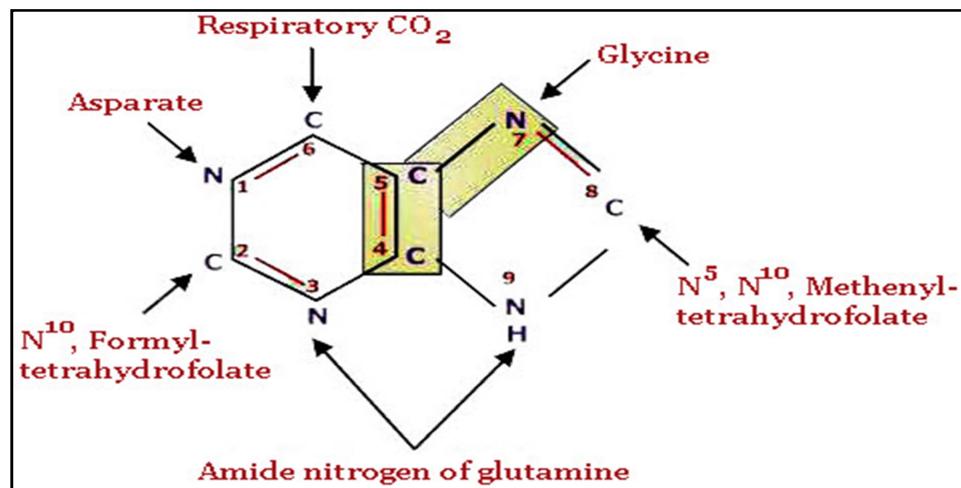


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PURINE METABOLISM

- Many compounds contribute to the **purine ring** of the nucleotides:
 - N_1 of purine is derived from amino group of aspartate.
 - C_2 and C_8 arise from formate of N^{10} -formyl THF.
 - N_3 and N_9 are obtained from amide group of glutamine.
 - C_4 , C_5 and N_7 are contributed by glycine.
 - C_6 directly comes from CO_2

SOURCES FOR PURINE RING



- This information was obtained from isotopic experiments with ^{14}C - or ^{15}N -labeled precursors. Formate is supplied in the form of N^{10} -formyltetrahydrofolate. Origin of the carbon and nitrogen atoms of the purine ring system, as determined by **John Buchanan** using **isotopic tracer experiments in birds**.
- Purine bases are not synthesized as such, but they are formed as ribonucleotides. The purines are built upon a pre-existing ribose 5-phosphate. Liver is the major site for purine nucleotide synthesis. Erythrocytes, polymorphonuclear leukocytes and brain cannot produce (*De novo* synthesis) purines.
- Purines and pyrimidines are **Non-essential** in the diet as dietary nucleic acids are degraded by pancreatic ribonuclease and deoxyribonuclease to mononucleotides. They are then converted to nucleosides by mononucleotidase and finally to free bases by nucleosidases. The phosphate and sugar produced by the digestion of nucleic acids are reused. Very little dietary purines and pyrimidines are incorporated into Nucleic acids.
- Most of the purine and pyrimidine bases are catabolized and excreted. Purines are converted to uric acid.

BIOSYNTHESIS OF PURINE NUCLEOTIDES

- The detailed pathway of purine biosynthesis was worked out primarily by Buchanan and G. Robert Greenberg in the 1950s.

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PURINE METABOLISM

- Purine nucleotides are synthesized by two pathways:
 - ***De novo synthesis:***
In *de novo* synthesis, purines and pyrimidines are synthesized from the smaller precursor molecules such as glycine, aspartic acid, glutamine, CO₂ and tetrahydrofolic acid. This pathway is expensive; several reactions require ATP.
 - **Salvage pathway:**
In salvage pathway the free bases and nucleosides released during the nucleic acid breakdown are reused.
- Both types of pathways are important.

