# Nucleotides, Purine and Pyrimidine Biosynthesis and Catabolism

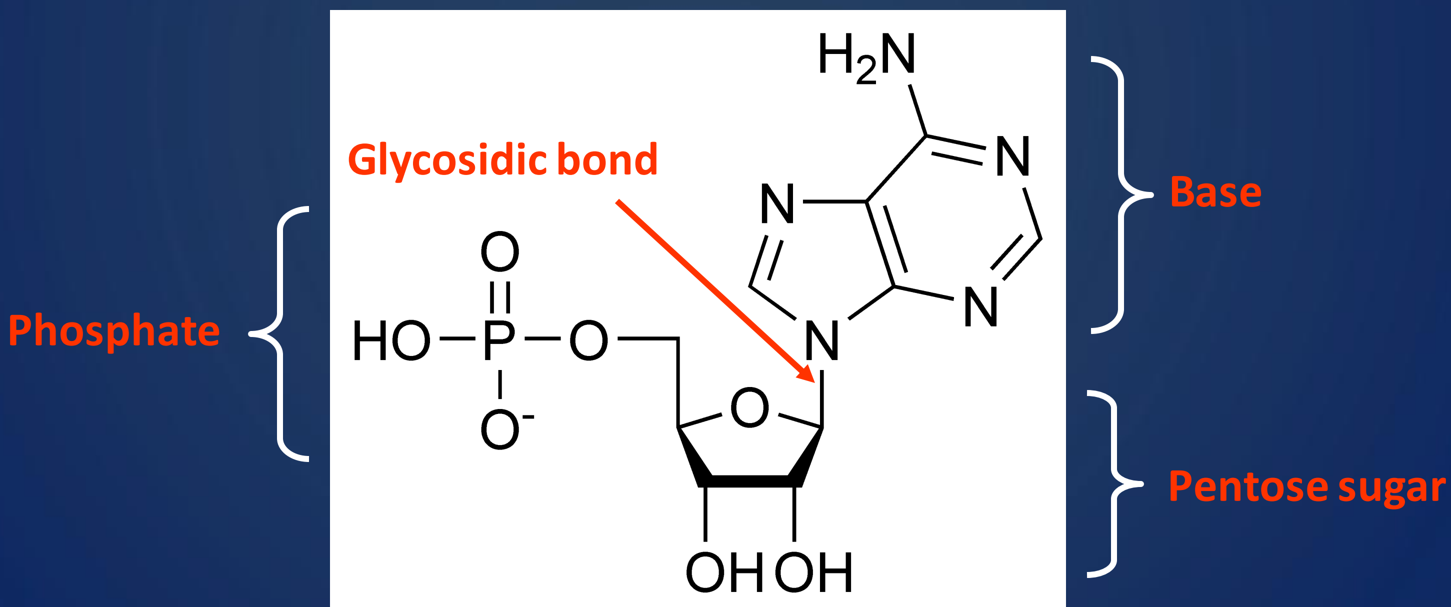
## LEARNING POINTS

1. Understand nucleosides*, nucleotides, and their function in DNA and RNA
2. Understand the structure and function of purines
3. Understand the origin of atoms in the purine ring
4. Understand the essential features of purine metabolism and catabolism
5. Understand clinical aspects of purine metabolism and deficiencies

*Keywords are highlighted in yellow

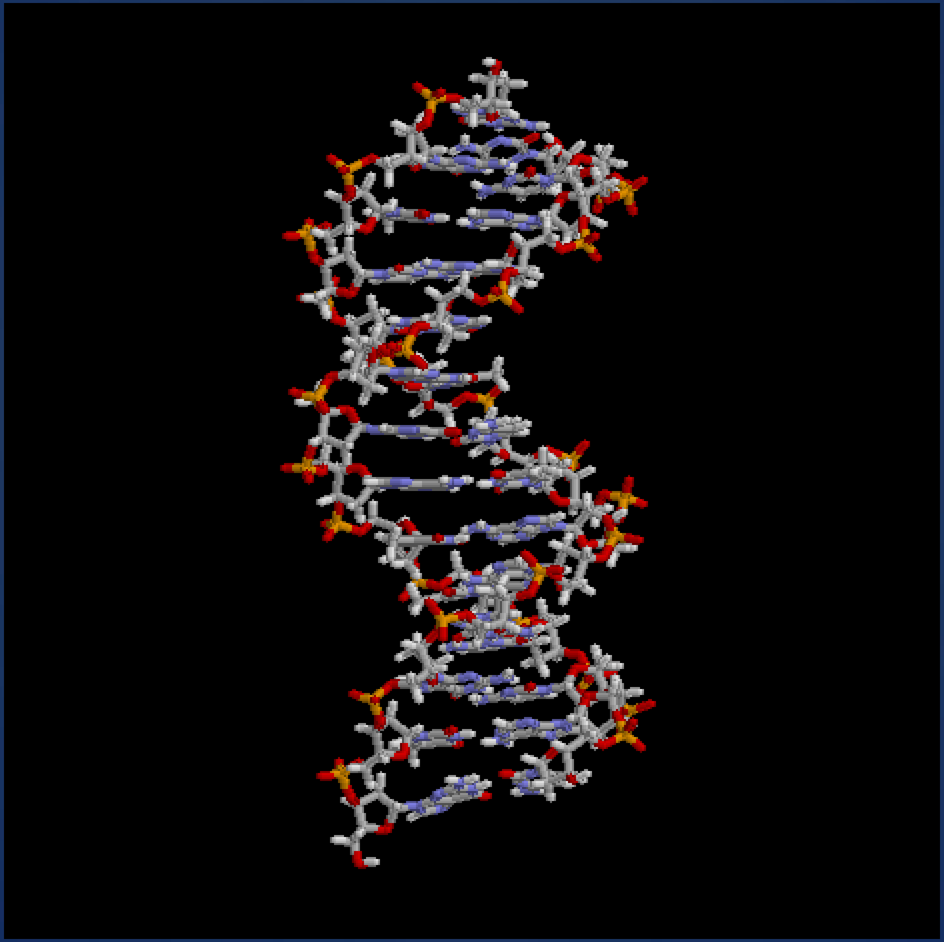
# Nucleotides

Chemical compound composed of three components: (1) heterocyclic base; (2) sugar (usually a pentose); and (3) one or more phosphate groups

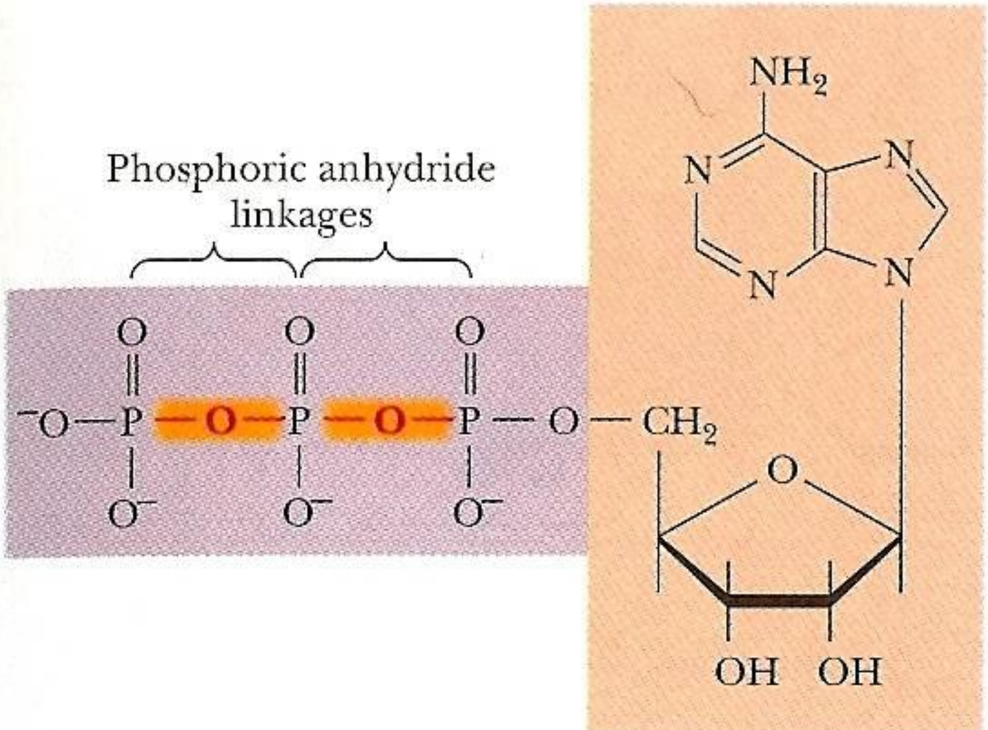



Adenosine monophosphate (AMP)

# Building blocks for DNA and RNA

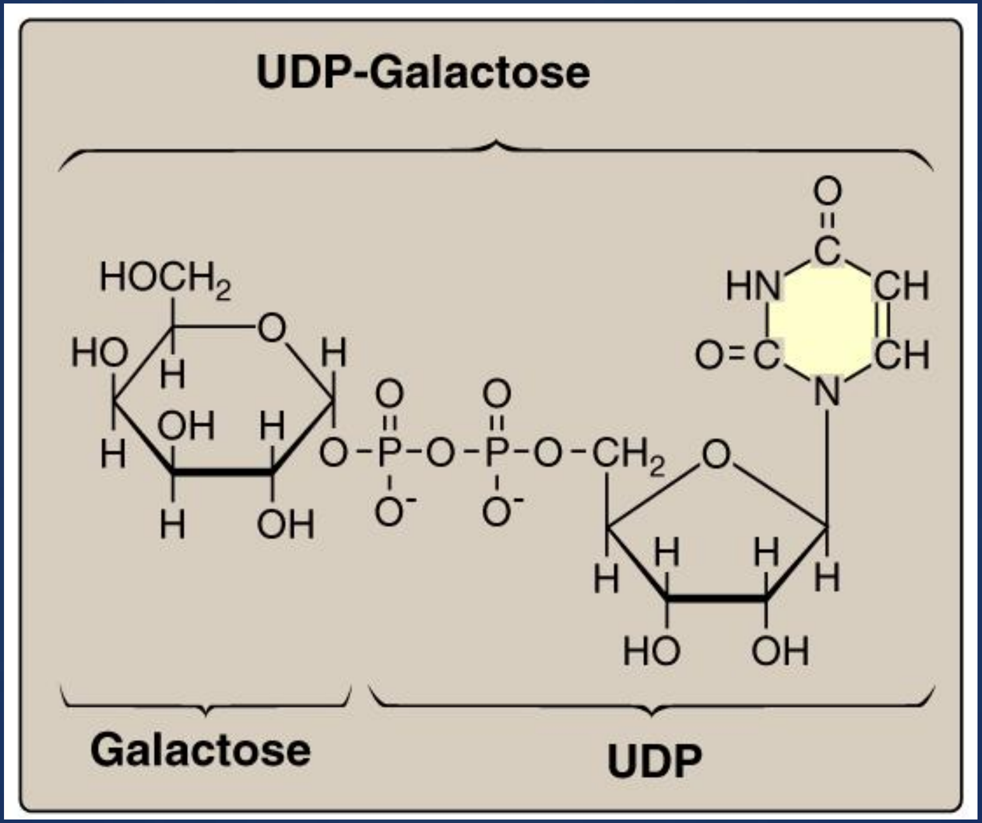


# Energy Currency

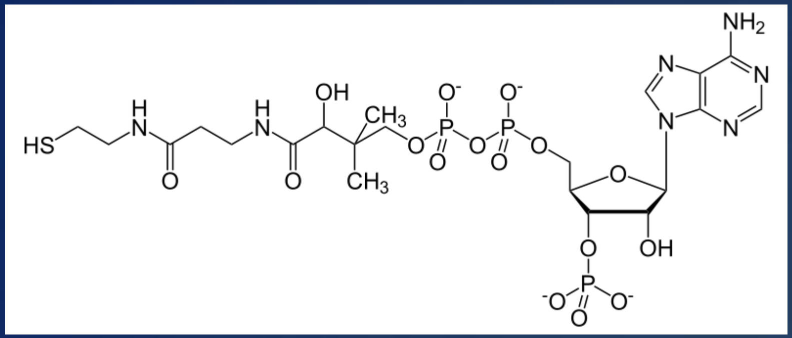



**ATP**
(adenosine-5′-triphosphate)

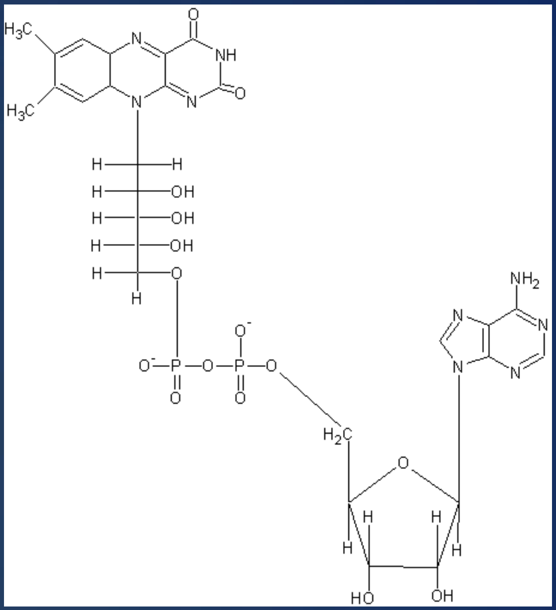
# Carriers for Activated Intermediates



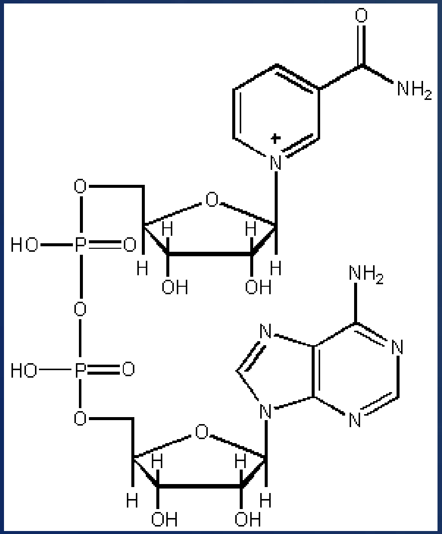
# Structural Components of:



