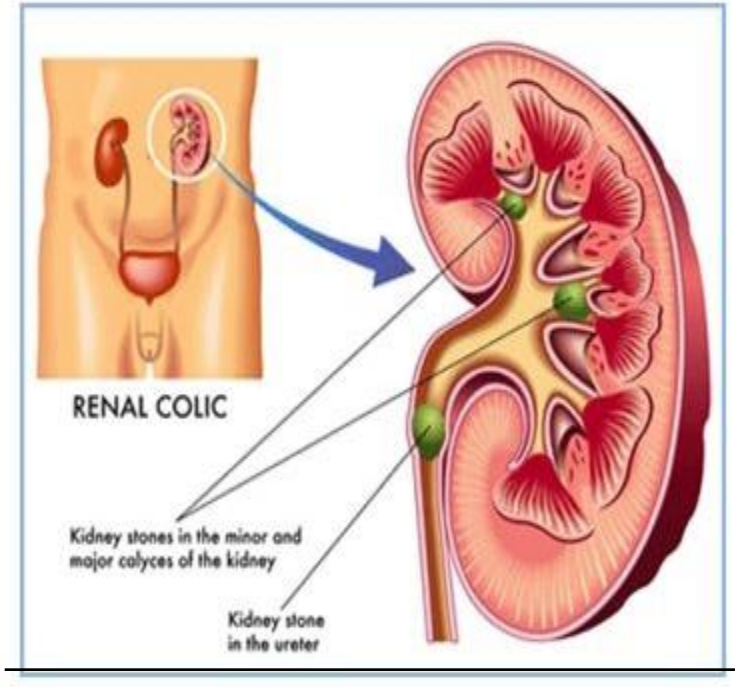
- **self mutilation**
- spasticity,
- aggressiveness,
- **mental retardation**