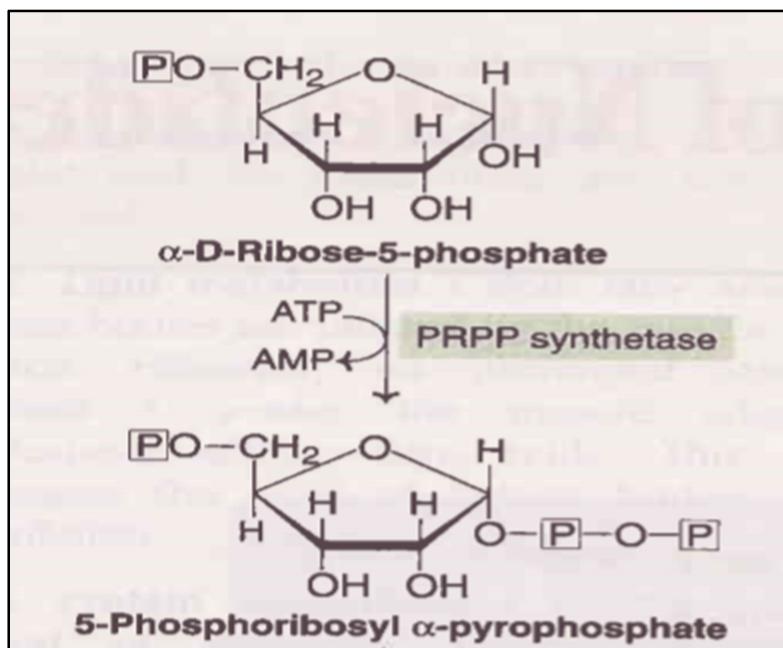
De Novo Synthesis of purines

- All enzymes of purine metabolism are found in **cytoplasm**.
- Purine ring structure is not synthesized as a free base but as a substituent of ribose-5-phosphate, which comes from **5-phosphoribosyl-1-pyrophosphate (PRPP)**. The PRPP is formed from ribose 5-phosphate and ATP by PRPP synthetase. PRPP then donates the ribose 5-phosphate, which serves as base upon which purine structure is built.
- The first step is the reaction of PRPP with **glutamine** to form **5-phosphorybosylamine**. The C-1 is changed from α to β configuration. This step can be inhibited by azaserine, an antimetabolite of glutamine. The enzyme PRPP glutamyl amidotransferase is controlled by feedback inhibition of nucleotides (IMP, AMP and GMP). This reaction is the '**committed step**' in purine nucleotide biosynthesis.
- **Glycine** is added to ribosylamine, forming glycinamide ribosyl 5-phosphate. This reaction requires ATP. The carbons 4,5 and nitrogen 7 are donated by glycine.
- Next, a formyl group is transferred from N¹⁰-formyltetrahydrofolate to the amino group of glycine to produce formylglycinamide ribosyl 5-phosphate (Carbon 8).
- The amide group of glutamine is transferred to the aldehyde derivative forming formylglycinamide ribosyl 5-phosphate (Nitrogen 3).
- The next reaction is the ring closure that produces an imidazole ring. This reaction requires ATP.
- CO₂ is incorporated by attachment to the carbon that becomes C-6 of the purine. This reaction requires **neither biotin nor ATP**. The product is aminoimidazolecarboxylate ribosyl 5-phosphate.
- The next step is the condensation of amino group of aspartate with the newly added carboxylate to form an amide, aminoimidazole succinyl carboxamide ribosyl 5-phosphate.
- Fumarate is eliminated by a non-hydrolytic cleavage. This results in the transfer of an amino group to become N-1 of IMP.
- N¹⁰-formyltetrahydrofolate donates a formyl group to the amino group forming formimidooimidazole carboxamide ribosyl 5-phosphate (Carbon 2). Therefore, **Folic acid is essential for the synthesis of purine nucleotide. Folic acid analogs (methotrexate) are employed to control cancer**.

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PURINE METABOLISM

- Ring closure occurs by the condensation of amide nitrogen with the formyl group to produce IMP.
- The two-purine nucleotides AMP and GMP are subsequently formed from IMP by two different enzymatic reactions.
- The amino group of aspartate condenses with the keto group of IMP to give adenylosuccinate. The energy for this reaction is supplied by GTP.
- The adenylosuccinate is cleaved to produce AMP and fumarate.
- In the conversion of IMP to GMP, C-2 is oxidized to give xanthosine monophosphate in the presence of NAD^+ . Glutamine donates its amide nitrogen to C-2 in an ATP dependant reaction yielding GMP, AMP and PPi.