**Coenzyme A**

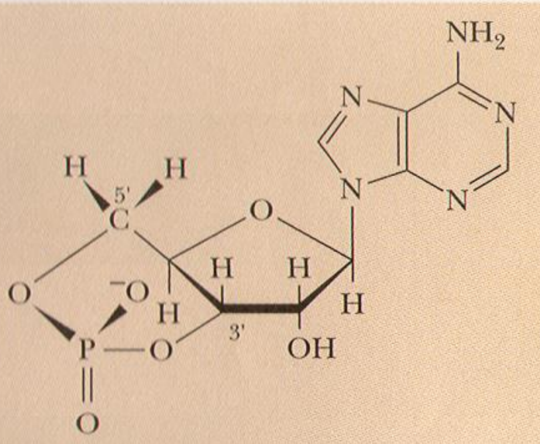


**Flavin adenine dinucleotide (FAD)**

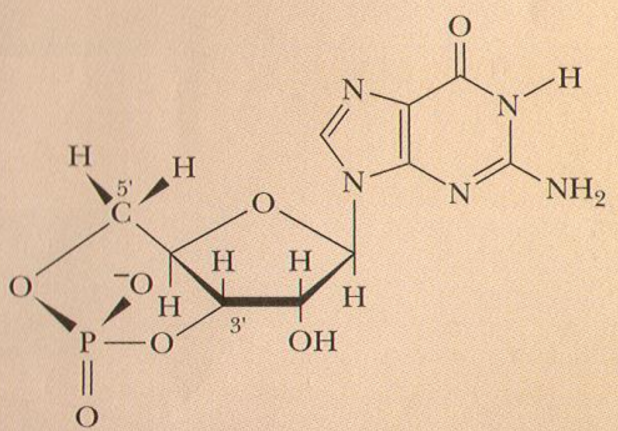


**$NAD(P)^+$**

# Signaling Molecules

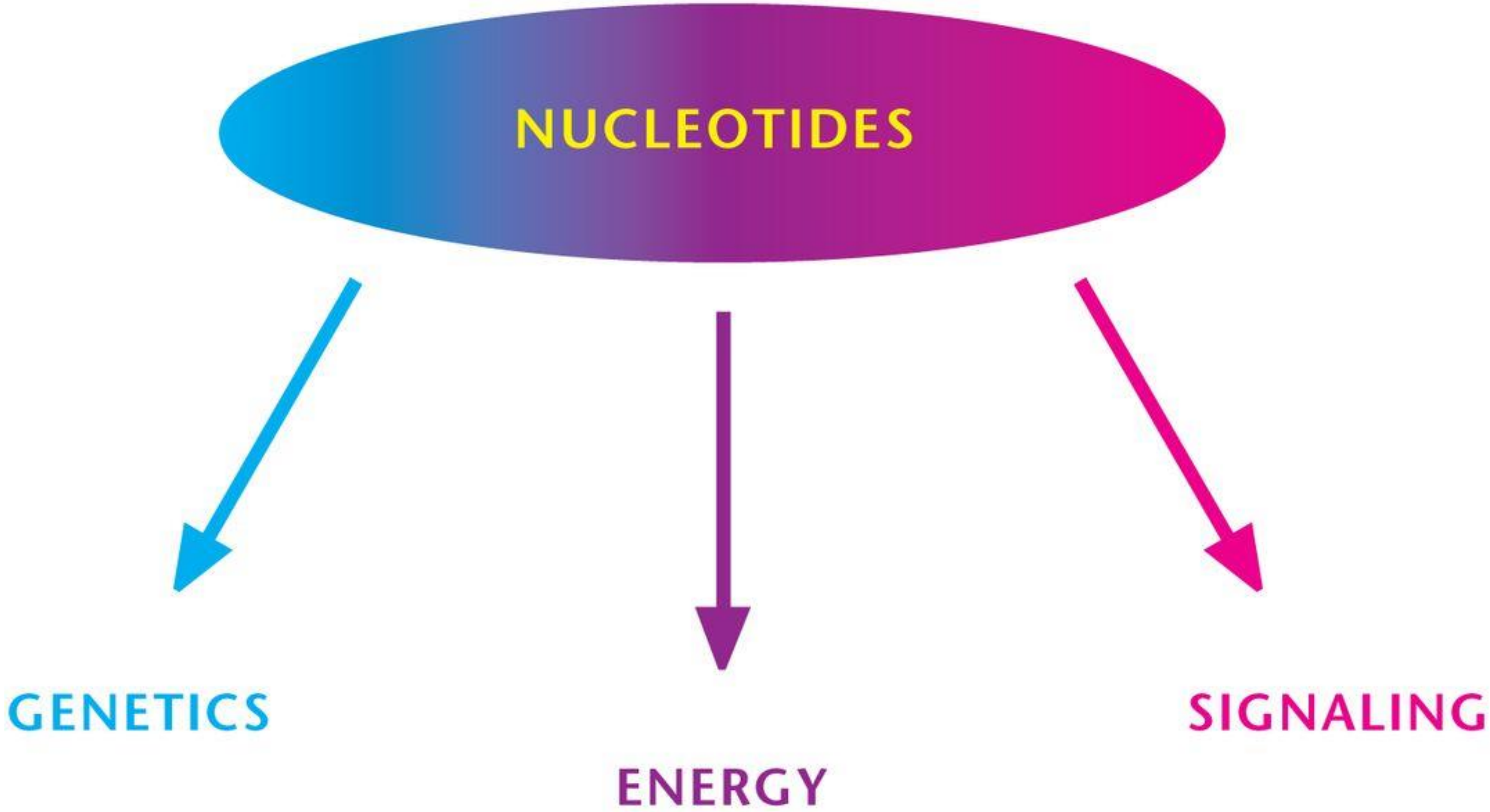


3',5'-Cyclic AMP

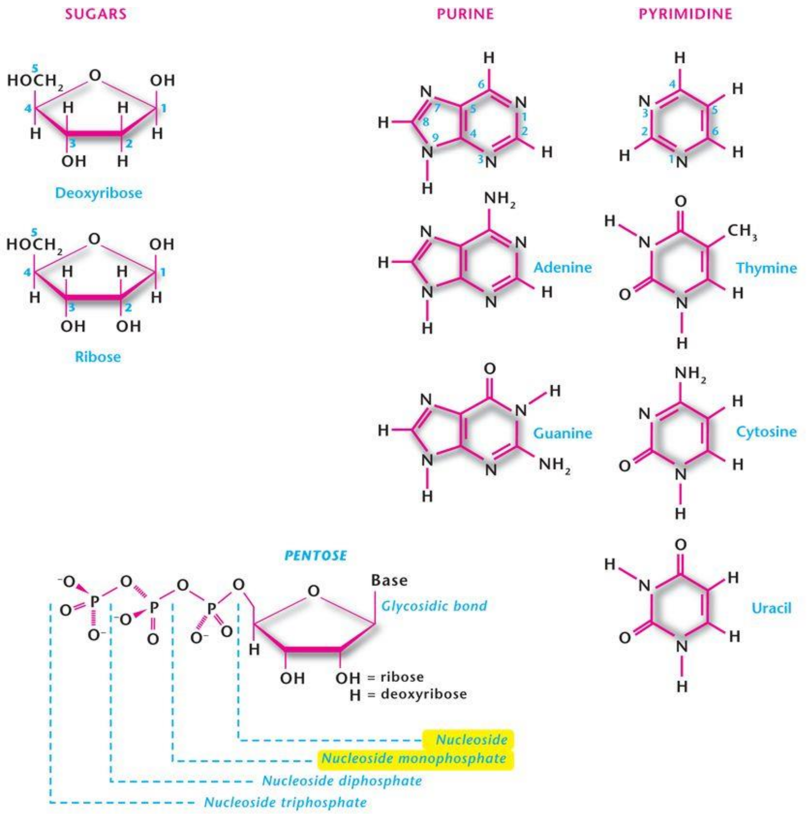


3',5'-Cyclic GMP

# Overview of nucleotide metabolism.



# Structure of purines and pyrimidines in a nutshell




| | Ribo-nucleoside | Deoxyribo-nucleoside | NMP dNMP | NDP dNDP | NTP dNTP |
|---|---|---|---|---|---|
| **PURINES** | | | | | |
| Adenine | Adenosine | Deoxyadenosine | AMP dAMP | ADP dADP | ATP dATP |
| Guanine | Guanosine | Deoxyguanosine | GMP dGMP | GDP dGDP | GTP dGTP |
| **PYRIMIDINES** | | | | | |
| Cytosine | Cytidine | Deoxycytidine | CMP dCMP | CDP dCDP | CTP dCTP |
| Thymine | Thymidine | Deoxythymidine | TMP dTMP | TDP dTDP | TTP dTTP |
| Uracil | Uridine | Deoxyuridine | UMP dUMP | UDP dUDP | UTP dUTP |

# The Nitrogenous Bases

In DNA:
- Adenine
- Guanine
- *Thymine*
- Cytosine

In RNA:
- Adenine
- Guanine
- *Uracil*
- Cytosine

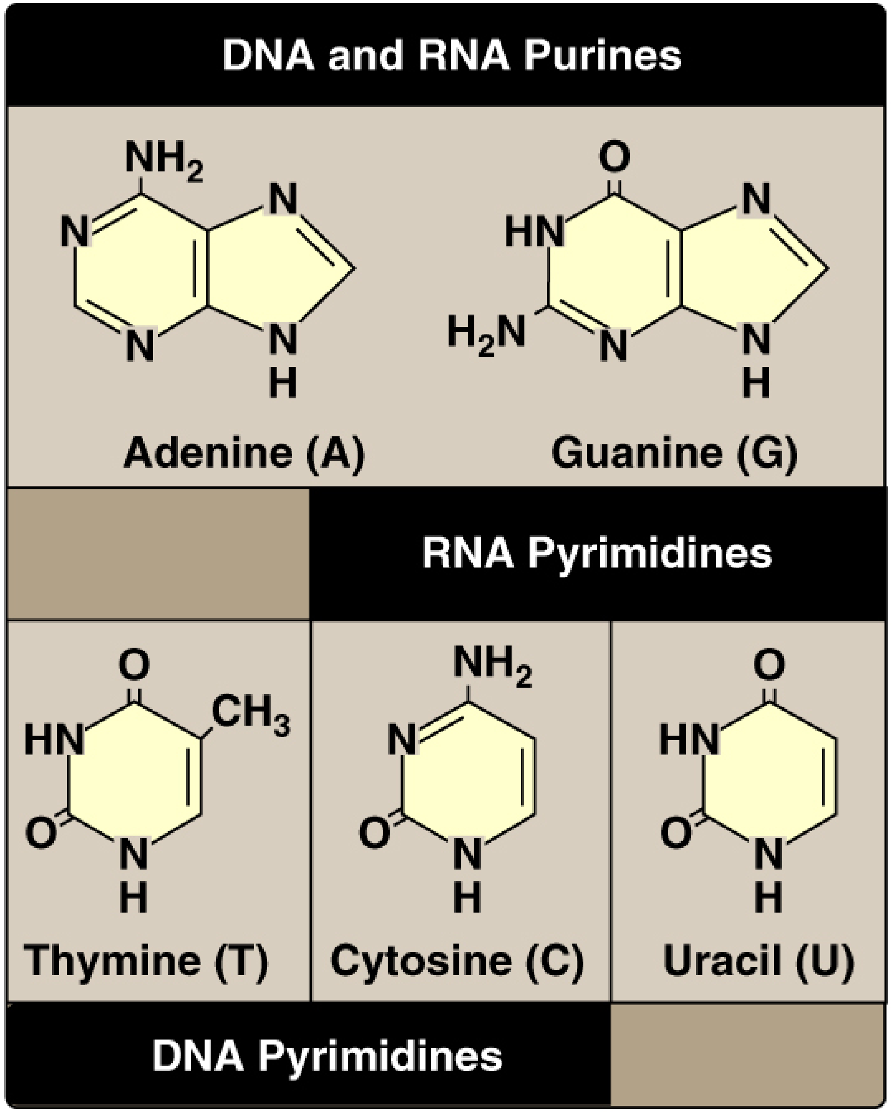
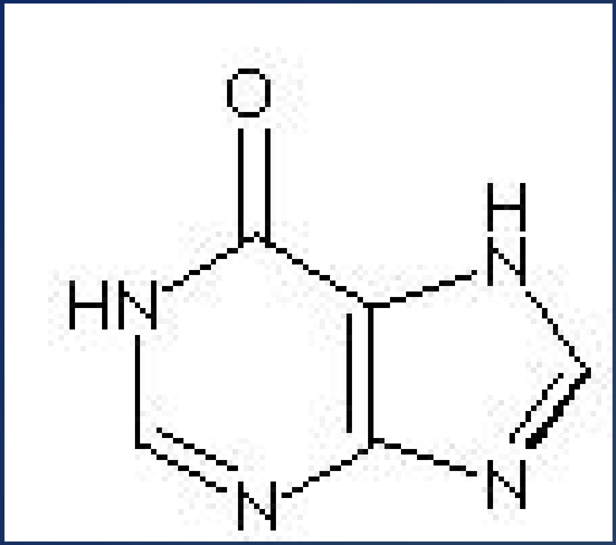
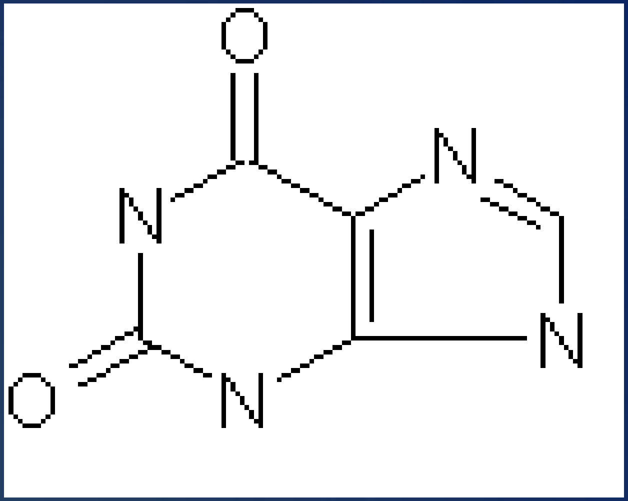



**Figure 22.1**
Purines and pyrimidines commonly found in DNA and RNA.

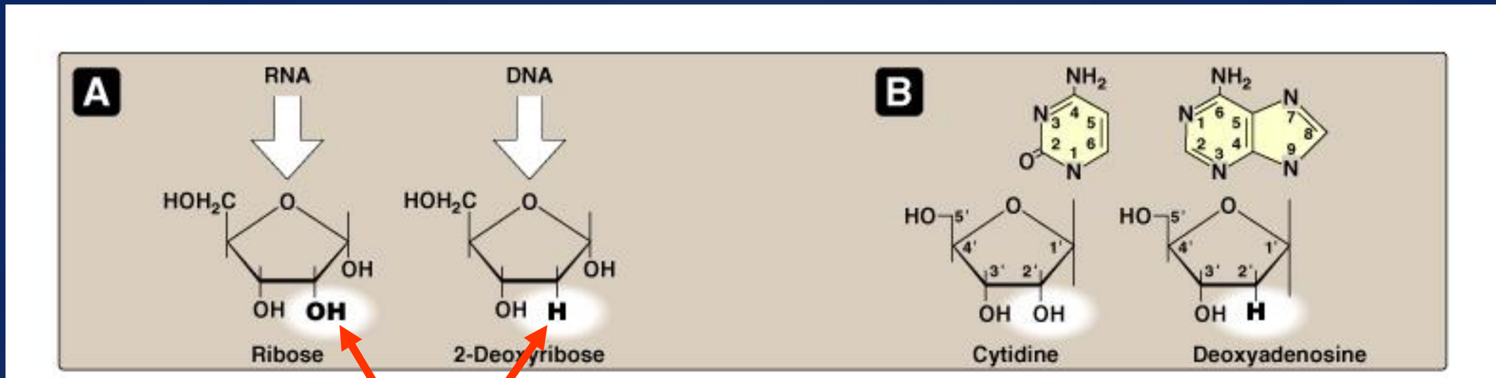



Hypoxanthine




Xanthine

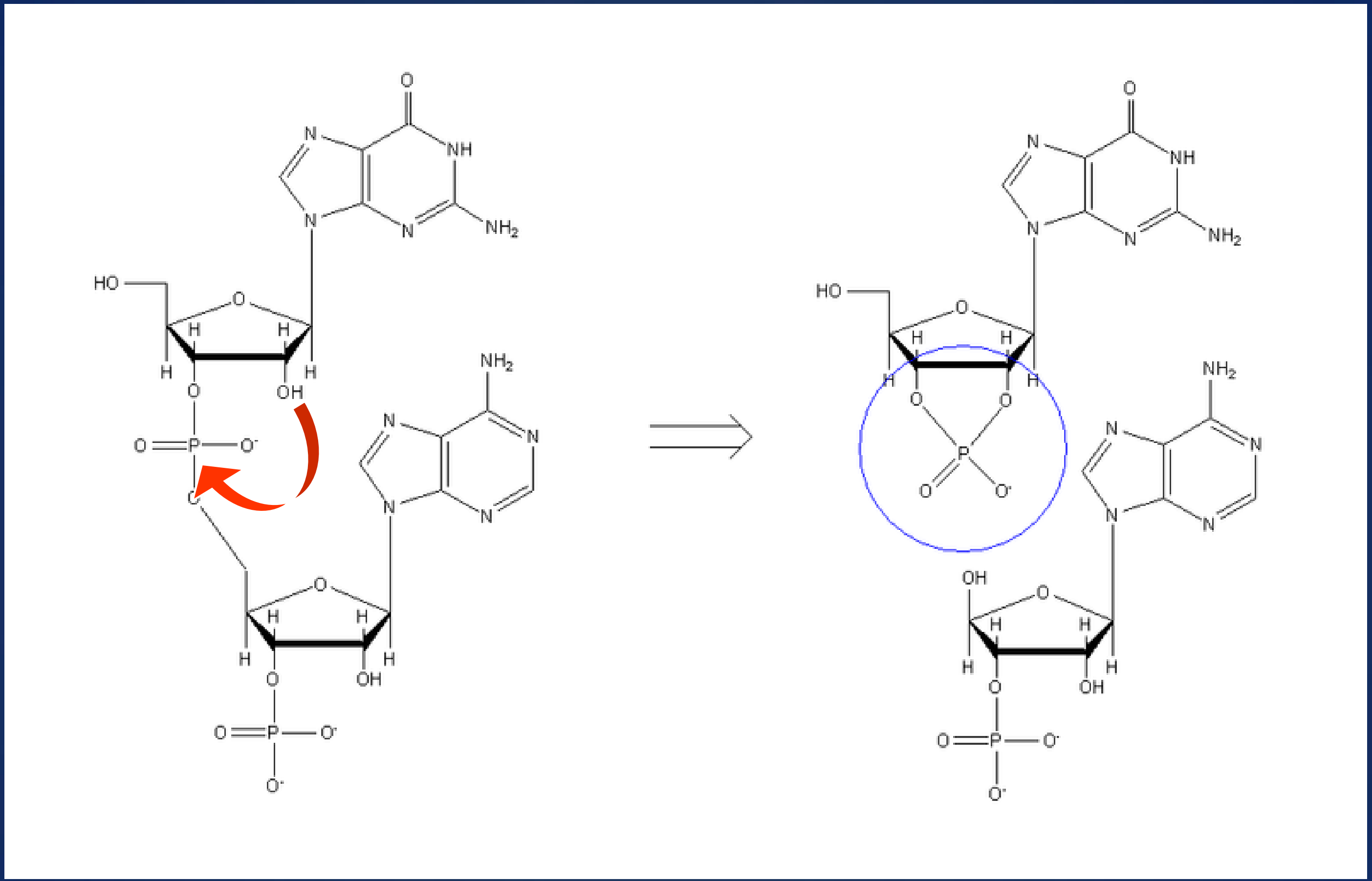
Not typically found in DNA or RNA, but are important metabolic intermediates.



