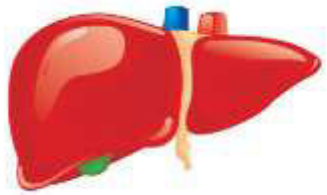
Biosynthesis of Purine & Pyrimidine

Ms. Charmy D. Pandya
Assistant Professor,
Department of Pharmaceutical Chemistry & Quality Assurance
Saraswati Institute of Pharmaceutical Sciences, Dhanap,
Gandhinagar - 382355

Introduction



- **Biosynthesis** is a multi-step, enzyme-catalyzed process where substrates are converted into more complex products in living organisms.
- In **biosynthesis**, simple compounds are modified, converted into other compounds, or joined together to form macromolecules.
- This process often consists of metabolic pathways.
- The purines are built upon a pre-existing ribose 5-phosphate.
- **Liver is** the major site for purine nucleotide synthesis.
- Erythrocytes, polymorphonuclear leukocytes & brain cannot produce purines.

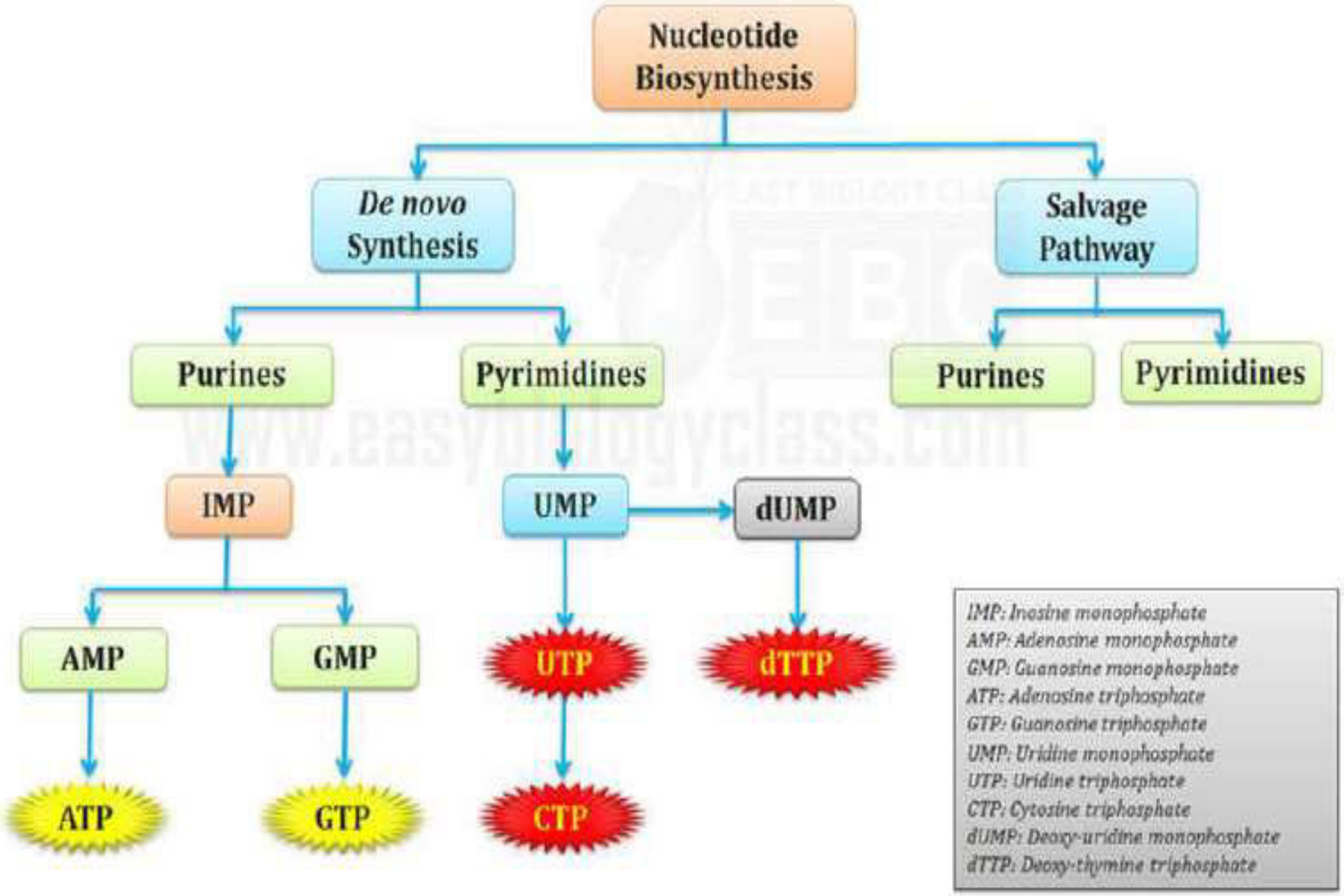
Pathways

- There are **Two pathways** for the synthesis of nucleotides:

1. ***De-novo* synthesis:** Biochemical pathway where nucleotides are synthesized from new simple precursor molecules
2. **Salvage pathway:** Used to recover bases and nucleotides formed during the degradation of RNA and DNA.