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PURINE METABOLISM

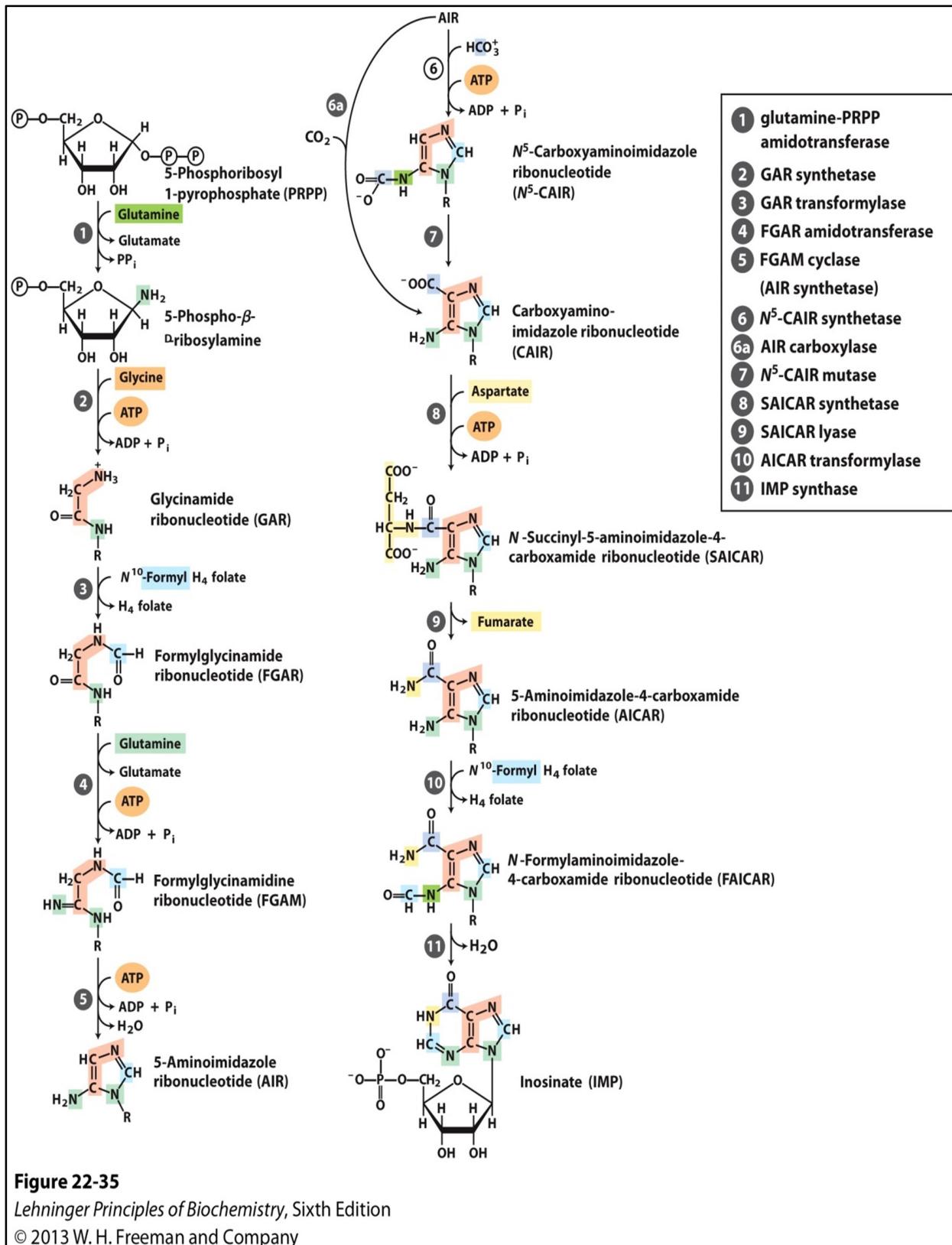


Figure 22-35

Lehninger Principles of Biochemistry, Sixth Edition

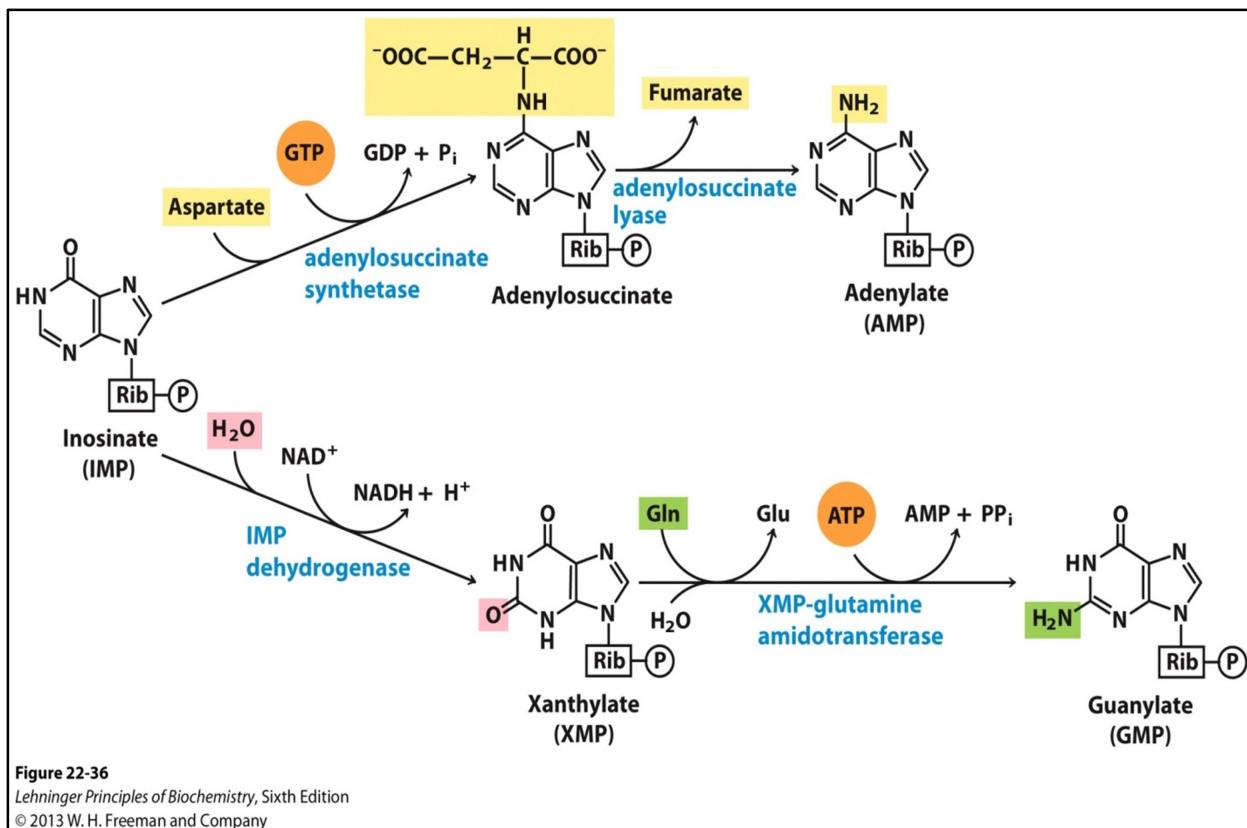
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R- Ribose 5-phosphate

Source: "Lehninger's Principles of Biochemistry" by David Nelson and Fox, 4th edition.

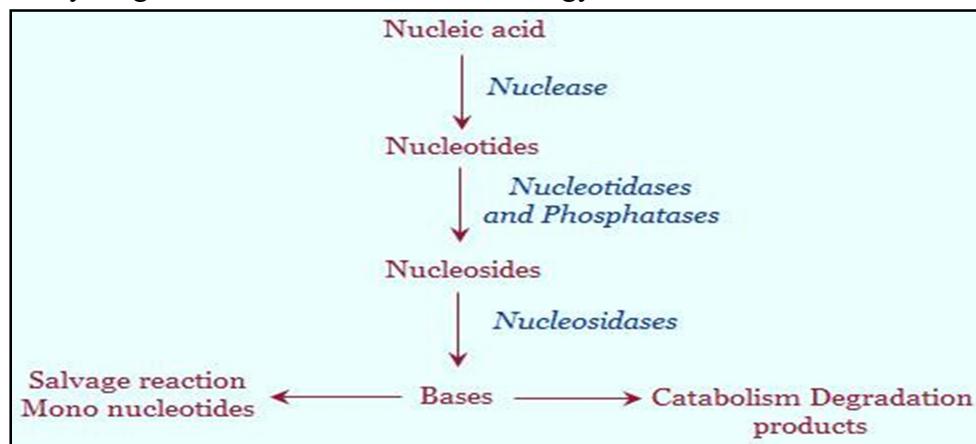
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PURINE METABOLISM



