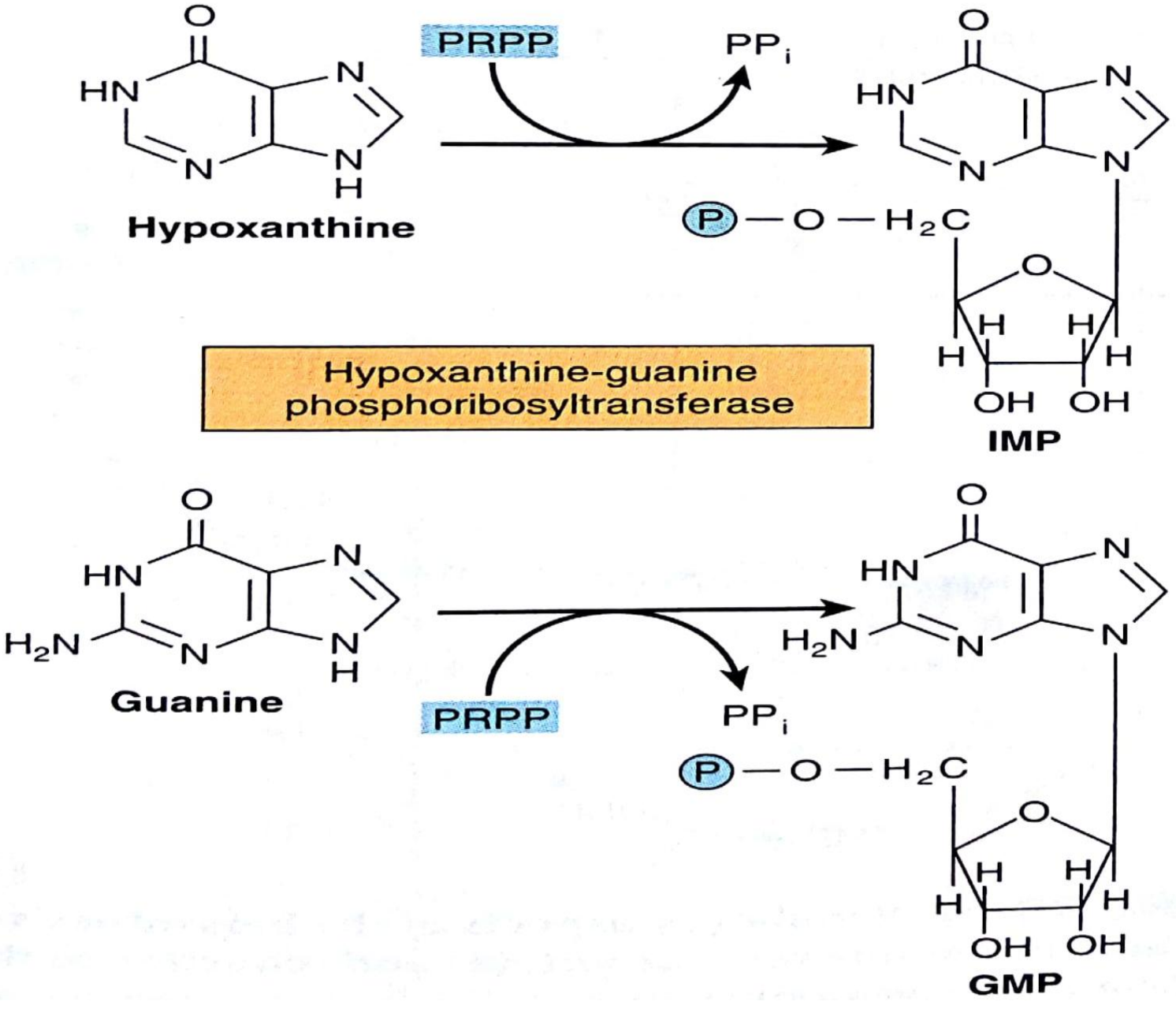
Lesch-Nyhan Syndrome

- 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

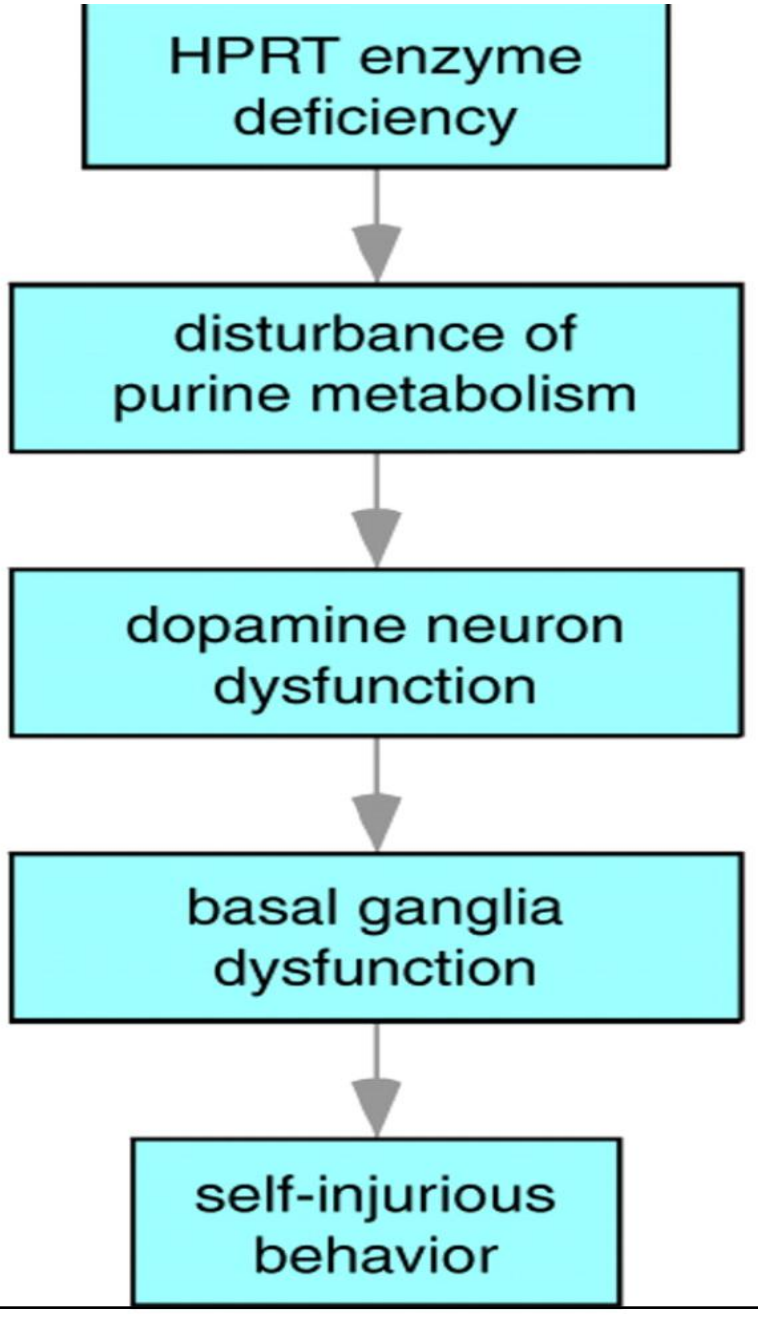
↓

increases their degradation to uric acid

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

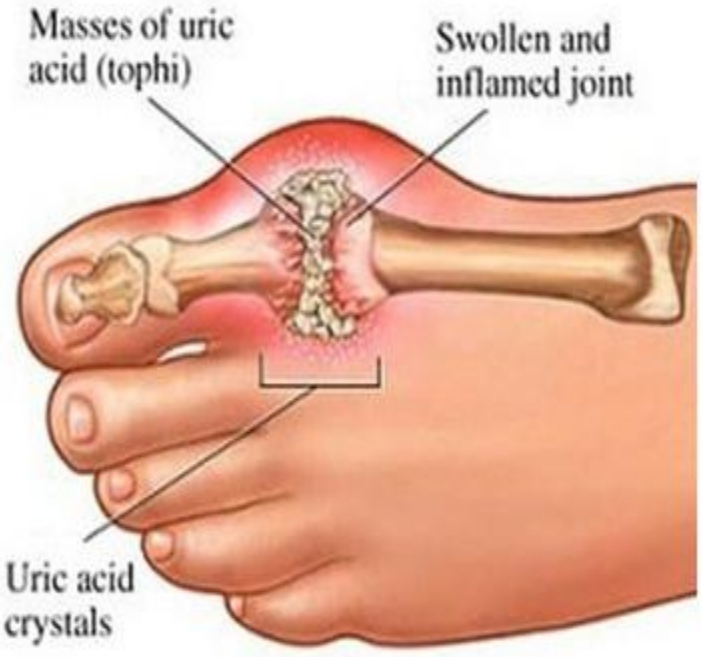
Neurological symptoms

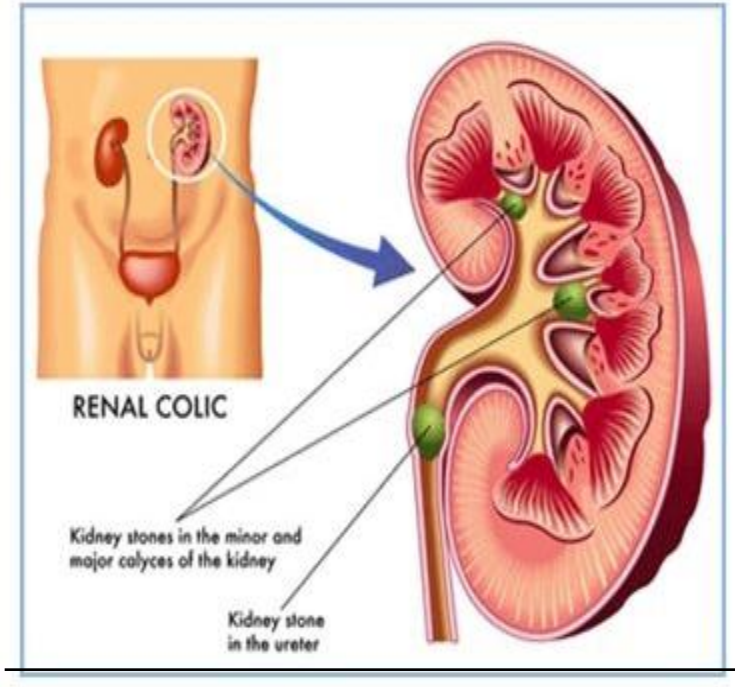
- **self mutilation**
- spasticity,
- aggressiveness,
- **mental retardation**