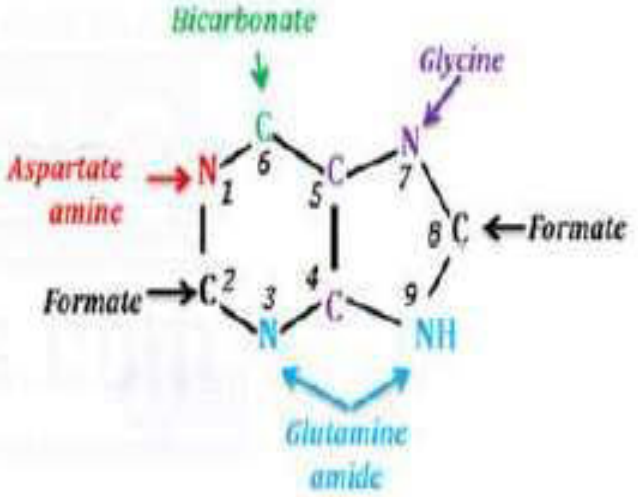
Mind-Map

(Nucleotide Biosynthesis)



De-novo synthesis of purines:

- The image shows the source of different atoms in a **purine skeleton** (identified by radio labeling studies)
  - **N1** - from amino group of **Aspartate**
  - **C2** & **C8** - from **Formate**
  - **N3** & **N9** - from amide group of **Glutamine**
  - **C4, C5** & **N7** - from **Glycine**
  - **C6** - from HCO_3^- (bicarbonate)

