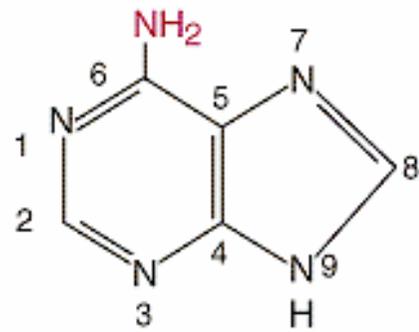


Nomenclature of Nucleotides and Nucleosides

		Purines		Pyrimidines	
		Adenine (A)	Guanine (G)	Cytosine (C)	Uracil (U) Thymine (T)
Nucleosides	RNA	Adenosine	Guanosine	Cytidine	Uridine
	DNA	Deoxyadenosine	Deoxyguanosine	Deoxycytidine	Deoxythymidine
Nucleotides	RNA	Adenylate (AMP)	Guanylate (GMP)	Cytidylate (CMP)	Uridylate (UMP)
	DNA	Deoxyadenylate (dAMP)	Deoxyguanylate (dGMP)	Deoxycytidylate (dCMP)	Thymidylate (dTMP)

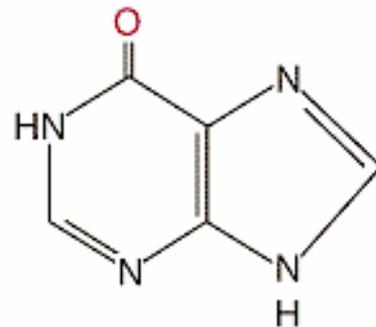
Purine bases



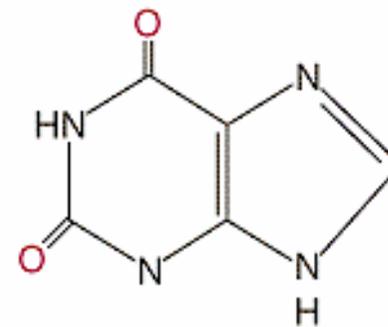
Adenine



Guanine

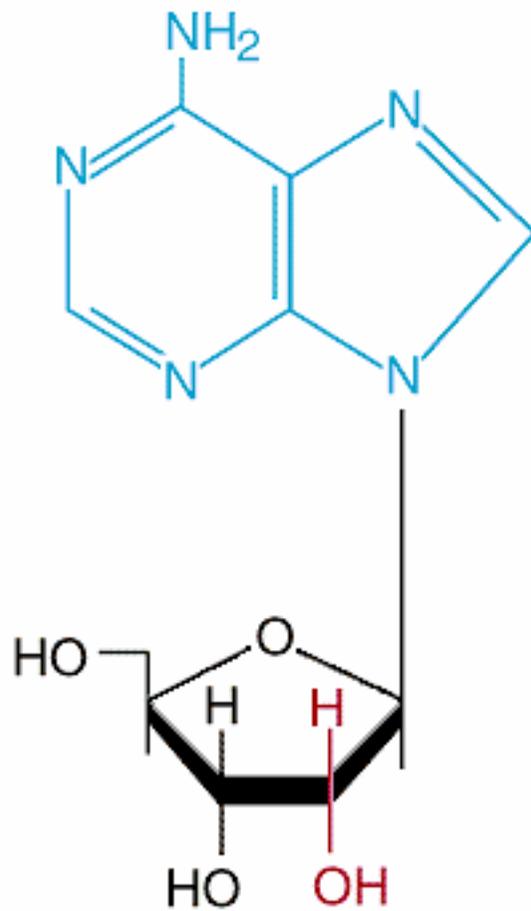


Hypoxanthine

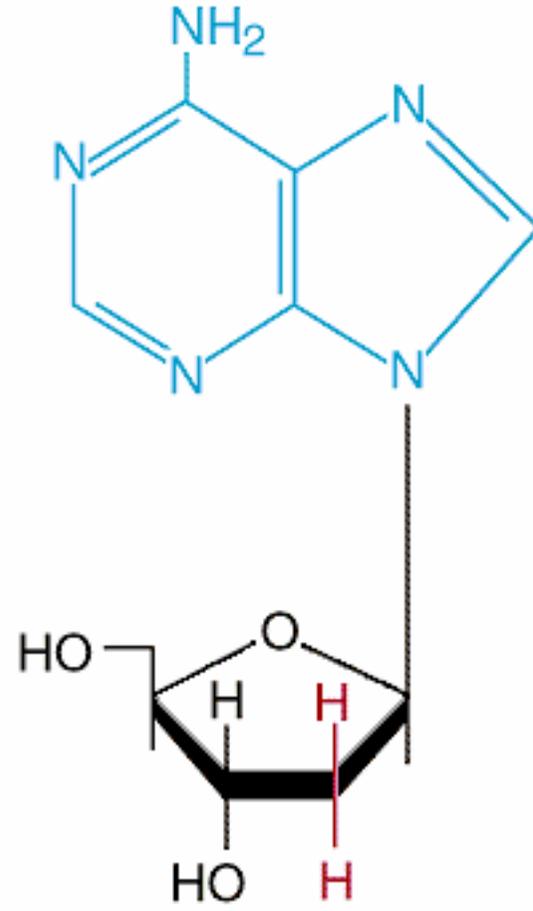


Xanthine

Copyright © 1997 Wiley-Liss, Inc.

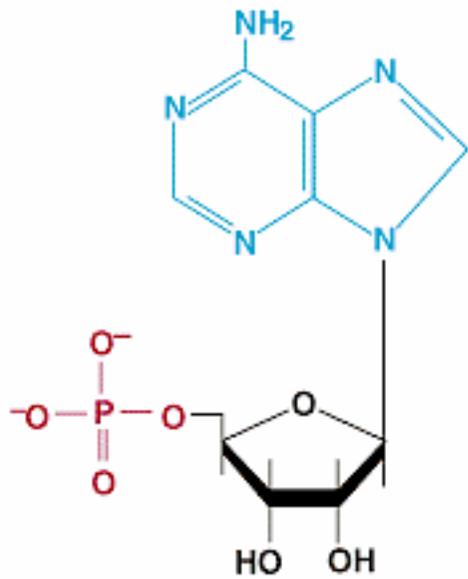


Adenosine

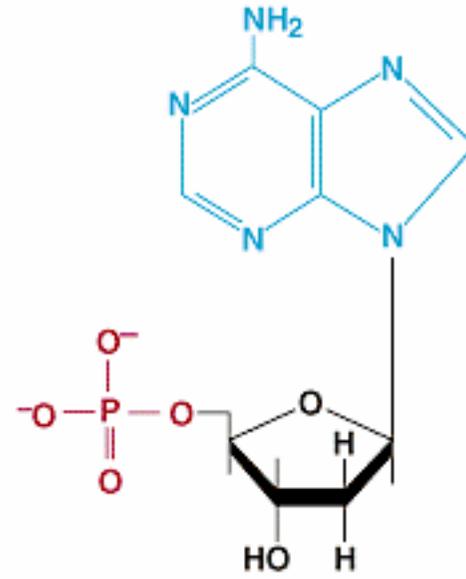


Deoxyadenosine

Copyright © 1997 Wiley-Liss, Inc.

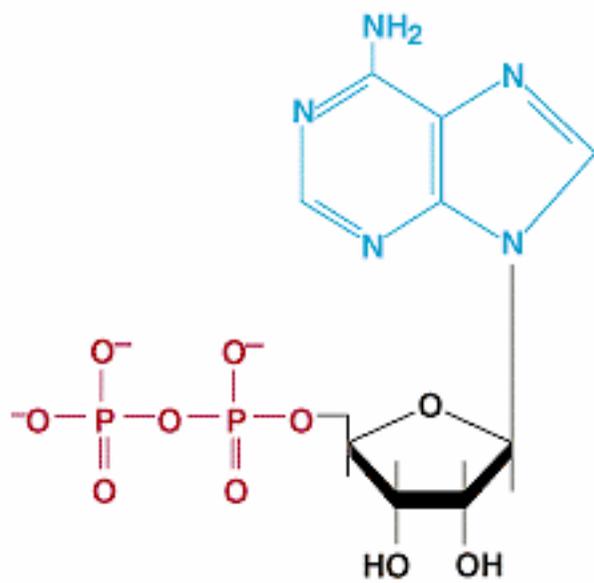


Adenosine 5'-monophosphate (AMP)

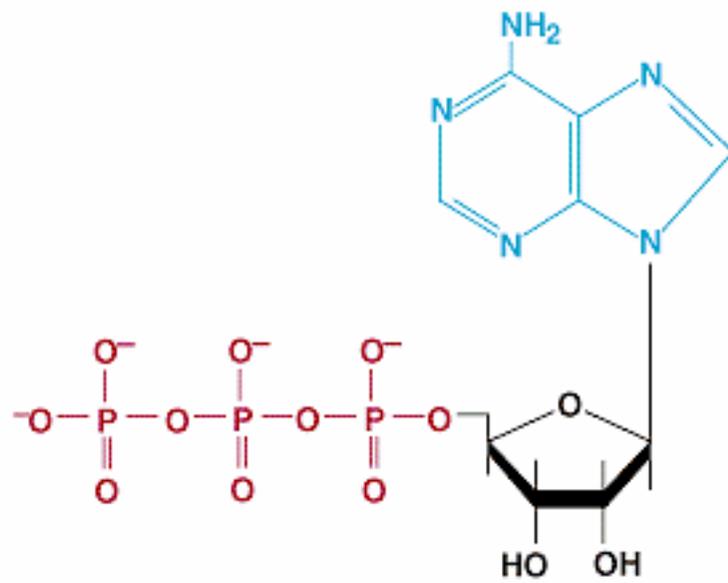


Deoxyadenosine 5'-monophosphate (dAMP)

Copyright © 1997 Wiley-Liss, Inc.