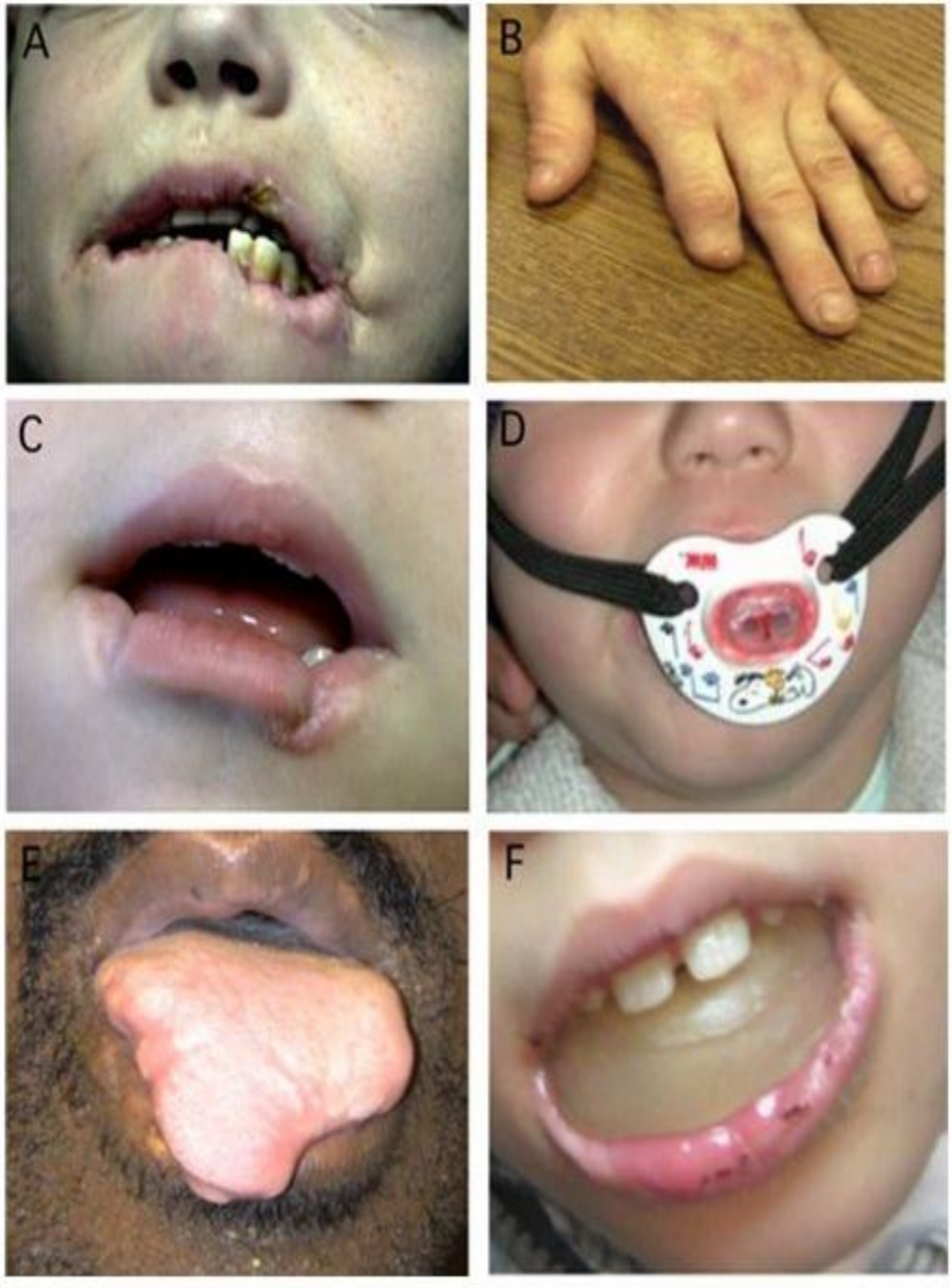


# DIAGNOSIS

- Increase urinary urate / creatinine ratio
- Absent / reduced enzyme activity in lymphocytes or fibroblast
- Mutation analysis of Hypoxanthine-Guanine phosphoribosyl transferase (HGPRT) gene.