Salvage Pathway

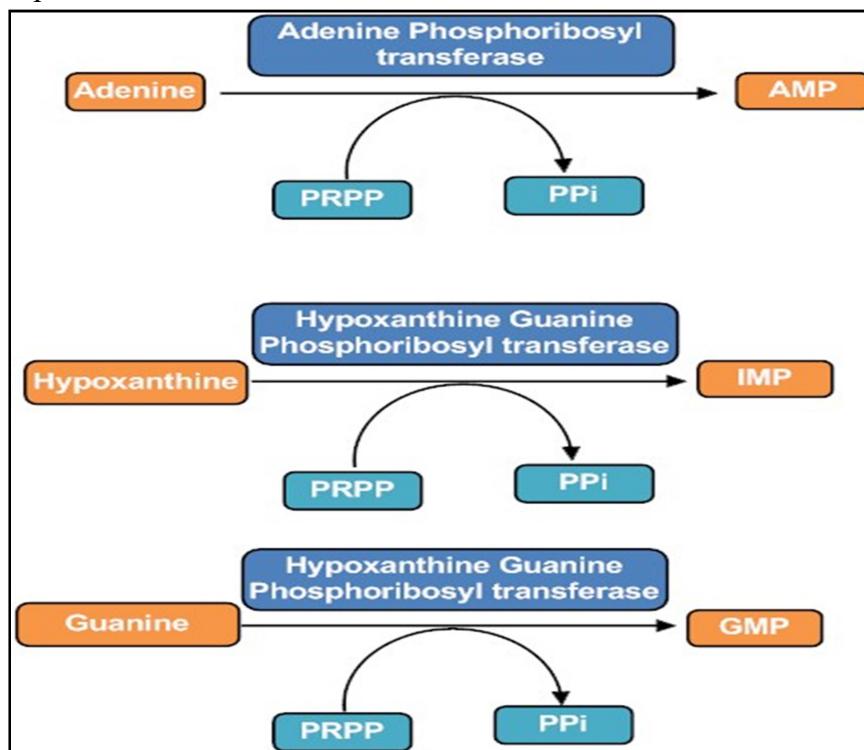
- It is a metabolic pathway that utilizes compounds formed in catabolism for biosynthetic reactions. The salvage pathway, involving the direct conversion of purines to nucleotides, is important in tissues- brain and erythrocytes.
- During cellular metabolism and during digestion in animals nucleic acids are degraded to mononucleotides, nucleosides and finally to free bases. Some of the purines and pyrimidines formed in this way are further degraded. But considerable amount is salvaged by reacting with PRPP to reform nucleotides.
- The recycling of bases conserves cellular energy.



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PURINE METABOLISM

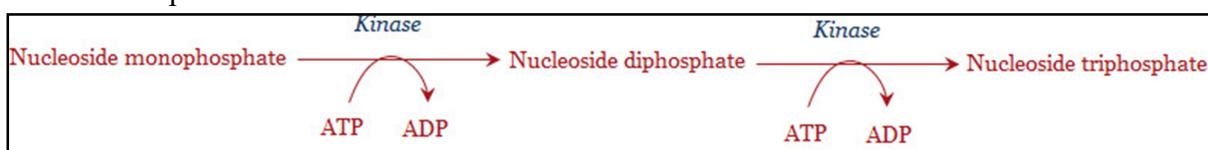
- Two specific salvage enzymes participate for the utilization of adenine, hypoxanthine and guanine.
- Adenine phosphoribosyltransferase (APRT) catalyzes the reaction of adenine with PRPP to form AMP and PPi.
- Hypoxanthine-guanine phosphoribosyltransferase (HGPRT) catalyzes the conversion of hypoxanthine to IMP and guanine to GMP with concomitant formation of pyrophosphates.



- Lesch-Nyhan syndrome is caused by the deficiency of HGPRT. In this condition, purine bases cannot be salvaged; instead they are degraded, forming excess amounts of uric acid. Individual with this syndrome suffer from mental retardation. They are prone to chewing of their fingers and performing other acts of self-mutilation.