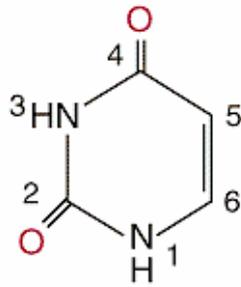
Adenosine 5'-diphosphate (ADP)



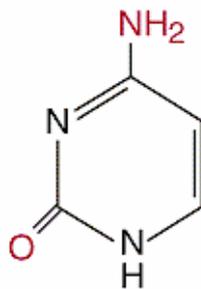
Adenosine 5'-triphosphate (ATP)

Copyright © 1997 Wiley-Liss, Inc.

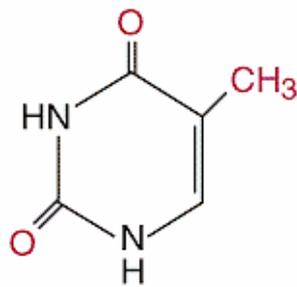
Pyrimidine bases



Uracil

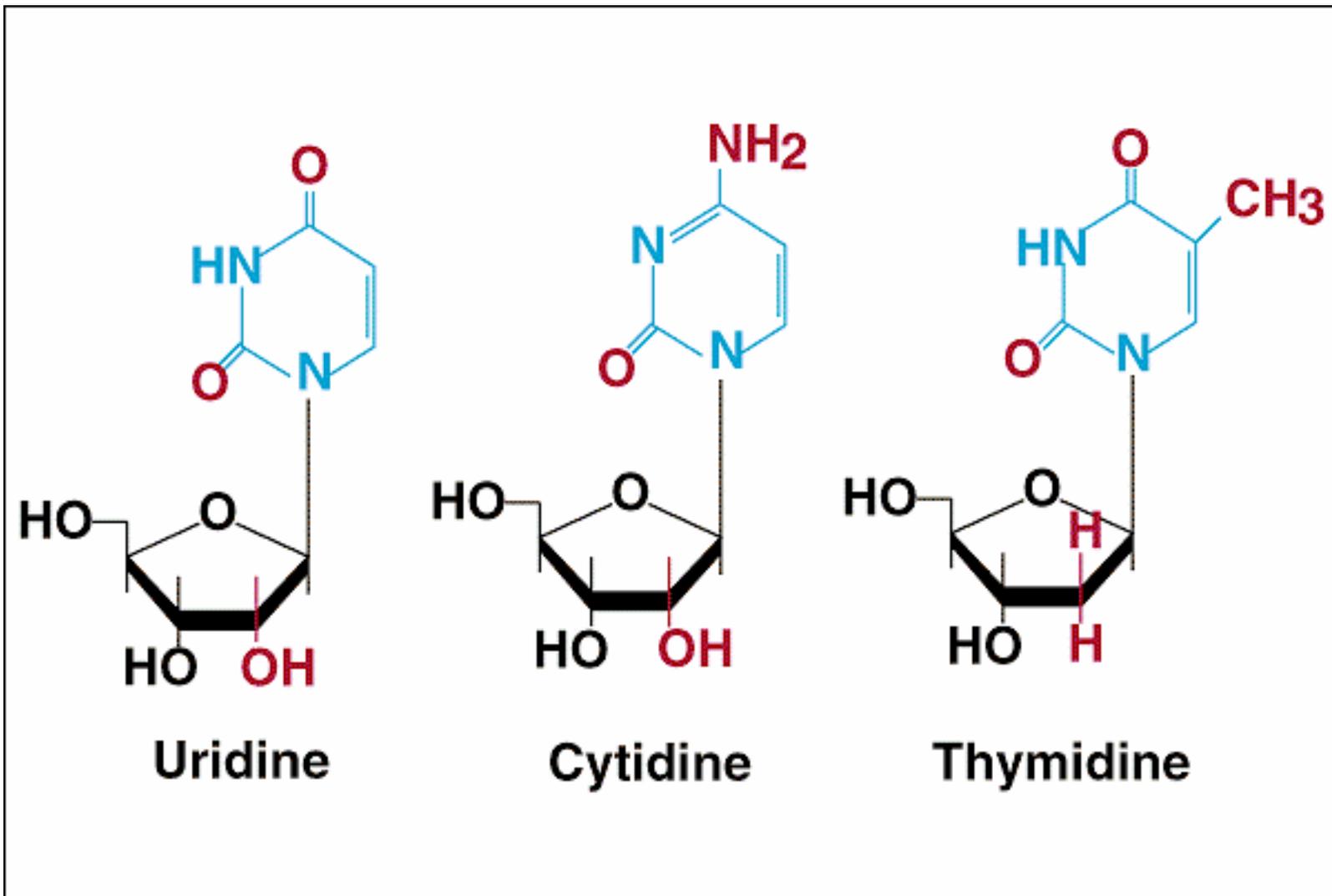


Cytosine

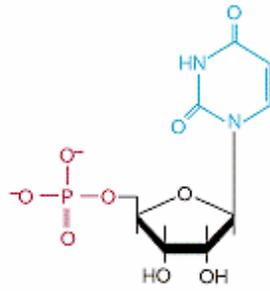


Thymine

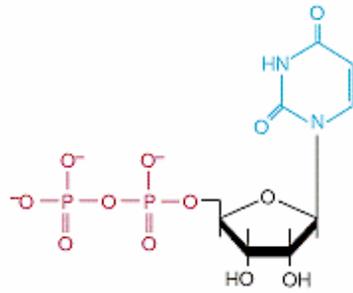
Copyright © 1997 Wiley-Liss, Inc.



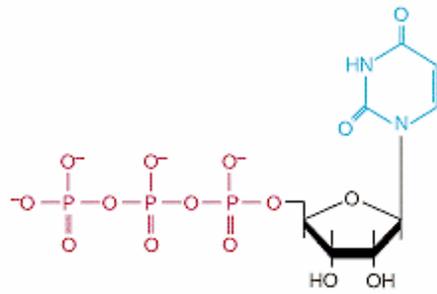
Copyright © 1997 Wiley-Liss, Inc.



Uridine 5'-monophosphate (UMP)



Uridine 5'-diphosphate (UDP)



Uridine 5'-triphosphate (UTP)

Copyright © 1997 Wiley-Liss, Inc.

5'-Phosphoribosyl-1 -pyrophosphate (PRPP) is an intermediate of major significance in nucleotide metabolism.

1. Formation. PRPP is formed from ribose-5-phosphate and adenosine triphosphate (ATP).



2. There are several sources of ribose-5-phosphate.

a. Glucose metabolism

b. Nucleoside degradation generates ribose-1-phosphate, which can be converted to ribose-5-phosphate.

3. PRPP is required in:

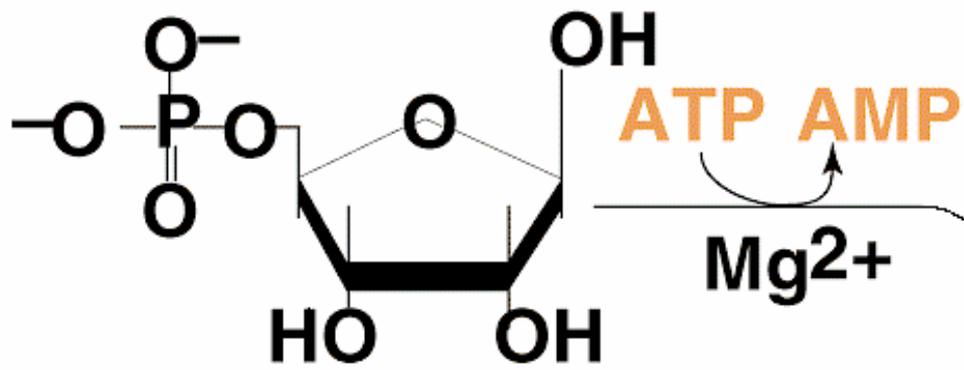
a. The de novo synthesis of pyrimidine and purine nucleotides

b. The salvage pathways for purine nucleotides

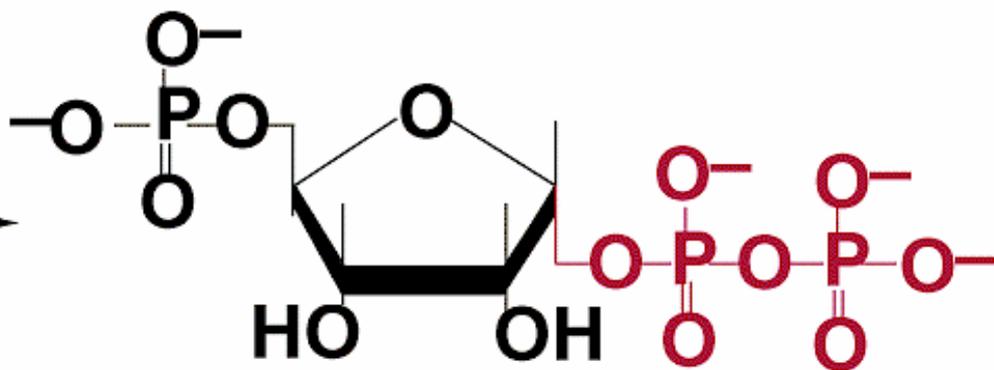
c. The biosynthesis of nucleotide coenzymes

