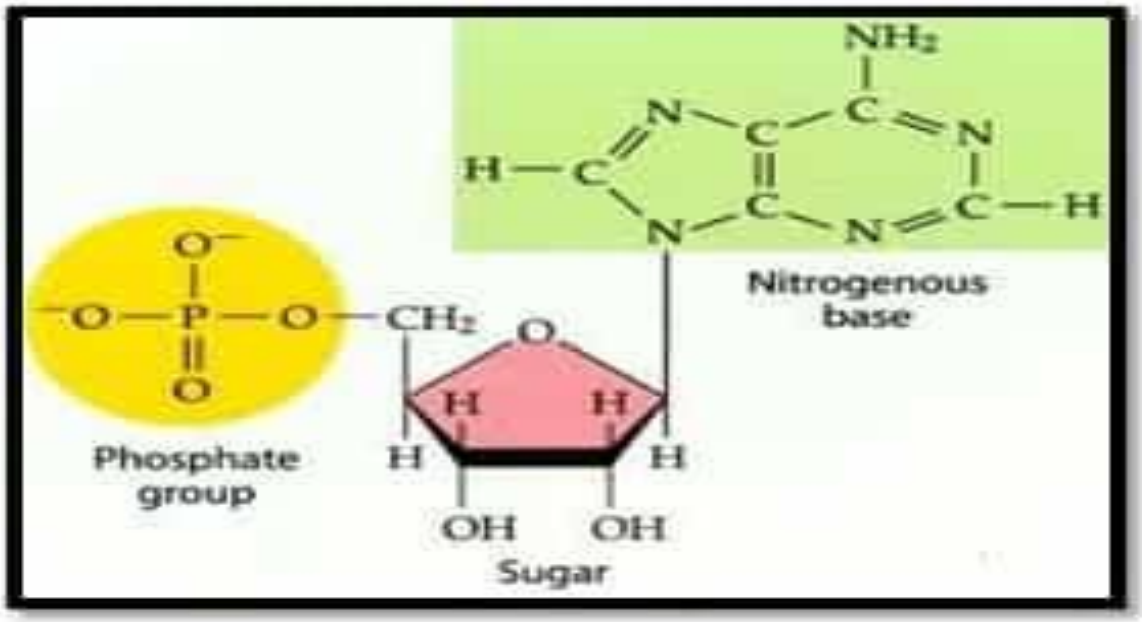
# Nucleotide Biosynthesis (Purine & Pyrimidine Synthesis)

Nucleotides are the biomolecules that form the basis of nucleic acid namely RNA and DNA. Nucleotides are composed of three elements viz, ribose or deoxyribose sugar, nucleobase (adenine, thymine, cytosine, guanine, and uracil), and a phosphate group.




**Nucleotide synthesis:** The pathways for the biosynthesis of nucleotides fall into two classes

- *de novo* pathways, and
- *salvage* pathways.



In de novo (means anew) pathways, the nucleotide bases are assembled from simpler compounds. The framework for a pyrimidine base is assembled first and then attached to ribose. In contrast, the framework for a purine base is synthesized piece by piece directly onto a ribose-based structure. In salvage pathways, preformed bases are recovered and reconnected to a ribose unit.

***Salvage pathway***

5-phosphoribosyl-l-pyrophosphate (PRPP) + Base à Nucleotide

***de novo pathway***

PRPP + Amino acids + ATP + CO2 + à Nucleotide

Both de novo and salvage pathways lead to the synthesis of ribonucleotides. All deoxyribonucleotides are synthesized from the corresponding ribonucleotides. The deoxyribose sugar is generated by the reduction of ribose within a fully formed nucleotide. Furthermore, the methyl group that distinguishes the thymine of DNA from the uracil of RNA is added at the last step in the pathway.