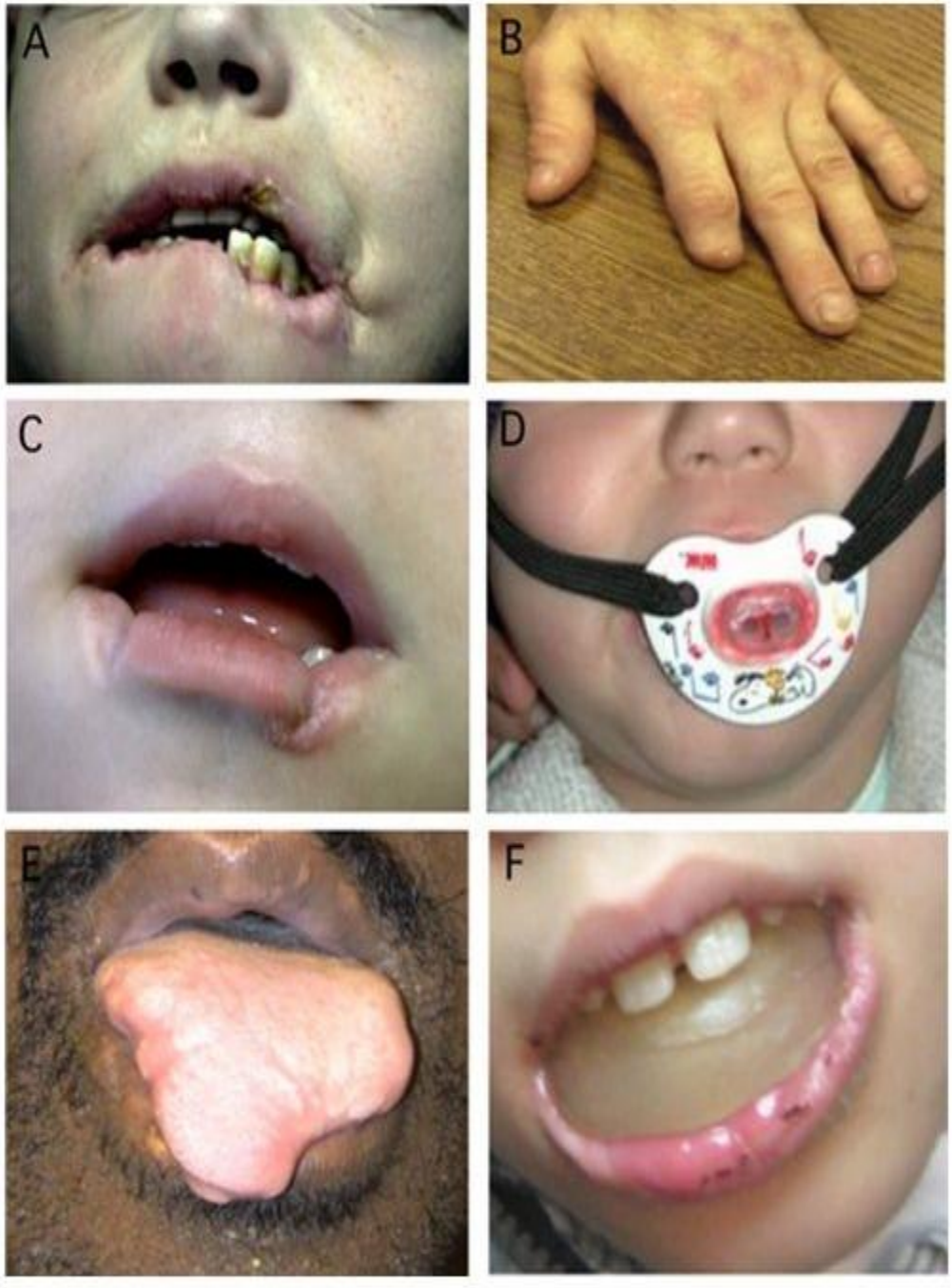


DIAGNOSIS

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

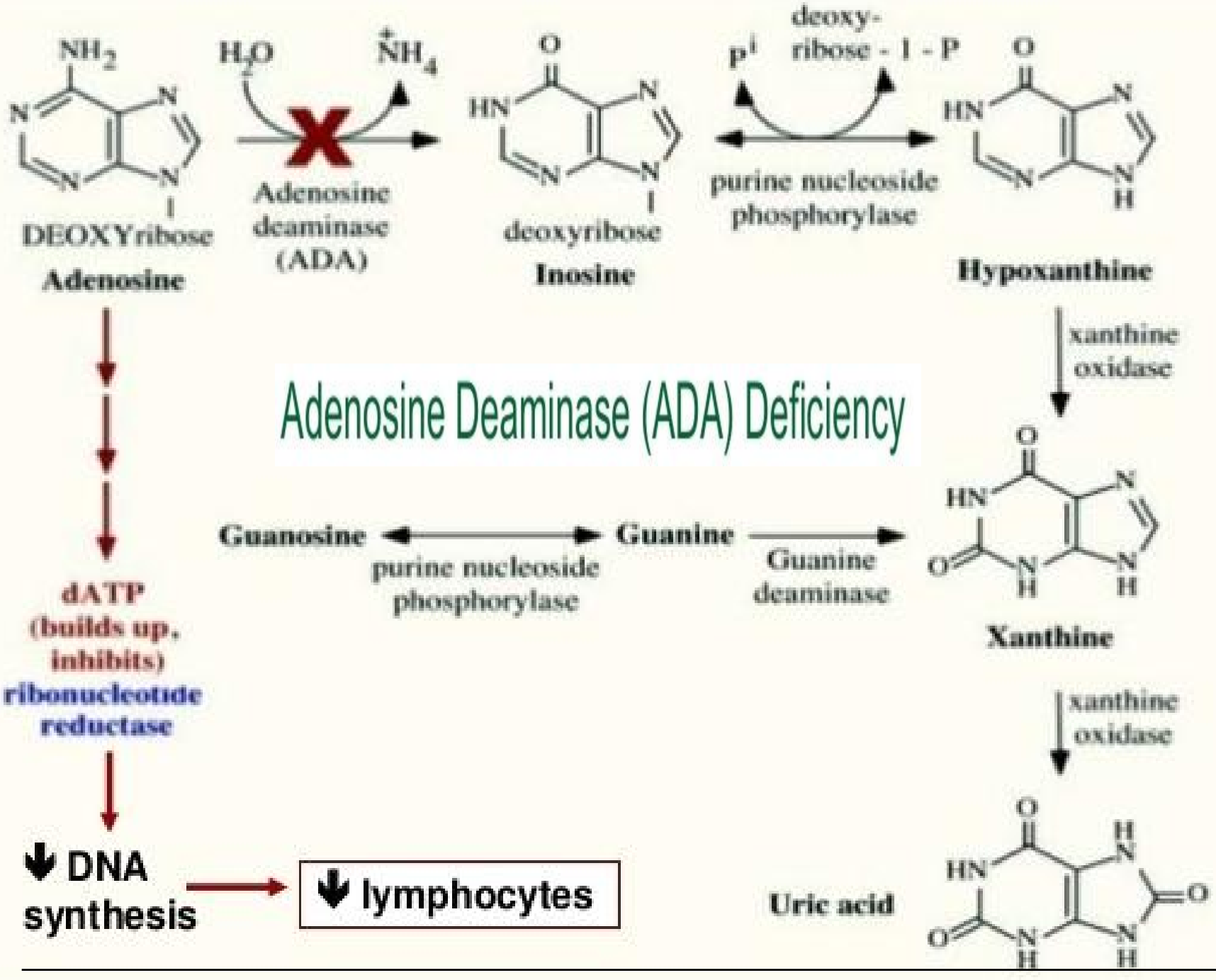






Severe combined immunodeficiency (SCID)

- 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.