4. The cellular concentrations of PRPP are closely regulated and usually are low.

The formation of PRPP is catalyzed by **PRPP synthetase**. **Adenosine diphosphate (ADP) and 2,3-bisphosphoglycerate** inhibit PRPP synthetase as well as adenosine 5'-monophosphate (AMP), guanosine 5'-monophosphate (GMP), and inosine 5'-monophosphate (IMP).



Ribose 5-phosphate

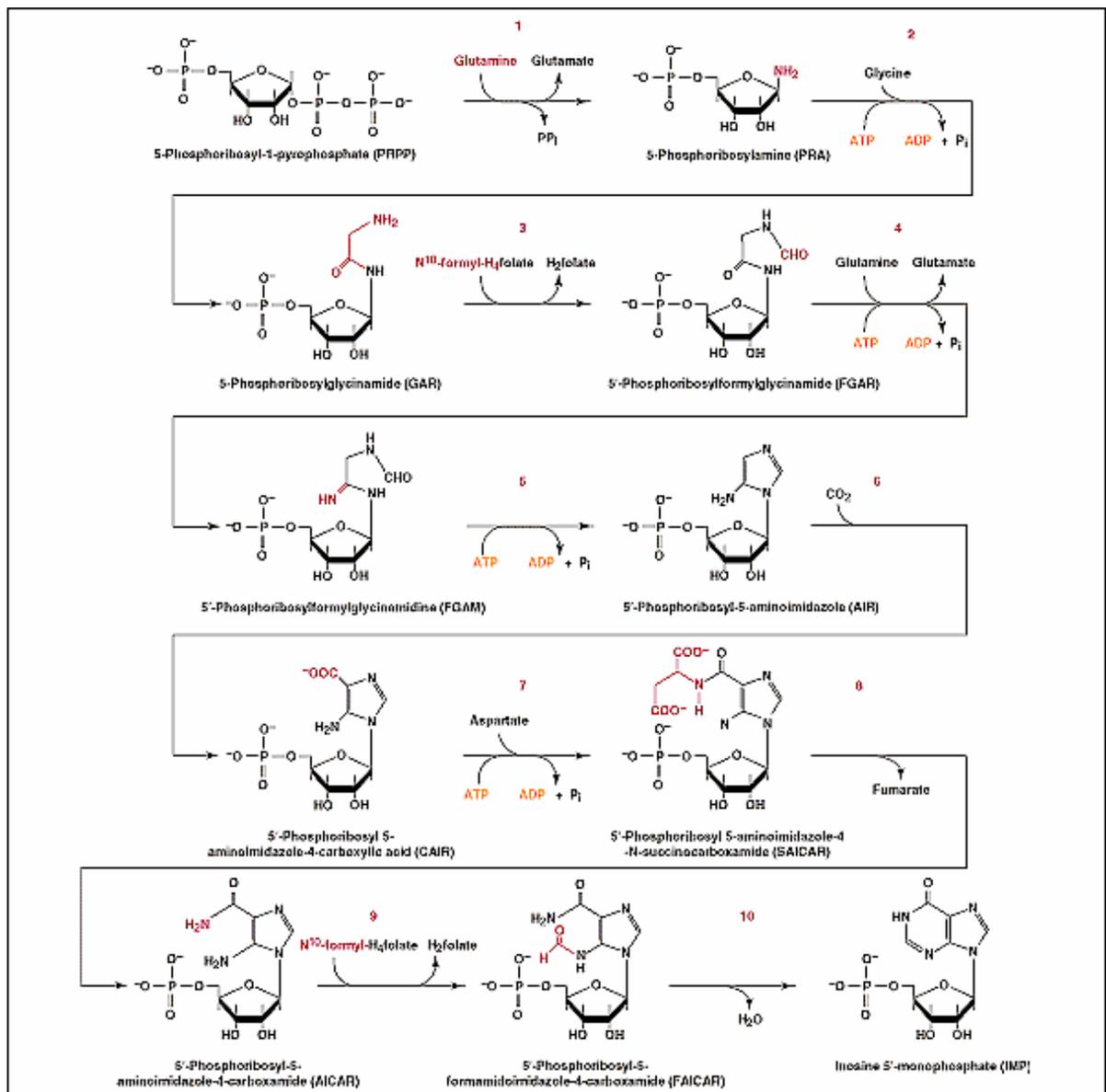


5-Phosphoribosyl-1-pyrophosphate (PRPP)

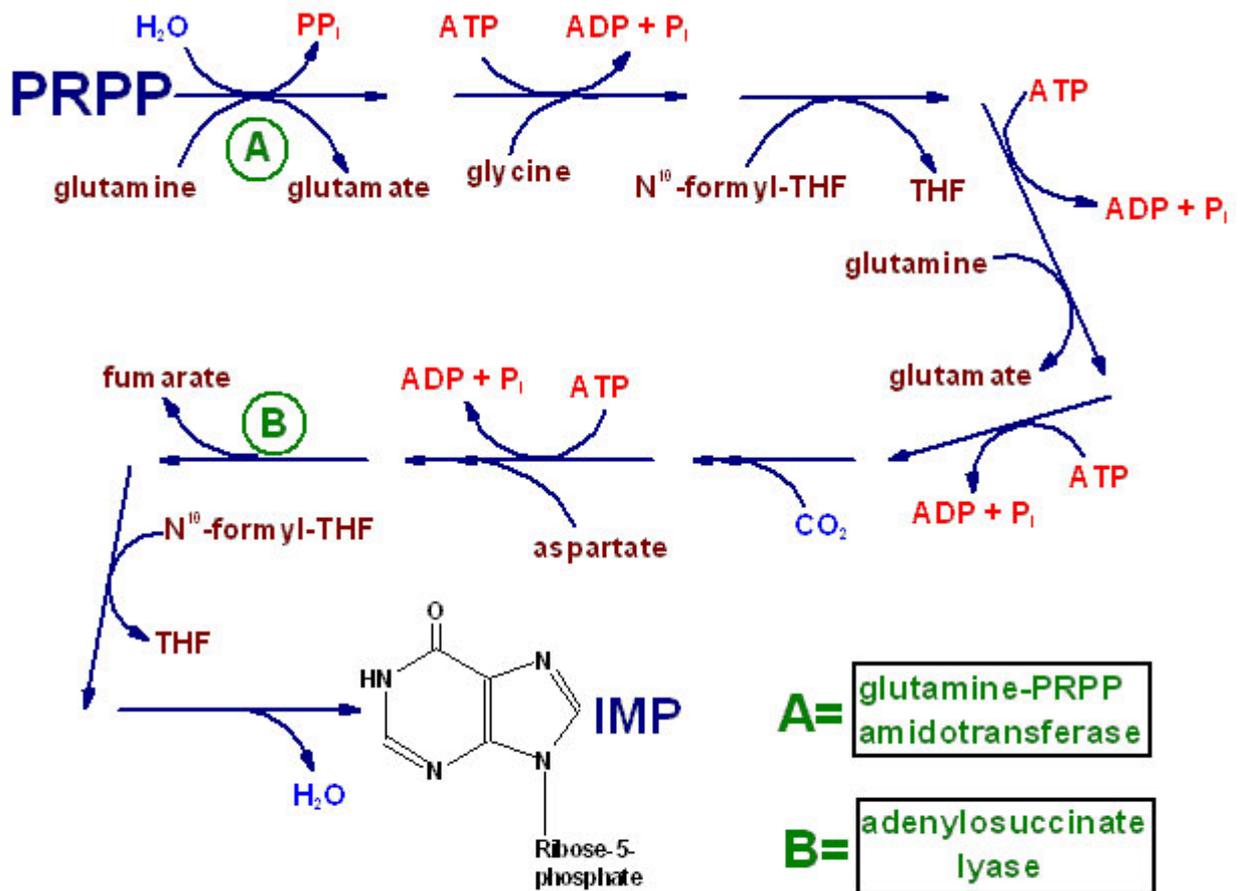
Copyright © 1997 Wiley-Liss, Inc.