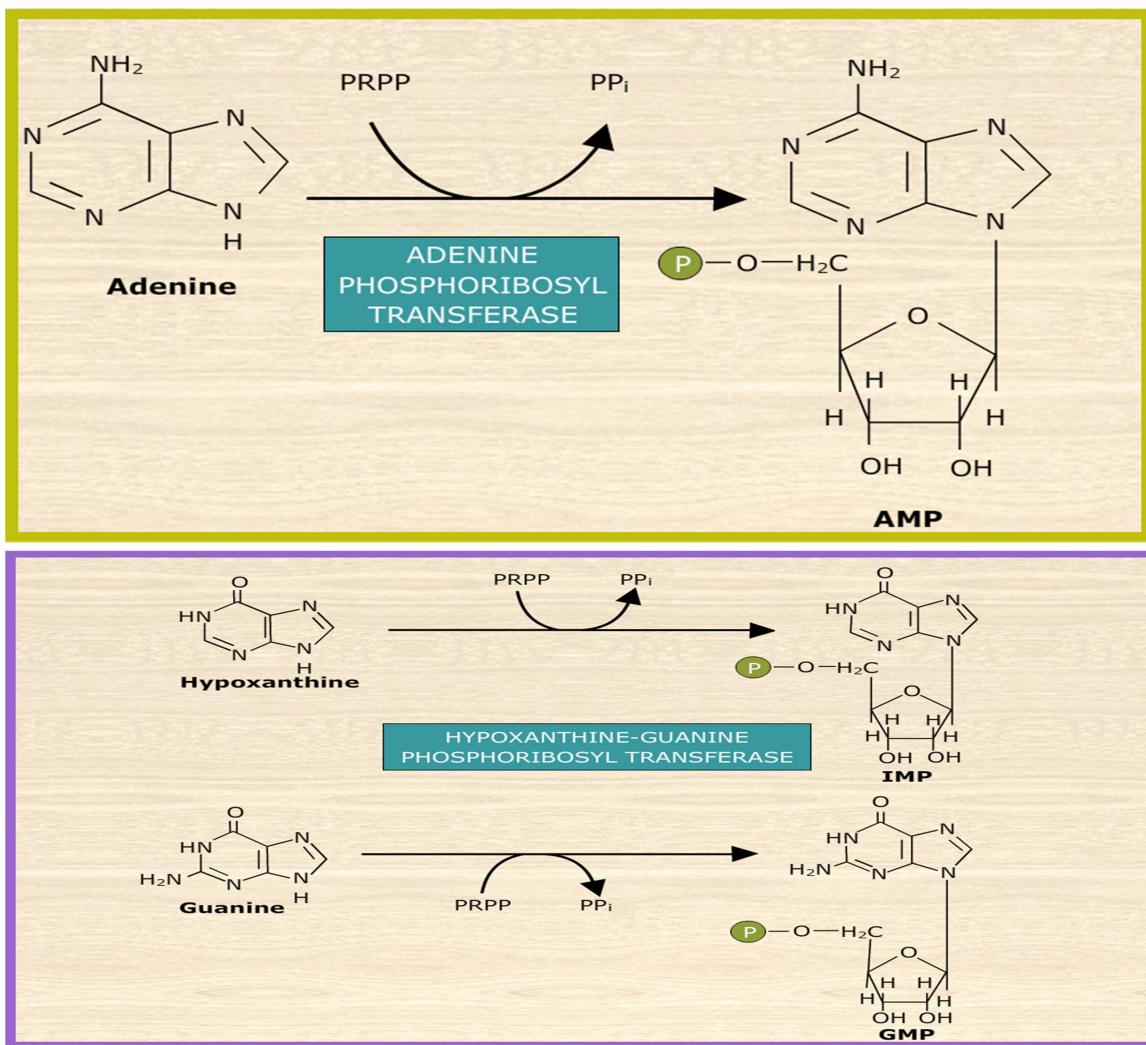
NMP to NDP to NTP

- Conversion of nucleotide monophosphate to diphosphate and triphosphate occurs with the help of kinases.



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PURINE METABOLISM



Conversion of Ribonucleotides to Deoxyribonucleotides

The synthesis of purine and pyrimidine deoxyribonucleotides occurs from ribonucleotides by a reduction at the C₂ of ribose moiety. This reaction is catalysed by a multisubunit (two B₁ and two B₂ subunits) enzyme, **rihonucleotide reductase**.

Supply of reducing equivalents: 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 oxidized thioredoxin to reduced form which can be recycled again and again. **Thioredoxin thus serves as a protein cofactor in an enzymatic reaction.**

Regulation of deoxyribonucleotide synthesis: Deoxyribonucleotides are mostly required for the synthesis of DNA. The activity of the enzyme ribonucleotide reductase maintains the adequate supply of deoxyribonucleotides.

Ribonucleotide reductase is a complex enzyme with multiple sites (active site and allosteric sites) that control the formation of deoxyribonucleotides.