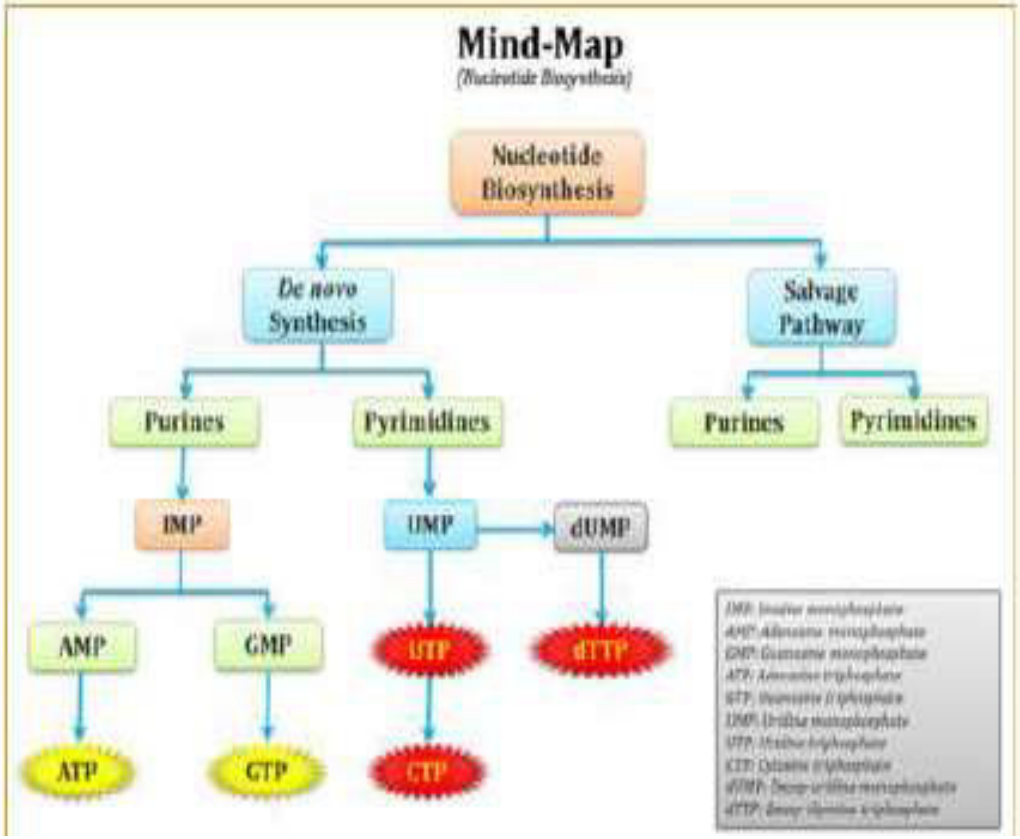


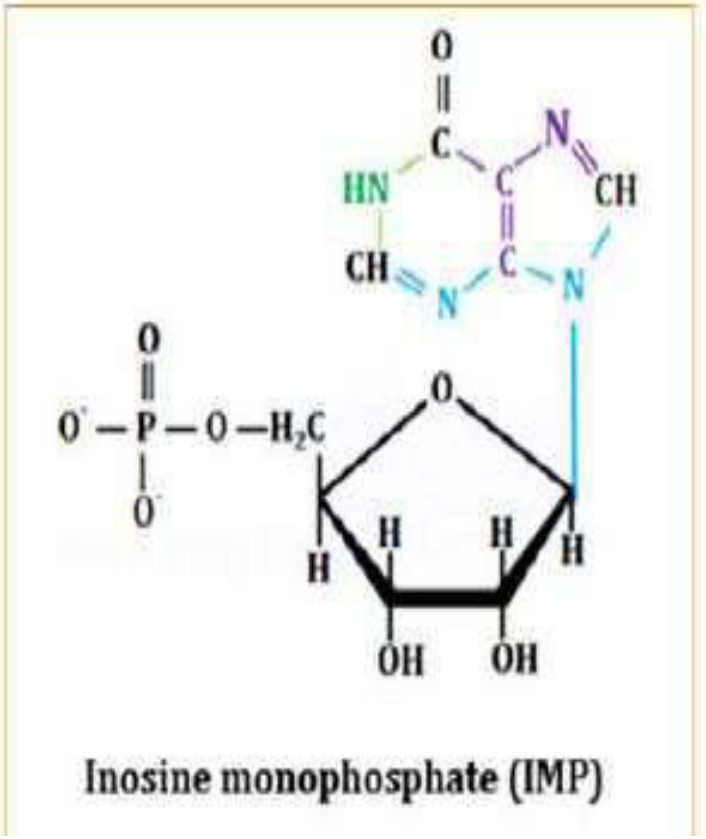
Purine Nucleus
Showing the source of different atoms

- ***Thus, Aspartate, Formate, Glutamine, Glycine and Bicarbonate acts as the building blocks for purine synthesis***

- Purines (adenine and guanine) are derived from **inosine-5′-monophosphate** (IMP)

- ***Thus purine synthesis starts with IMP synthesis*** *(mind map)*





Step involved in purine biosynthesis (Adenine & Guanine)

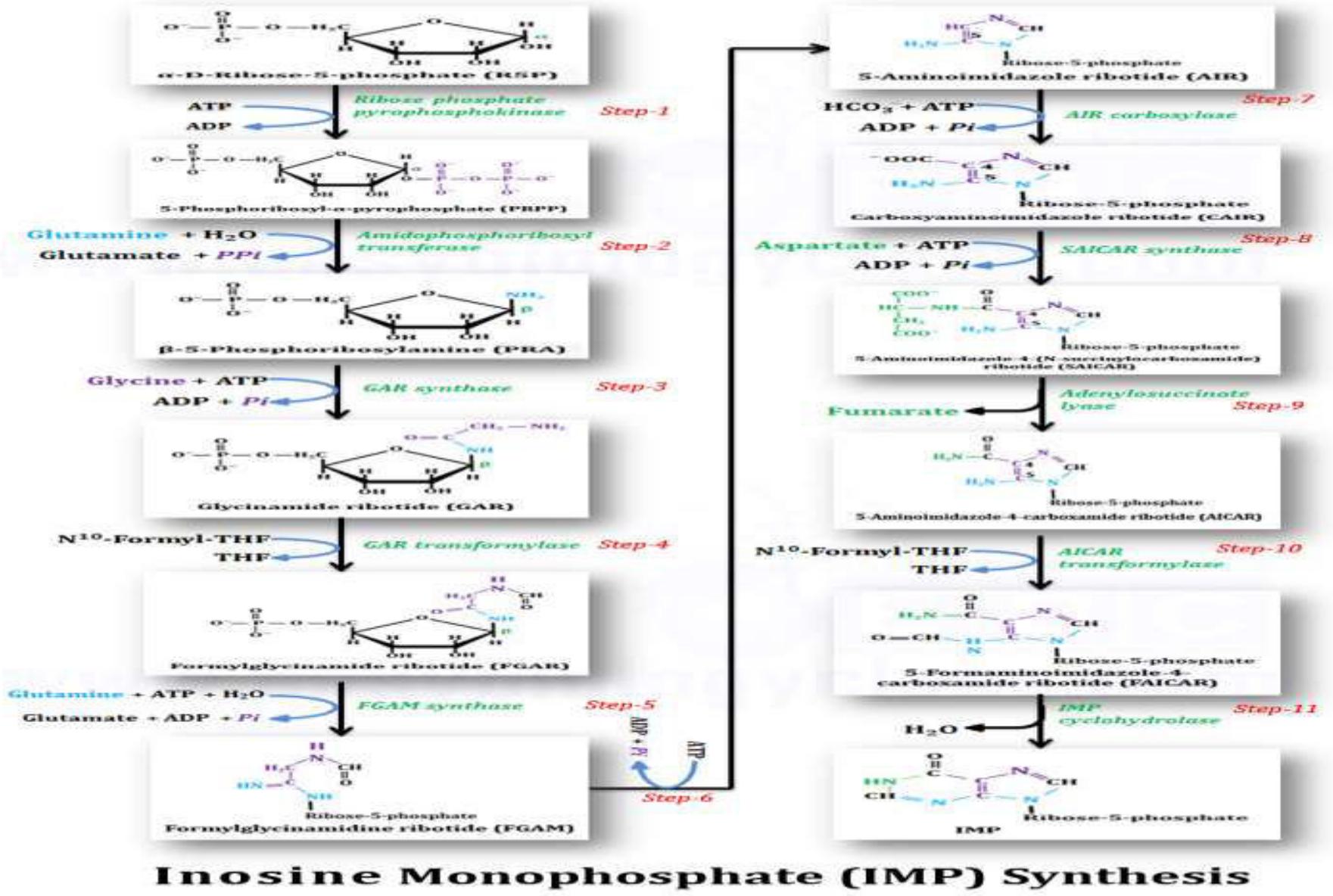
- Ribose-5-phosphate, of carbohydrate metabolism is the starting material for purine nucleotide synthesis.
- It reacts with ATP to form phosphoribosyl pyrophosphate (PRPP).
- Glutamine transfers its amide nitrogen to PRPP to replace pyrophosphate & produce 5-phosphoribosylamine. Catalysed by PRPP glutamyl amidotransferase.
- This reaction is the committed.