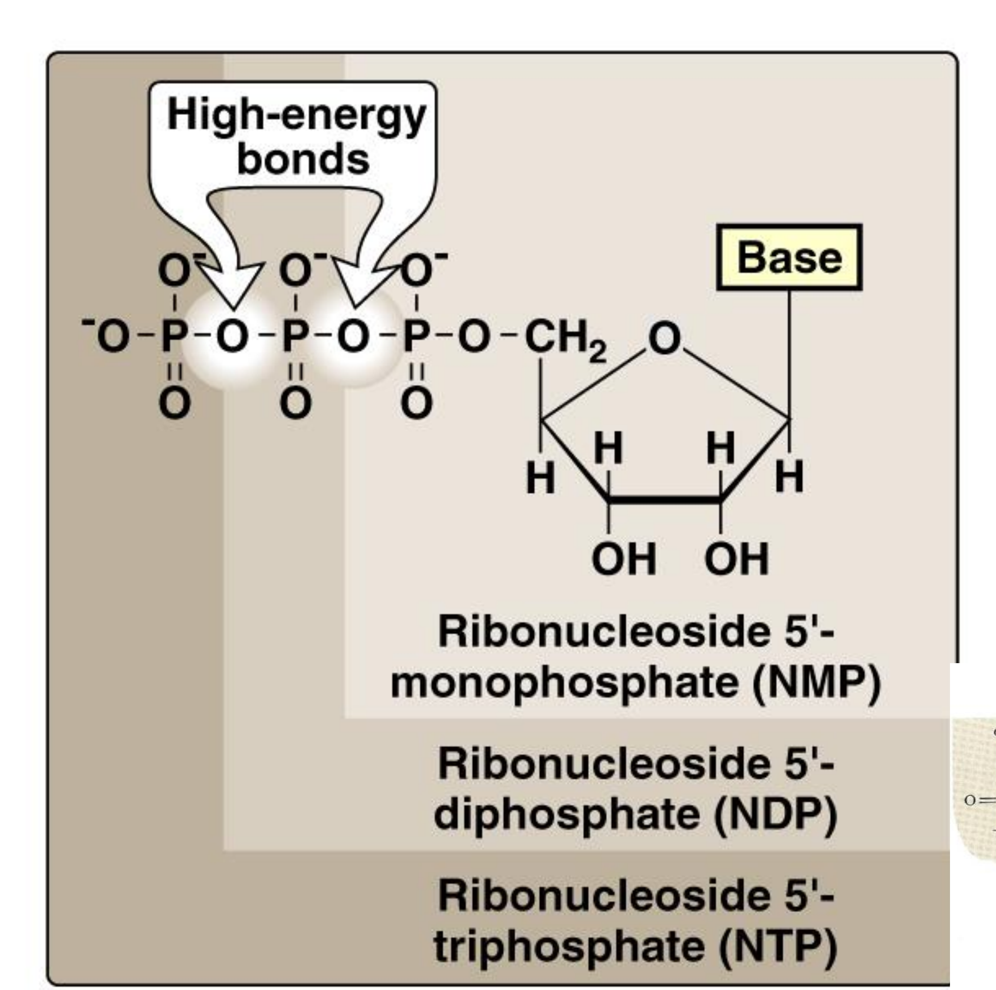

RNA is sensitive to alkaline degradation

| Base | Ribonucleoside | Ribonucleotide | Deoxyribonucleotide |
|---|---|---|---|
| Adenine | Adenosine | Adenylate | Deoxyadenylate |
| Guanine | Guanosine | Guanylate | Deoxyguanylate |
| Cytosine | Cytidine | Cytidylate | Deoxycytidylate |
| Thymine | Thymidine | Ribothymidylate | Thymidylate |
| Uracil | Uridine | Uridylate | Deoxyuridylate |
| Hypoxanthine | Inosine | Inosinate | Deoxyinosinate |
| Xanthine | Xanthosine | Xanthylate | Deoxanthylate |

# Mechanism of RNA Hydrolysis

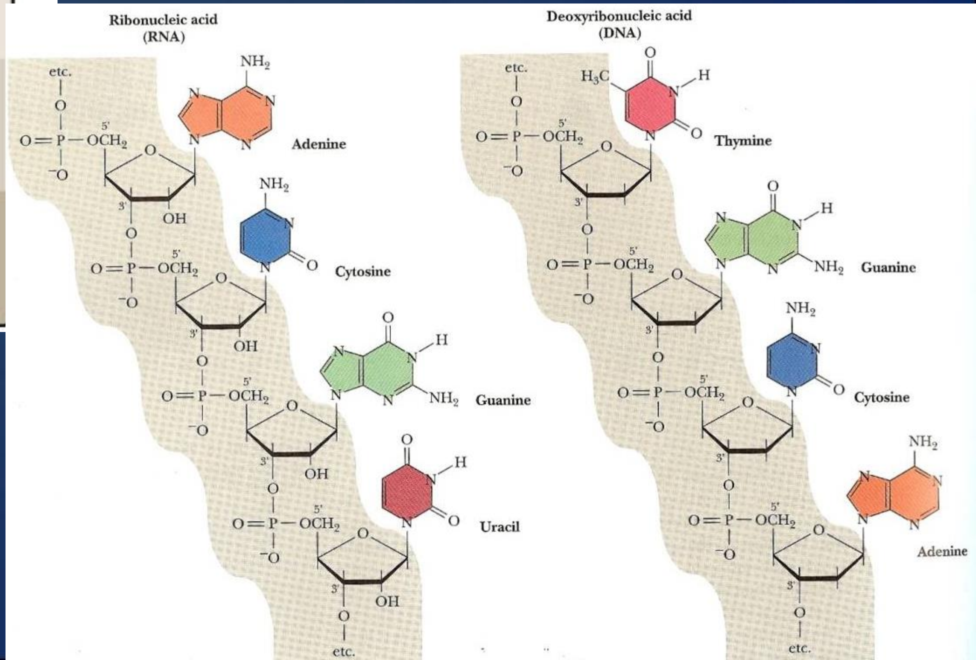


**Hydrolysis occurs by nucleophilic attack of the 2'-hydroxyl group on the polarized phosphate to yield a 2'-3' cyclic phosphodiester intermediate (circled) that subsequently spontaneously hydrolyzes to a mix of 2'- and 3'-phosphomonoesters.**

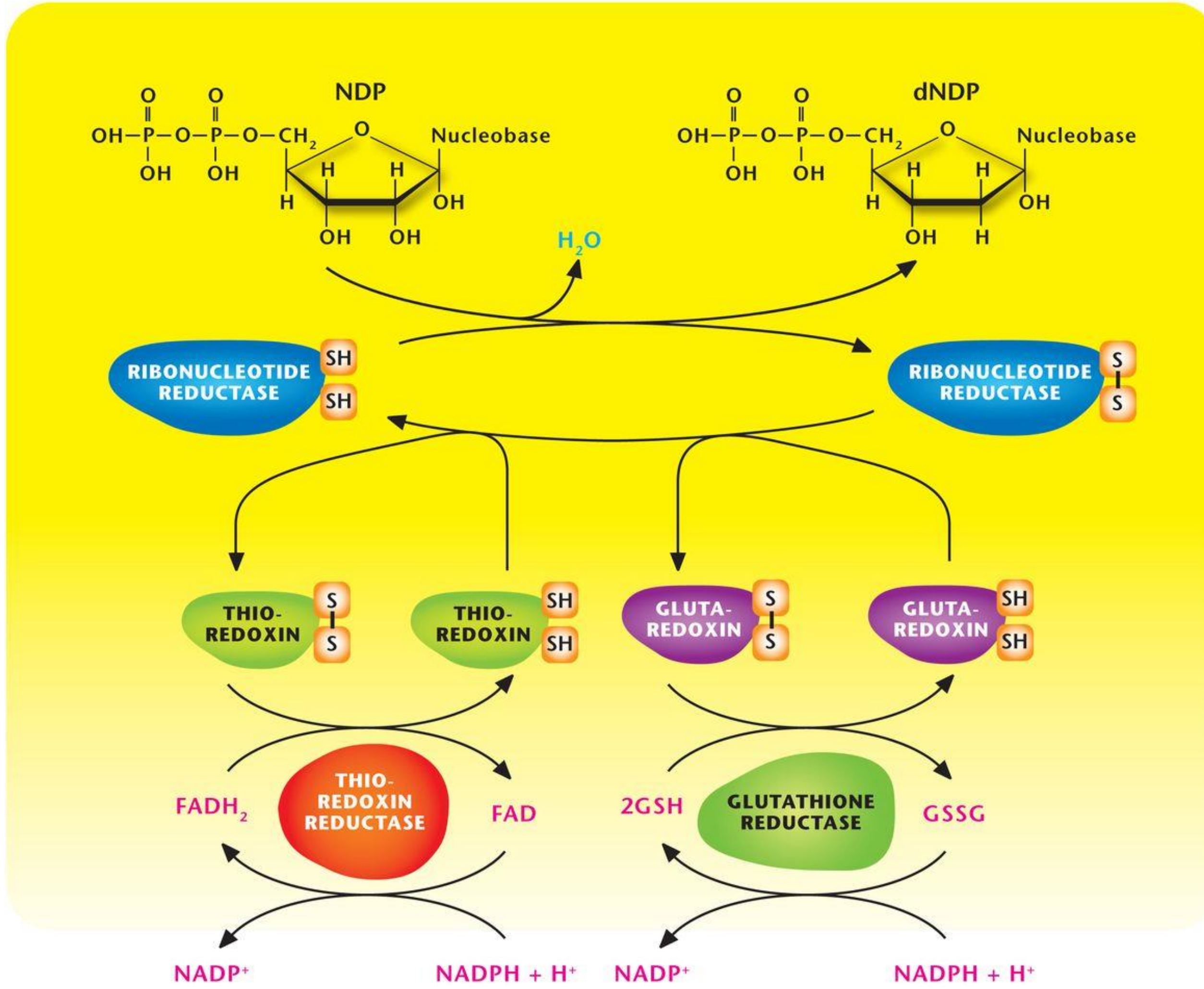



# Two Important Points

1. The phosphate groups are responsible for the net negative charge associated with DNA and RNA.
2. The hydroxyl group at the 2'-position accounts for the greater ease with which RNA is degraded by alkali.

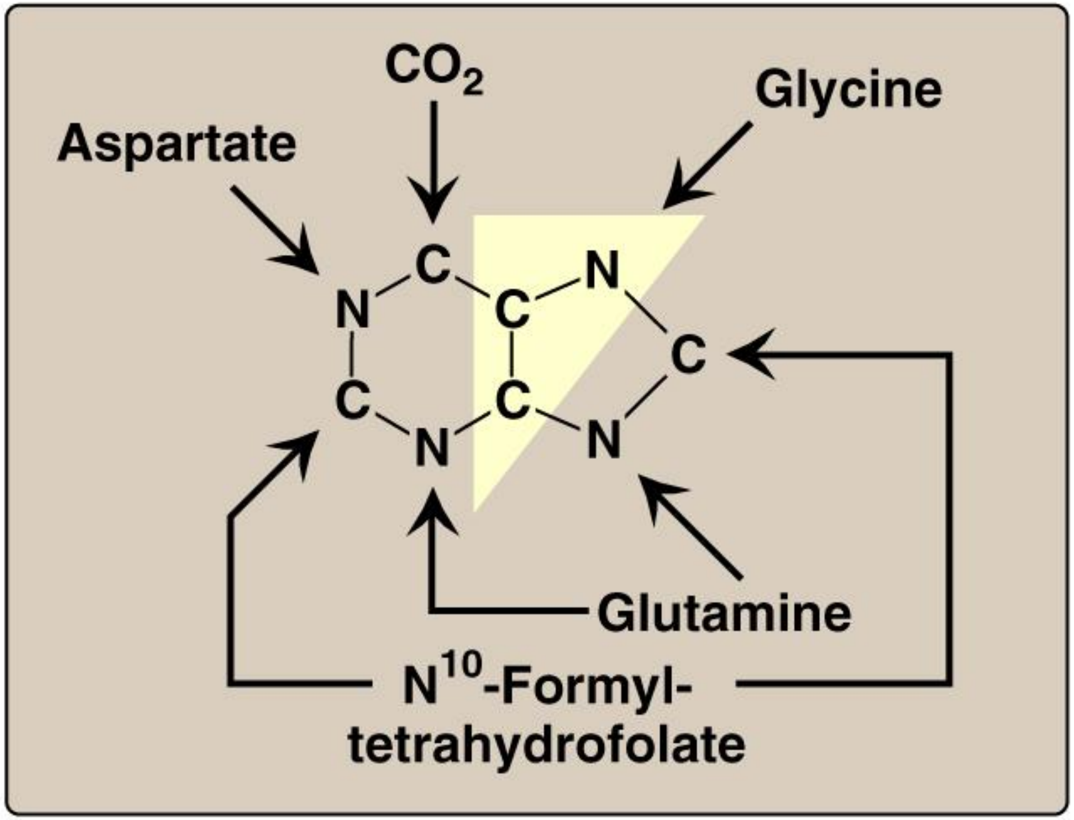


# Ribonucleotide reductase (RR) produces dNDPs




Nucleotide diphosphate (NDP) kinase use ATP to phosphorylate dNTP to dNTP triphosphate