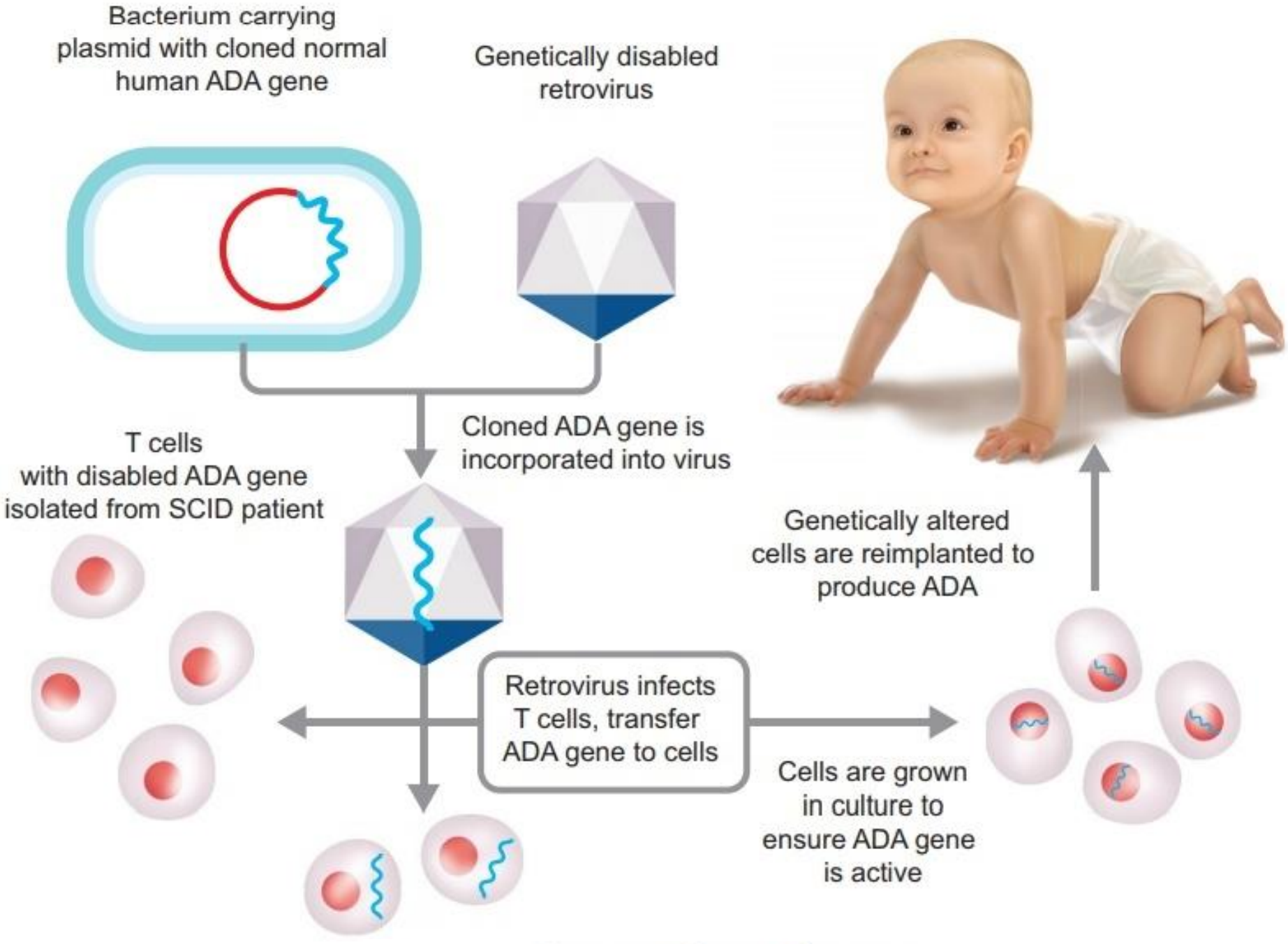
SCID

- 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.

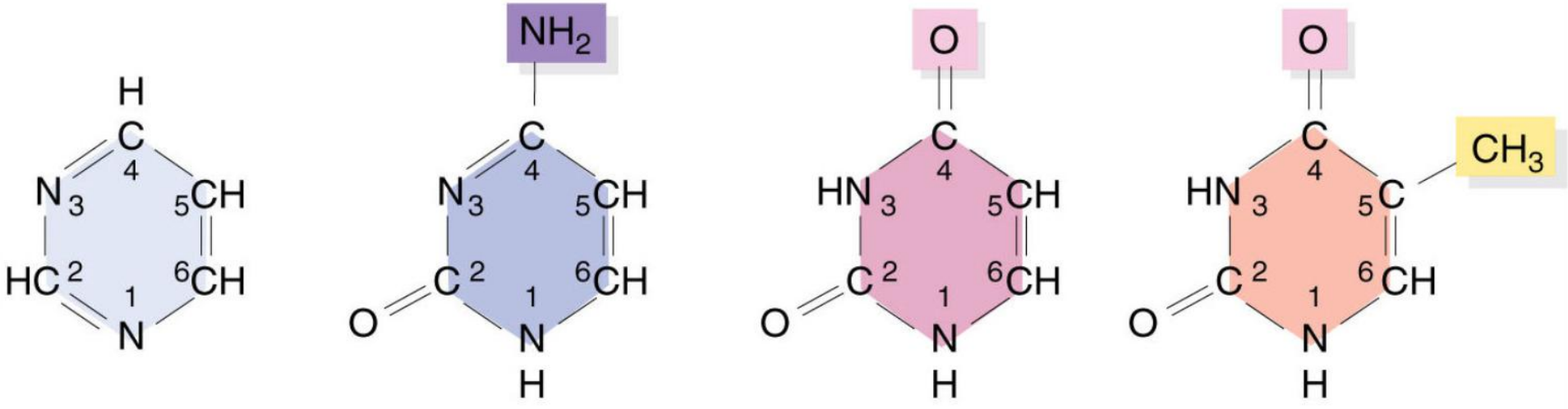


Process of gene therapy

Purine Nucleoside Phophorylase Deficiency

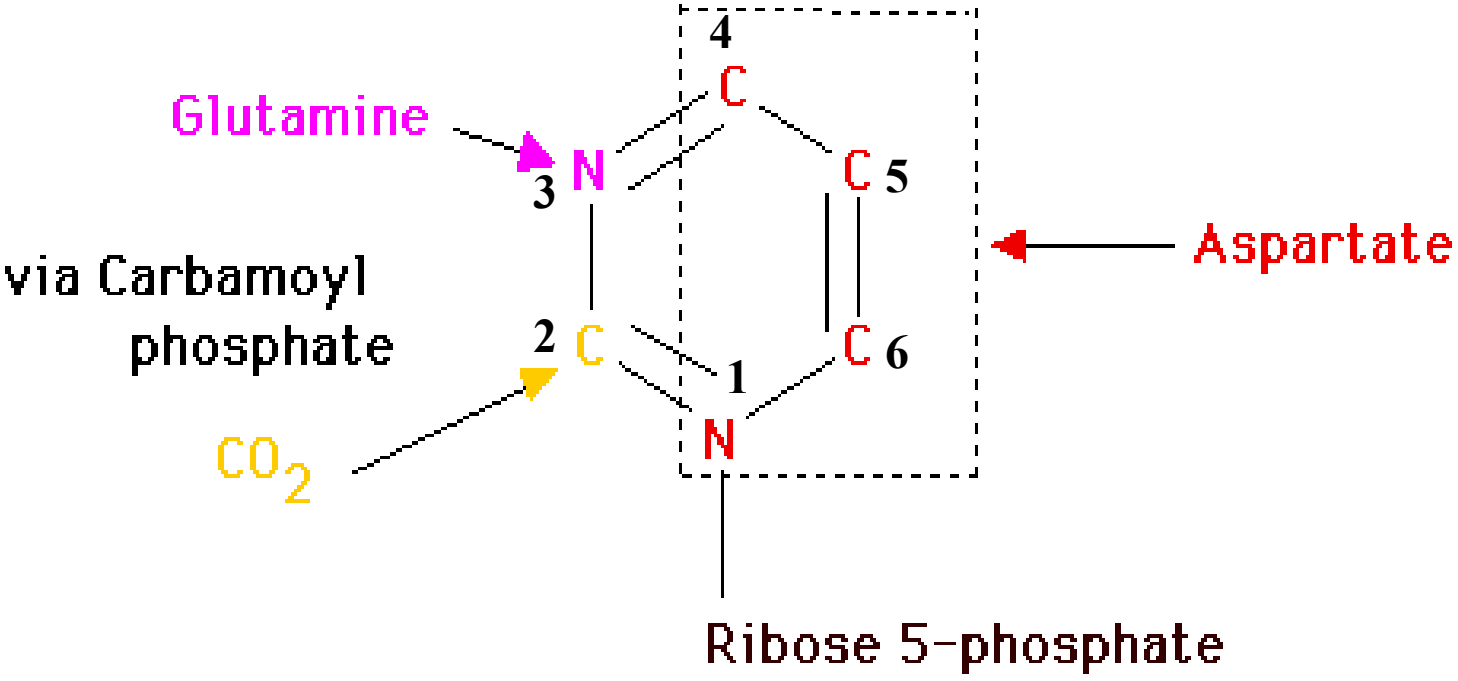
- Less severe than ADA deficiency
- Associated with severe deficiency of T- cells but apparently normal B- cell function.
- Immune dysfunction appear to result from accumulation of dGTP, and dATP, which inhibit ribonucleotide reductase and thereby deplete cells of DNA precursors.

METABOLISM OF PYRIMIDINE



DR. SUDHANSHU SHEKHAR
ASSOCIATE PROFESSOR
DEPT. OF BIOCHEMISTRY

Pyrimidine is a heterocyclic ring.

- 

Sources of different atoms of pyrimidine rings

Synthesis of pyrimidine nucleotides

A. Denovo synthesis

Denovo synthesis of pyrimidine nucleotide refers to the formation of pyrimidine ring structure followed by the addition of ribose phosphate

B. Salvage pathway

Formation of pyrimidine nucleotides from pyrimidine bases

Denovo synthesis

- The synthesis of pyrimidines is a much simpler process compared to that of purines.
- Aspartate, Glutamine and bicarbonate contribute to atoms in the formation of pyrimidine ring.
- Pyrimidine ring is first synthesized and then attached to ribose 5-phosphate.
- This is in contrast to purine nucleotide synthesis where in purine ring is built upon a pre-existing ribose-5-phosphate.