- Phosphoribosylamine reacts with glycine in the presence of ATP to form glycinamide ribosyl 5-phosphate or glycinamide ribotide (GAR).Catalyzed by synthetase.
- N10-Formyl tetrahydrofolate donates the formyl group & the product formed is formylglycinamide ribosyl 5-phosphate. Catalyzed by formyltransferase.
- Glutamine transfers the second amido amino group to produce formylglycinamidine ribosyl 5-phosphate. Catalyzed by synthetase.

- The imidazole ring of the purine is closed in an ATP dependent reaction to yield 5-aminoimidazole ribosyl 5-phosphate. Catalyzed by synthetase.
- Incorporation of CO2 (carboxylation) occurs to yield aminoimidazole carboxylate ribosyl 5-phosphate. Catalyzed by carboxylase.
- Does not require the vitamin biotin or ATP.
- Aspartate condenses with the aminoimidazole carboxylate ribosyl 5- phosphate to form aminoimidazole 4- succinylcarboxamide ribosyl 5-phosphate. Catalyzed by synthetase.

- Adenosuccinatelyase cleaves off fumarate & only the amino group of aspartate is retained to yield aminoimidazole 4-carboxamide ribosyl 5-phosphate.
- N10-Formyl tetrahydrofolate donates one carbon moiety to produce 5- formaminoimidazole 4-carboxamide ribosyl 5- phosphate. Catalyzed by formyltransferase.
- The final reaction catalyzed by cyclohydrolase leads to ring closure with an elimination of water molecule.
- The product obtained is **Inosine Monophosphate (IMP),** the parent purine nucleotide from which other purine nucleotides can be synthesized.

Purine biosynthesis



- https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/pyrimidine-nucleotides

Synthesis of AMP & GMP from IMP

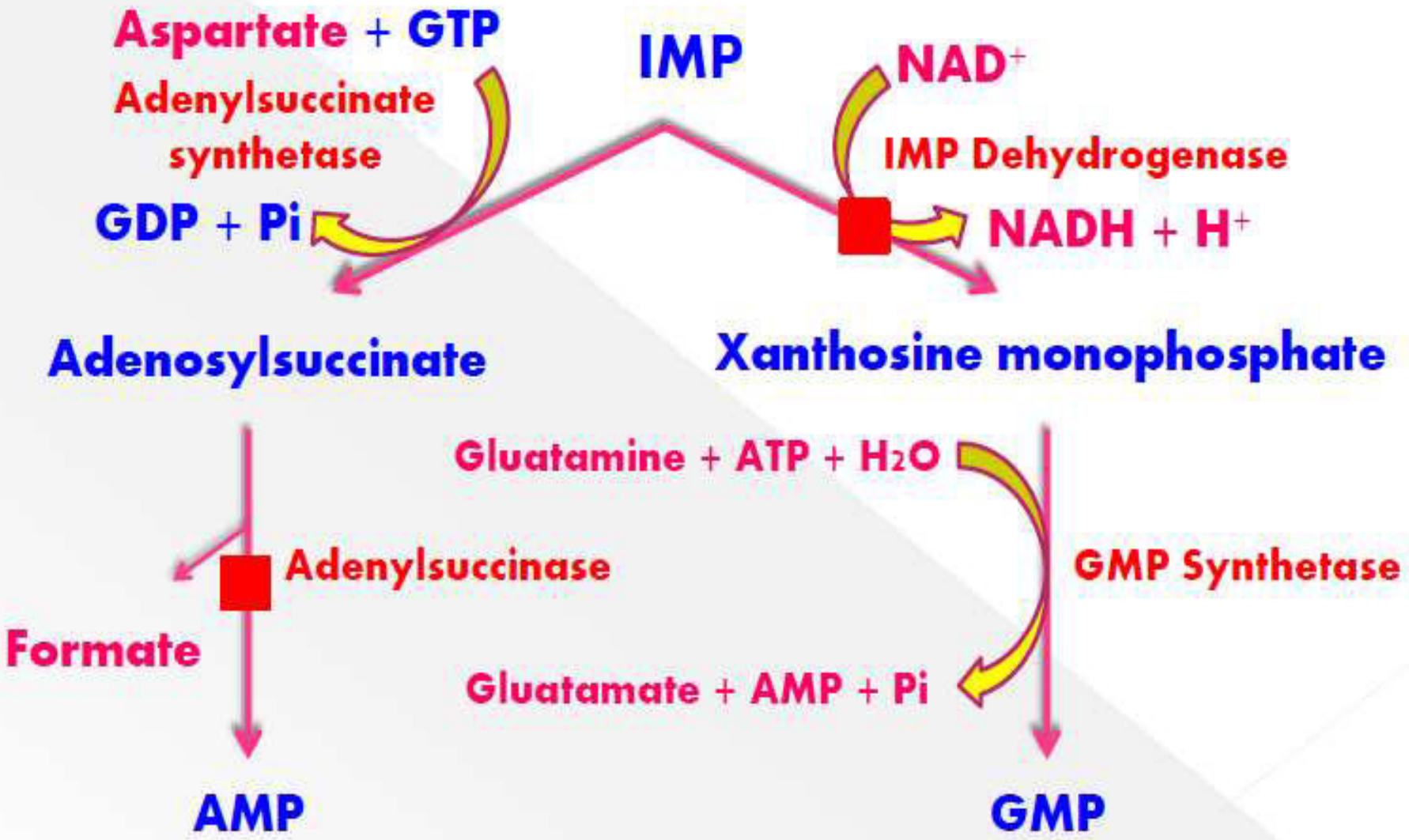
Synthesis of AMP:

- Ionosine monophosphate (IMP) is the immediate precursor for the formation of AMP & GMP.
- Aspartate condenses with IMP in the presence of GTP to produce adenylsuccinate which, on cleavage, forms AMP.

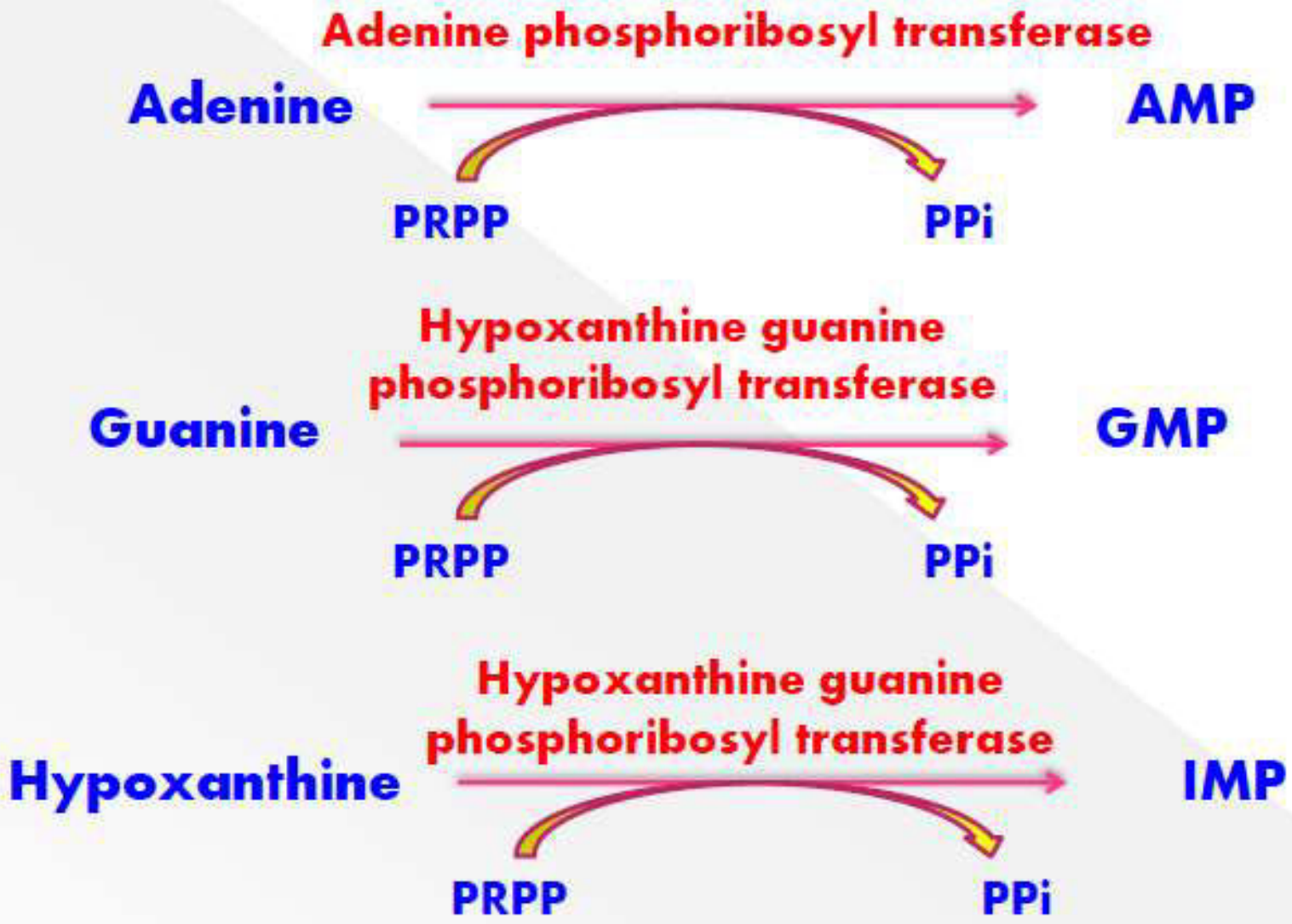
Synthesis of GMP:

- IMP undergoes NAD+ dependent dehydrogenation to form xanthosine monophosphate (XMP).
- Glutamine then transfers amide nitrogen to xanthosine monophosphate (XMP) to produce GMP.
- **6-Mercaptopurine is an inhibitor of the synthesis of AMP & GMP.**
- It acts on the enzyme adenylsuccinase (of AMP pathway) & IMP dehydrogenase (of GMP pathway).