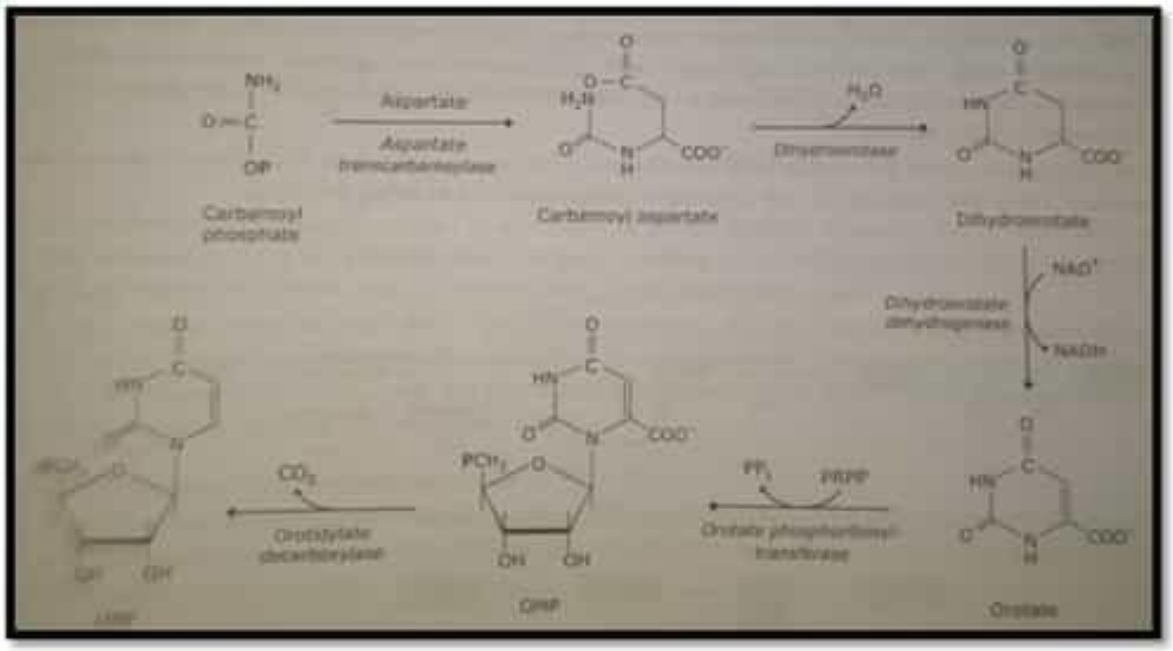
***De novo Synthesis:*** **Pyrimidine ribonucleotide**

In de novo synthesis of pyrimidines, the six-membered ring is synthesized first, and then it is attached to ribose-5-phosphate to form a pyrimidine nucleotide. UMP is synthesized as a pyrimidine base to which ribose-5-phosphate is added. CTP and UTP are derived from UMP. Pyrimidine rings are synthesized from carbamoyl phosphate and aspartate. Isotopic labeling experiments have shown that atoms N1, C4, C5, and C6 of the pyrimidine ring are derived from aspartic acid. The C2 and N3 are contributed by *carbamoyl phosphate*.

The precursor of carbamoyl phosphate is bicarbonate and glutamine. The synthesis of carbamoyl phosphate from bicarbonate and the amide nitrogen of glutamine occurs in a multistep process, requiring the cleavage of two molecules of ATP. This reaction is catalyzed by cytosolic carbamoyl phosphate synthetase II.

Carbamoyl phosphate reacts with aspartate to form carbamoyl aspartate in a reaction catalyzed by aspartate transcarbamoylase. Carbamoylaspartate then cyclizes to form dihydroorotate which Is then oxidized to form orotate.