Synthesis of pyrimidine nucleotides

Tissue and site of synthesis

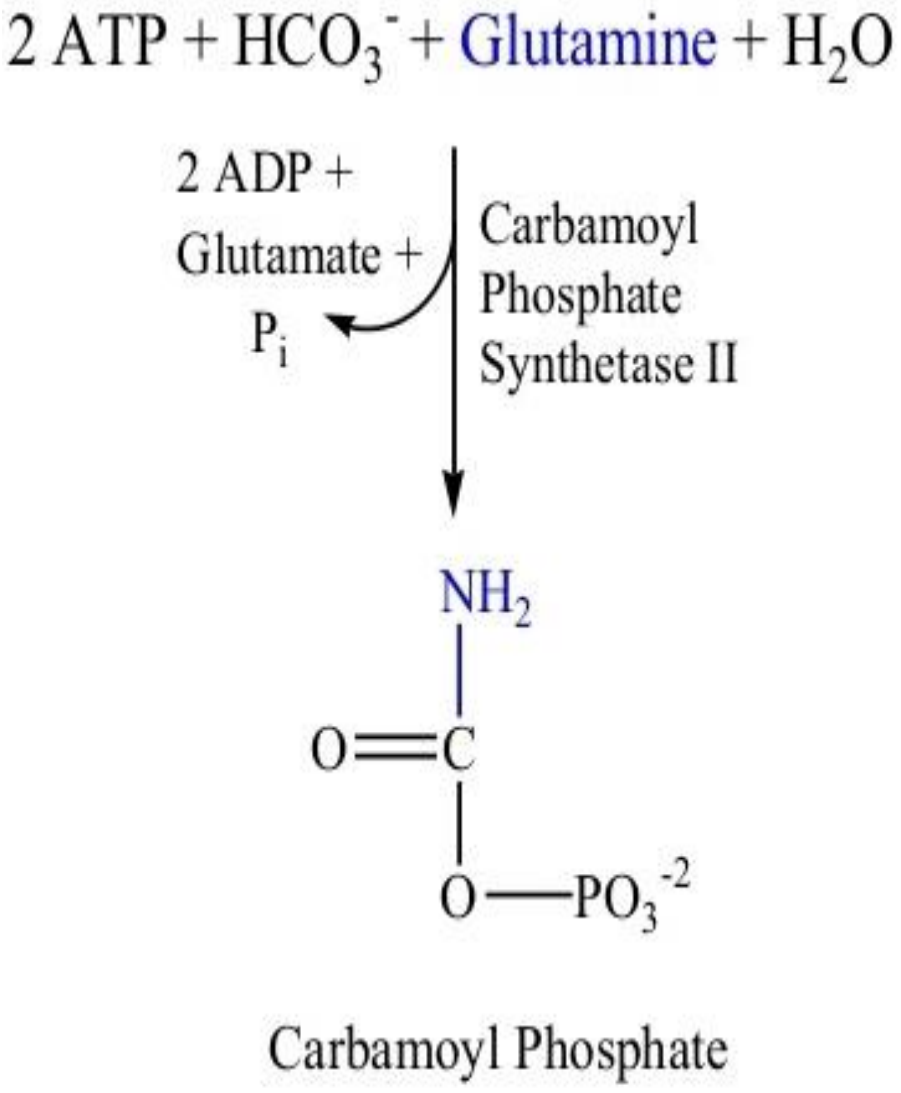
Mainly occurs in the liver.

The reaction occurs in cytosol and mitochondria.

The formation of orotate from dihydroorotate occurs ie mitochondria and all other reactions occur in the cytosol.

Step 1: Carbamoyl Phosphate Synthesis

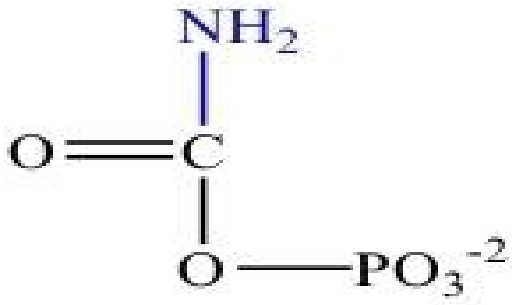
- The reaction occurs in cytoplasm (in urea synthesis, the reaction is in mitochondria).
- The nitrogen of glutamine, ATP and bicarbonate react to form carbamoyl phosphate (step 1).
- The enzyme is carbamoyl phosphate synthetase II (CPS II). N



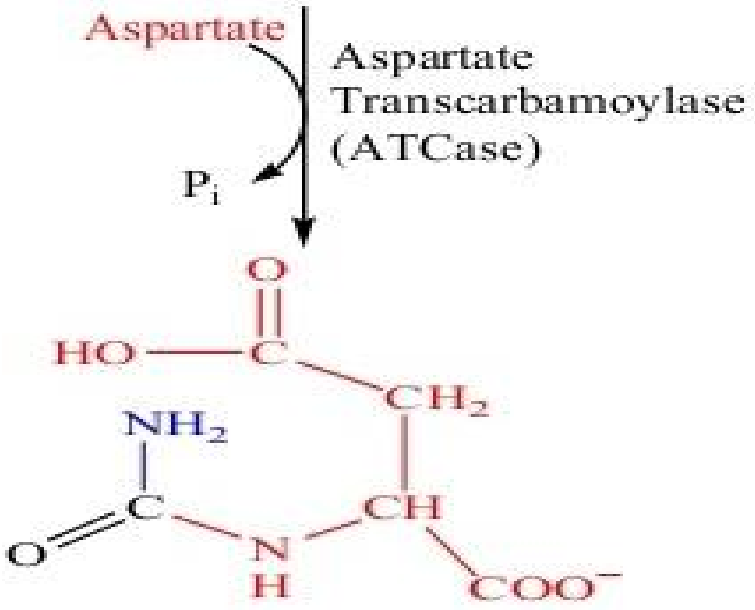
Carbamoyl Phosphate

Step 2: Rate Limiting Step :Condensation

- Carbamoyl phosphate and aspartate combine to form carbamoyl aspartate
- The enzyme is aspartyl trans carbamoylase (ATC), which is allosterically regulated
- The atoms C2 and N3 are derived from carbamoyl phosphate and the rest are from aspartate.



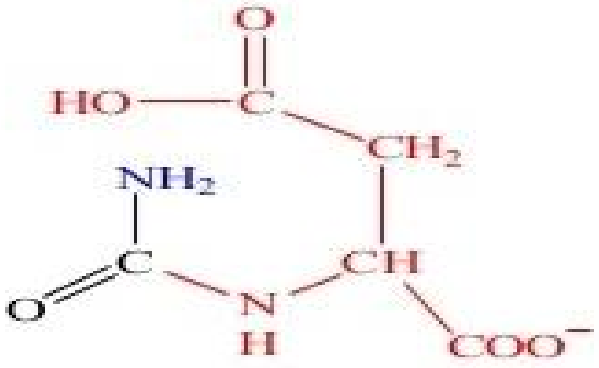
Carbamoyl Phosphate



Carbamoyl Aspartate

Step 3: Formation of Pyrimidine Ring

- The 3rd nitrogen and 4th carbon are joined by a covalent bond and carbamoyl aspartate is cyclized.
- Dihydo orotic acid is produced.
- The enzyme is dihydro orotase (DHOase)



Carbamoyl Aspartate

Ring closure:

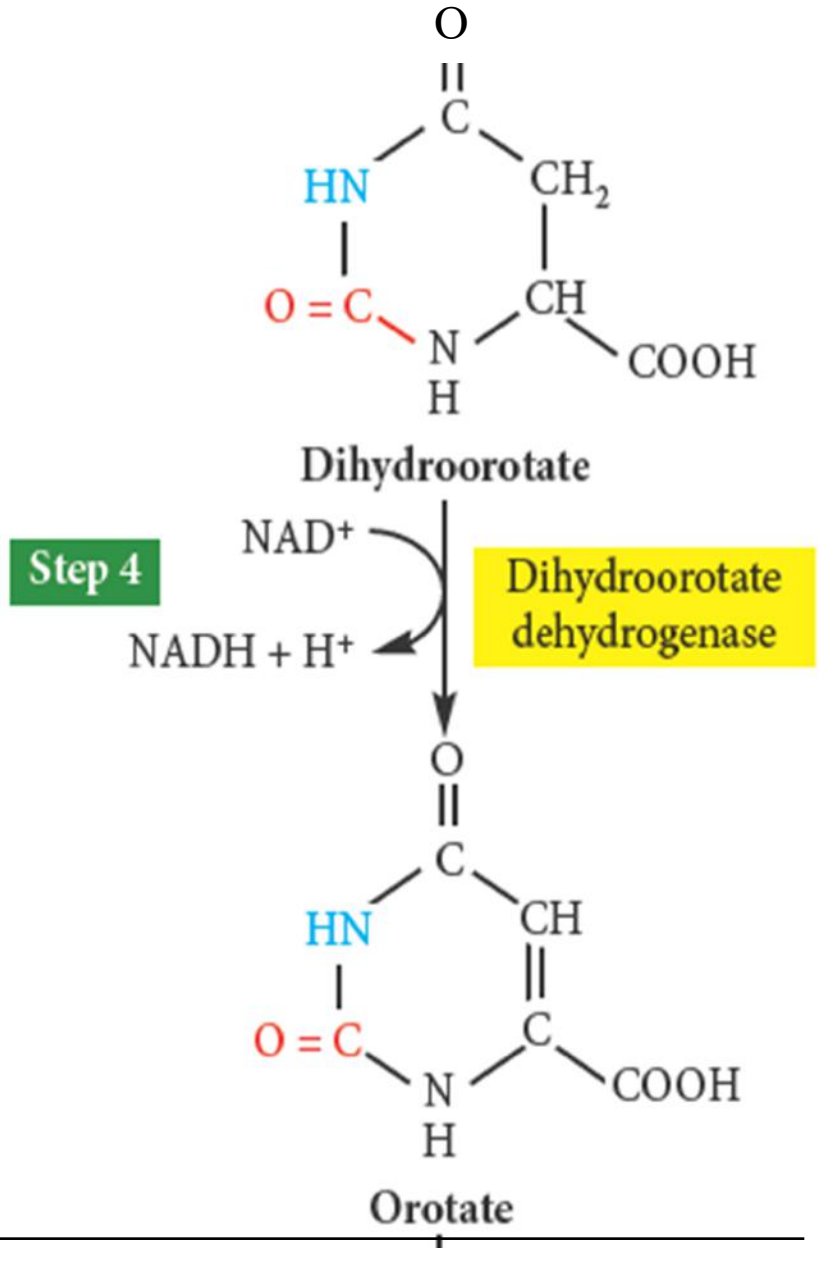
Dihydroorotase

H_2O

Dihydroorotate

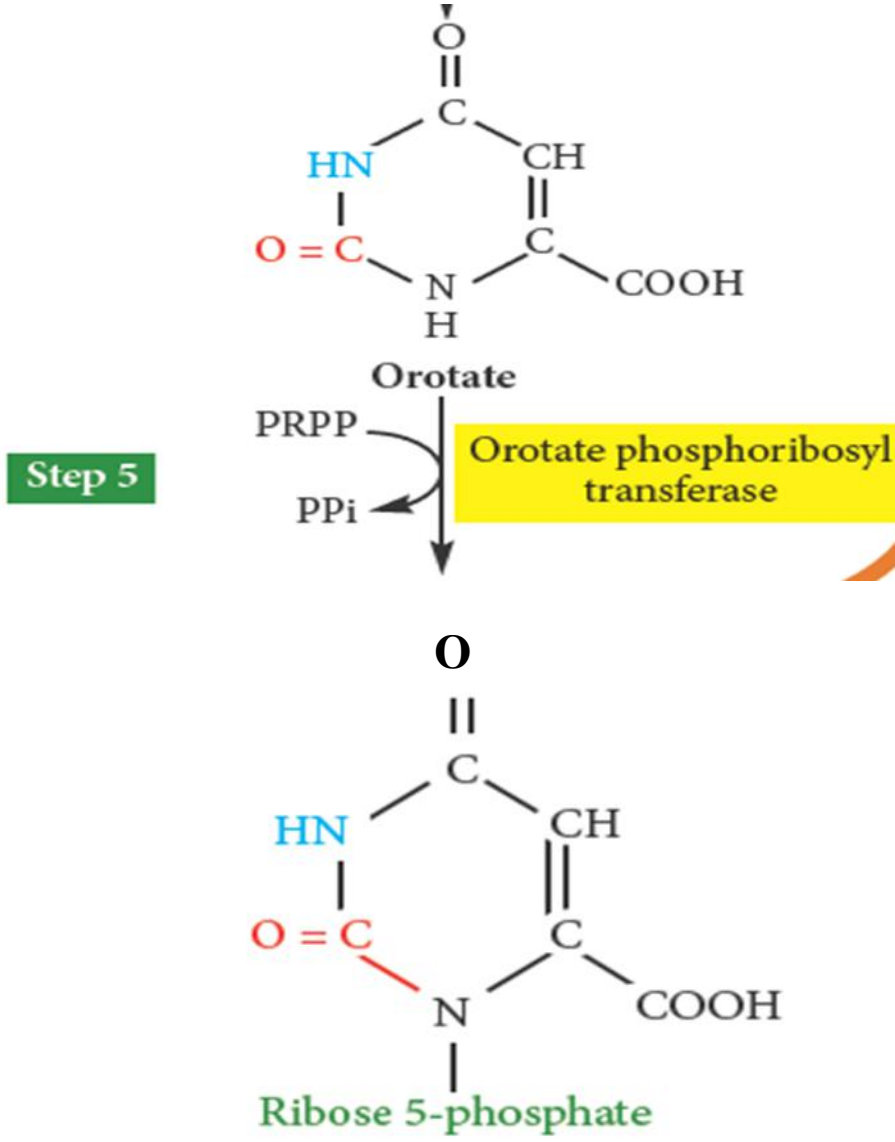
Step 4: Oxidation

- Hydrogen atoms are removed from C5 and C6 positions, so that orotic acid is produced
- Enzyme is dihydro orotate dehydrogenase (DHODH).
- It requires NAD as co-enzyme.



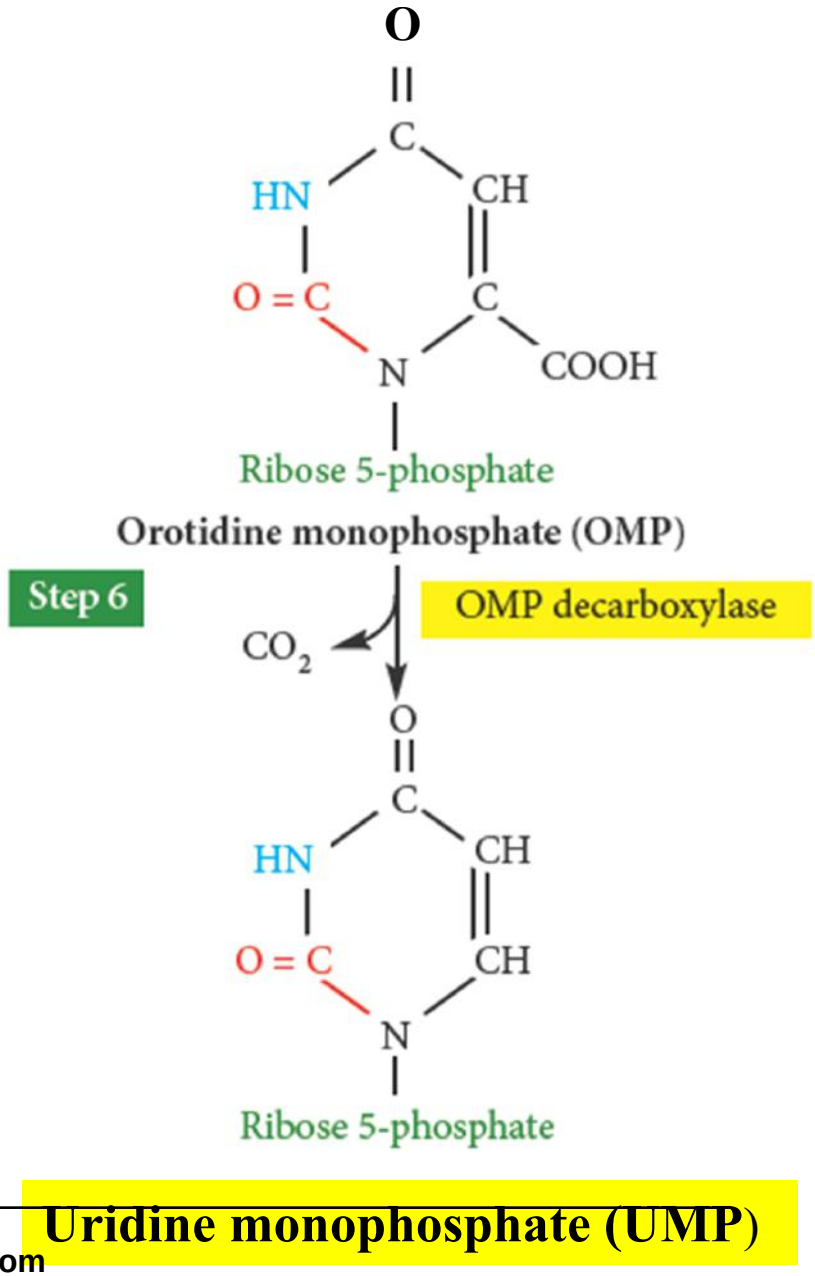
Step 5:Transfer of ribose phosphate & Formation of OMP

- Ribose-5-phosphate is added to orotic acid, so as to produce orotidylic acid or orotidine monophosphate (OMP).
- PRPP is the donor of ribose-5-P.
- The enzyme is orotate phosphoribosyl transferase (OPRTase)