De novo synthesis of purine nucleotides

1. **Origin of the carbons and nitrogens of the purine ring**
 - a. **Base.** The purine ring is built on a **molecule of PRPP**.
 - b. **The precursors of the ring are PRPP, glutamine, glycine, carbon dioxide (CO₂), aspartate, and two one-carbon fragments from the one-carbon folate pool.**
2. **Synthesis of IMP.** The formation of IMP is a 10-step, energetically expensive process that uses 6 high-energy phosphate bonds.

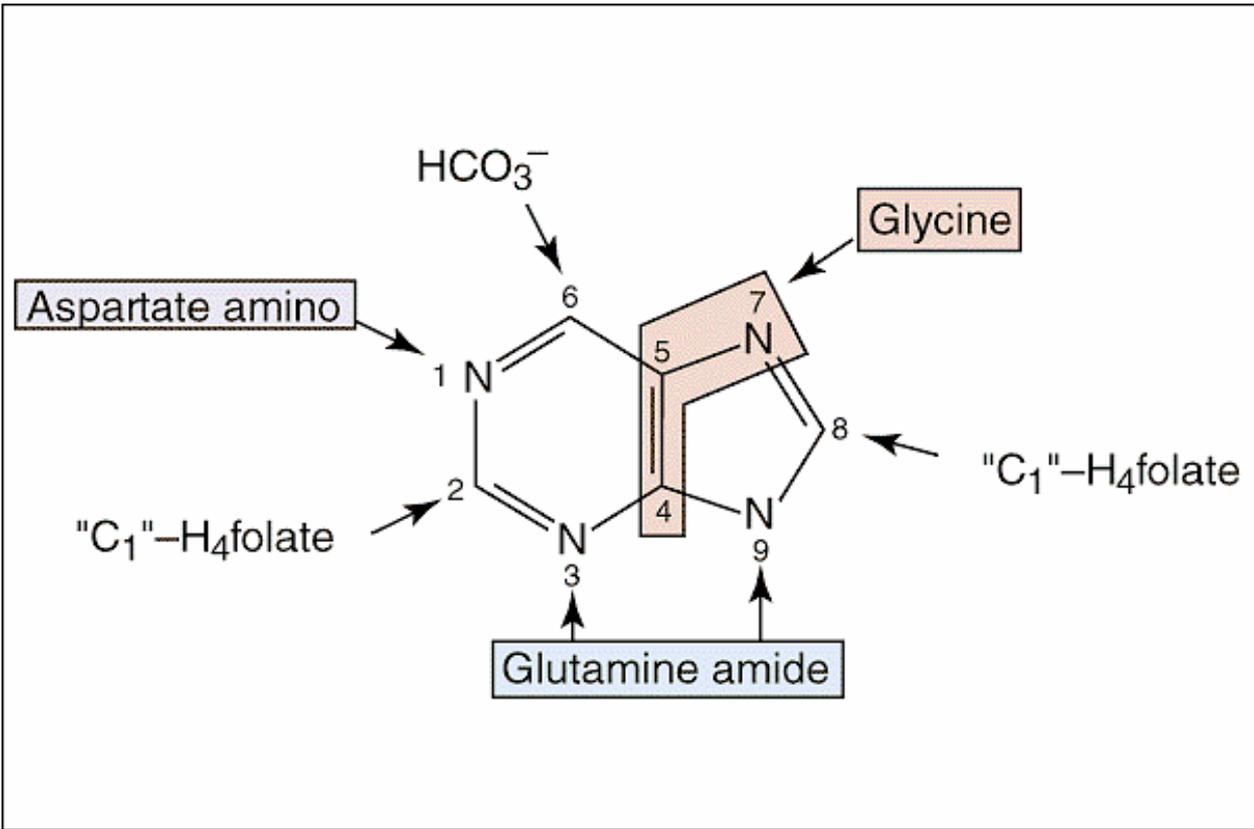


Copyright © 1997 Wiley-Liss, Inc.