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PURINE METABOLISM

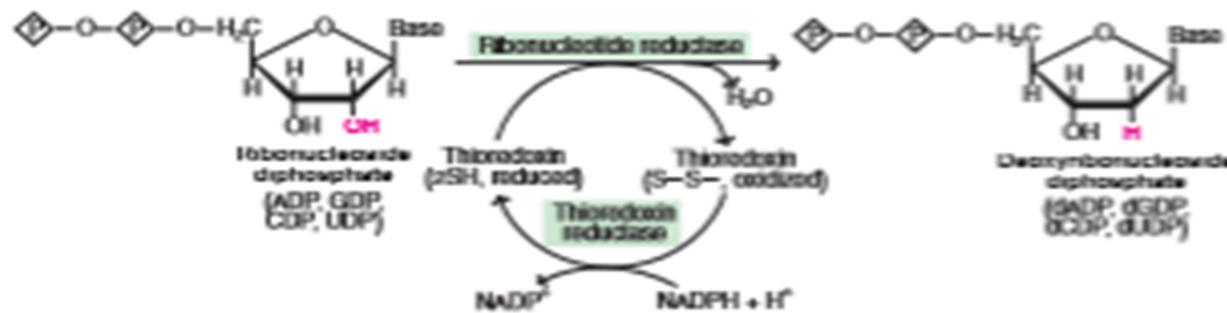


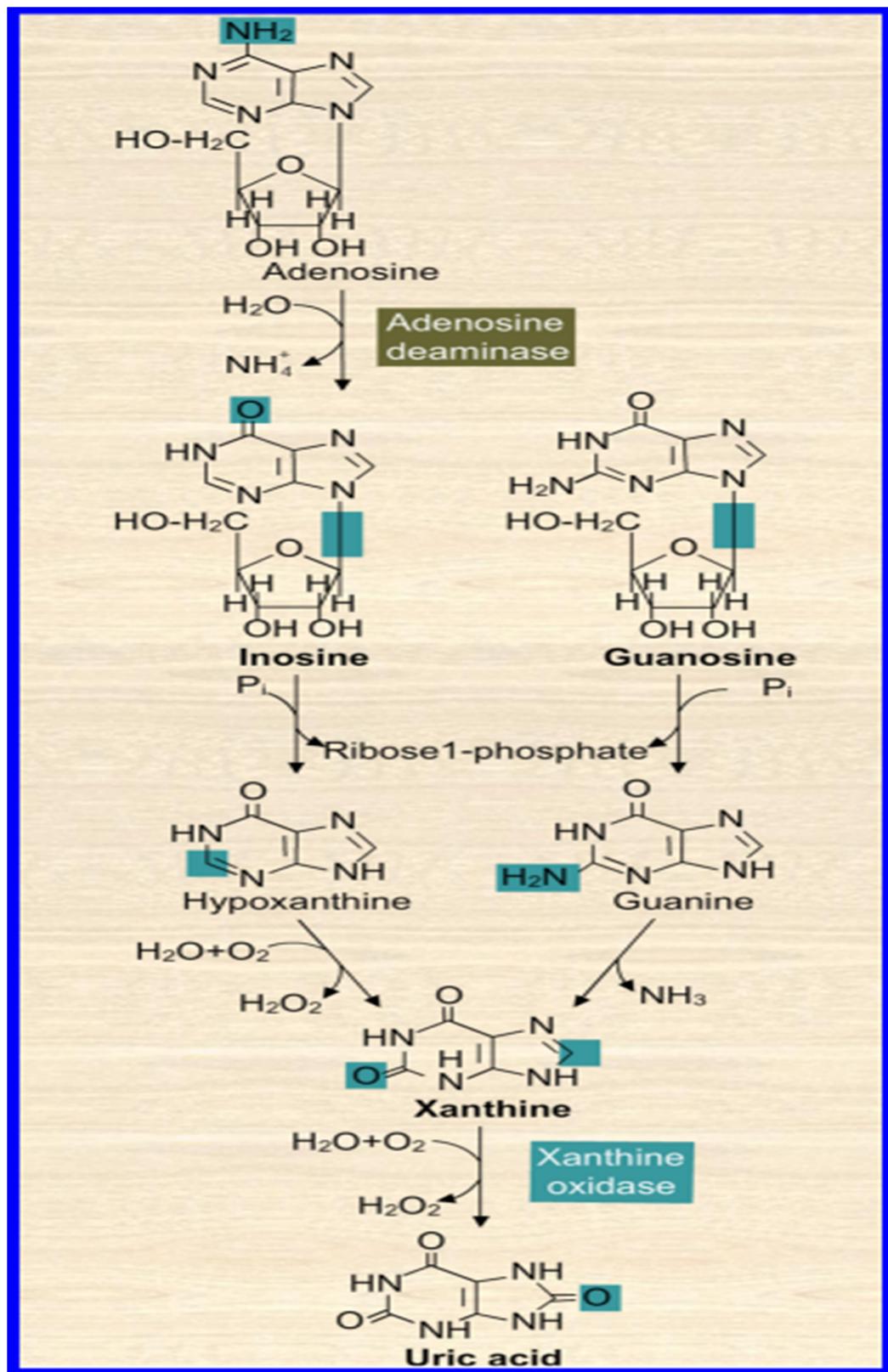
Fig. 17.6 : Formation of deoxyribonucleotides from ribonucleotides.

Source: "Biochemistry by U. Satyanarayana"

PURINE CATABOLISM

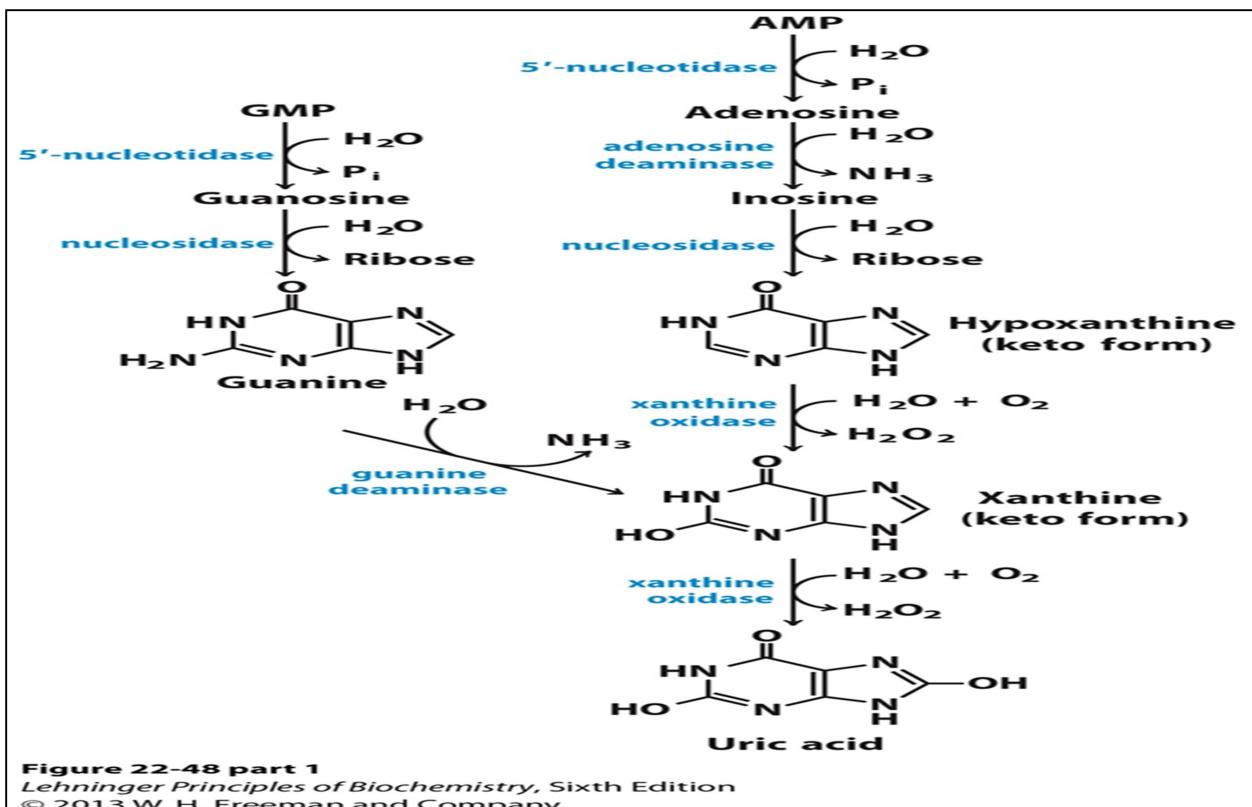
- The first enzyme in AMP degradation is **AMP deaminase**, which catalyzes the conversion of AMP to IMP and ammonia.
- IMP is hydrolyzed to inosine by the removal of inorganic phosphate followed by the removal of ribose as ribose 1-phosphate catalyzed by the enzyme purine nucleoside phosphorylase to produce hypoxanthine.
- **Xanthine oxidase catalyzes** the conversion of hypoxanthine to xanthine. This enzyme contains FAD, molybdenum and iron, and is exclusively found in liver and small intestine. Xanthine oxidase liberates H₂O₂ which is harmful to the tissues. Catalase cleaves H₂O₂ to H₂O and O₂.
- Guanine nucleotide also follows the similar pathway (removal of phosphate and ribose sugar) to produce guanine, which is then deaminated to xanthine catalyzed by guanase or guanine deaminase.
- Finally xanthine is converted to uric acid by the enzyme xanthine oxidase.