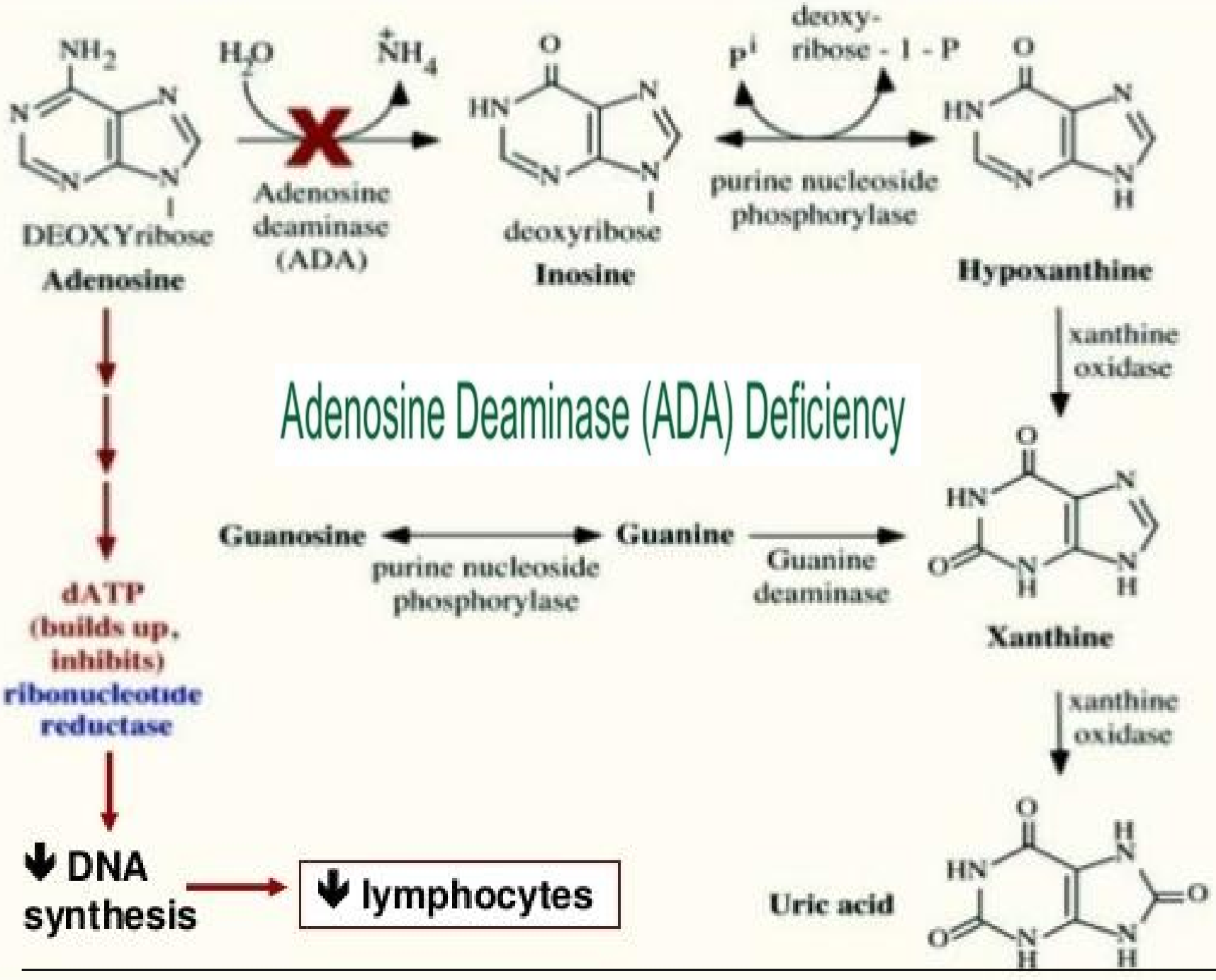


# Severe combined immunodeficiency (SCID)

- The deficiency of adenosine deaminase (ADA) causes severe combined immunodeficiency (SCID) involving T-cell and usually B-cell dysfunction.
- ADA deficiency results in the accumulation of dATP.
- dATP is an inhibitor of ribonucleotide reductase which causes reduced synthesis of other dNTPs and therefore DNA synthesis and cell replication is inhibited.
- Thus proliferation and differentiation of immune cells is compromised.