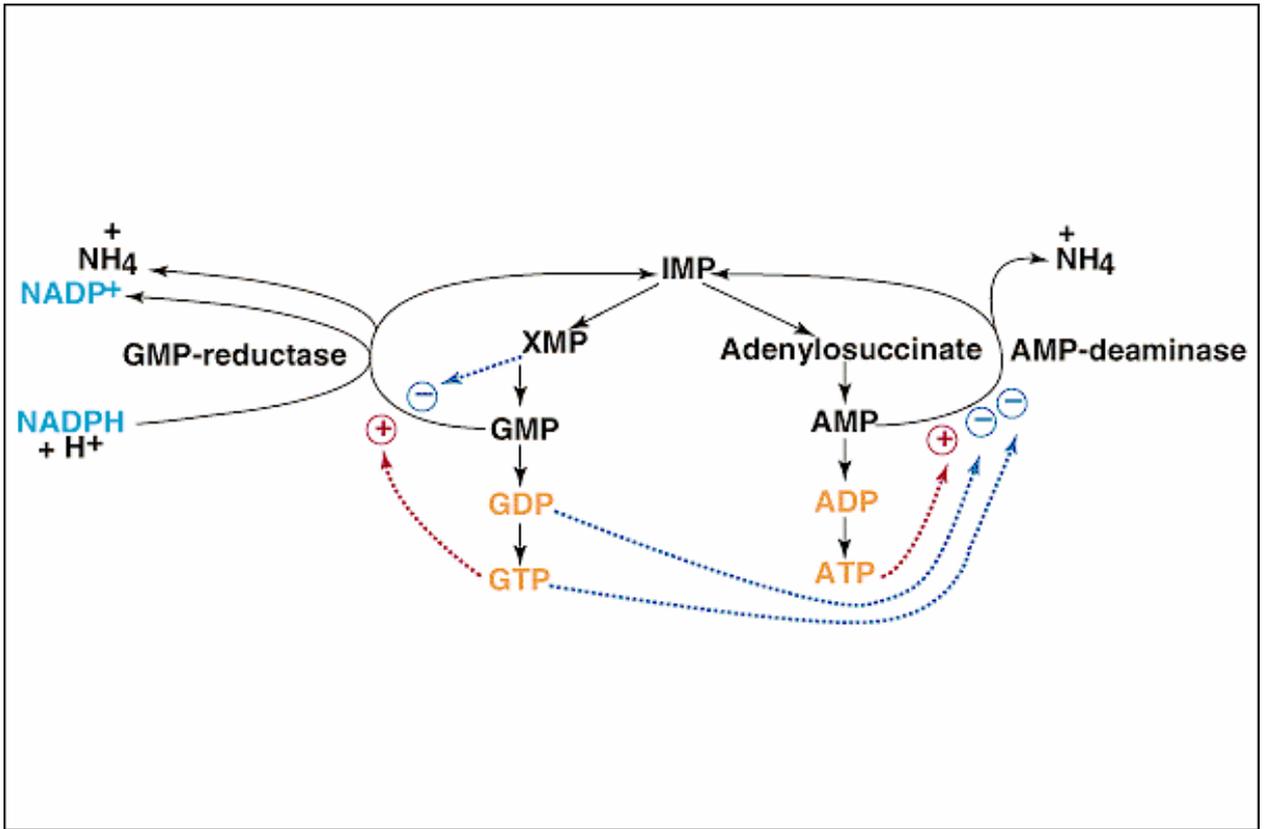


copyright 2003 M.W.King

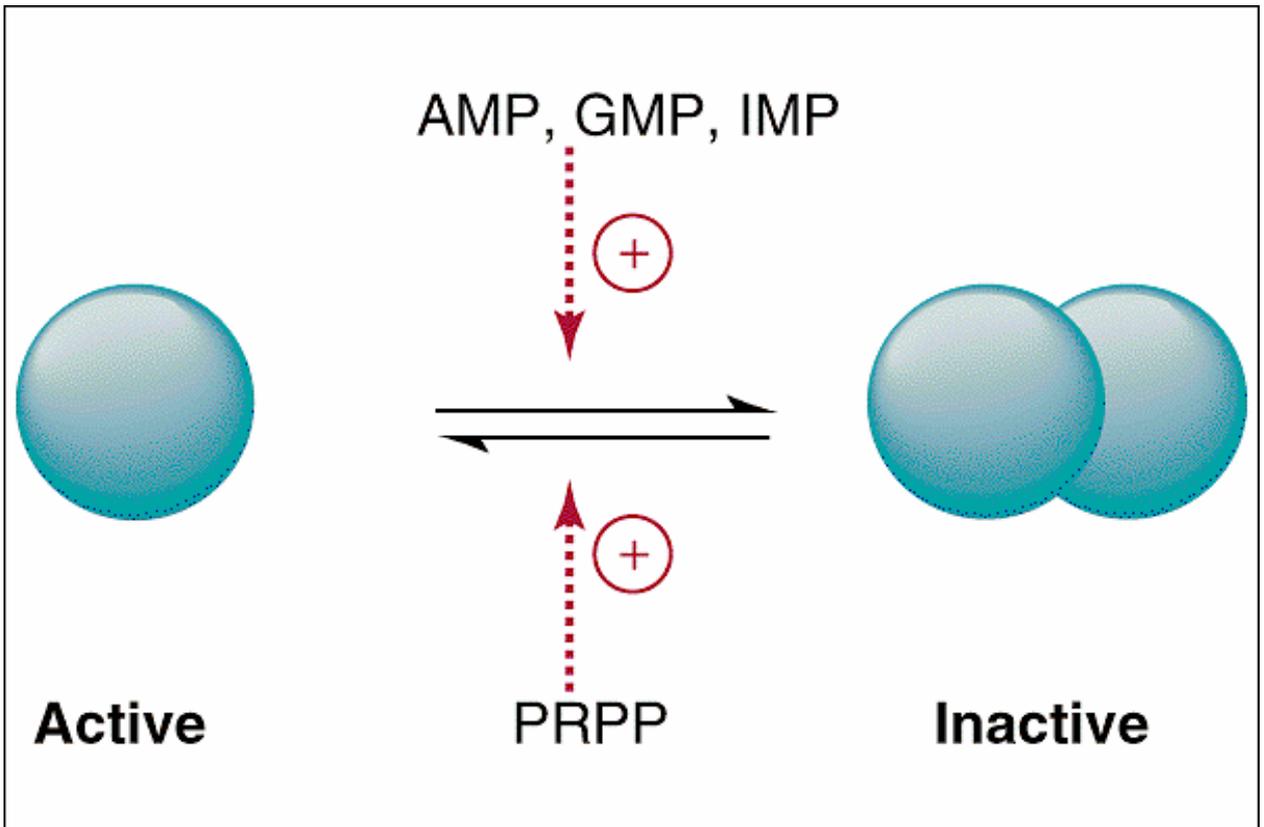
Synthesis of the first fully formed purine nucleotide, **inosine monophosphate, IMP** begins with **5-phospho- α -ribosyl-1-pyrophosphate, PRPP**. Through a series of reactions utilizing ATP, tetrahydrofolate (THF) derivatives, glutamine, glycine and aspartate this pathway yields IMP. The two indicated enzymes (A and B) are those catalyzing the rate limiting step and the reaction necessary for the **purine nucleotide cycle**, respectively. The structure of the nucleobase of IMP (hypoxanthine) is shown.



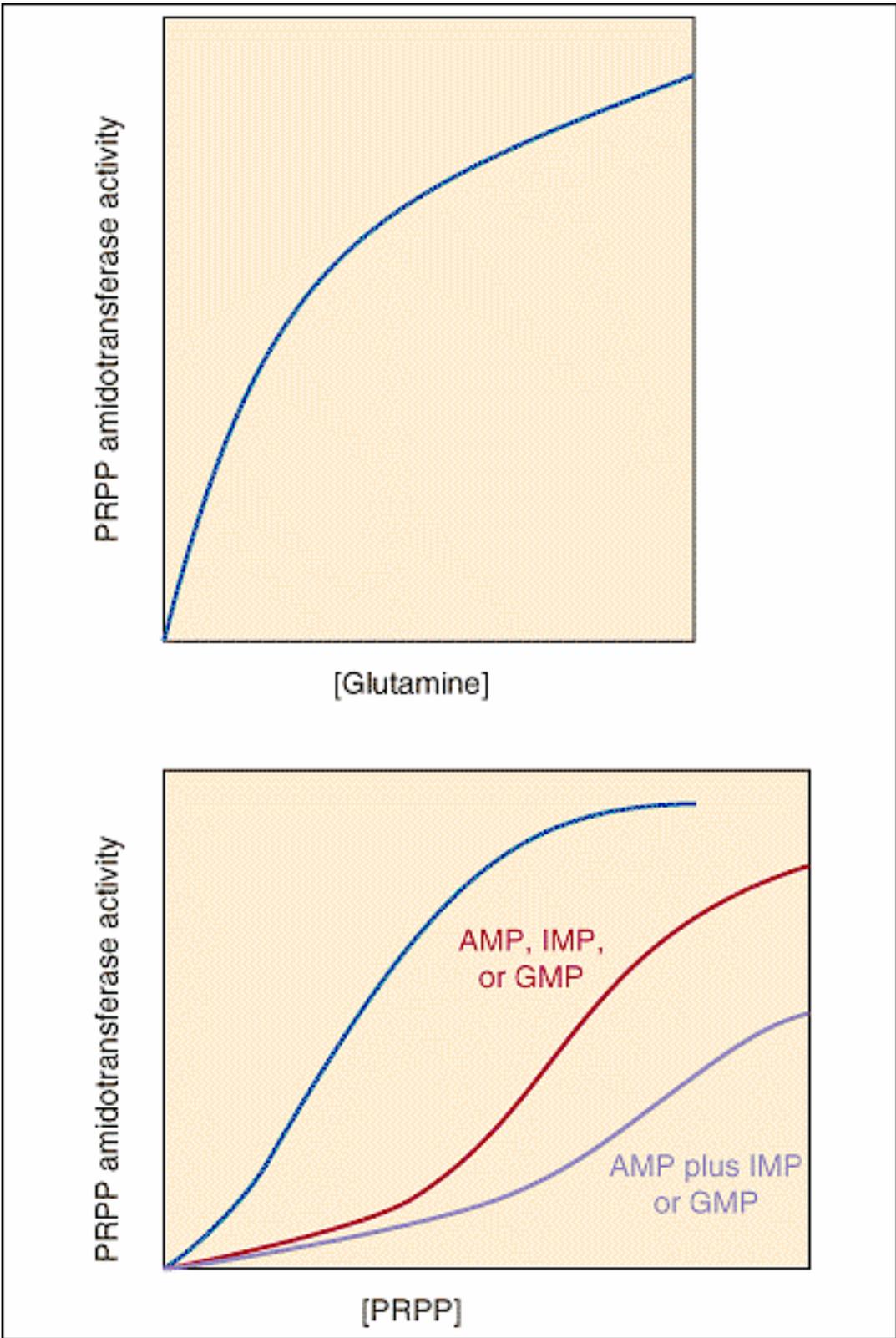
Copyright © 1997 Wiley-Liss, Inc.



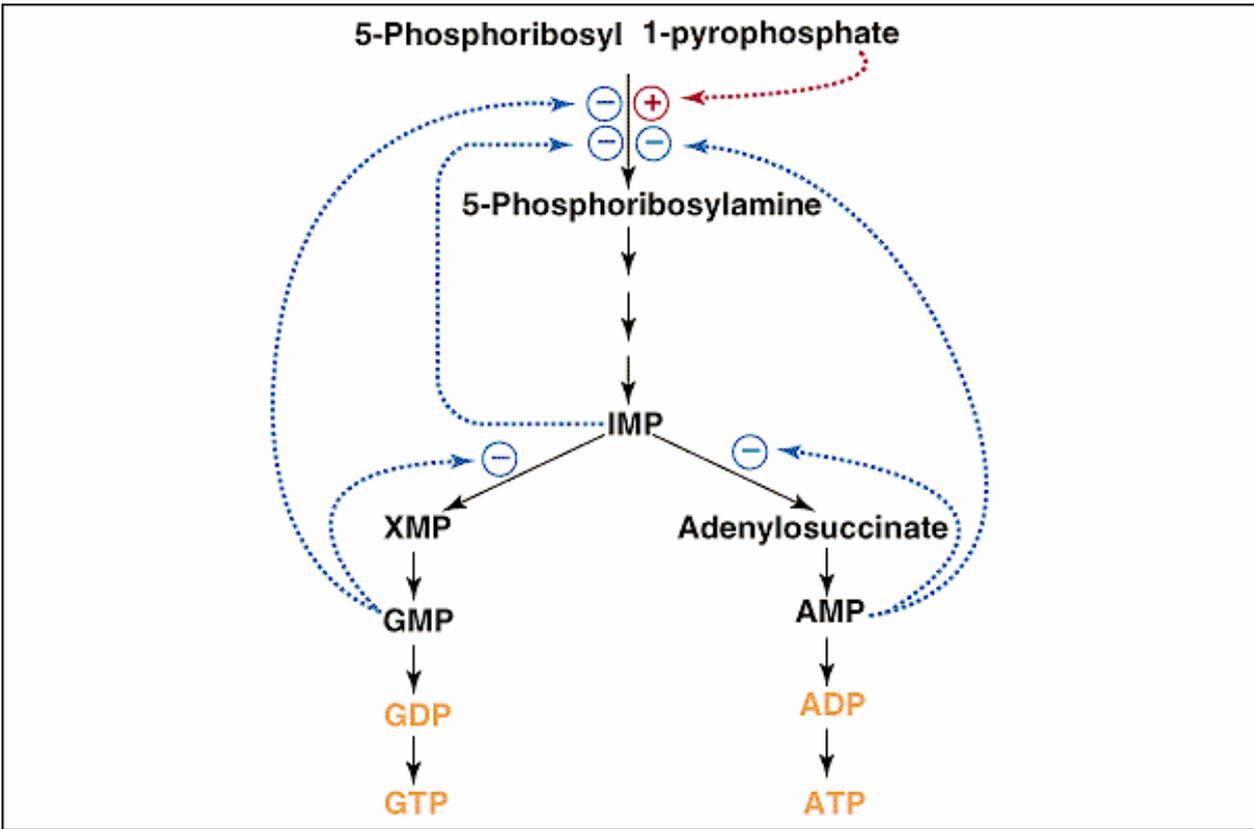
Copyright © 1997 Wiley-Liss, Inc.