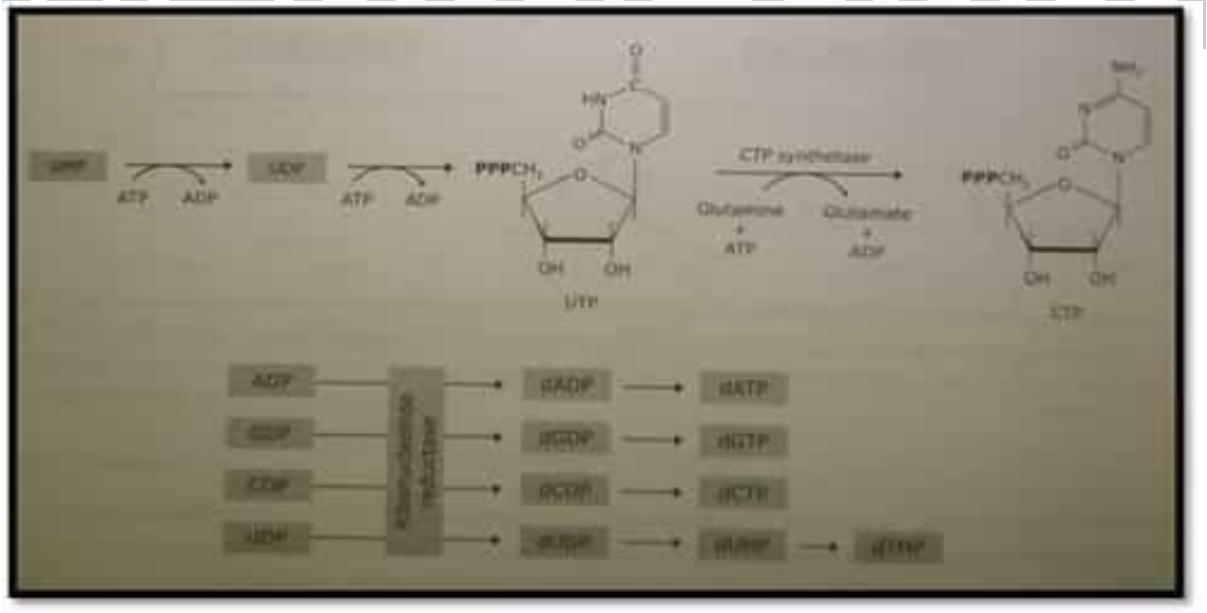


Orotate couple to ribose, in the form of 5-phosphoribosyl-1-pyrophosphate (PRPP), a form of ribose activated to accept nucleotide bases (PRPP Is synthesized from ribose-5-phosphate, formed by the pentose phosphate pathway, by the addition of pyrophosphate from ATP). Orotate reacts with PRPP to form orotidylate (OMP), a pyrimidine nucleotide. This reaction is driven by the hydrolysis of pyrophosphate. The enzyme that catalyzes this addition, *orotate phosphoribosyltransferase*, is homologous to a number of other *phosphoribosyl-transferases* that add different groups of PRPP to form the other nucleotides. Orotidylate is then decarboxylated to form uridylate (UMP), a major pyrimidine nucleotide that is a precursor to RNA. This reaction Is catalyzed by orotidylate decarboxylase. Deoxyribonucleotides are synthesized by the reduction of ribonucleotides

Deoxyribonucleotides, the precursors of DNA, are formed by the reduction of ribonucleoside diphosphates. These conversions are catalyzed by ribonucleotide reductase. Electrons are transferred from NADPH to sulfhydryl groups at the active sites of this enzyme by thioredoxin or glutaredoxin. TMP is formed by methylation of dUMP. The donor of a methylene group and a hydride in this reaction is N5, N10-methylenetetrahydrofolate, which is converted into dihydrofolate. Tetrahydrofolate is regenerated by the reduction of dihydrofolate by NADPH in the presence of the enzyme *dihydrofolate reductase.*

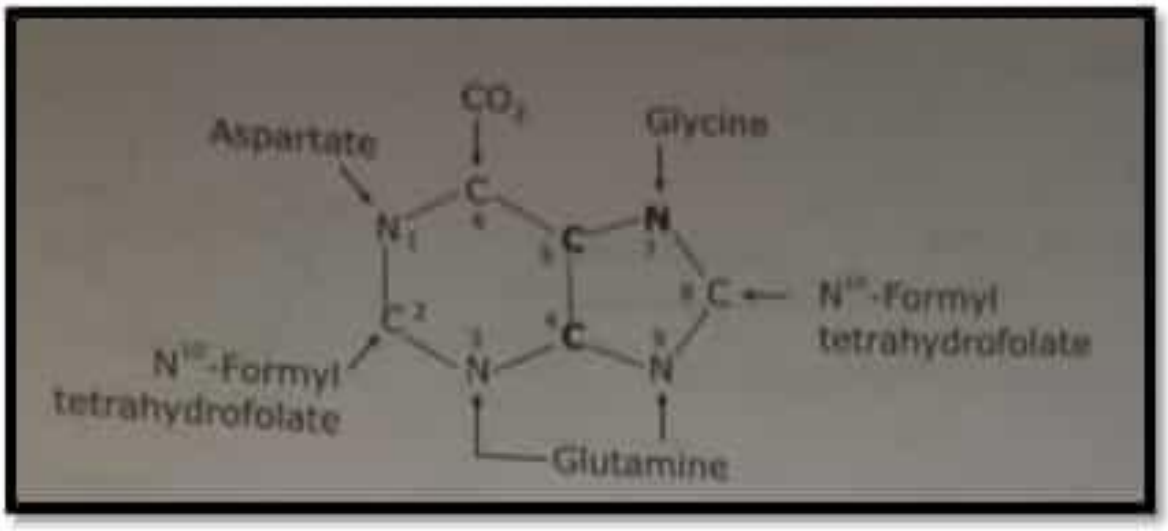
Chemotherapeutic agents like **methotrexate** (amethopterin) and **aminopterin** inhibit the activity of dihydrofolate reductase. These folate analogs act as competitive inhibitors and used as anticancer drugs.