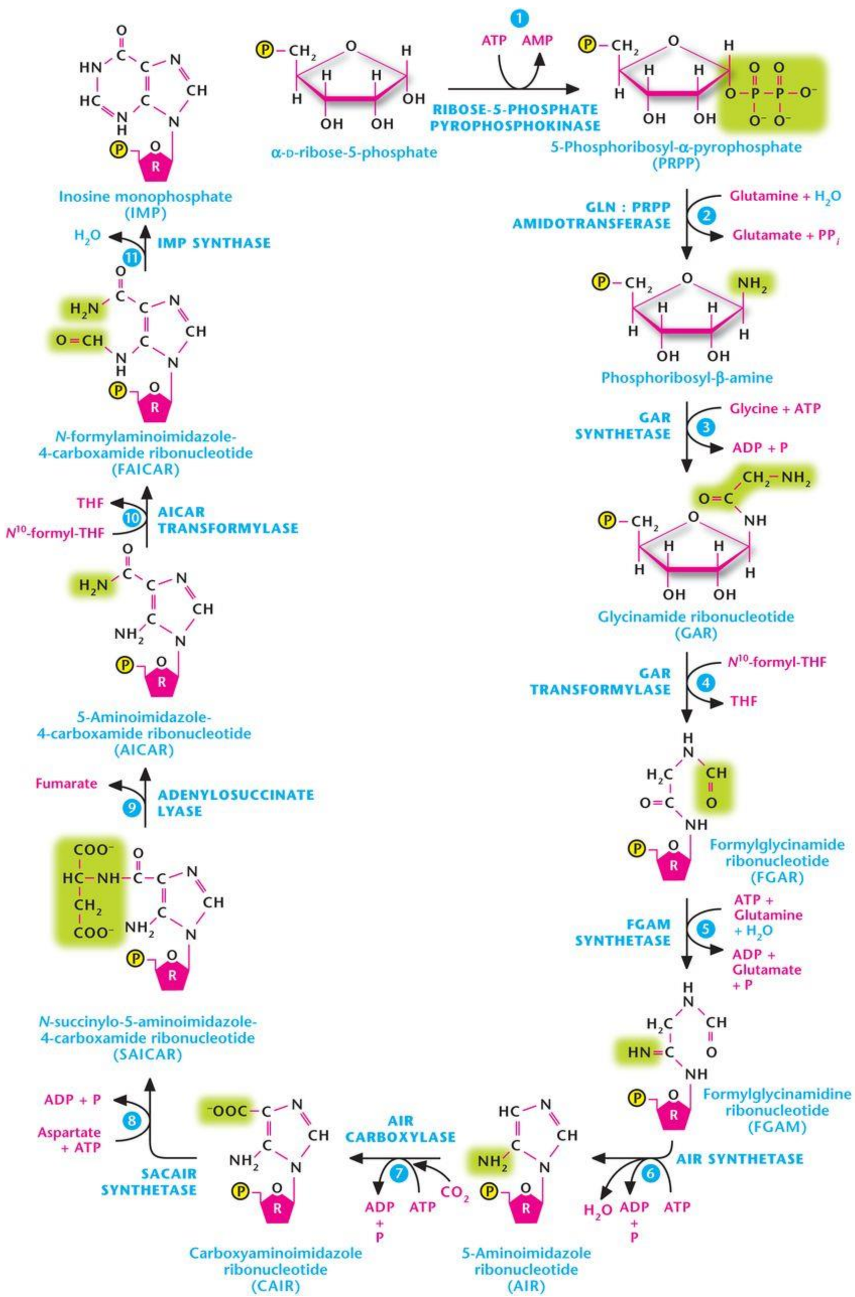
# *De novo* purine synthesis



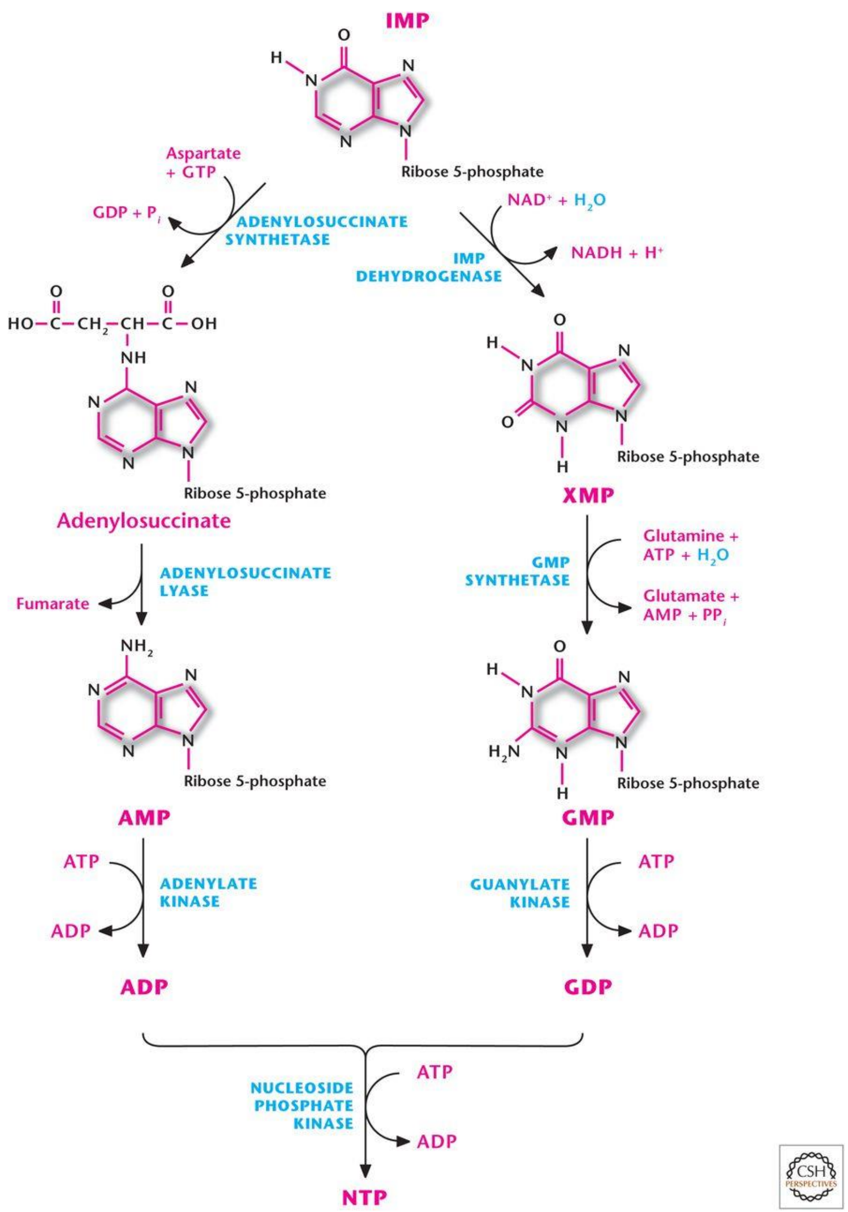
# *De novo* purine synthesis

- The purine ring is synthesized by a series of reactions that add the carbon and nitrogen atoms to a pre-formed ribose-5-phosphate.
- The ribose-5-phosphate is synthesized as part of the Pentose Phosphate Pathway (or Hexose MonoPhosphate pathway). PPP is a metabolic pathway that runs parallel to glycolysis.
- In humans, all necessary enzymes are found in the cytoplasm of the cell.

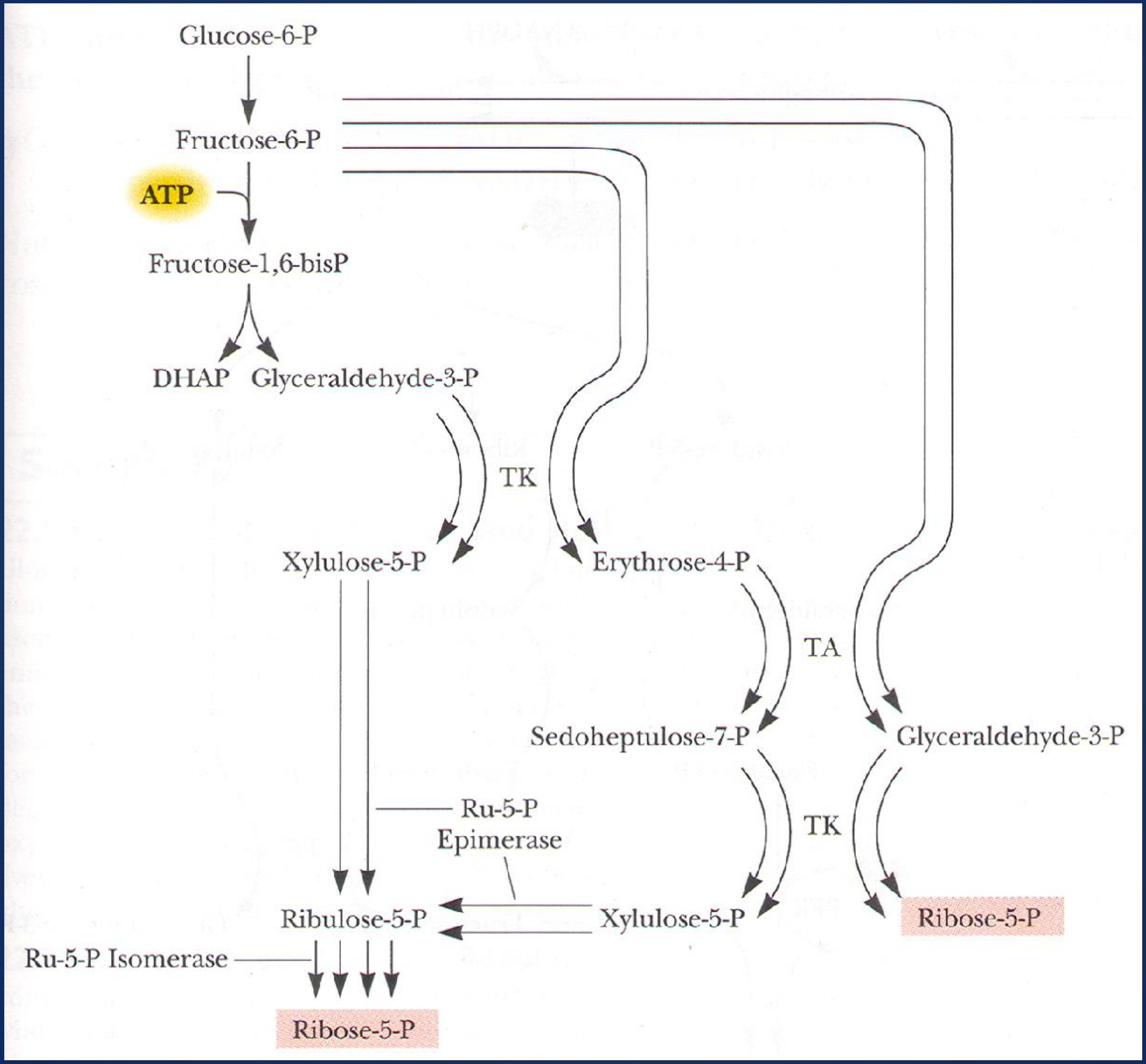
# The purine synthesis pathway



# Purine nucleotide production



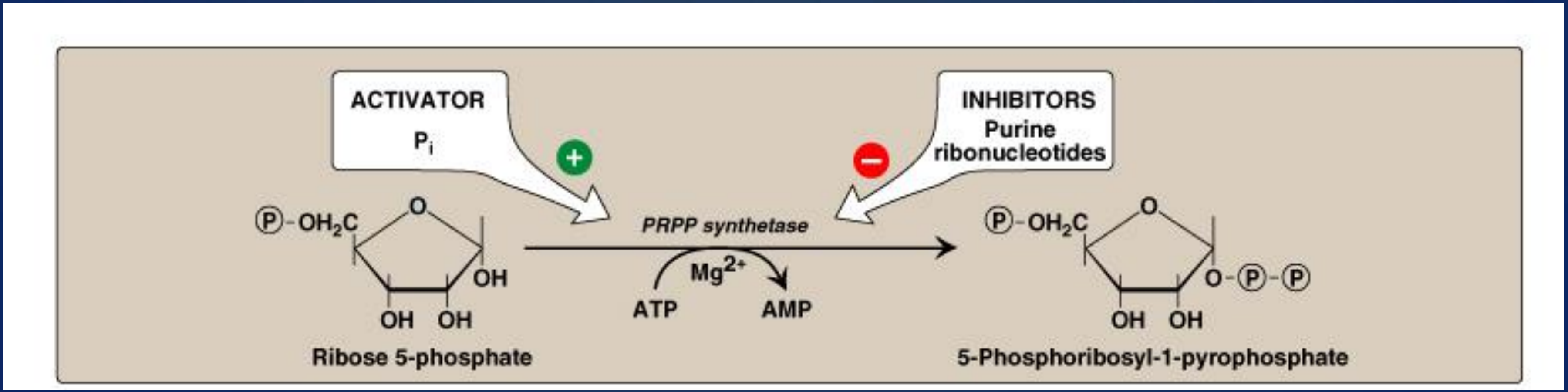
# Source For Ribose-5-Phosphate

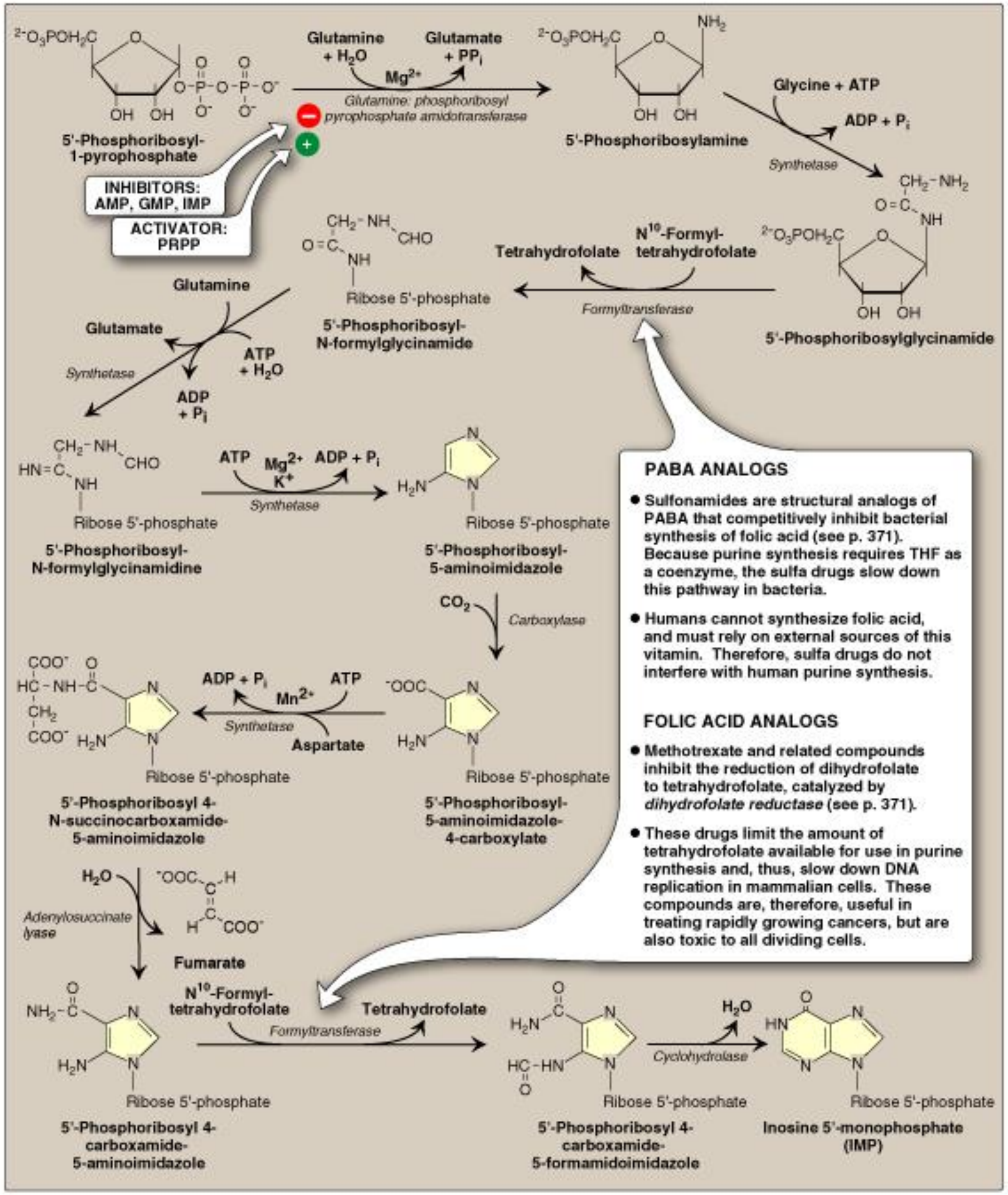


# Conversion of Ribose-5-phosphate to PRPP

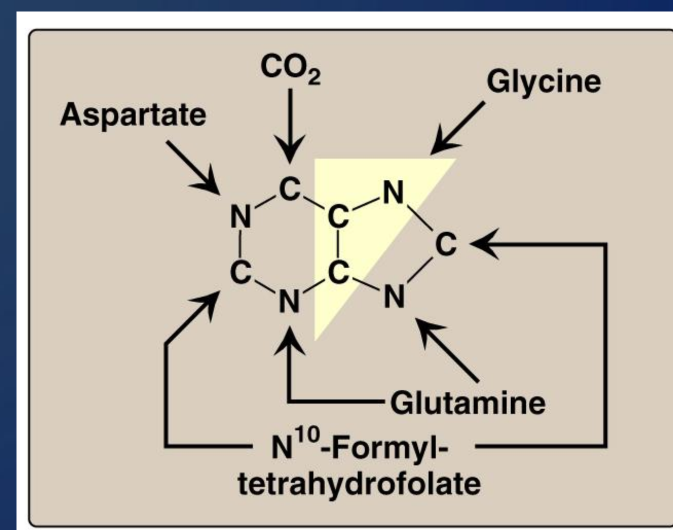
•The pentose sugar is always a ribose, which may be reduced to deoxyribose after nucleotide synthesis is complete.

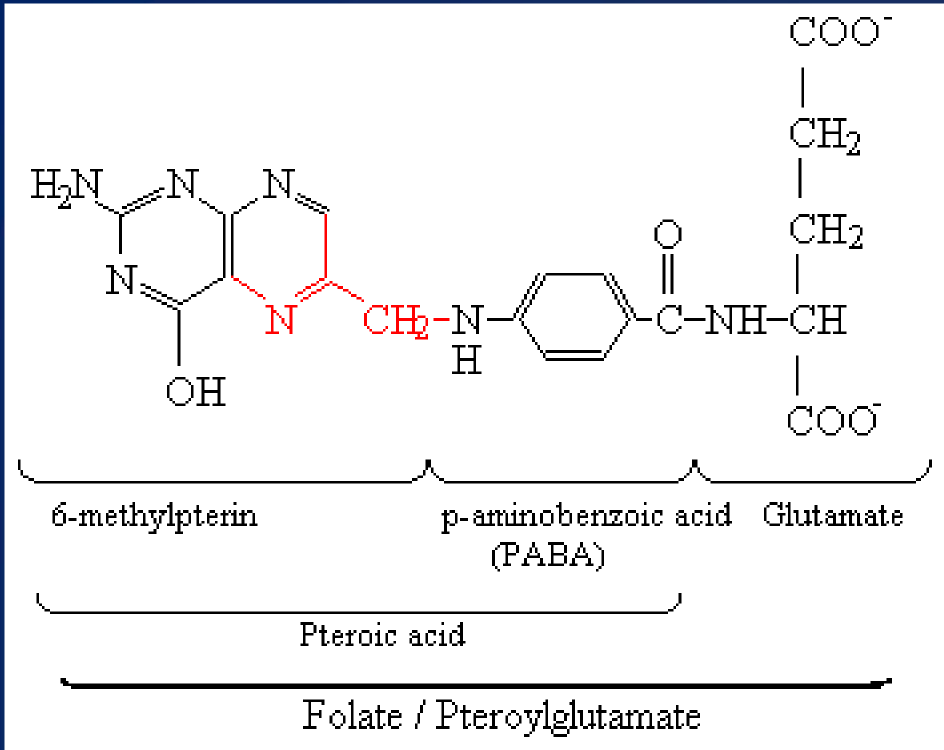
•5-Phosphoribosyl-1-pyrophosphate (PRPP) is also involved in synthesis of pyrimidine nucleotides, $NAD^+$, and histidine biosynthesis.