Synthesis of AMP & GMP



Salvage Pathway



Inhibitor of purine biosynthesis

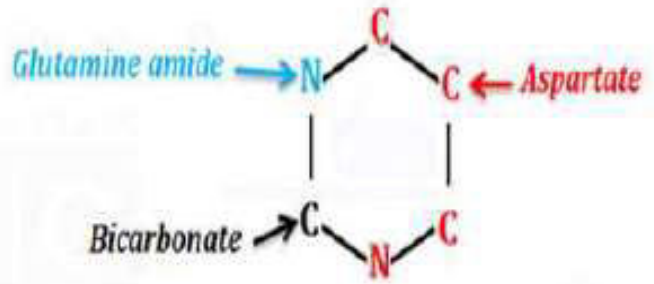
- Folic acid (THF) is essential for the synthesis of purine nucleotides.
- Sulfonamides are the structural analogs of Para-aminobenzoic acid (PABA).
- These sulfa drugs can inhibit the synthesis of folic acid by microorganisms. This indirectly reduces the synthesis of purines & nucleic acids (DNA & RNA).
- The structural analogs of folic acid (e.g. methotrexate), used to control cancer.

- Azaserine (diazo acetyl-L-Serine) is a glutamine antagonist & inhibits reactions involving glutamine.
- Other synthetic nucleotide analogues used as anticancer agents are 6-thio guanine & 8- aza guanine.

Biosynthesis of pyrimidine
(Uracil, Cytosine & Thymine)

De novo synthesis of pymiridine:

- Biosynthesis of pyrimidine is simple than that of purine
- Following diagram shows the source of different atoms in a pyrimidine skeleton (identified by radio labeling studies)
  - N1, C6, C5 and C4 from Aspartate
  - N3 from Glutamine
  - C2 from HCO3- (bicarbonate)

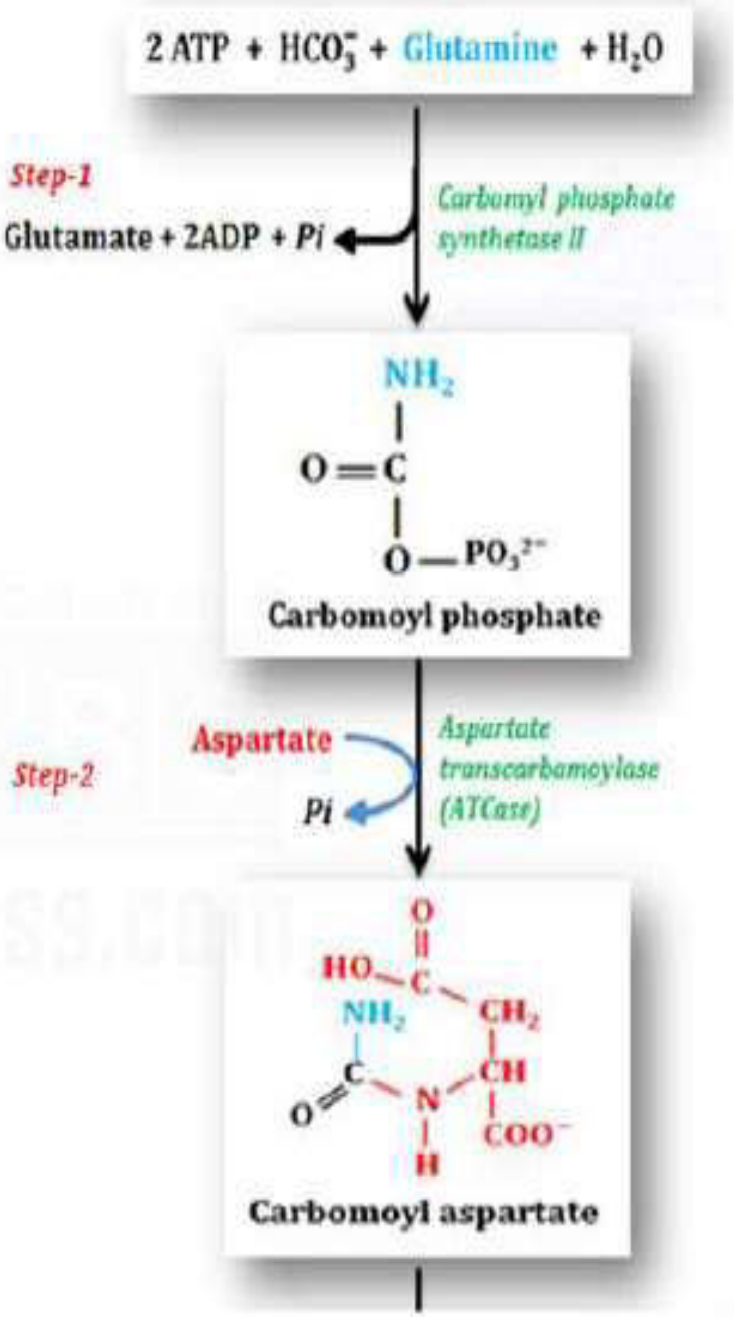


Pyrimidine Nucleus

- In pyrimidine nucleotide synthesis, the ring is completed before being linked to ribose-5-phosphate