**Purine ribonucleotide**

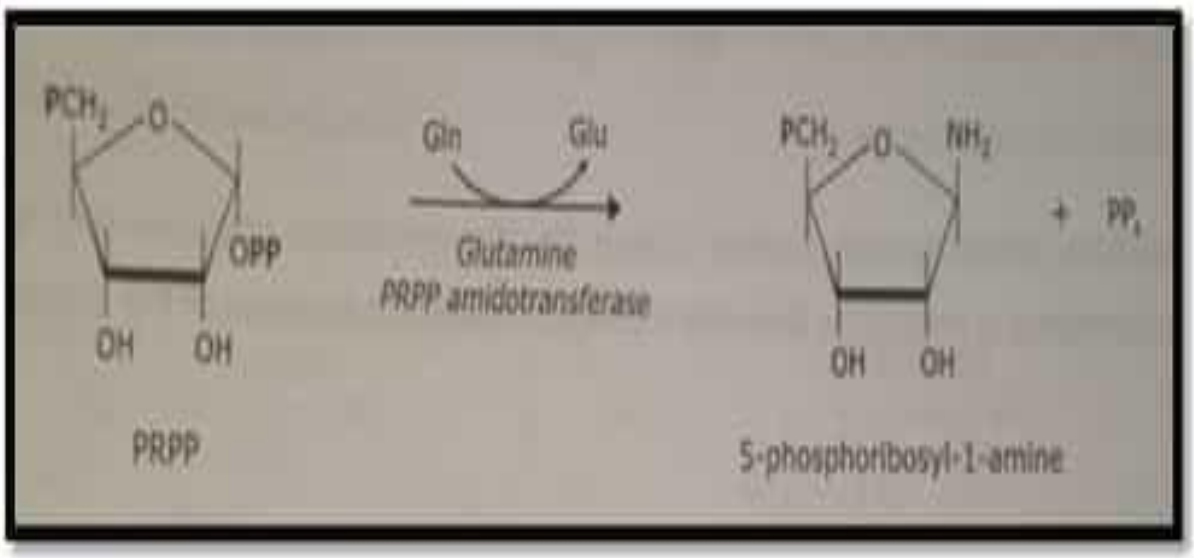
The purine ring is assembled from a variety of precursors: glutamine (N3 and N9), glycine (C4, C5, N7), aspartate (N1), N10- formyltetrahydrofolate (C2 and C8), and CO2 (C6).




***De novo* synthesis** of purine begins with simple starting materials such as amino acids and bicarbonate. Unlike the case for pyrimidines, the purine bases are assembled onto a ribose ring.

***De novo* purine biosynthesis**, like pyrimidine biosynthesis, requires PRPP, but for purines, PRPP provides the foundation on which the bases are constructed step by step. The initial committed step is the displacement of pyrophosphate by ammonia, rather than by a preassembled base, to produce 5-phosphoribosyl-1-amine.



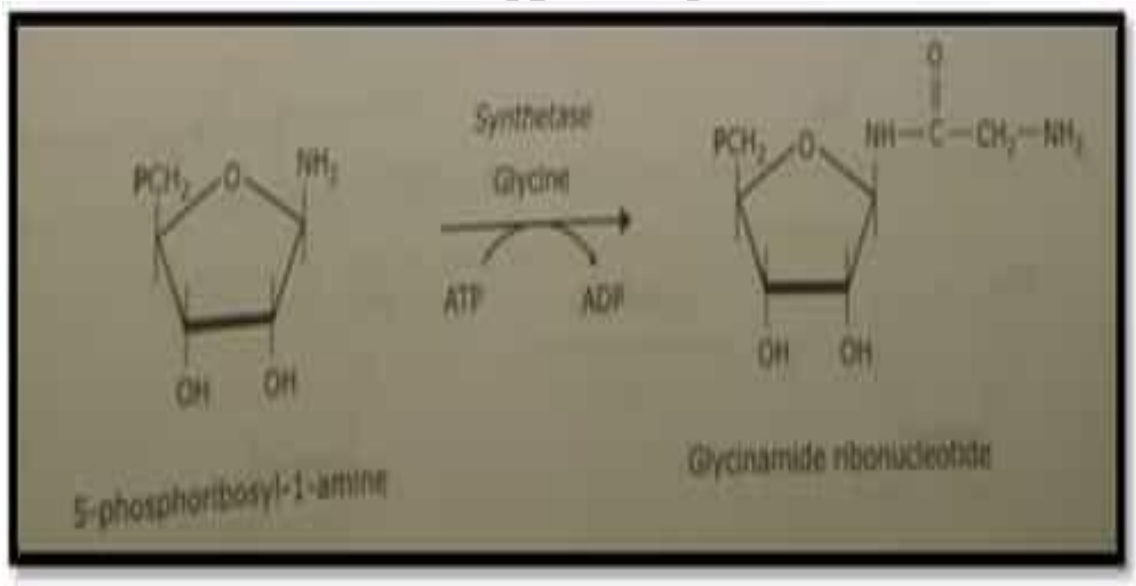


Glutamine PRPP amidotransferase catalyzes this reaction. To prevent wasteful hydrolysis of either substrate, the amidotransferase assumes the active configuration only on the binding of both PRPP and glutamine. This enzyme, activity is inhibited by glutamine analog azaserine, which is used as an antitumor agent.