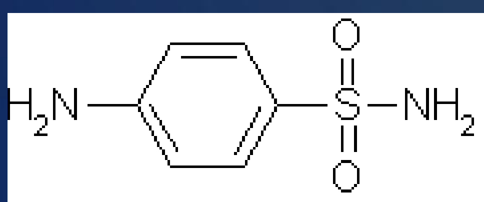




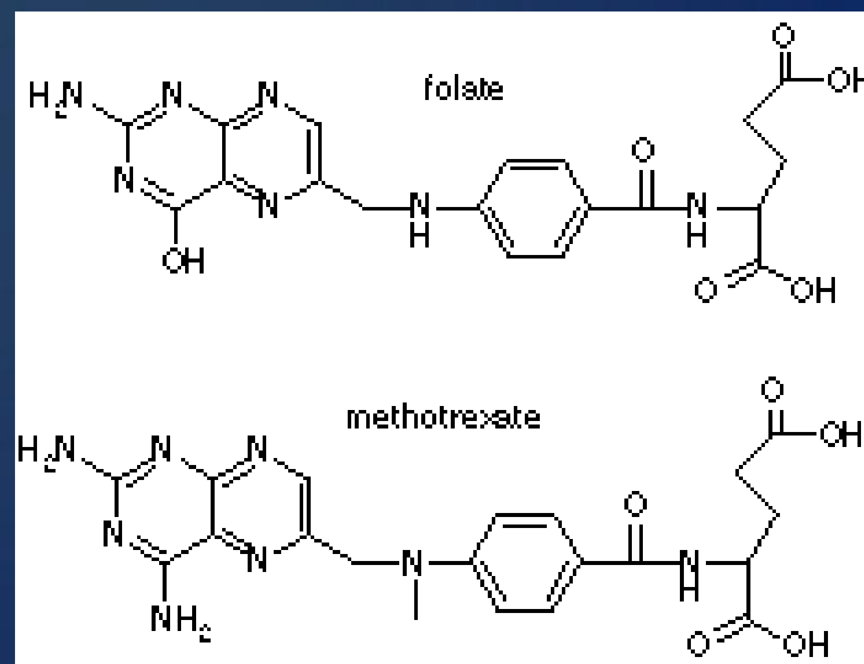

1. First step of purine synthesis is committed step and rate limiting step
2. Intracellular concentrations of glutamine and PRPP control the reaction rate
3. Inhibited by AMP, GMP, and IMP
4. Requires 4 ATP molecules



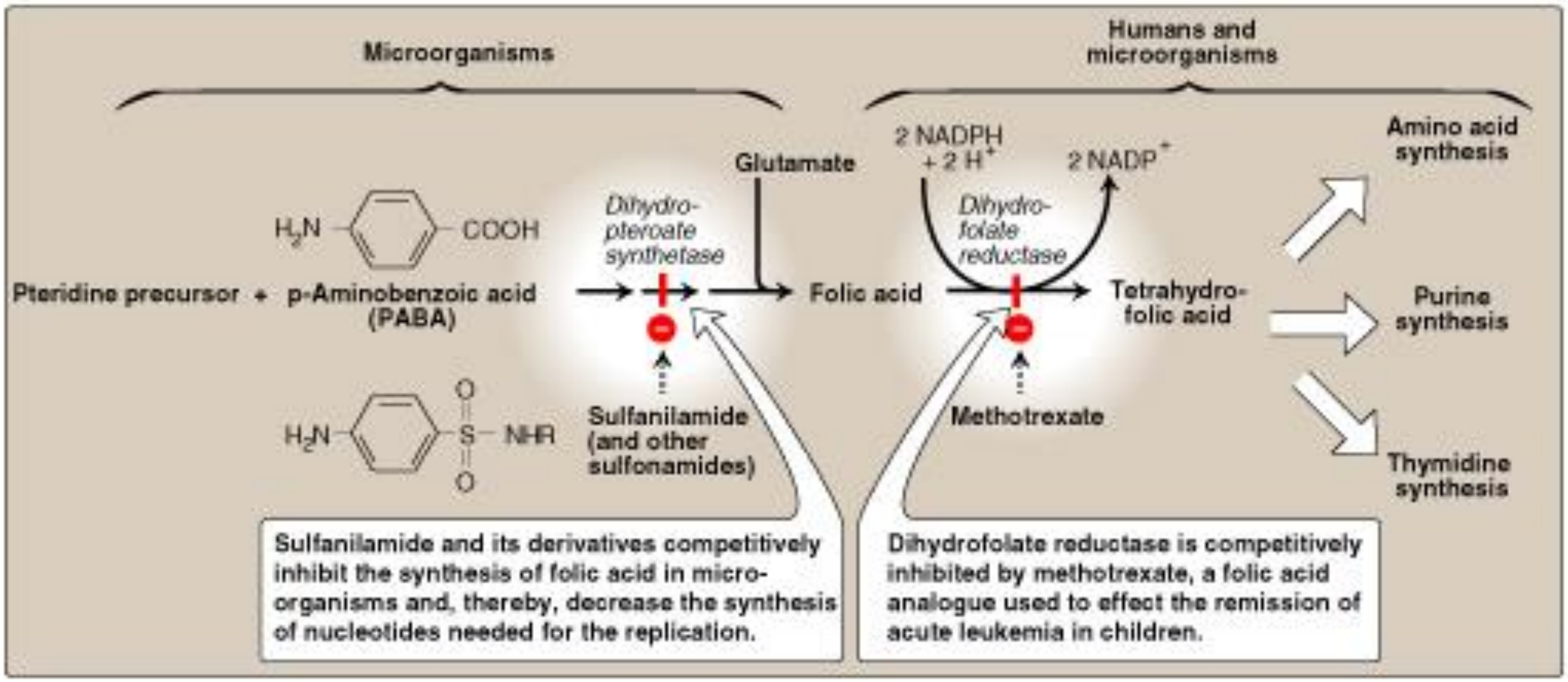


Sulfonamide
(PABA analogue)

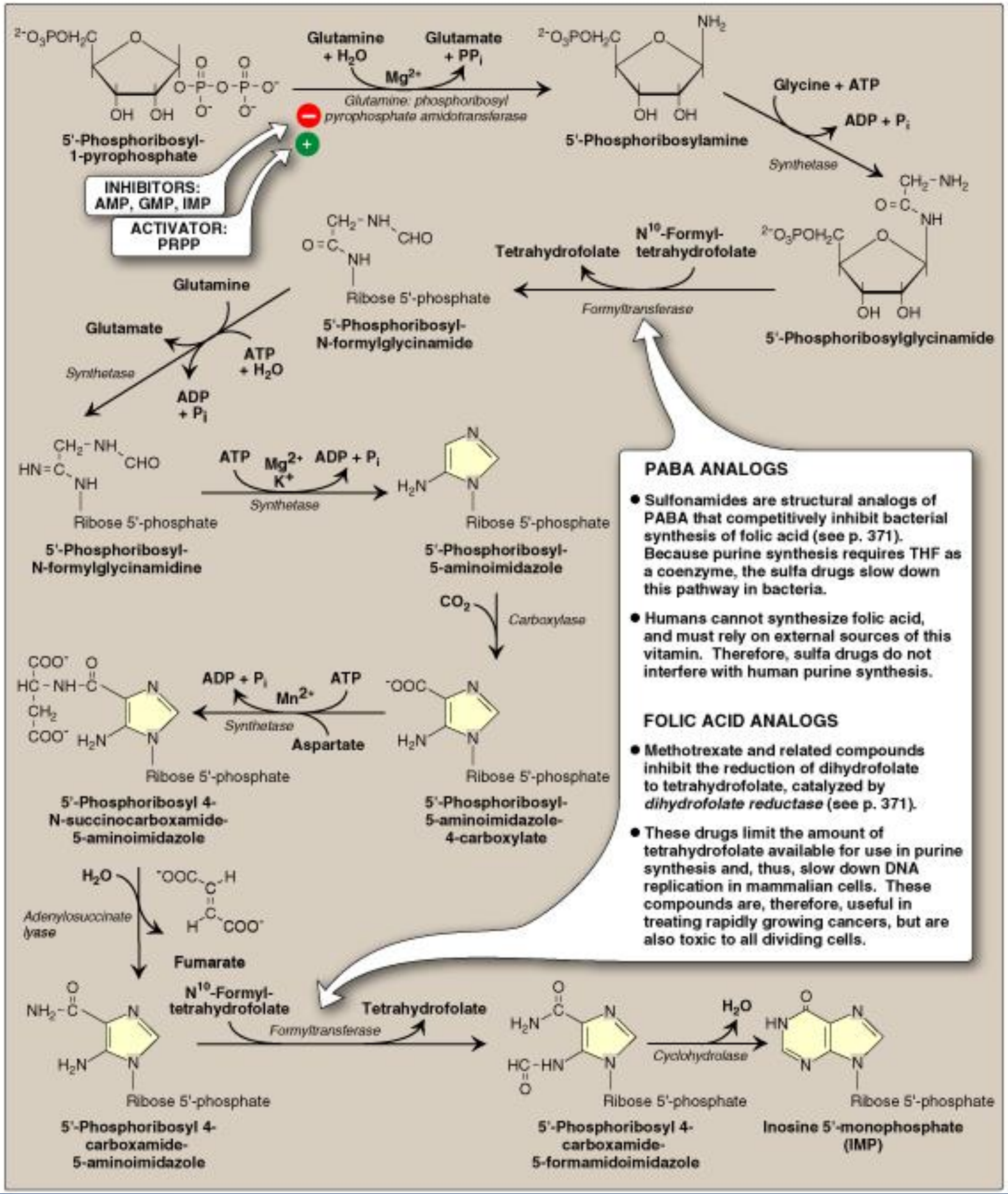


Can synthesize folate    Cannot synthesize folate



# Methotrexate and Cancer

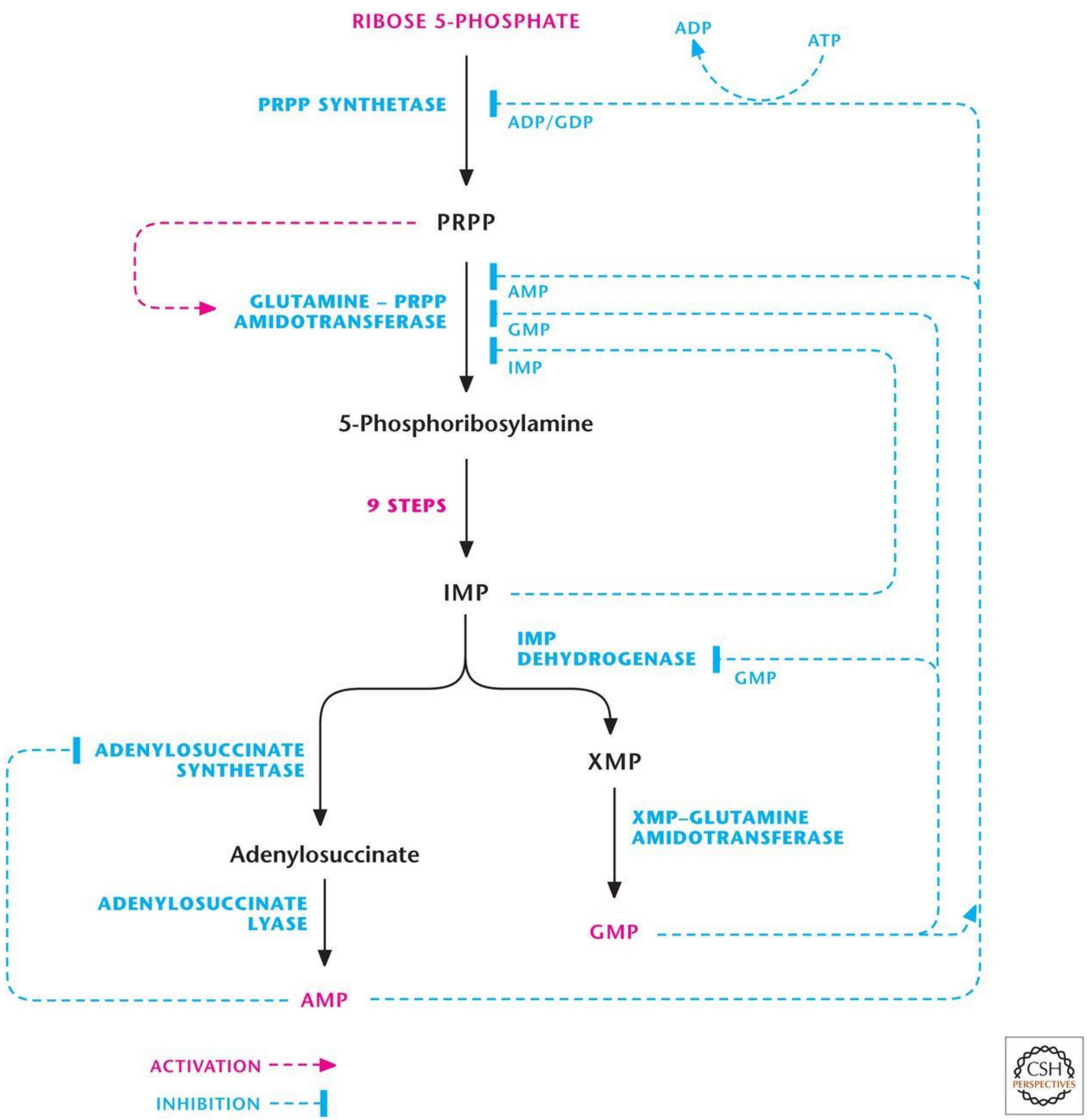
- Affects rapidly growing cells
- Adverse events include anemia, scaly skin, GI tract disturbances (diarrhea), and baldness
- Resistance to MTX is caused by amplification of dihydrofolate reductase gene
- Also used for treatment of rheumatoid arthritis and psoriasis at lower doses, though site of action is not through DHFR but inhibition of salvage pathways that lead to increased adenosine that inhibits T cell activation.