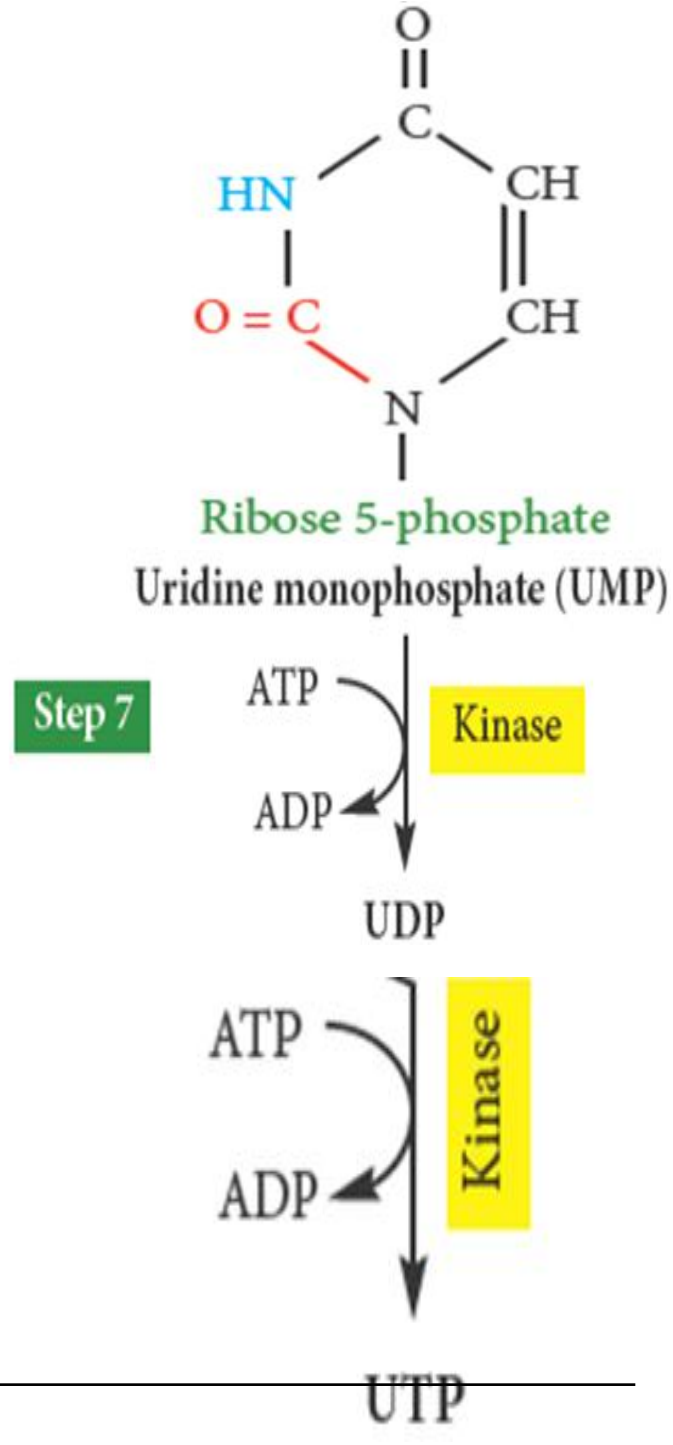
Step 6: Decarboxylation

- The C7 of OMP is removed as carbon dioxide, so that uridine monophosphate (UMP) is produced
- This is the first pyrimidine that is synthesized.
- The enzyme is OMP-decarboxylase (OMPDC).
- 6-aza-uridine inhibits this step, and so used as an anticancer drug



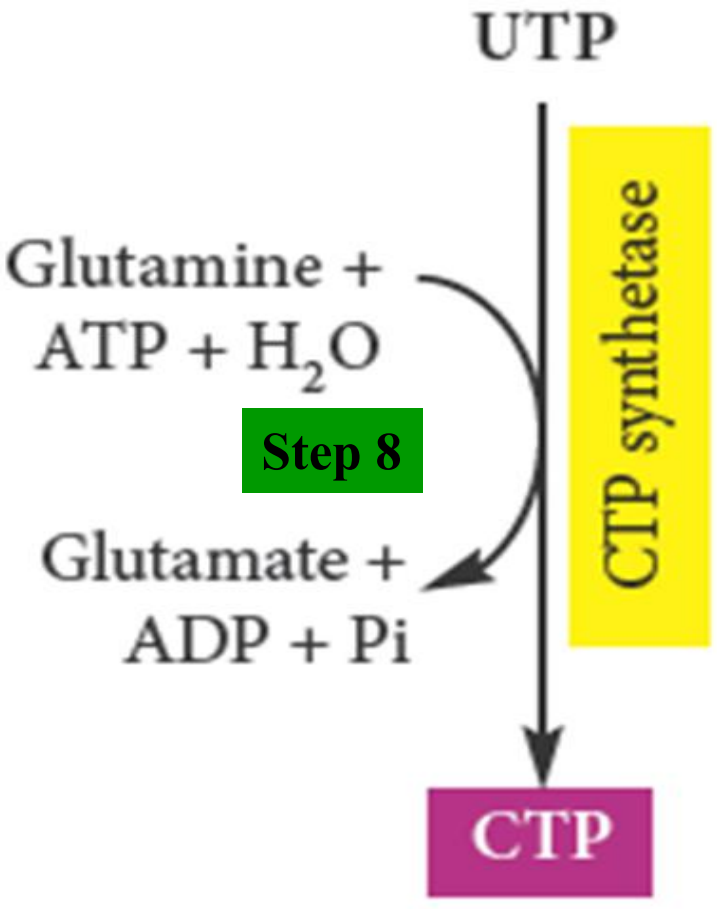
Step 7: Synthesis of Triphosphates

- UMP is phosphorylated to form UDP (uridine diphosphate) with the help of ATP
- The enzyme is nucleoside monophosphatekinase (UMP kinase).
- The UDP is phosphorylated to UTP (uridine triphosphate) with the help of ATP
- The enzyme is nucleoside diphosphate kinase

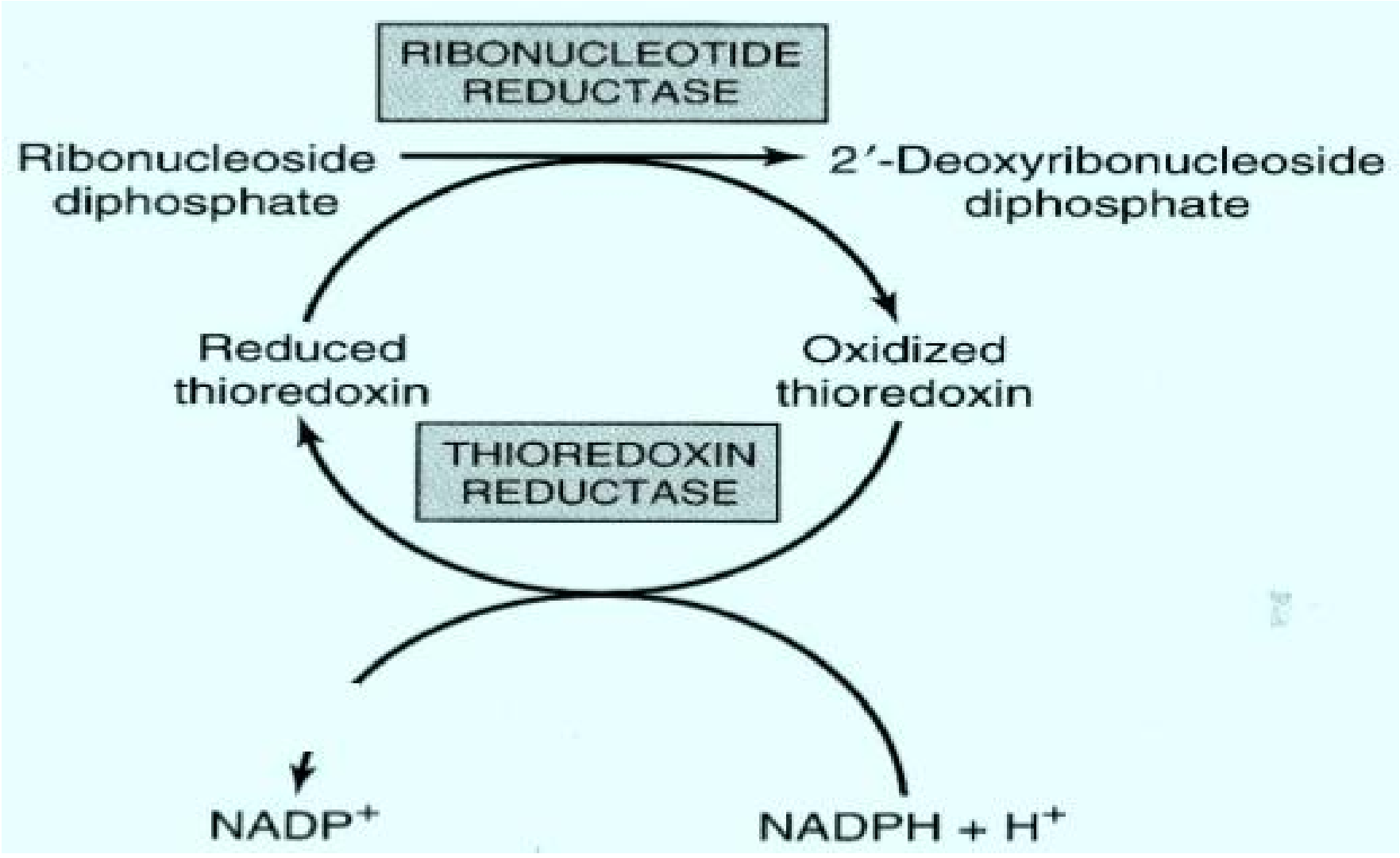


Step 8: Formation of CTP

- UTP is converted to CTP by adding an amino group from glutamine catalyzed by CTP synthetase.
- It needs ATP