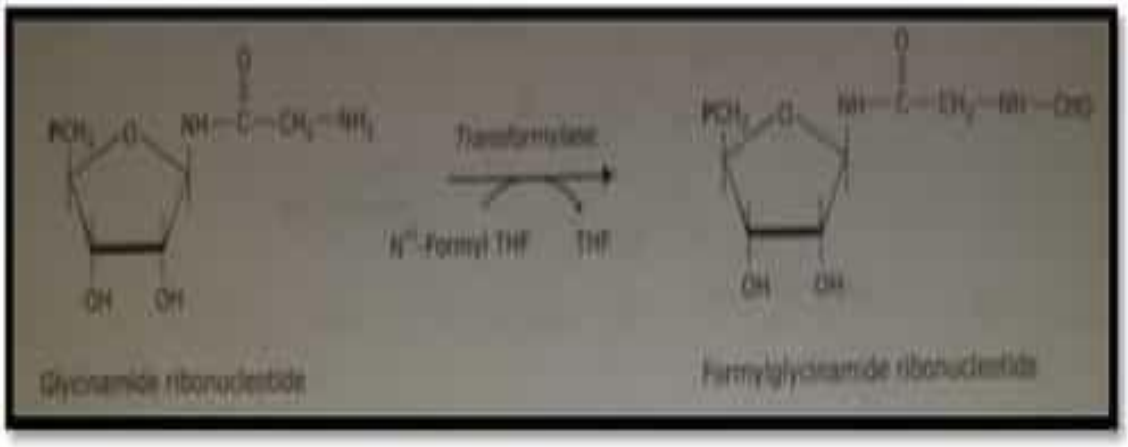
Later, the addition of glycine, followed by formylation, amination, and ring closure, yields 5-aminoimidazole ribonucleotide. This intermediate contains the completed five-membered ring of the purine skeleton. The addition of CO2, the nitrogen atom of aspartate, and a formyl group, followed by ring closure, yields inosinate (IMP), a purine ribonucleotide. AMP and GMP are formed from the IMP.

***de novo purine biosynthesis proceeds as follows:***

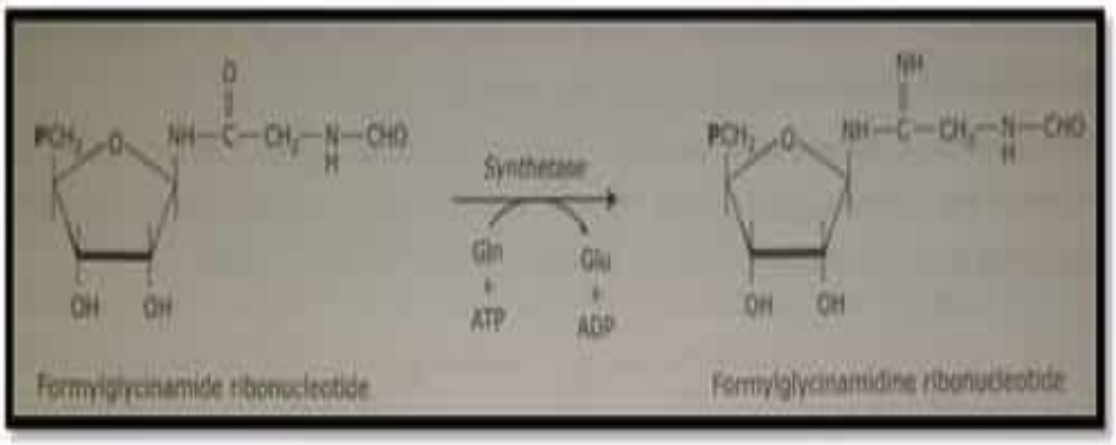
1. The carboxylate group of a glycine residue is activated by phosphorylation and then coupled to the amino group of 5-phosphoribosyl-1-amine. A new amide bond is formed while the amino group of glycine is free to act as a nucleophile in the next step.




2. Formate is activated and then added to this amino group to form formyl glycinamide ribonucleotide. In some organisms, two distinct enzymes can catalyze this step. One enzyme transfers the formyl group from N10-formyltetrahydrofolate. The other enzyme activates formate as formyl phosphate, which is added directly to the glycine amino group.