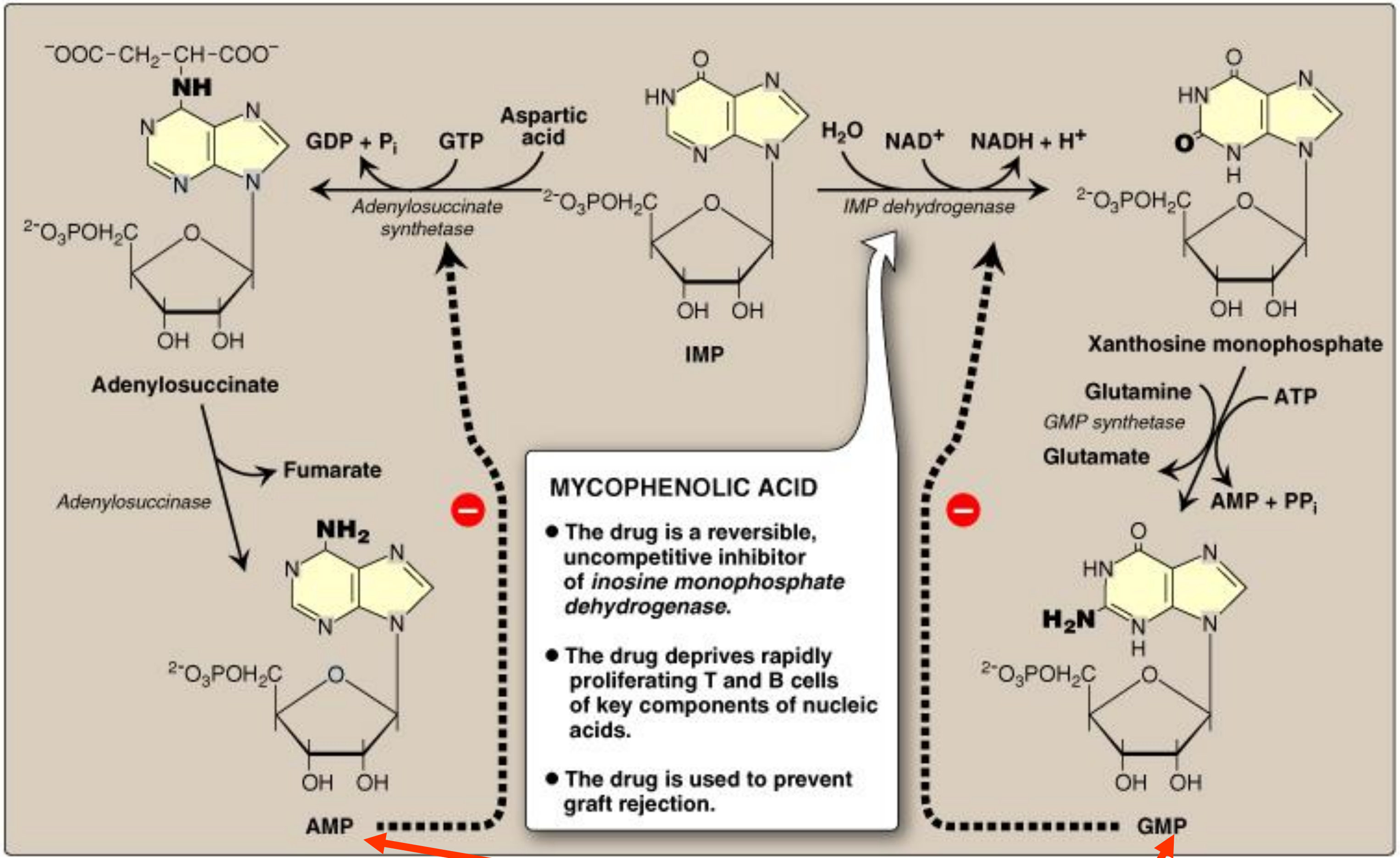


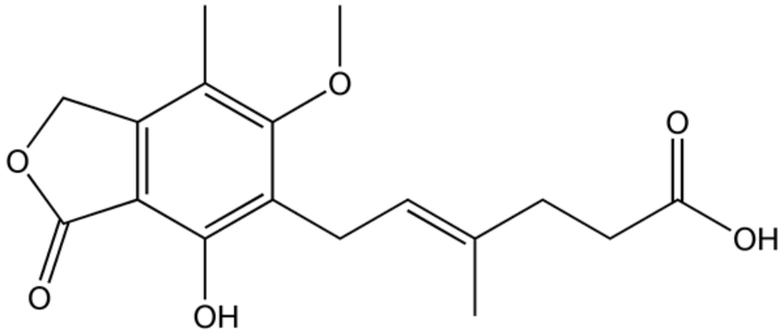

# Need to Know

1. That sulfonamides inhibit purine synthesis in bacteria by interfering with folate synthesis.
2. That methotrexate inhibits purine synthesis by inhibiting dihydrofolate reductase.
3. That IMP is the end product of *de novo* purine synthesis.
4. AMP, GMP, and IMP inhibit the reaction. PRPP is an activator.
5. Rate limiting step of the pathway and source of atoms for the purine ring

# Regulation of purine biosynthesis





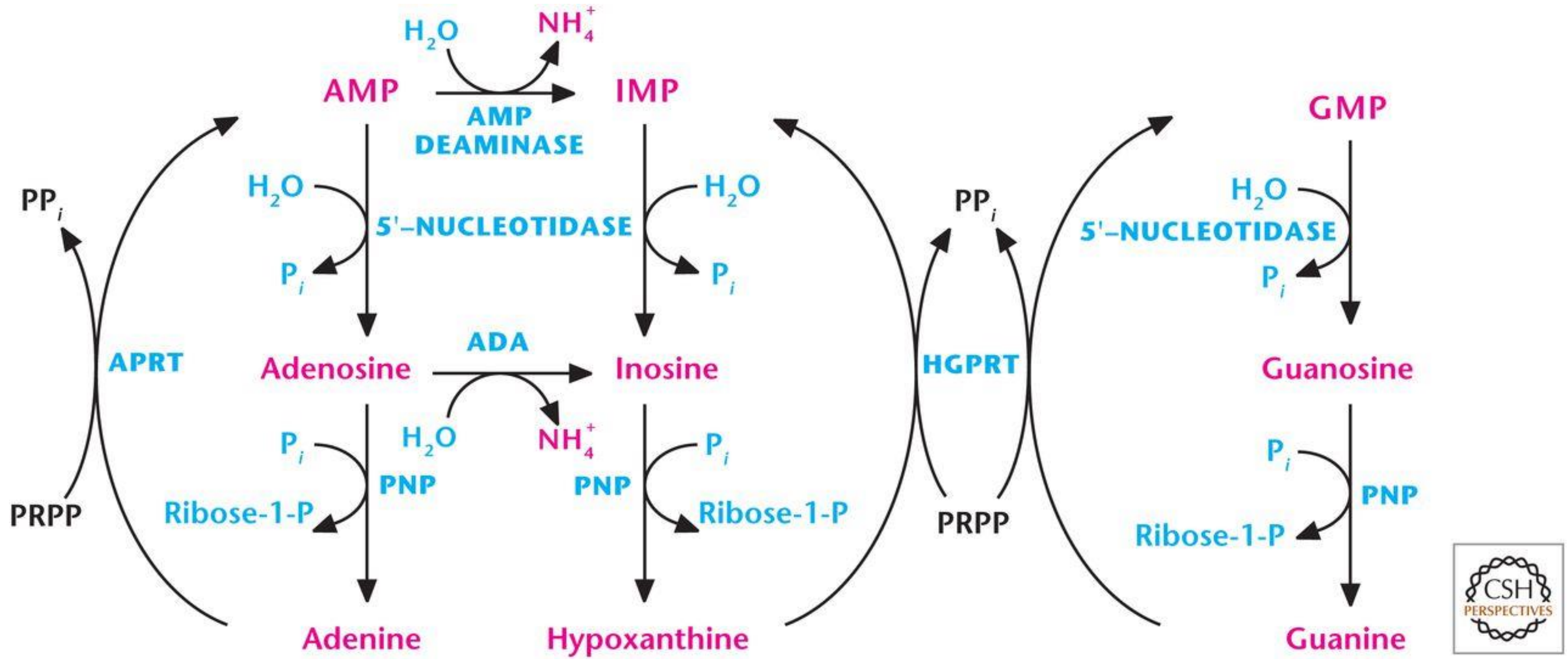
Mycophenolic acid

High levels shut down *de novo* purine synthesis

# Purine Salvage Pathway

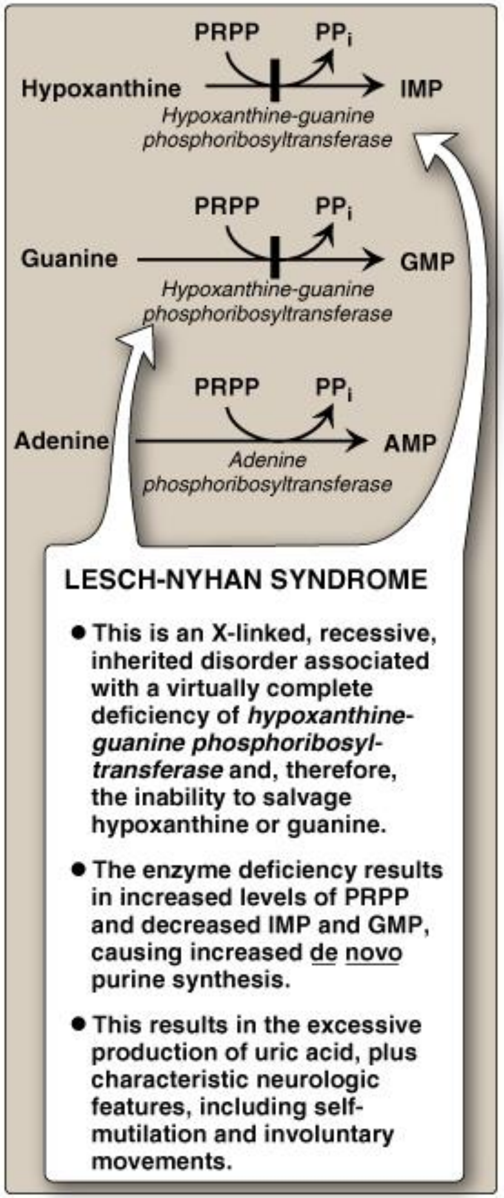
- Purines from normal turnover of cellular nucleic acids
- Purines obtained from the diet
- Nucleotides can also be synthesized from the purine bases and purine nucleosides in a series of steps referred to as salvage pathways.

# Purine salvage pathway.



# Lesch-Nyhan Syndrome (deficiency of HGPRT)

- Build up of hypoxanthine and guanine
- Degradation of hypoxanthine and guanine results in increased uric acid
- Excess uric acid in urine often results in orange crystals in the diaper of affected children
- Severe mental retardation
- Self-mutilation
- Involuntary movements
- Gout



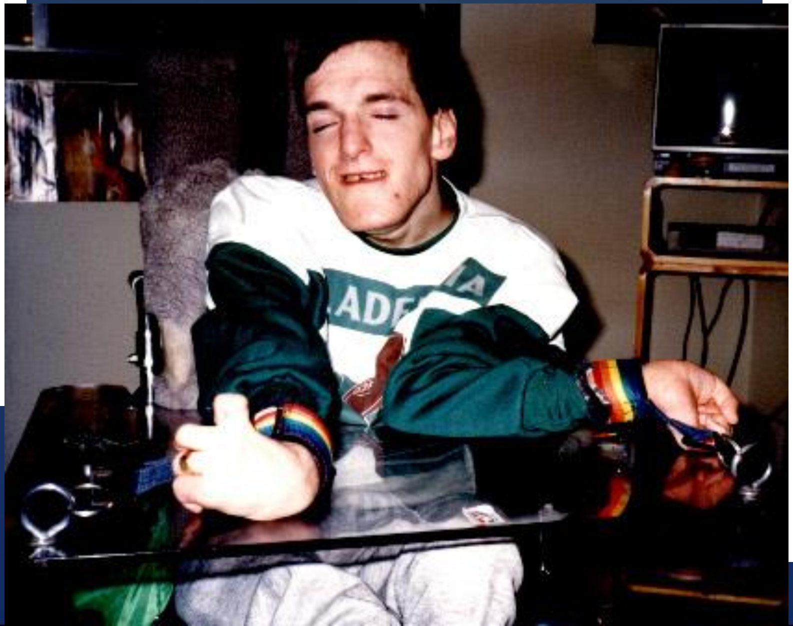
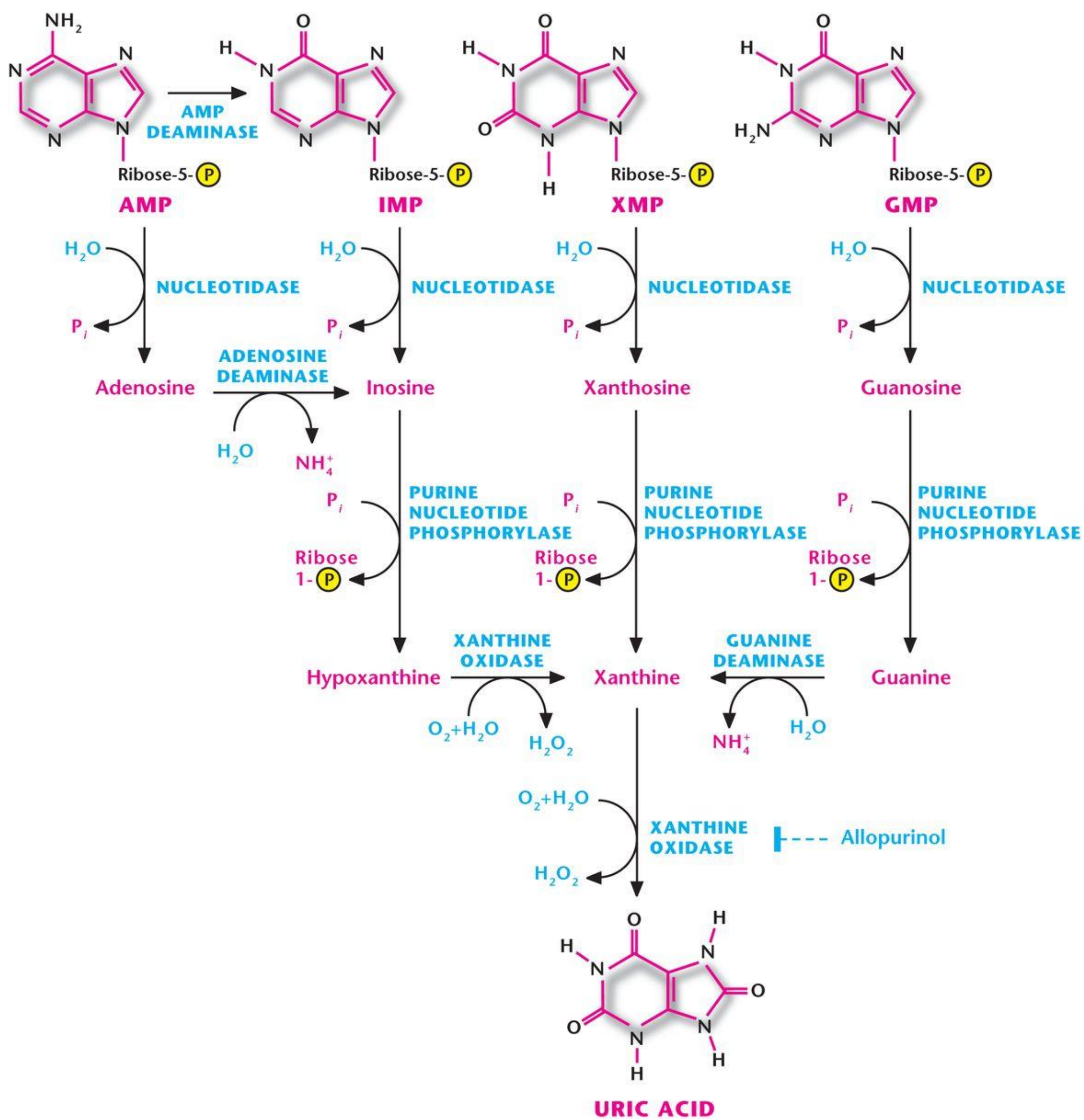
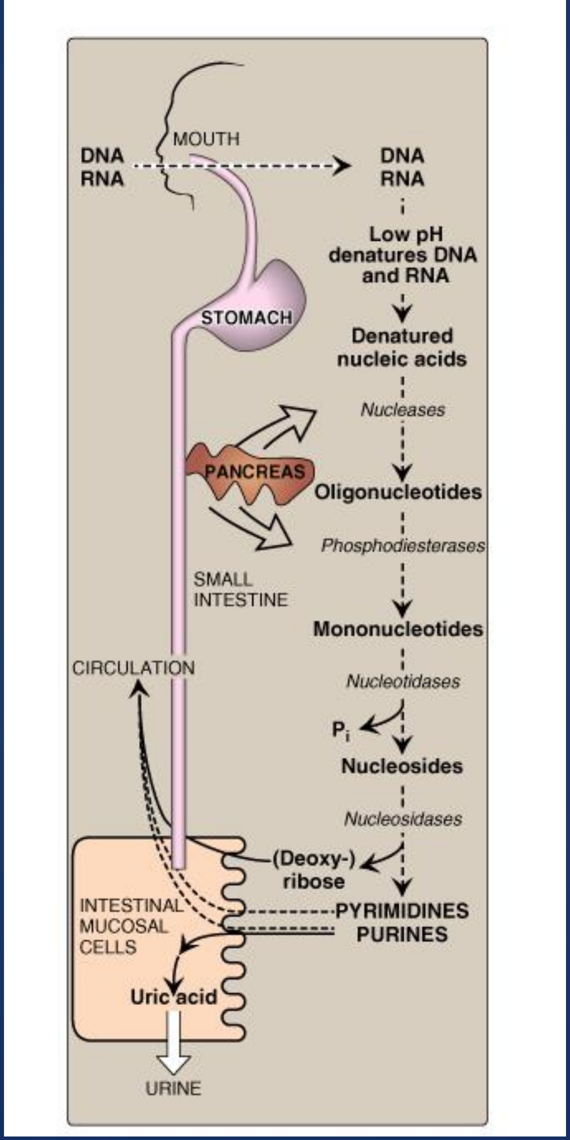