Copyright © 1997 Wiley-Liss, Inc.



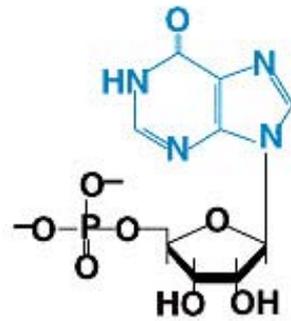
Copyright © 1997 Wiley-Liss, Inc.



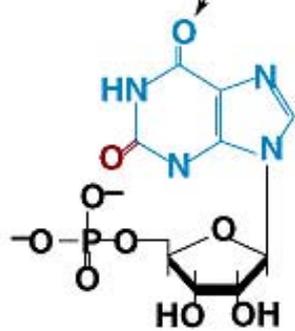
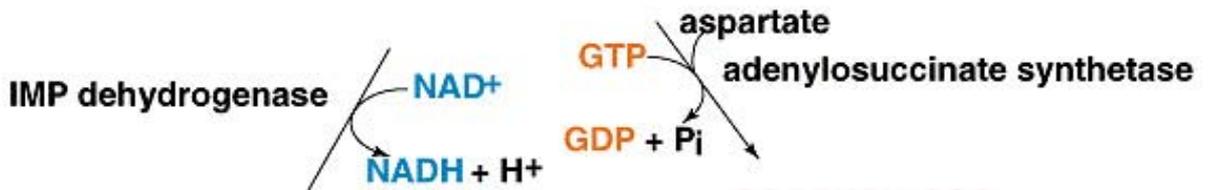
Copyright © 1997 Wiley-Liss, Inc.

3. Synthesis of AMP and GMP from IMP

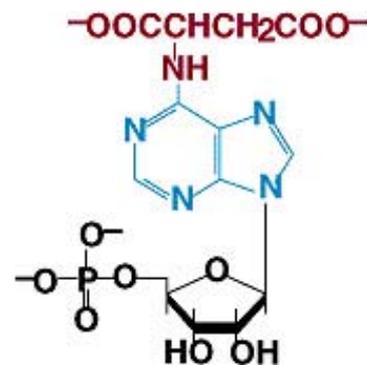
- a. **Adenylosuccinate** is formed by the addition of aspartate to IMP. **Fumarate** is then cleaved to yield AMP (adenylate).
- b. **Xanthosine 5'-monophosphate** (xanthylate; XMP) is formed by the oxidation of IMP. An amino group from glutamine is then added to yield GMP (guanylate).
- c. **Guanosine triphosphate** (GTP) is cleaved in the synthesis of AMP, and ATP is cleaved in the synthesis of GMP. This reciprocity helps to balance the levels of adenine and guanine nucleotides.



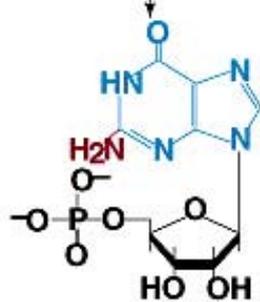
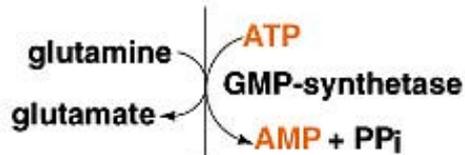
Inosine 5'-monophosphate (IMP)



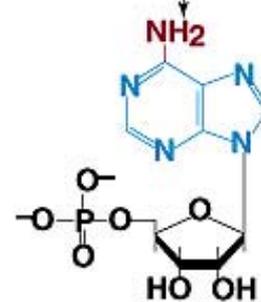
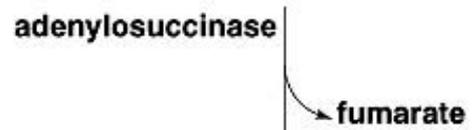
Xanthosine 5'-monophosphate (XMP)



Adenylosuccinate



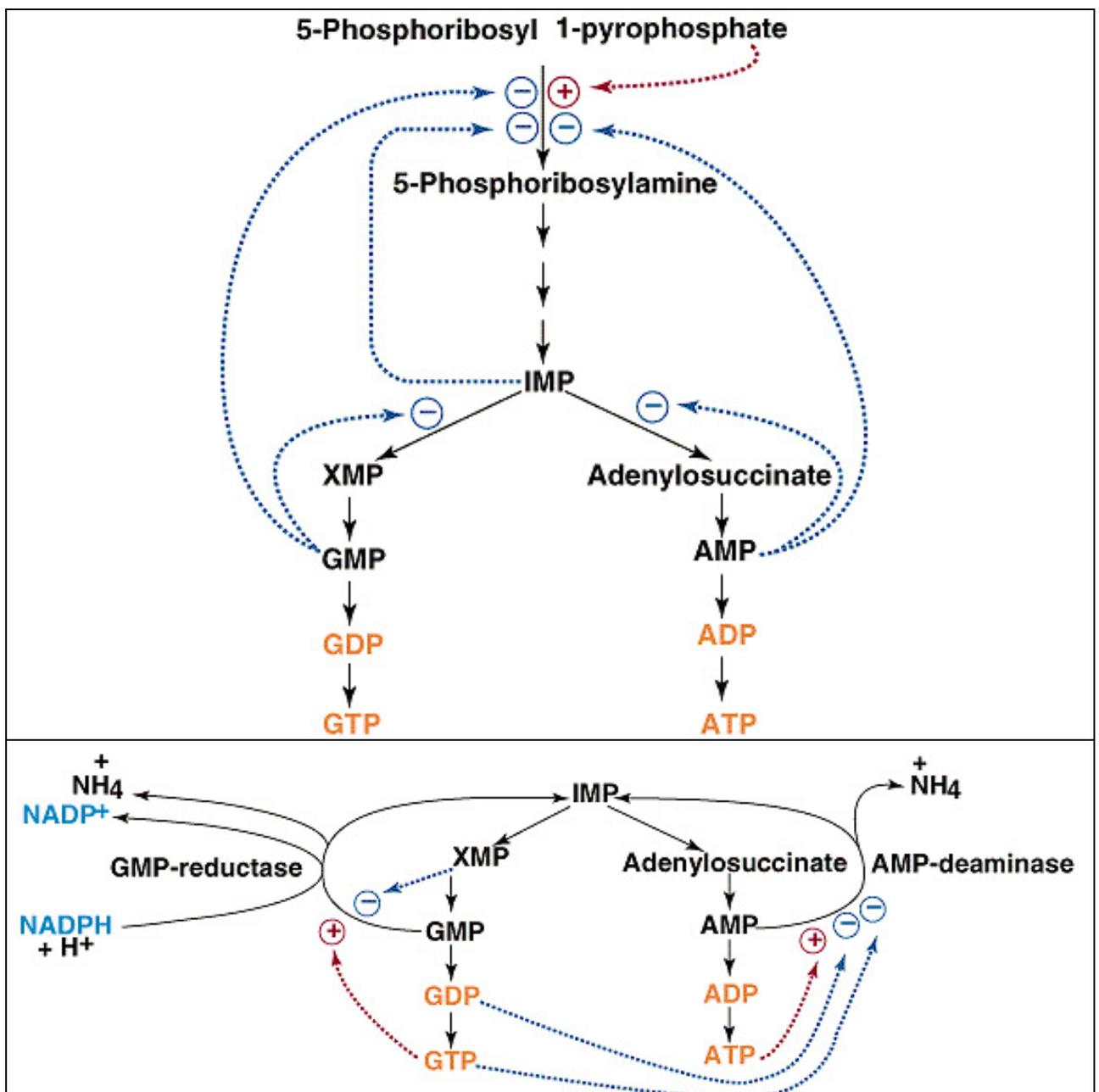
Guanosine 5'-monophosphate (GMP)



Adenosine 5'-monophosphate (AMP)

4. **De novo synthesis of purine nucleotides is regulated by feedback inhibition.**

- a. Both of the **enzymes** that catalyze the first two steps of IMP synthesis—**PRPP synthetase** and **PRPP amidotransferase**—are inhibited by IMP, GMP, and AMP.
- b. **PRPP amidotransferase has two allosteric sites:** one for IMP or GMP, and one for AMP. If both sites are occupied, then inhibition is synergistic.
- c. **Inhibition.** The synthesis of adenylosuccinate from IMP is inhibited by AMP, and the synthesis of XMP is inhibited by GMP.