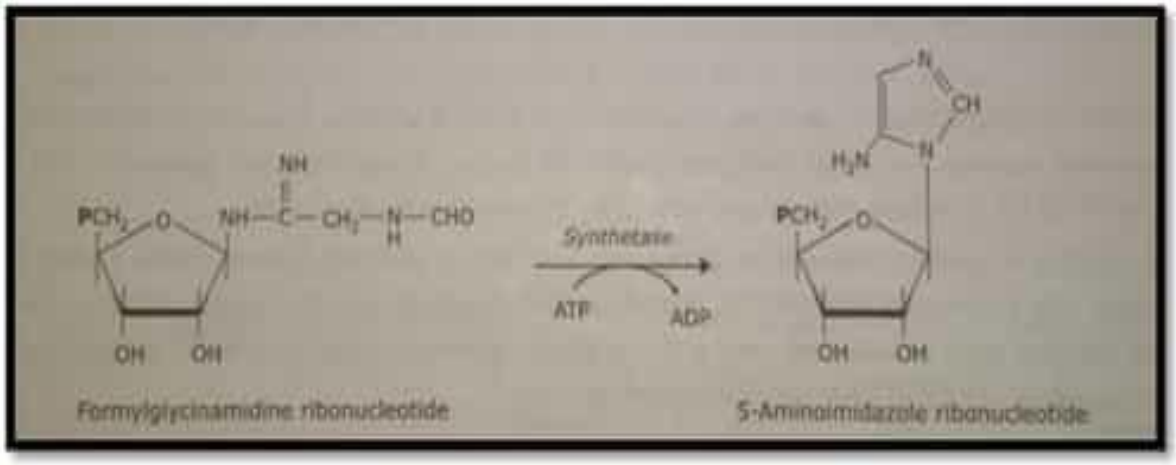



3. The inner amide group is activated and then converted into an amidine by the addition of ammonia derived from glutamine.

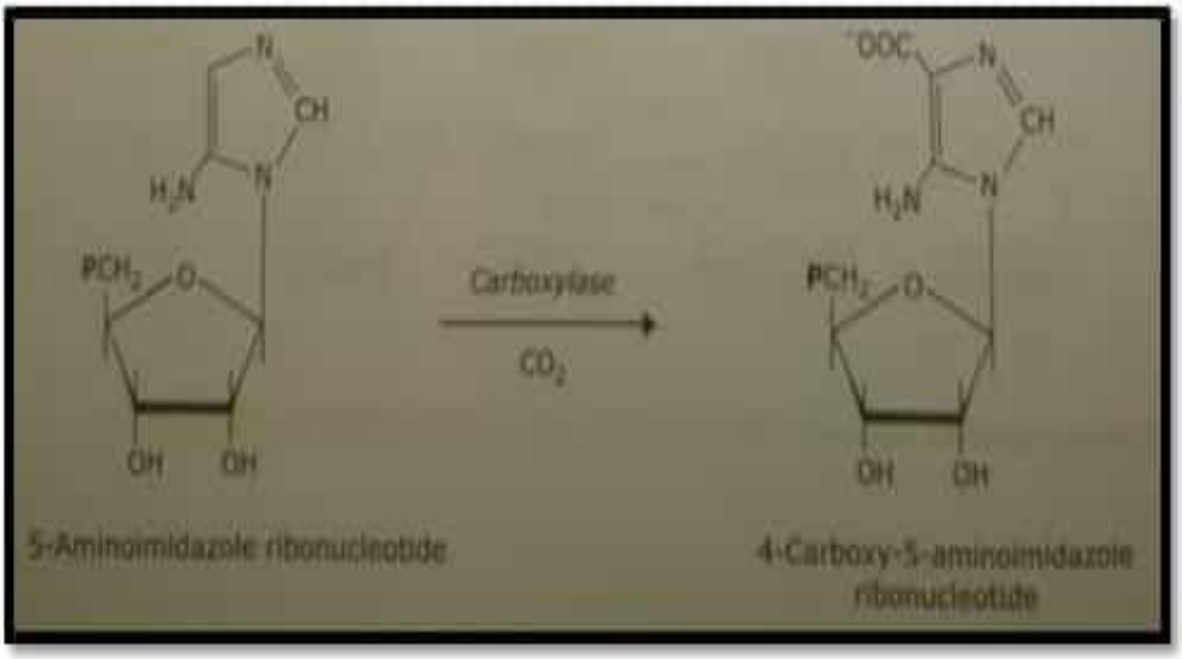





4. Formylglycinamide ribonucleotide, cyclizes to form the five-membered imidazole ring found in purines. Although this cyclization is likely to be favorable thermodynamically, a molecule of ATP is consumed to ensure irreversibility.