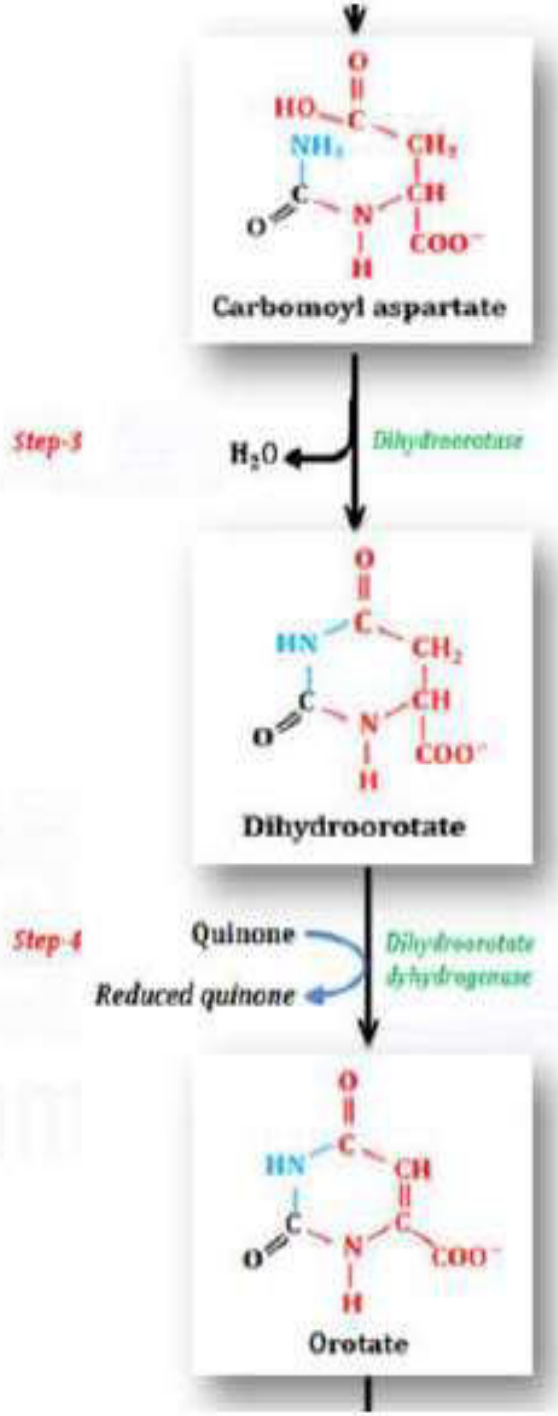
De-novo synthesis of UMP (Uridine monophosphate)

- UMP is also act as the precursor of CMP
- UMP is synthesized in 6 steps
- Step-1: **Synthesis of carbamoyl phosphate:** With the hydrolysis of two ATPs, bicarbonate and amide nitrogen of glutamine combine to form **carbamoyl phosphate**
- Step-2: **Synthesis of carbamoyl aspartate**: Carbamoyl phosphate reacts with aspartate to yield **carbamoyl aspartate**.



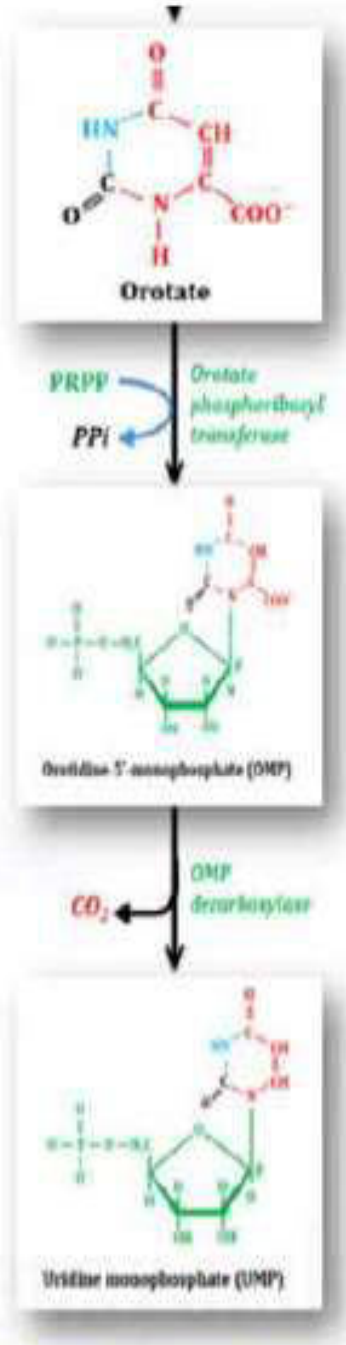
De-novo synthesis of UMP (Uridine monophosphate)

- **Step-3: Ring closure & dihydroorotate formation**: By the elimination (condensation) reaction, the carbamoyl aspartate is converted to a ring compound **dihydroorotate**
- One molecule of water is eliminated in Step-3
- **Step-4: Oxidation of dihydroorotate:** Dihydroorotate is dehydrogenated to form **orotate**