**Figure 22.11**
Lesions on the lips of Lesch-Nyhan patients caused by self-mutilation.

# Catabolism of purines



# Degradation of Purines



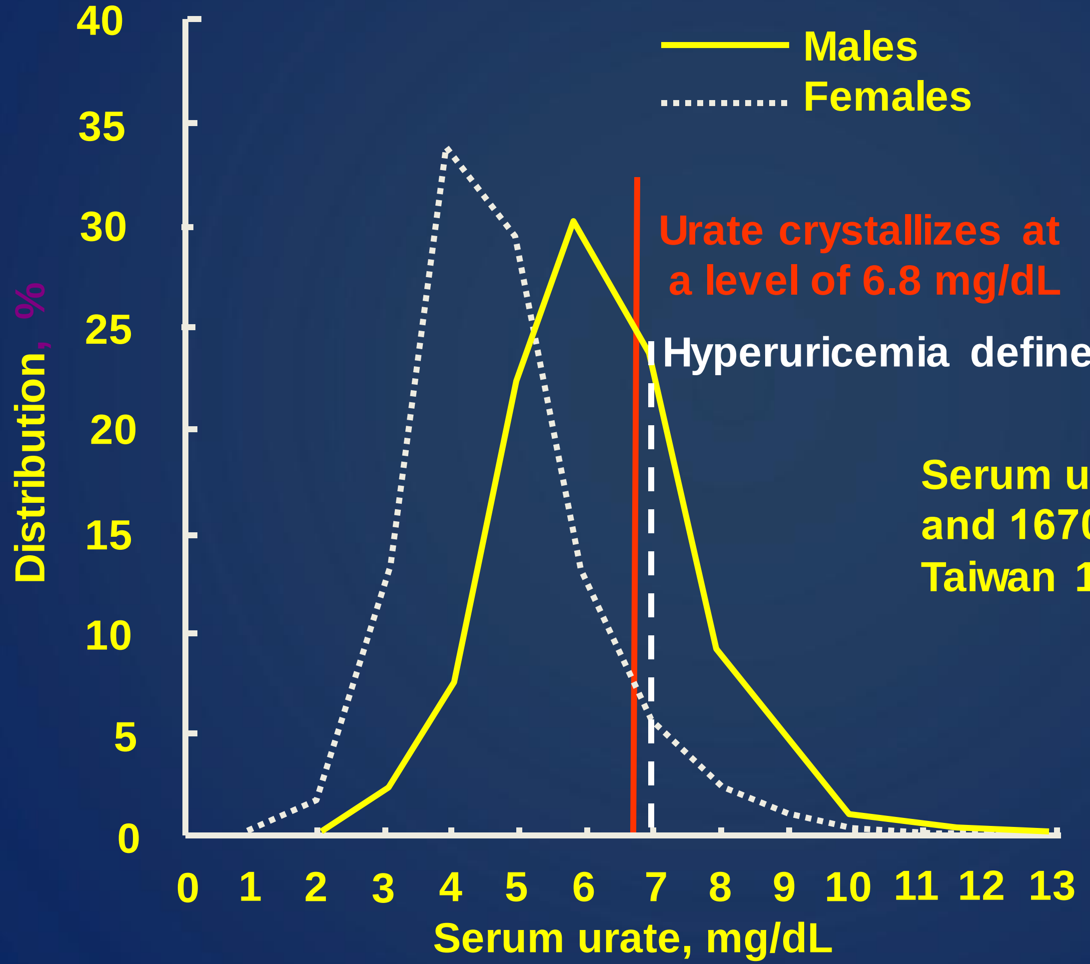
# Clinical aspects of purine metabolism and deficiencies

# Gout

- Characterized by hyperuricemia and acute arthritic joint inflammation by deposition of uric acid crystals
- Primary gout is genetic and mainly affects men over 30
- Secondary gout is associated with leukemia, polycythemia, HGPRT deficiency, renal insufficiency, lifestyle (rich foods)


**Figure 22.17**
Gout can be diagnosed by the presence of negatively birefringent monosodium urate crystals in aspirated synovial fluid examined by polarized-light microscopy. Here, crystals are within polymorphonuclear leukocytes.

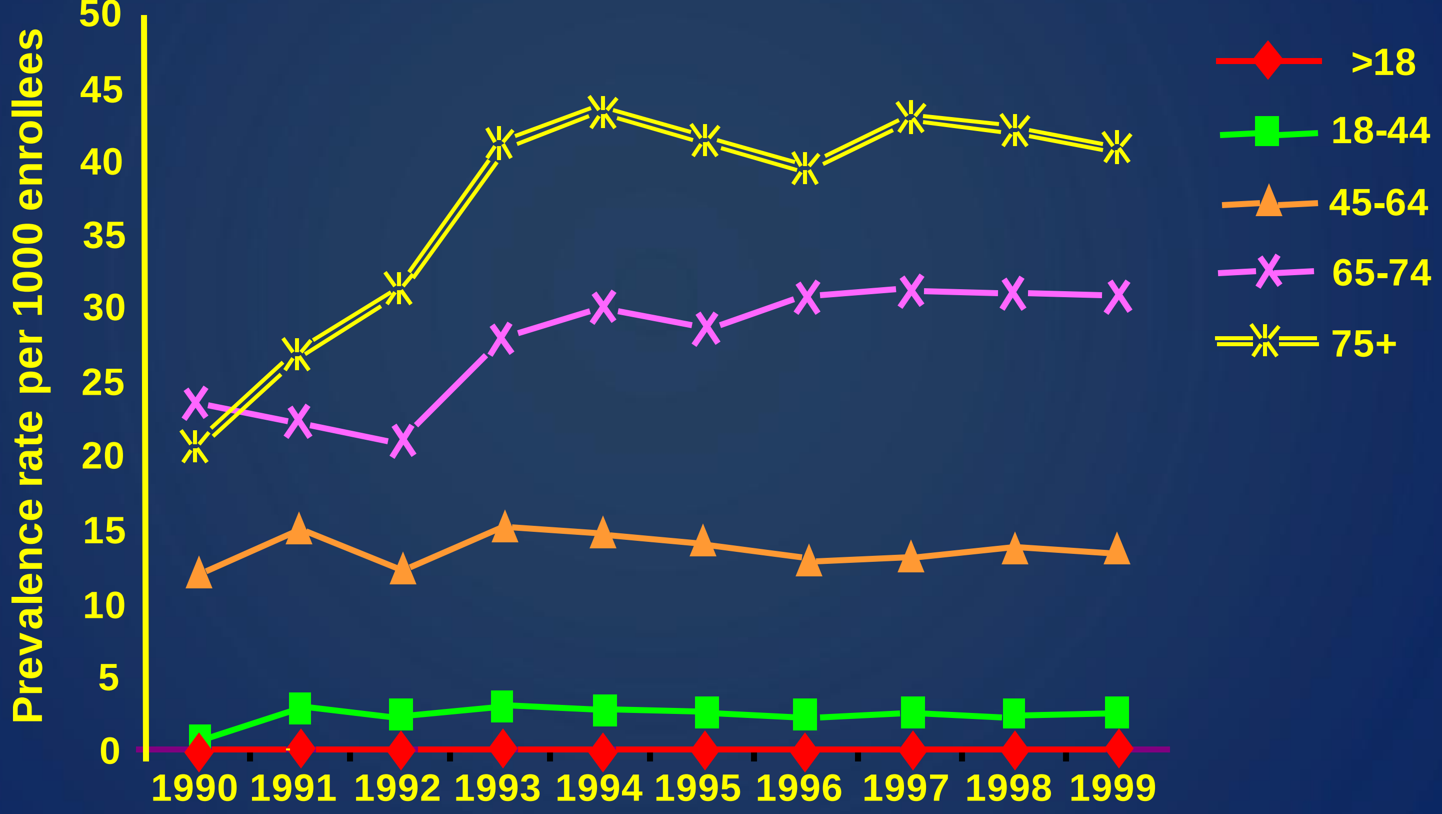
# Distribution of Serum Urate Values




Serum urate levels in 1515 men and 1670 women aged ≥30 in Taiwan 1991-1992

Lin *et al. J Rheumatol*. **27**, 1045-1050 (2000)

# Higher Prevalence of Gout and Clinically Significant Hyperuricemia in Higher Age Groups

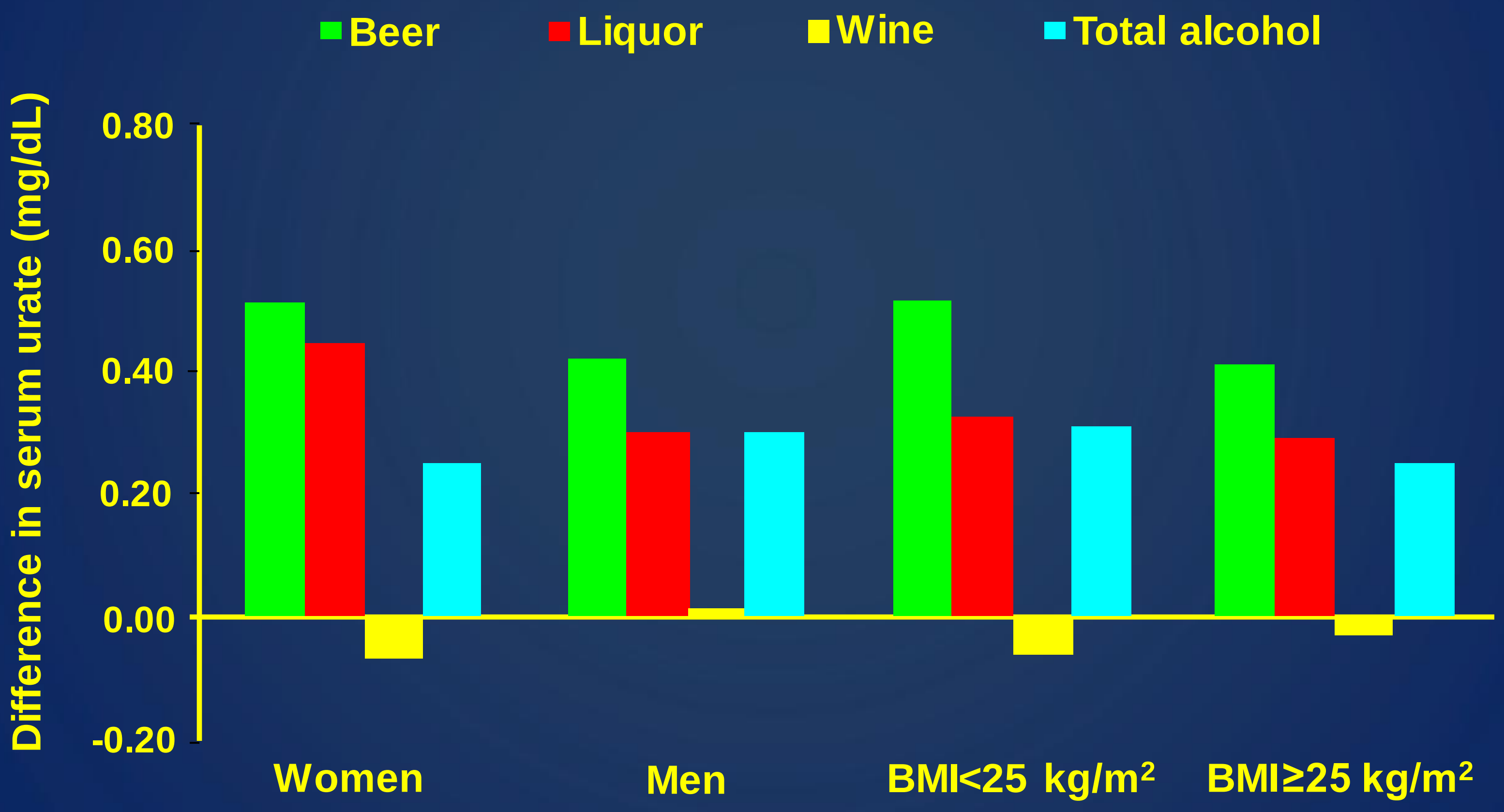



Wallace *et al*. *J Rheum.* **31**, 1582-1587 (2004).

# Common Foods With High Purine Content

| Very high | High |
|---|---|
| Brewer's yeast | Bacon |
| Anchovies | Beer |
| Herring | Liver |
| Sardines | Lobster |
| Mussels | Salmon |
| Clams | Sweetbreads (pancreas) |
| | Turkey |
| | Veal |

# Differences in Serum Urate Among Alcoholic Beverages

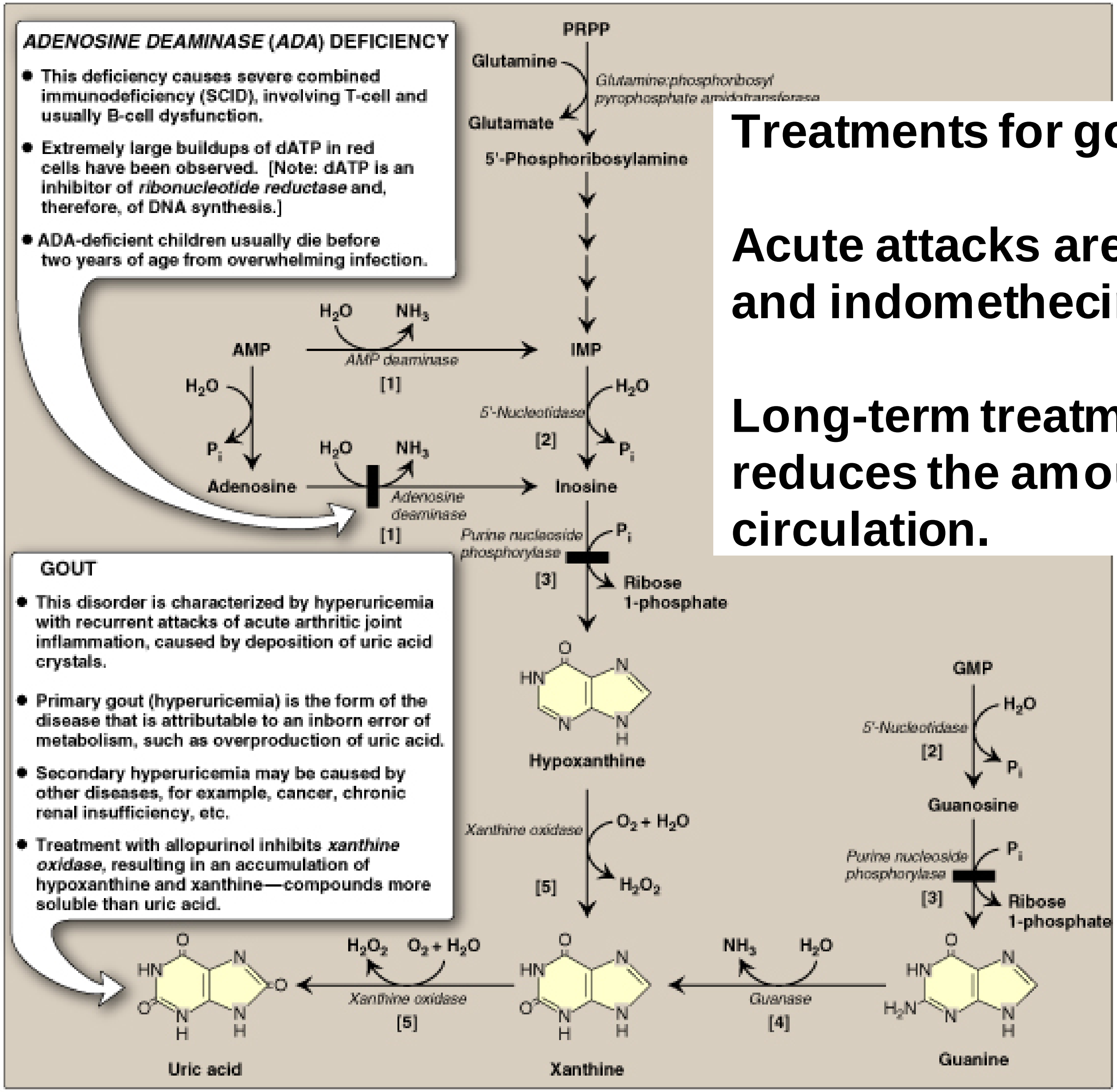



Choi *et al*. *Arthritis Rheum.* **50**, S480 (2004)

# Drugs That Promote Gout

| | |
|---|---|
| Diuretics | Leads to increased uric acid reabsorption |
| Low-dose aspirin | Over 6% increase in mean serum urate and 23% decrease in uric acid clearance[1] |
| Pyrazinamide<br>Ethambutol<br>Niacin | Gout observed at higher incidence |

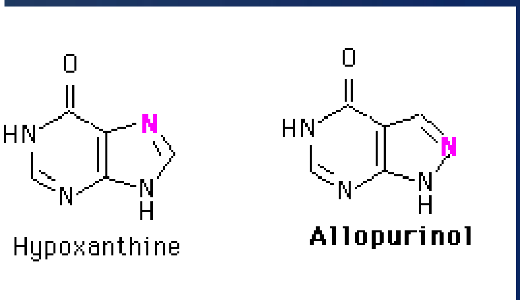
Caspi *et al*. *Arth Rheum*. **43**, 103-108 (2000)