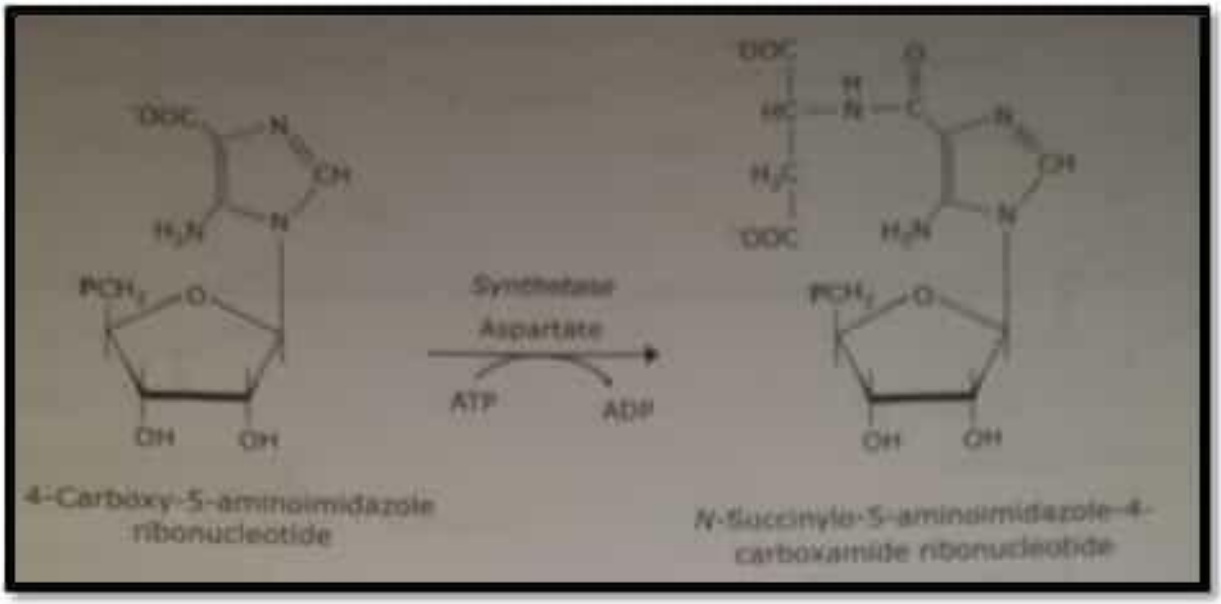


5. Bicarbonate is activated by phosphorylation and then attacked by the exocyclic amino group. The product of the reaction rearranges to transfer the carboxylate group to the imidazole ring. Interestingly, mammals do not require ATP for this step; bicarbonate apparently attaches directly to the exocyclic amino group and is then transferred to the imidazole ring.

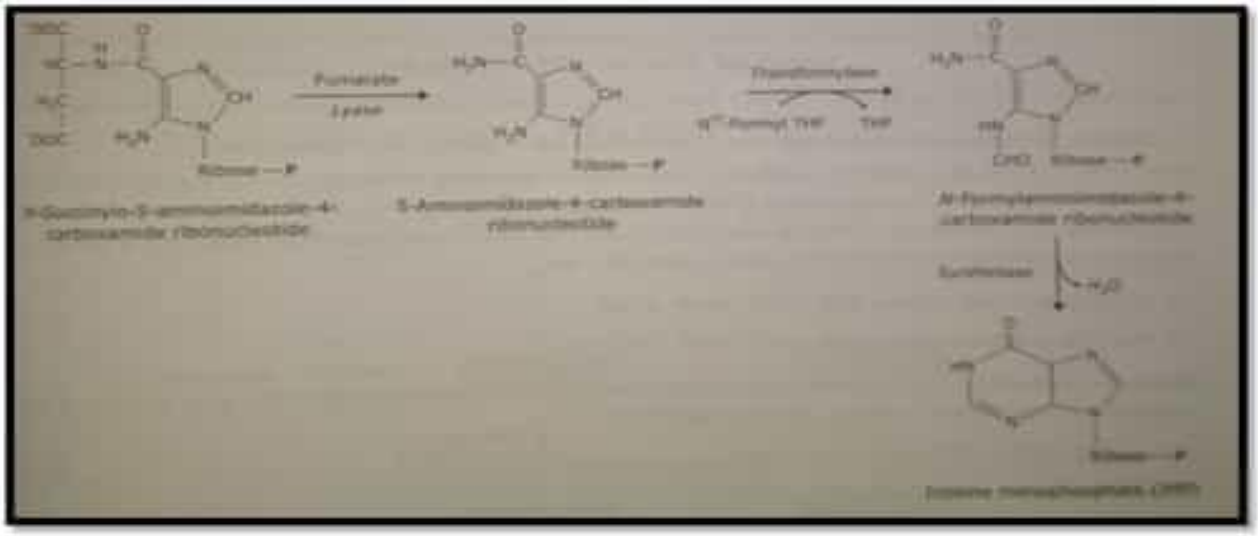



6. The imidazole carboxylate group is phosphorylated again, and the phosphate group is displaced by the amino group aspartate. Thus, a six-step process links glycine, formate, ammonia, bicarbonate, and aspartate to form an intermediate that contains all but two of the atoms necessary for the formation of the purine ring.





7. Three more steps complete the ring construction. Fumarate, an Intermediate in the citric acid cycle, is eliminated, leaving the nitrogen atom from aspartate joined to the Imidazole ring. The use of aspartate as an amino-group donor and the concomitant release of the fumarate are reminiscent of the conversion of citrulline into arginine in the urea cycle and these steps are catalyzed by homologous enzymes in the two pathways. A formyl group from N10-formyltetrahydrofolate is added to this nitrogen atom to form a final intermediate that cyclizes with the loss of water to form inosinate.