De-novo synthesis of UMP (Uridine monophosphate)

- Step-5: **Acquisition of the ribose phosphate moiety:** Orotate reacts with PRPP to produce **orotidine-5′-monophosphate** (OMP)
- Step-6: **Decarboxylation to form UMP:** OMP undergoes decarboxylation to form **UMP**



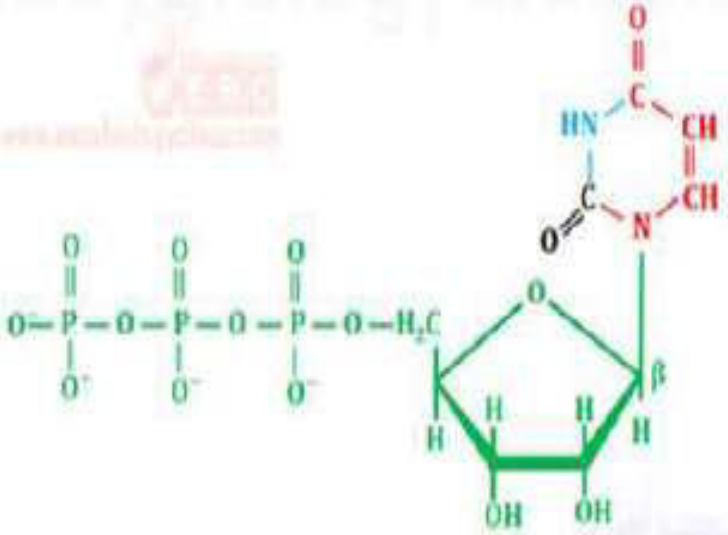
Uridine Monophosphate (UMP) Synthesis

Synthesis of UTP

- UMP is converted to UTP in two step kinase reaction
- Two ATP molecules are required

$$UMP + ATP \rightleftharpoons UDP + ADP$$

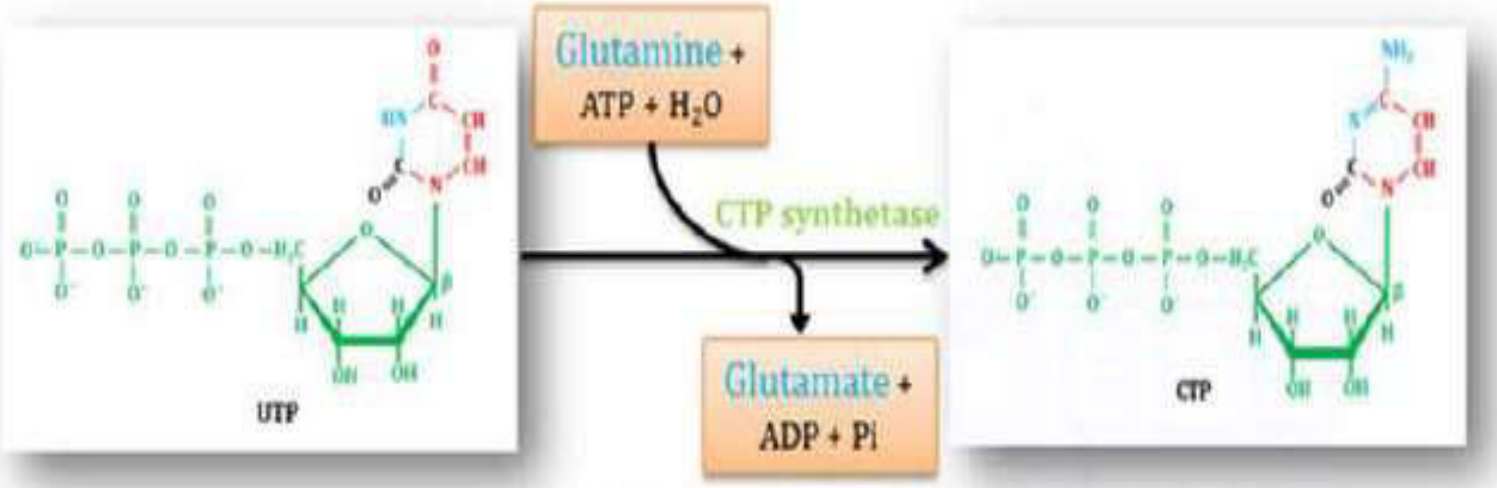
$$UDP + ATP \rightleftharpoons UTP + ADP$$



UTP

Synthesis of CTP

- CTP is synthesized by the **amination** of **UTP** by the enzyme *CTP synthase*
- In animals amino group is donated by **glutamine**
- In bacteria amino group is donated by **ammonia**



CTP is synthesized from UTP

Important links for study

- http://cancerres.aacrjournals.org/content/canres/37/9/3080.full.pdf
- http://www.jbc.org/content/202/1/241.full.pdf
- http://www.jbc.org/content/early/2018/01/03/jbc.M117.809459.full.pdf
- http://www.jbc.org/content/253/19/6794.full.pdf

Successful and unsuccessful people do not vary greatly in their *abilities*. They vary in their *desires to reach their potential*.

JOHN MAXWELL