Salvage pathways for purine nucleotides.

There are two specific enzymes that catalyze the transfer of the ribose phosphate from PRPP to free purine bases, which are formed by the degradation of nucleotides.

1. Hypoxanthine-guanine phosphoribosyltransferase (HGPRT) catalyzes the formation of nucleotides from either hypoxanthine or guanine. The enzyme is inhibited by high concentrations of its products, IMP and GMP.



2. Adenine phosphoribosyltransferase (APRT) catalyzes the formation of AMP from adenine. The enzyme is inhibited by high concentrations of its product, AMP.

De novo synthesis of pyrimidine nucleotides

1. Origin of the carbons and nitrogens of the pyrimidine ring

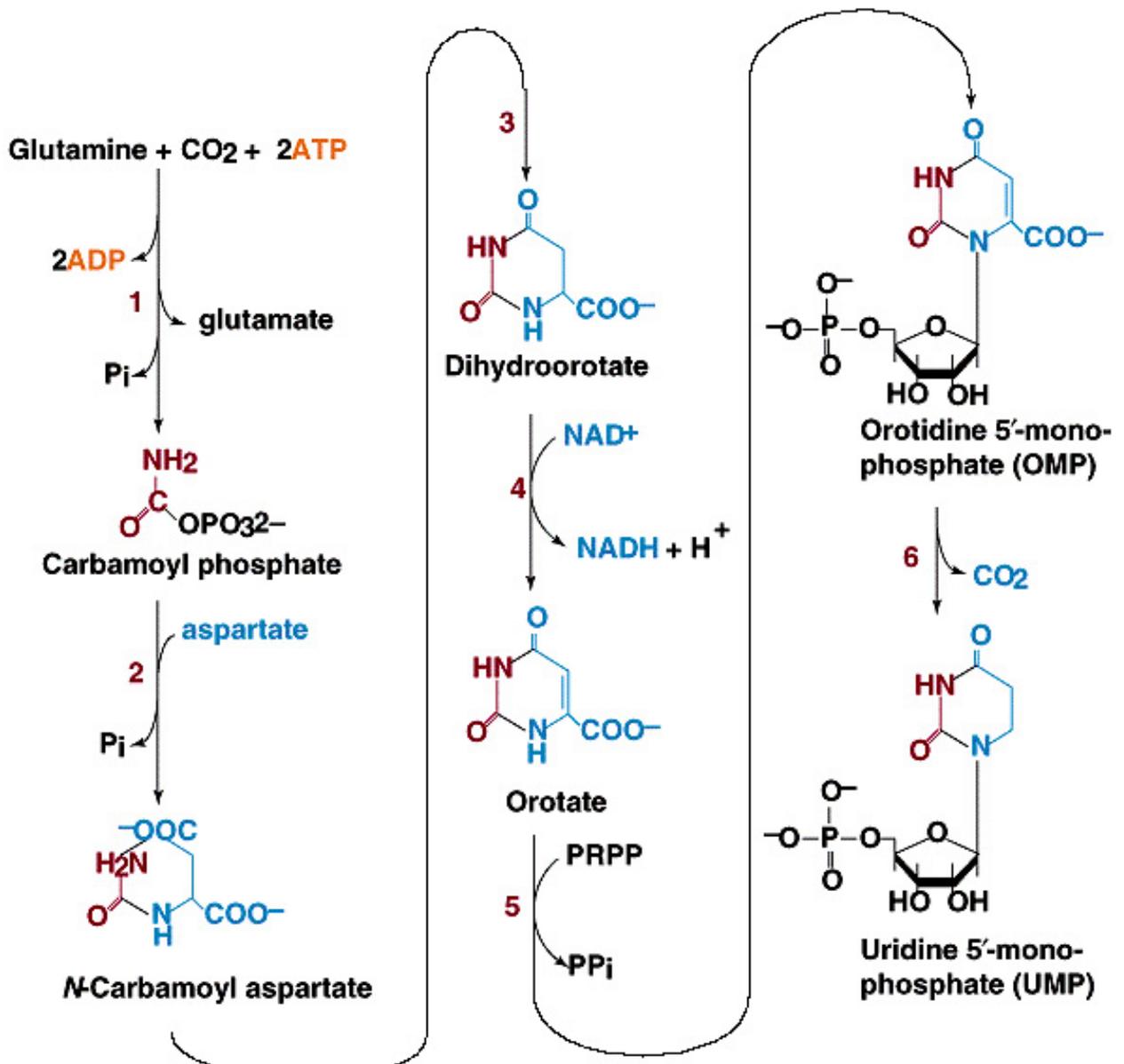
a. **Base.** The pyrimidine ring, unlike the purine ring, is not built on a molecule of PRPP. Instead, the pyrimidine ring is formed, and then it reacts with PRPP to form the nucleotide.

b. The precursors of the ring are **glutamine, aspartate, and CO₂**.

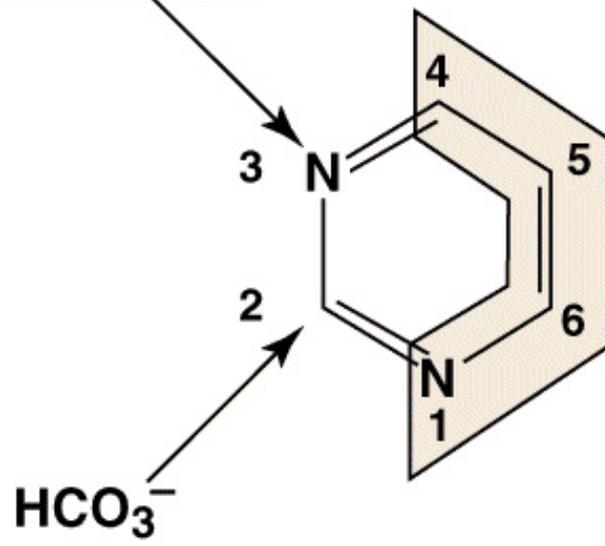
2. The formation of uridine 5'-monophosphate (uridylyate; UMP). The first compound in the pathway is **carbamoyl phosphate**.

a. Carbamoyl phosphate is synthesized in the **cytosol** from glutamine and CO₂ by the enzyme **carbamoyl phosphate synthetase II**.

b. Carbamoyl phosphate also is synthesized in the **liver** as an intermediate in urea synthesis, but this synthesis takes place in the mitochondria and is catalyzed by a different enzyme, carbamoyl phosphate synthetase I.