# SCID

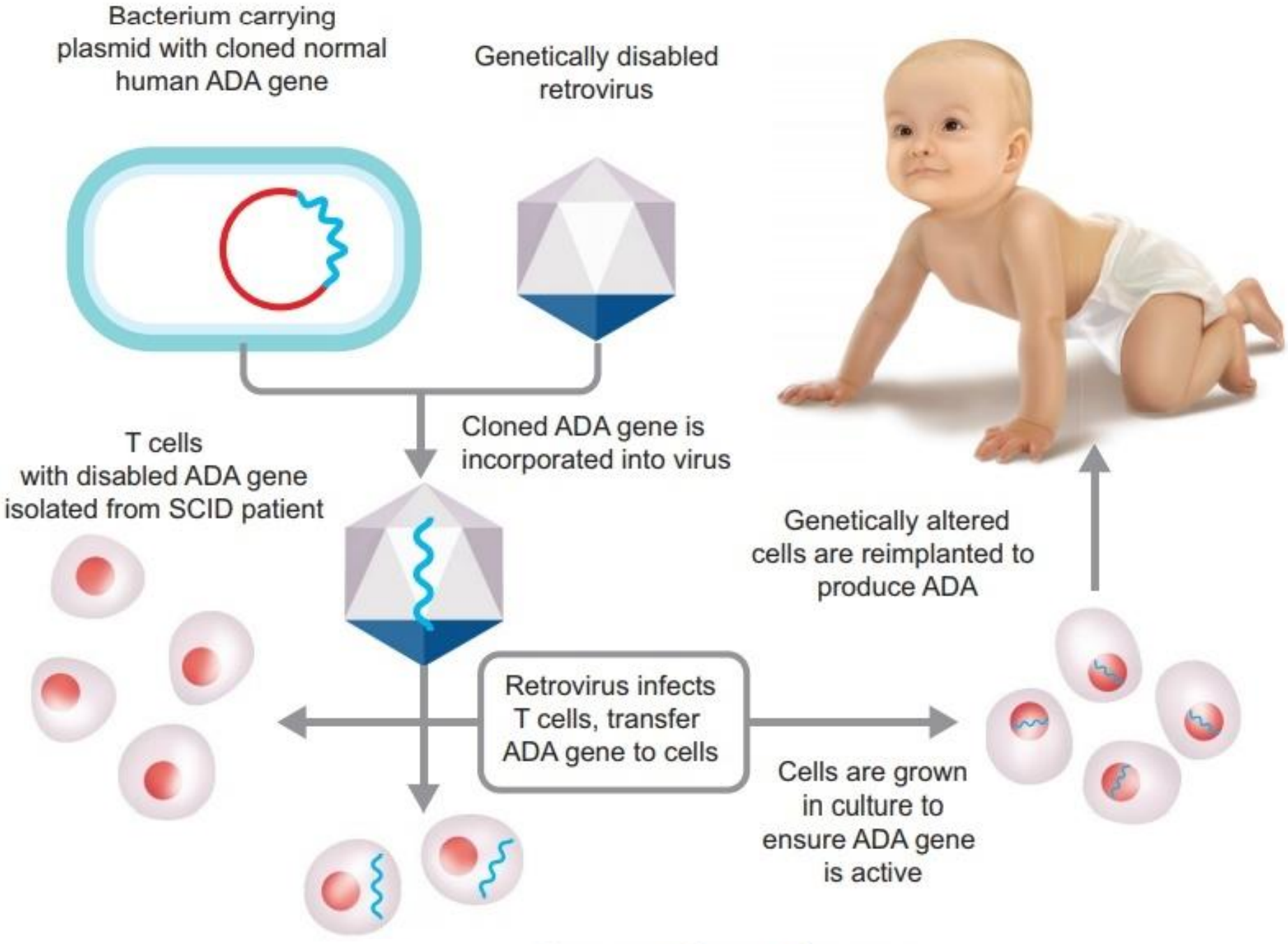
- Lymphocytes usually contain high levels of ADA.
- Therefore, ADA deficiency is mainly manifested as reduced lymphocytes.
- This leads to impaired cellular and humoral immunity.
- Hypouricemia is due to defective breakdown of purine nucleotides.



ADA estimation in CSF is used for the diagnosis of tuberculous meningitis.
ADA levels can be estimated in various body fluids like blood, CSF, pleural fluid, pericardial fluid, ascitic fluid, etc.

# SCID - Treatment

- Antibiotics and periodic injections of immunoglobulin will be lifesaving.
- Bone marrow stem cells will increase both T and B cells in the patients.
- Enzyme replacement therapy with ADA-Polyethylene glycol ( the first successful application of enzyme replacement therapy for an inherited disease.
- Gene therapy- recently, ADA gene has been successfully transfected into stem cells of ADA deficient children.




**Process of gene therapy**

# Purine Nucleoside Phophorylase Deficiency

- Less severe than ADA deficiency
- Associated with severe deficiency of T- cells but apparently normal B- cell function.
- Immune dysfunction appear to result from accumulation of dGTP, and dATP, which inhibit ribonucleotide reductase and thereby deplete cells of DNA precursors.