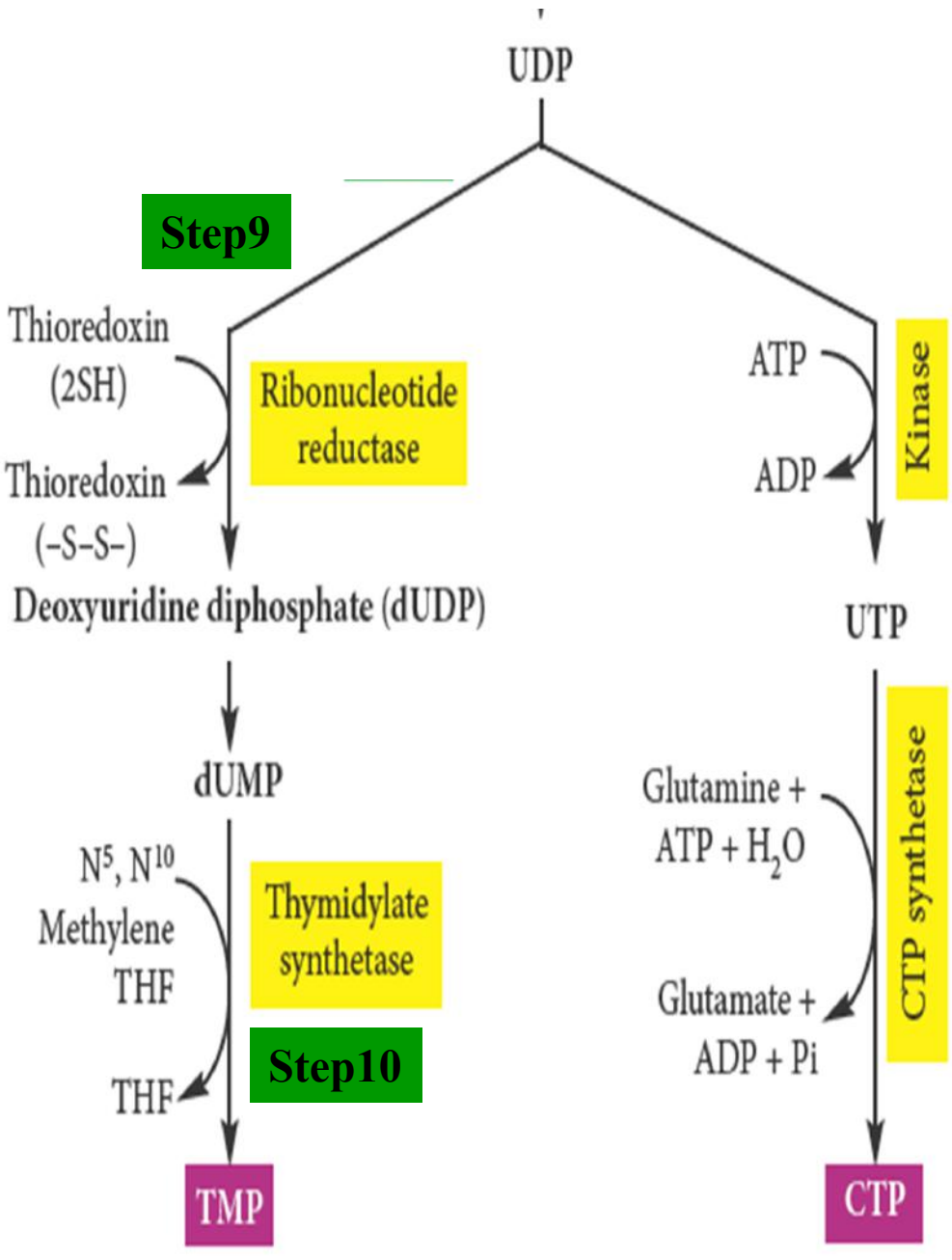


Step 9 .Reduction of ribonucleoside diphosphates to their corresponding dNDP's

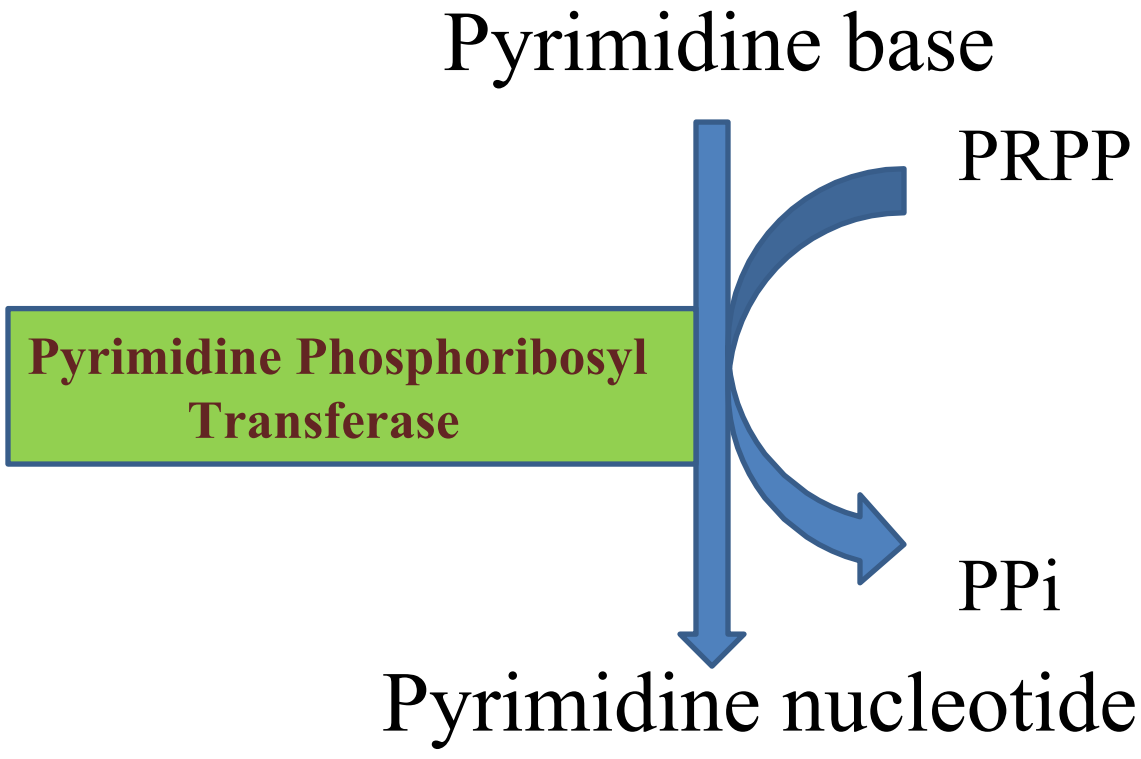


Step10.Formation of TMP from UDP

- dUMP is substrate for TMP synthesis.
- dUDP is dephosphorylated to dUMP
- Methylation of dUMP occurs at C5 by N^5,N^{10}methyleneTHF, forming TMP.
- This reaction is catalysed by Thymidylate synthase.



SALVAGE PATHWAY OF PYRIMIDINE SYNTHESIS



PROPERTY	CPS I	CPS II
Subcellular localization	Mitochondria (matrix) (along with ornithine transcarbamylase)	Cytosol (along with aspartate transcarbamylase)
Tissue distribution	Liver Gut, kidney (< 10% of activity in liver)	Variety of tissues (related to growth rate)
Substrate	NH_3 Km ~ 1 mM	Glutamine Km ~ 20 μM NH_3 Km ~ 5mM
Effector	N-acetyl-L-glutamate ↑ (essential for activity)	P-ribose-PP ↑ UTP ↓ glutamine analogues ↓
Activity	~ 1-3 μmoles/hr/mg	~ 0.001-0.05 μmoles/hr/mg
Metabolic role	Arginine & urea synthesis	Pyrimidine synthesis

*Arrows indicate that effector increases or decreases enzymatic activity. Specific activity is expressed as micromoles of product formed/hr/mg of protein.

Regulation of pyrimidine synthesis

CPSII and Aspartate transcarbomylase are main regulatory enzymes.

There is feedback regulation to maintain optimal pyrimidine nucleotide concentrations.