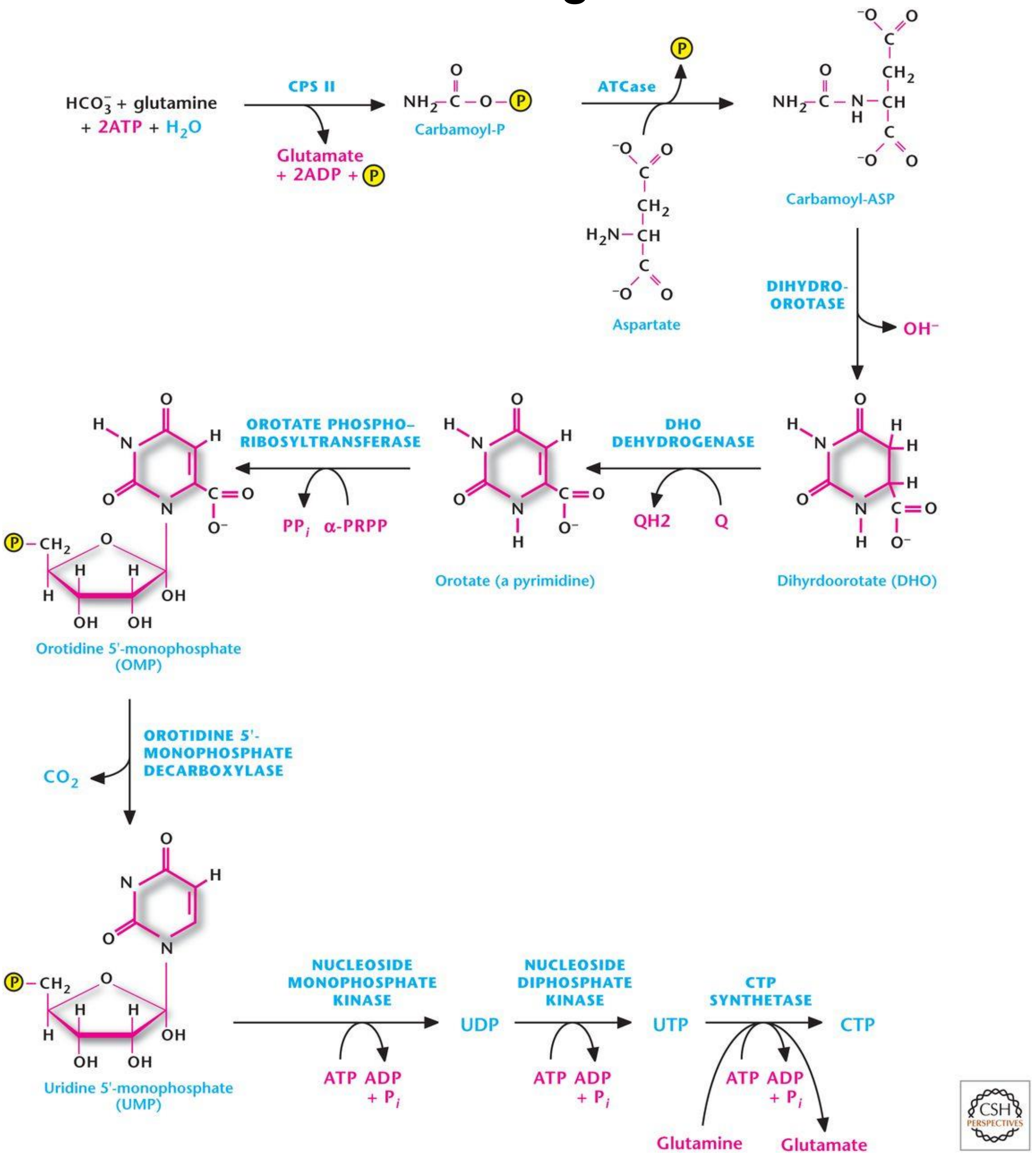

# Treatments for gout:

**Acute attacks are treated with colchicine and indomethecin for 3 weeks.**

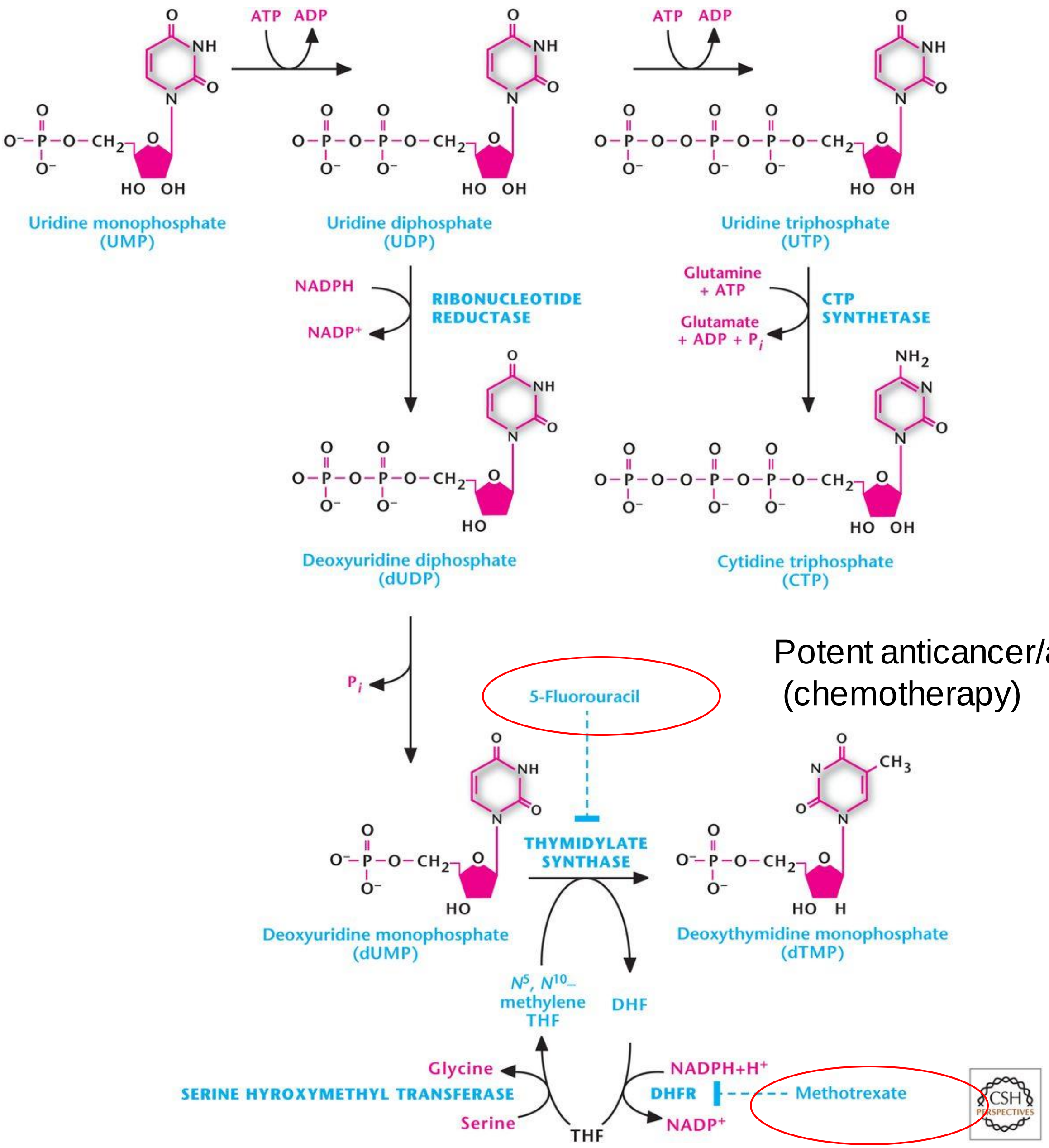
**Long-term treatment with allopurinol reduces the amount of uric acid in circulation.**



# Pyrimidine ring is fully synthesized before being attached to the ribose sugar.

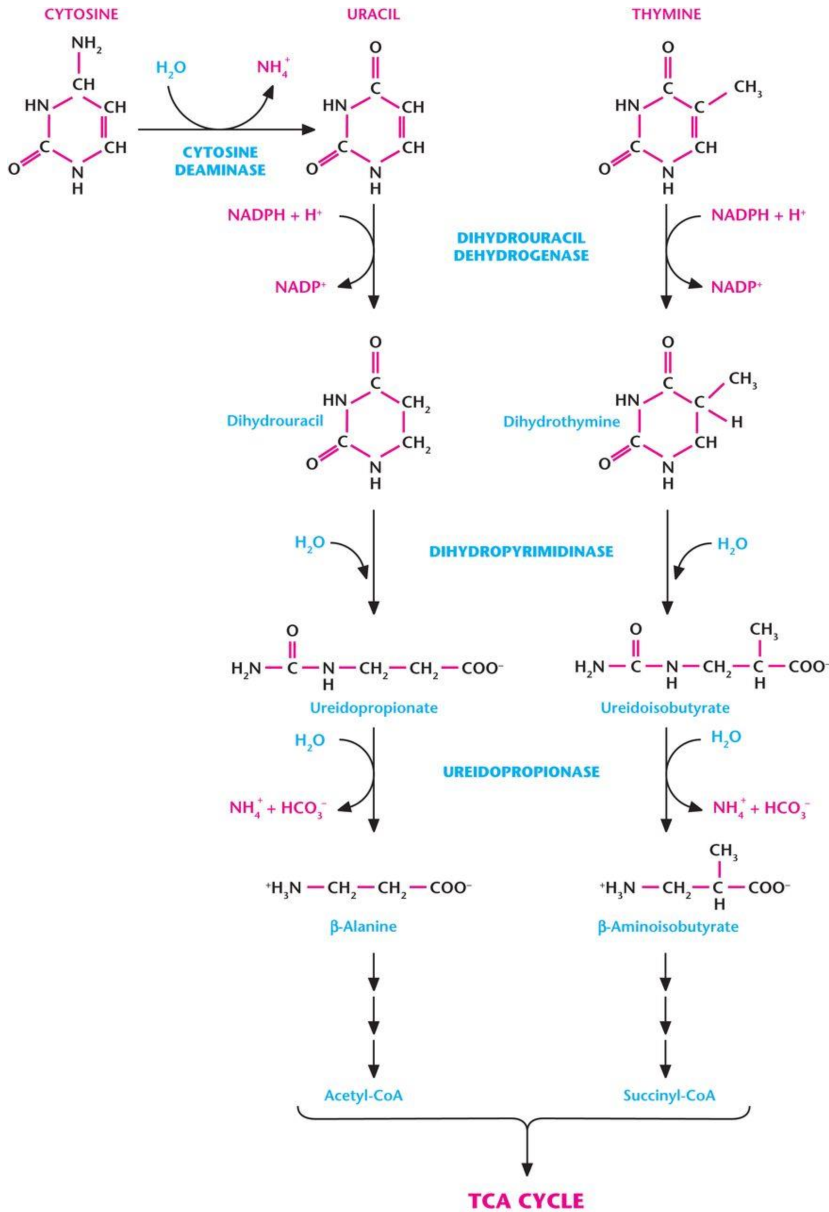


# Uridine nucleotides are also the precursors for de novo synthesis of the thymine nucleotides

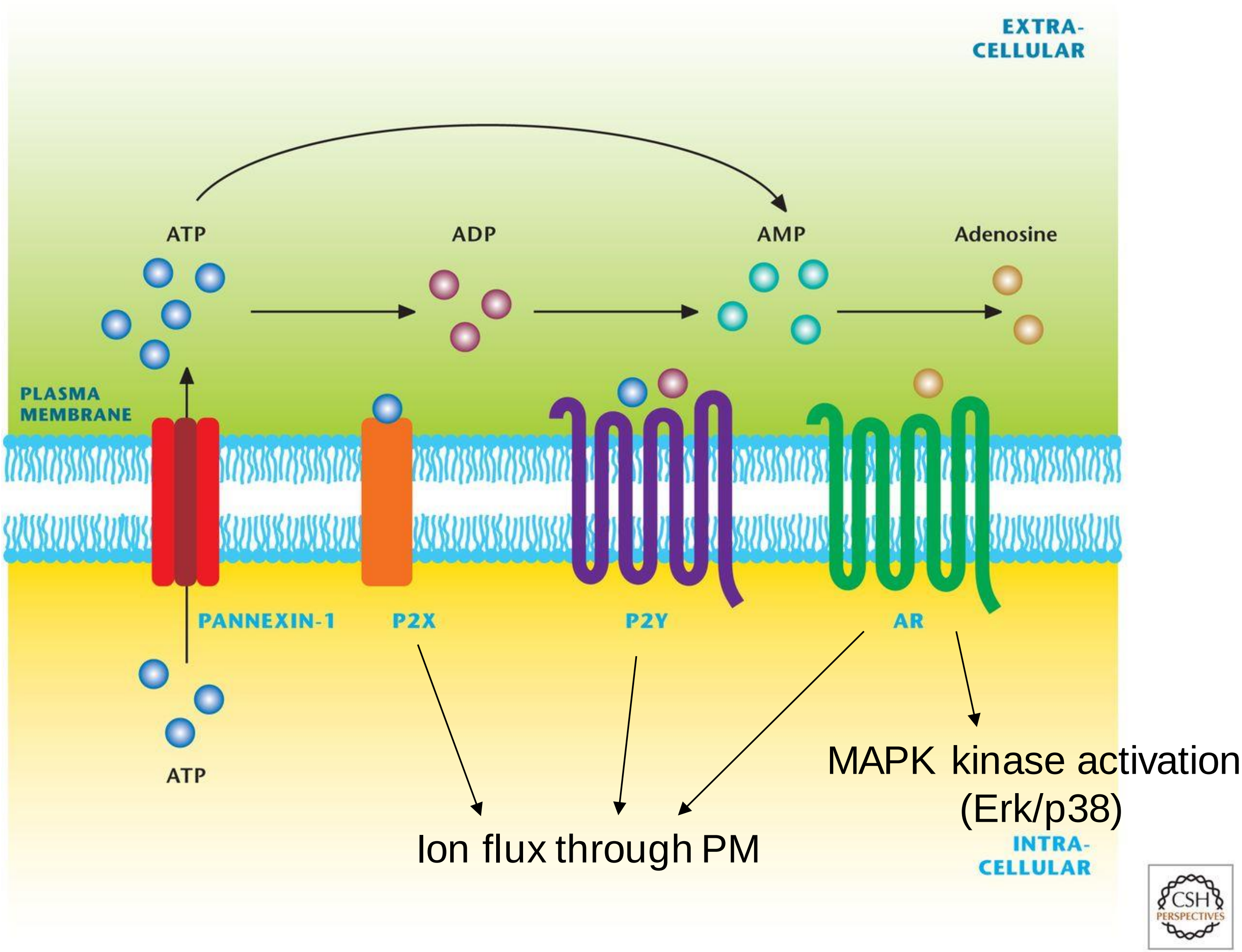



Potent anticancer/antiproliferative drug (chemotherapy)

# Catabolism of pyrimidine rings



# Nucleotide signaling



## Take home message

• Nucleosides have either a ribose or 2-deoxyribose bound to purine or pyrimidine. The addition of one or more phosphates to a nucleoside results in a nucleotide.
• Purines (adenine and guanine) are comprised of attached six-membered and five-membered nitrogen-containing rings.
• Pyrimidines (uracil, thymine, and cytosine) have only a six-membered nitrogen-containing ring.
• Ribonucleotide reductase (RR) generates deoxynucleoside diphosphate (dNDP) from ribonucleoside diphosphate (rNDPs). Nucleoside diphosphate (NDP) kinases use ATP to phosphorylate dNDP to produce deoxynucleoside triphosphates (dNTPs).
• Purine nucleotides synthesis begins with 5-phosphoribosyl-1-pyrophosphate (PRPP), which, through a series of reactions, generates the nucleotide inosine 50 -monophosphate (IMP). Subsequently, IMP can be converted into either AMP or GMP through distinct reactions. AMP or GMP can be converted to ADP or GDP, respectively.
• Pyrimidine nucleotides synthesis begins with carbamoyl phosphate and aspartate generating the pyrimidine base orotate. Succeeding steps attach PRPP to orotate to generate orotate monophosphate (OMP), which is then decarboxylated to UMP. UMP generates UDP and UTP, which can generate CTP.
• Humans cannot break down the purine ring. The catabolism of purine nucleotides results in a uric acid. In contrast, the pyrimidine ring can be completely degraded. Catabolism of the pyrimidine nucleotides leads, ultimately, to β-alanine or β-aminoisobutyrate production, as well as NH3 and CO2.
• Nucleotides are signaling molecules that regulate multiple physiological processes, including neurotransmission and inflammation.
• ATP activates a family of ionotropic receptors (P2X) and metabotropic receptors (P2Y) Extracellular adenosine activates G-protein-coupled cell-surface receptors, which are divided into four subtypes: A1, A2A, A2B, and A3.