**AMP and GMP are formed from the IMP**

The conversion of IMP to either AMP or GMP utilizes a two-step, energy-requiring pathway. Note that AMP requires GTP as an energy source, whereas the synthesis of GMP requires ATP.



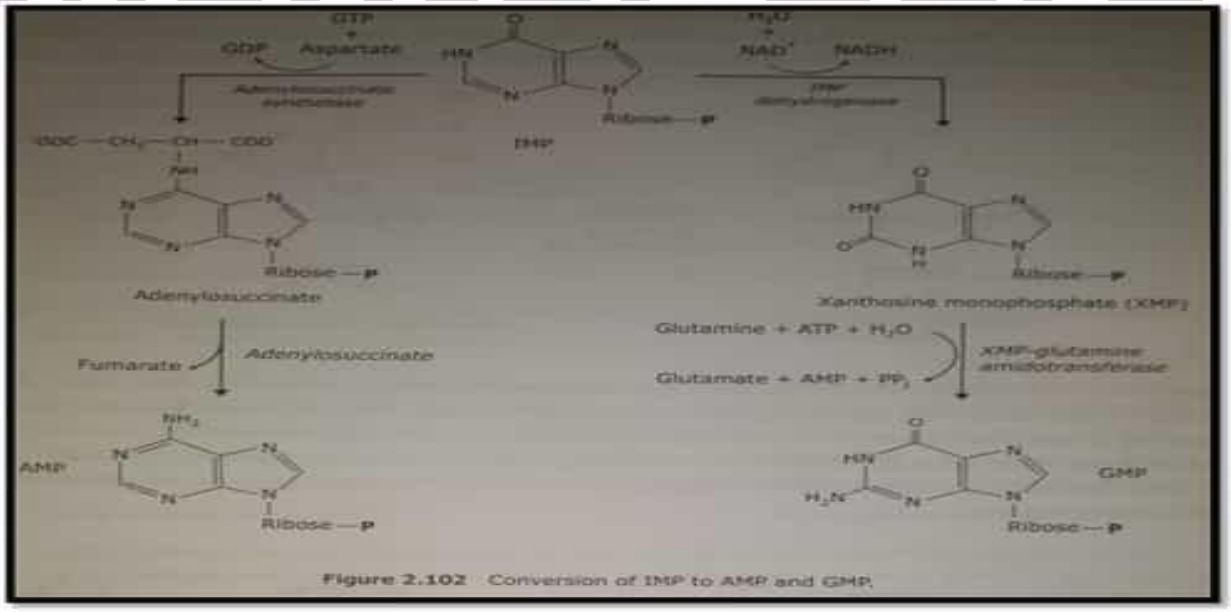



Figure 2.102 Conversion of IMP to AMP and GMP.

**Conversion of nucleoside monophosphates to nucleoside diphosphates and triphosphates** Nucleoside diphosphates (NDP) are synthesized from the corresponding nucleoside monophosphates (NMP) by base-specific nucleoside monophosphate kinases. (Note: These kinases do not discriminate between ribose or deoxyribose in the substrate). ATP is generally the source of the transferred phosphate because it is present in higher concentrations than the other nucleoside triphosphates.

For example, adenylate kinase: AMP + ATP à 2 ADP

For example, guanylate kinase: GMP + ATP à GDP + ADP

Nucleoside diphosphates and triphosphates are interconverted by nucleoside diphosphate kinase—an enzyme that, unlike the monophosphate kinases, has broad specificity.

For example, GDP + ATP à GTP + ADP

For example, CDP + ATP à CTP + ADP

**Salvage pathways for purines:** Purines that result from the normal turnover of cellular nucleic acids or that is obtained from the diet and not degraded can be reconverted into nucleoside triphosphates and used by the body. This is referred to as the salvage pathway for purines. This pathway involves two enzymes: adenine phosphoribosyltransferase (APRT) and hypoxanthine-guanine phosphoribosyltransferase (HGPRT). Both enzymes utilize PRPP as the source of the ribose 5-phosphate group.



***Adenine phosphoribosyltransferase catalyzes the formation of adenylate***

Adenine + PRPP à Adenylate + PPi

whereas hypoxanthine-guanine phosphoribosyltransferase (HGPRT) catalyzes the formation of guanylate as well as inosinate (inosine monophosphate, IMP), a precursor of guanylate and adenylate.

Guanine + PRPP à Guanylate + PPi

Hypoxanthine + PRPP à Inosinate + PPi

Similar salvage pathways exist for pyrimidines. Pyrimidine phosphoribosyltransferase will reconnect uracil, but not cytosine, to PRPP.