CPS II –

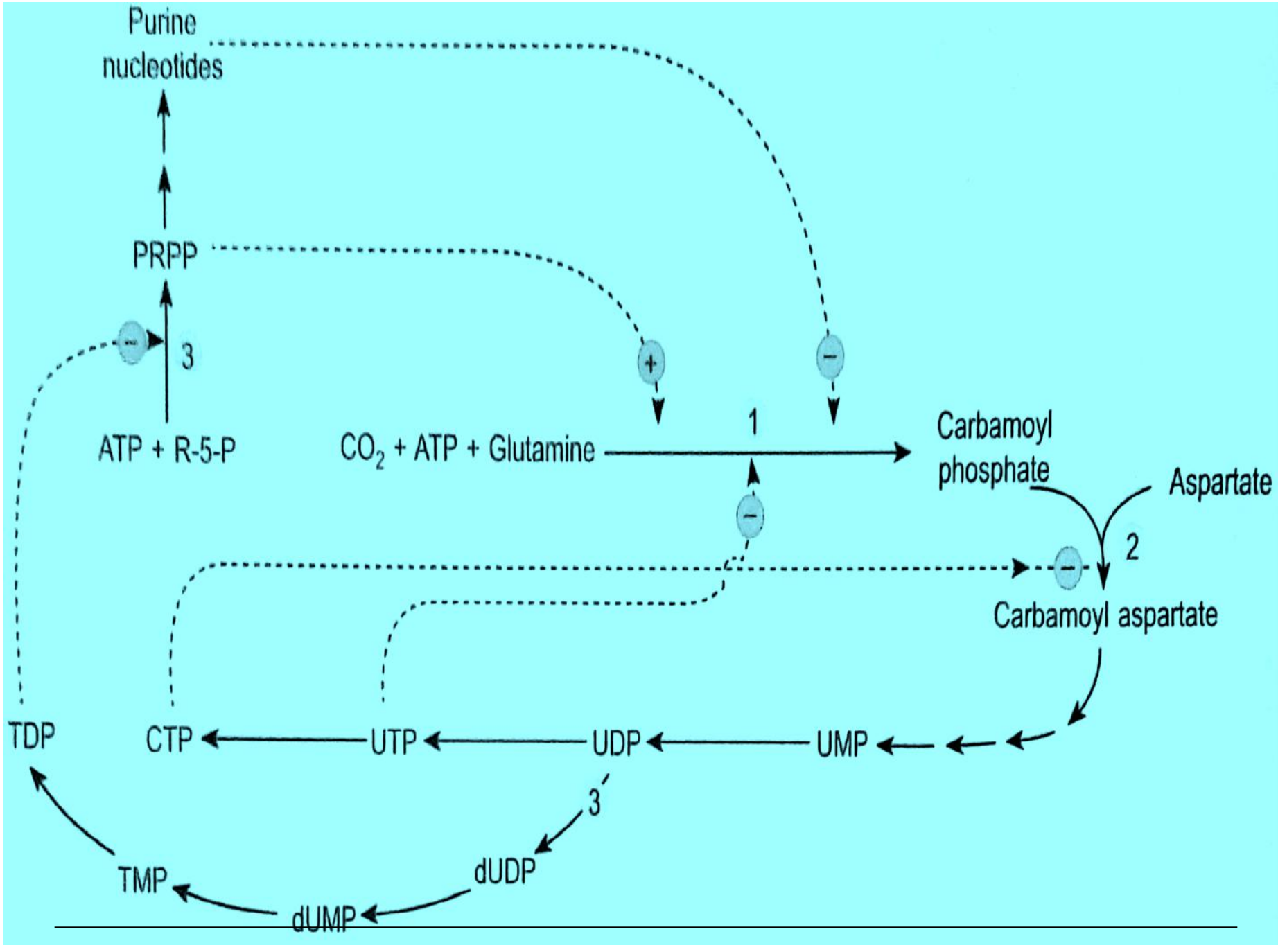
- inhibited by UTP .
- activated by PRPP

Aspartate transcarbomylase :

- inhibited by CTP
- activated by ATP

Cross Regulation of Purine and pyrimidine synthesis:

- PRPP is required for the synthesis of both purines and pyrimidines, so its regulation by both ensures coordinated purine and pyrimidine synthesis
- PRPP stimulates the purine and pyrimidine synthesis through amidotransferase and carbamoyl phosphate synthase respectively.
- So both purine and pyrimidine feedback inhibit PRPP synthase
- Increase synthesis of pyrimidines (TDP) leads to allosteric inhibition of PRPP synthase.
- Purine (ADP) also inhibit PRPP synthase.

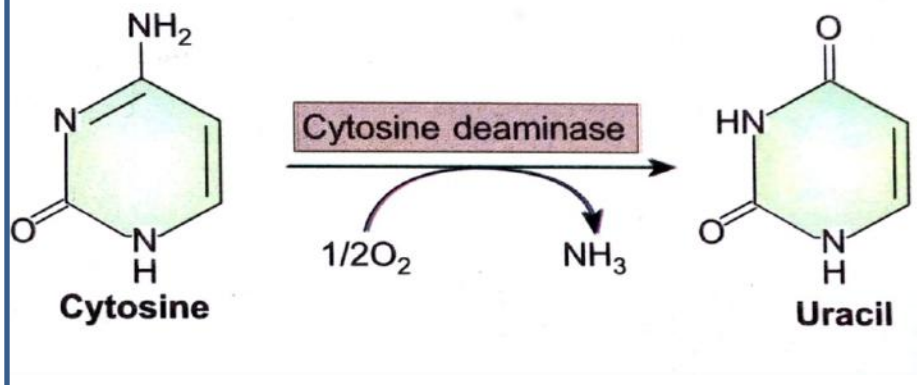


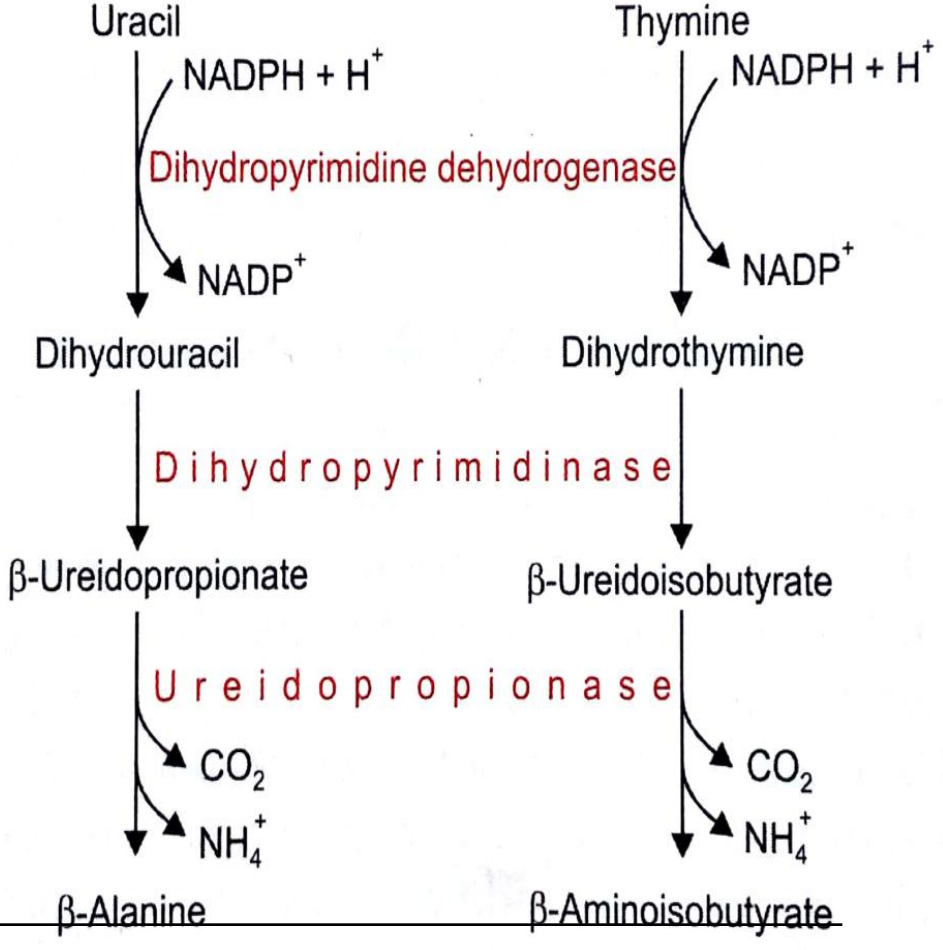
Degradation of pyrimidine nucleotides

•The pyrimidine nucleotides undergo similar reactions (dephosphorylation, deamination and cleavage of glycosidic bond) to liberate the nitrogenous bases cytosine, uracil and thymine.

•The bases are then degraded to highly soluble products β-alanine and β-aminoisobutyrate.

•These are the amino acid which undergo transamination and other reactions to finally produce acetyl CoA and succinyl CoA





DISORDERS OF PYRIMIDINE METABOLISM
1.OROTIC ACIDURIA

- Orotic aciduria type I – deficiency of
  a) Orotate phosphoribosyl transferase and
  b) OMP –decarboxylase.
- Orotic aciduria type II :
  - Rare
  - deficeincy of ONLY OMP decarboxylase.
- Both types are inherited as autosomal recessive disorders.

Features :

- Due to lack of feedback inhibition orotic acid production is excessive.(UMP inhibits OMP decarboxylase)

Rapidly growing cells are affected –

a) anemia
b) Retarded growth
c) Crystals excreted in urine causing urinary obstruction.

- Both types respond to uridine , as it is converted to UTP . This acts as feed back inhibitor

Other causes of orotic aciduria

1. Deficeincy of liver mitochondrial ornthine – trancarbomylase (X-linked).

↓

under utilised substrate carbomyl phosphate enters cytosol

↓

Stimulates pyrimidine nucleotide biosynthesis

↓

Leading to orotic aciduria

2. Drugs may precipitate orotic aciduria:

ALLOPURINOL , a purine analog is a substrate for Orotate phosphoribosyl transferase.

↓

It competes for phosphoribosylation with natural substrate, orotic acid.

↓

The resulting nucleotide product inhibits

↓

OMP DECARBOXYLASE

↓

leading to Orotic aciduria and orotiduniria

Reye's syndrome

- This is considered as a secondary orotic aciduria.
- It is believed that a defect in ornithine trascarbamoylase (or urea cycle) causes the accumulation of carbamoyl phosphate.
- This is then diverted for the increased synthesis and excretion of orotic acid.

Anti-Folate Drugs

- Cancer cells consume dTMP quickly for DNA replication
- Interfere with thymidylate synthase reaction to decrease dTMP production
- Fluorodeoxyuridylate – irreversible inhibitor – also affects rapidly growing normal cells (hair follicles, bone marrow, immune system, intestinal mucosa)
- Dihydrofolate reductase step can be stopped competitively (DHF analogs)
- Anti-Folates: Aminopterin, methotrexate, trimethoprim

Inhibitors

5-Flurouracil and methotrexate