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PURINE METABOLISM

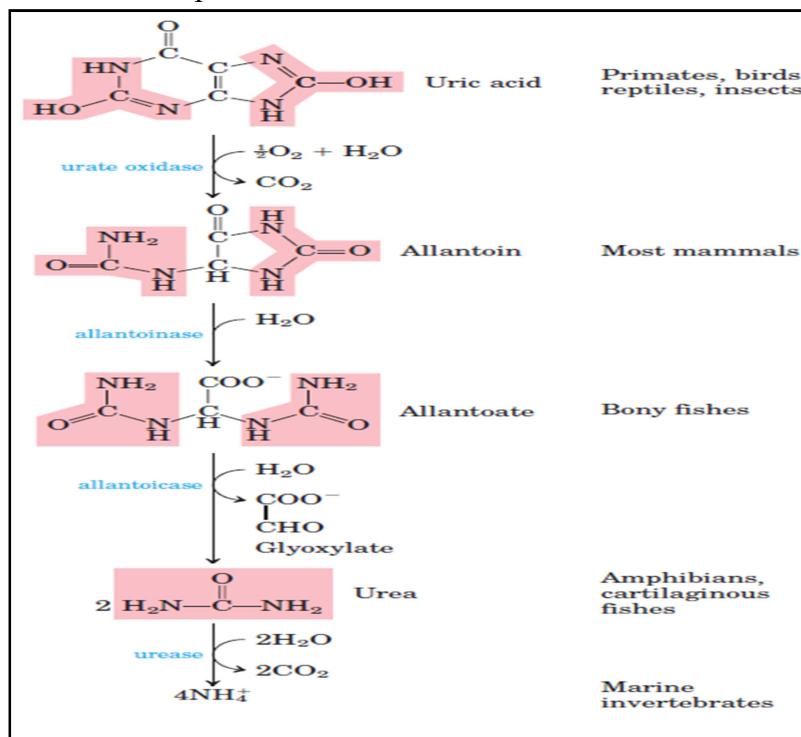


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PURINE METABOLISM



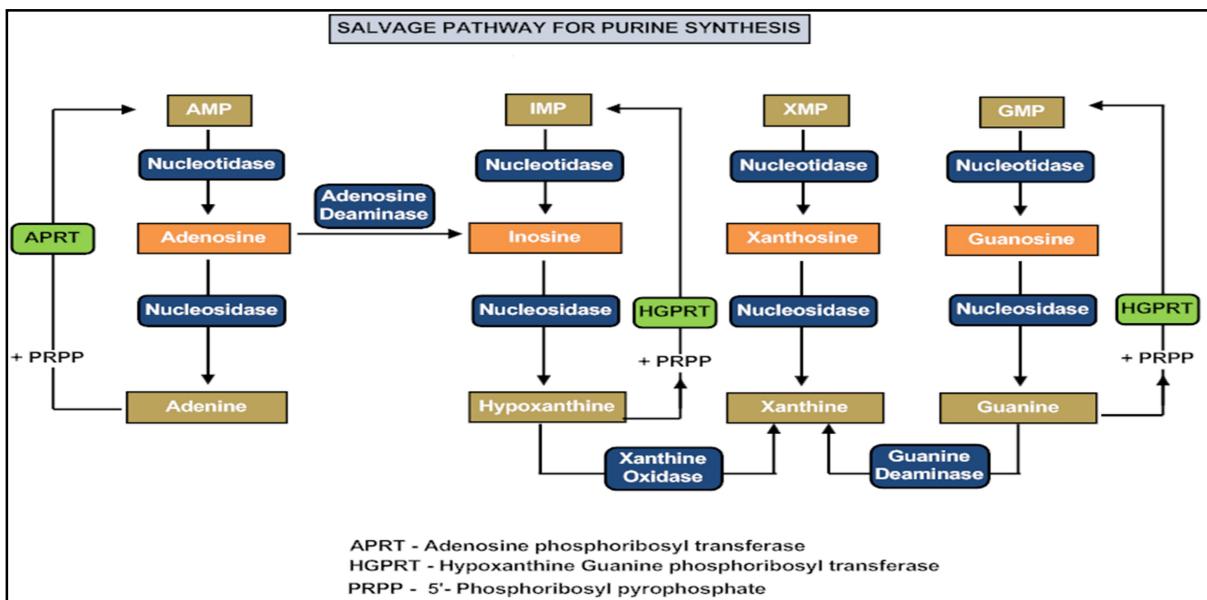
- Mammals other than primates and reptiles produce allantoin (highly water soluble) as their end product of purine catabolism. Such organisms contain the enzyme uricase that converts uric acid to allantoin. The following are the other end products of purine catabolism in different species.



Source: "Lehninger's Principles of Biochemistry" by David Nelson and Fox, 4th edition.

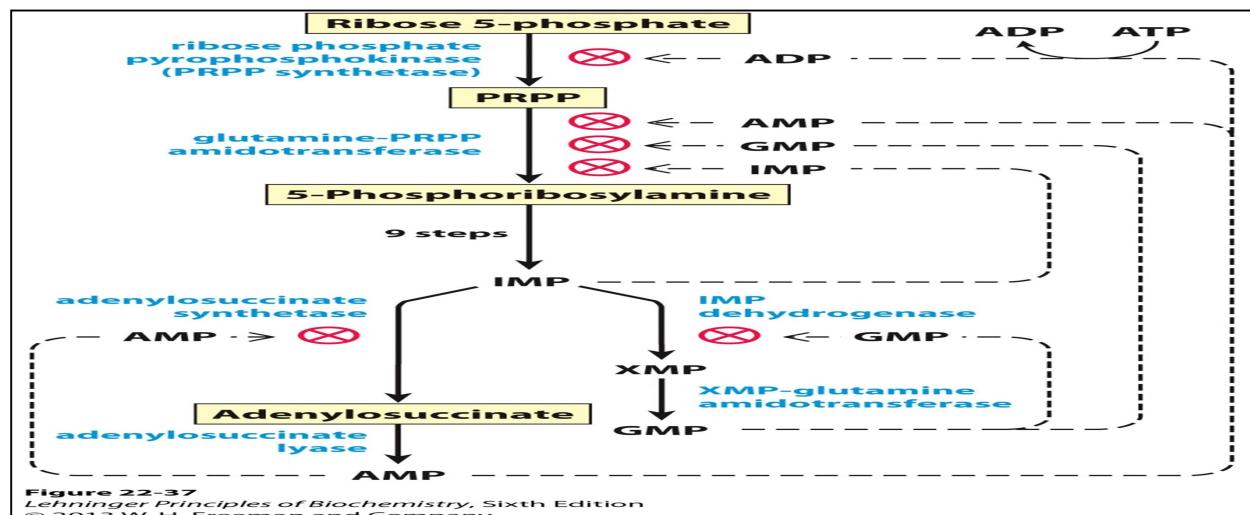
II B.V.Sc. & A.H. Veterinary Biochemistry (Unit-II) PURINE METABOLISM

OVERALL SALVAGE PATHWAY - PURINE SYNTHESIS & CATABOLISM



Regulation of purine nucleotide biosynthesis: The purine nucleotide synthesis is well coordinated to meet the cellular demands. The intracellular concentration of PRPP regulates purine synthesis to a large extent. This, in turn, is dependent on the availability of **ribose 5-phosphate and the enzyme PRPP synthetase**. PRPP glutamyl amidotransferase is controlled by a feedback mechanism by purine nucleotides. That is, if AMP and GMP are available in adequate amounts to meet the cellular requirements, their synthesis is turned off at the amidotransferase reaction. Another important stage of regulation is in the conversion of IMP to AMP and GMP. AMP inhibits adenylosuccinate synthetase while GMP inhibits IMP dehydrogenase. Thus, AMP and GMP control their respective synthesis from IMP by a feedback mechanism.

Regulatory mechanisms in the biosynthesis of adenine and guanine nucleotides (Purines) in *E. coli*



II B.V.Sc. & A.H. Veterinary Biochemistry (Unit-II)

PURINE METABOLISM

Disorders of Purine Metabolism:

- **Gout** is the disorder associated with the overproduction of uric acid, the end product of purine metabolism. At the physiological pH, uric acid is found in a more soluble form as sodium urate. 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 **gouty arthritis**. Sodium urate and/or uric acid may also precipitate in kidneys and ureters those results in renal damage and stone formation. Gout is of two types-primary and secondary.
 1. **Primary gout:** It is an inborn error of metabolism due to overproduction of uric acid. This is mostly related to increased synthesis of purine nucleotides. . The following are the important metabolic defects (enzymes) associated with primary gout:
 - **Increased activity of PRPP synthetase and PRPP glutamylamidotransferase** lead to the increased production of purines.
 - **Glucose 6-phosphatase deficiency:** In type I glycogen storage disease (**von Gierke's disease**) resulting in elevated levels of ribose 5-phosphate and PRPP and, ultimately, purine overproduction.
 - **Elevation of glutathione reductase** generates more NADP⁺ which is utilized by HMP shunt. This causes increased ribose 5-phosphate and PRPP synthesis.
 - **HGPRT deficiency (Lesch-Nyhan syndrome)** leads to increased synthesis of purine nucleotides.
 2. **Secondary gout:** Secondary hyperuricemia is due to various diseases causing increased synthesis or decreased excretion of uric acid. Increased degradation of nucleic acids (hence more uric acid formation) is observed in various cancers (leukemias, polycythemia, lymphomas, etc.) psoriasis and increased tissue breakdown (trauma, starvation etc.).
- **Allopurinol** is the drug of choice for the treatment of gout.
- **Pseudogout:** The clinical manifestations of pseudogout are similar to gout. But this disorder is caused by the deposition of calcium pyrophosphate crystals in joints. Further serum uric acid concentration is normal in pseudogout.
- **Lesch-Nyhan syndrome:** It is caused by a defect in the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT), an enzyme of purine salvage pathway. It was first described in 1964 by Michael Lesch (a medical student) and William L. Nyhan (his teacher). Lesch-Nyhan syndrome is a sex-linked metabolic disorder since the structural gene for HGPRT is located on the X-chromosome. It affects only the males and is characterized by excessive uric acid production (often Gouty arthritis), and neurological abnormalities such as mental retardation, aggressive behaviour, learning disability etc. **The patients of this disorder have an irresistible urge to bite their fingers and lips, often causing self-mutilation.**
- A defect in the enzyme **adenosine deaminase (ADA)** results in severe combined immunodeficiency (**SCID**) involving both T-cell and B-cell dysfunction. A girl suffering

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PURINE METABOLISM

from SCID was cured by transferring ADA gene (in 1990) and that was the first attempt for **gene therapy** in modern medicine.

Source: "Biochemistry by U. Satyanarayana"