Glutamine amide

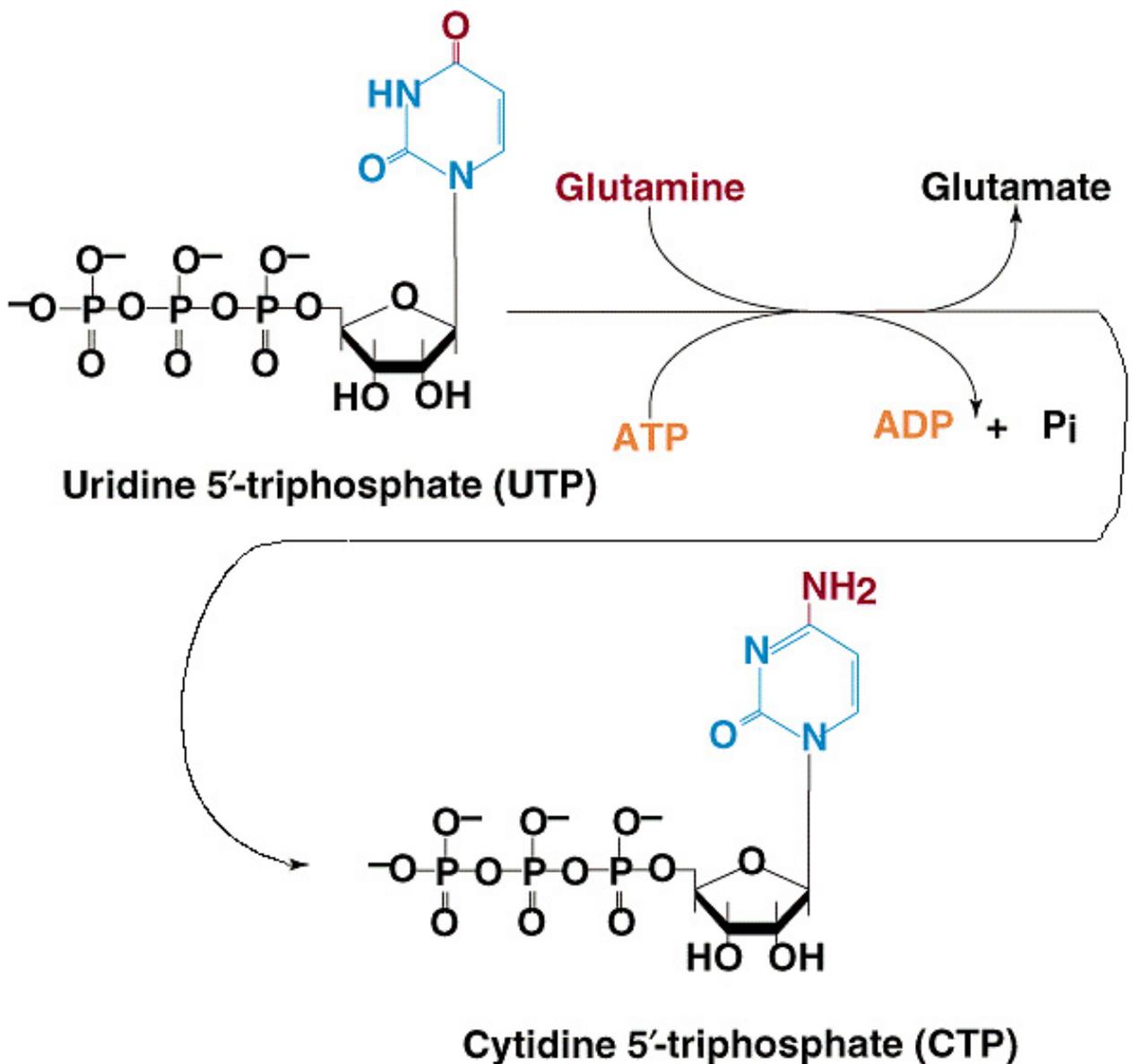


← Aspartate

3. **UMP is converted to uridine triphosphate (UTP) in two steps.**
- The first step** is catalyzed by a specific nucleoside monophosphate kinase, **UMP kinase**.
 - The second step** is catalyzed by **nucleoside diphosphate kinase**, an enzyme that has broad specificity for nucleoside diphosphates.



4. **An amino group from glutamine is donated to UTP to form cytidine triphosphate (CTP).** This reaction is catalyzed by CTP synthetase.

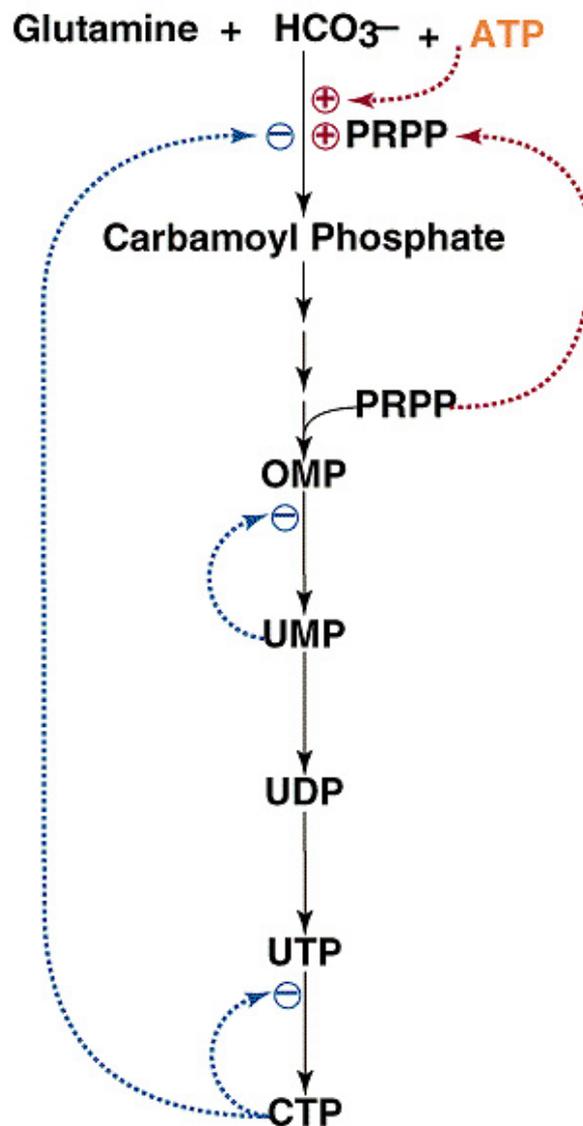


5. Regulation of pyrimidine synthesis

a. In bacteria, the enzyme **aspartate carbamoyl transferase**, which catalyzes the committed step in pyrimidine synthesis (i.e., the formation of N-carbamoyl aspartate) is **inhibited by UTP**.

b. Mammals

- (1) The enzyme that catalyzes the synthesis of carbamoyl phosphate (carbamoyl phosphate synthetase II) is a single polypeptide called **CAD**, which also contains the enzymes aspartate carbamoyl transferase and dihydroorotase. DTP inhibits the carbamoyl phosphate synthetase II activity of this multienzyme polypeptide.
- (2) The enzymes that catalyze the last two steps of the synthesis of UMP-orotate phosphoribosyltransferase and orotidine 5'-monophosphate (OMP) decarboxylase, also are linked in a single polypeptide. UMP and CMP inhibit the activity of this multienzyme.
- (3) CTP feedback inhibits CTP synthetase.



Salvage pathways for pyrimidine nucleotides are not as significant in mammals as are those for purine nucleotides.

1. Pyrimidine phosphoribosyltransferase can convert the pyrimidines, orotate, uracil, and thymine, to pyrimidine nucleoside monophosphates.



2. Uracil can be converted to UMP by two sequential reactions.

a. The first reaction is the reverse of a reaction that occurs in degradation of nucleotides. It is catalyzed by a nucleoside phosphorylase, uridine phosphorylase.



b. The second reaction is catalyzed by uridine kinase.



A deoxycytidine kinase can convert deoxycytidine to deoxycytidine monophosphate (dCMP).

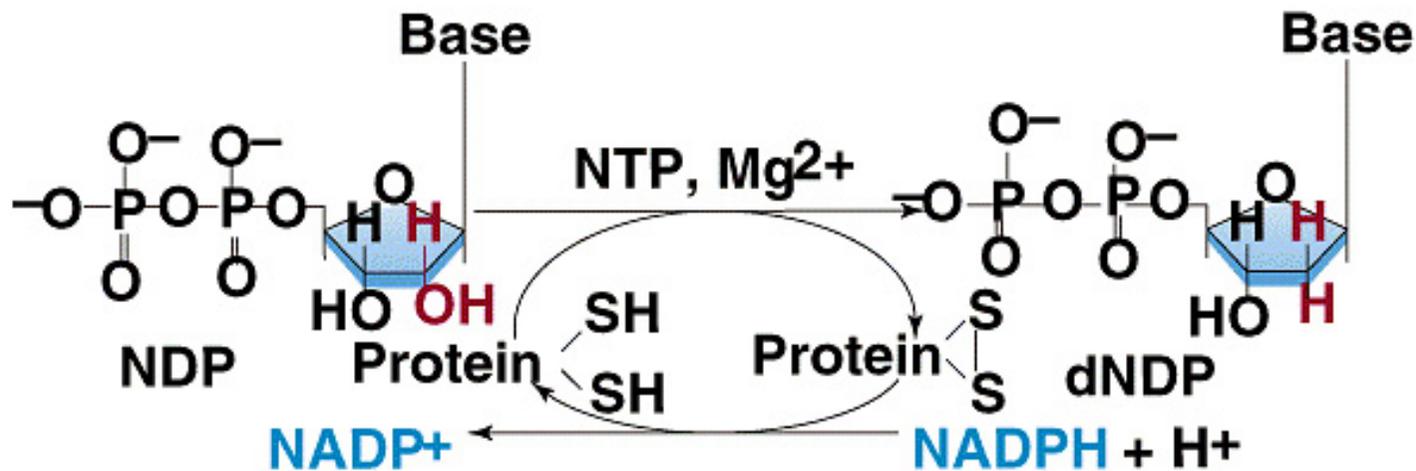


DEOXYRIBONUCLEOTIDES are found in DNA. The cellular levels of deoxyribonucleotides are ordinarily low, and they increase at the time of DNA replication.

Formation of deoxyribonucleotides. Deoxyribonucleoside diphosphates are formed by the **reduction of ribonucleoside diphosphates**.

Enzyme. The reduction of ribonucleoside diphosphates is catalyzed by **ribonucleotide reductase**.

- 1. Regulation.** Ribonucleotide reductase is regulated by ribo- and deoxyribonucleotides in a complex fashion. The pattern of regulation ensures that the proper ratios and levels of deoxyribonucleotides are present for the synthesis of DNA.
- 2. Oxidized thioredoxin.** During the reduction of ribonucleoside diphosphates, thioredoxin, the electron donor, is oxidized. Oxidized thioredoxin is reduced by thioredoxin reductase and reduced nicotinamide adenine dinucleotide phosphate (NADPH).



Deoxyribonucleotides that are formed directly by **ribonucleotide reductase** are deoxyadenosine diphosphate (dADP), deoxycytidine diphosphate (dCDP), deoxyguanosine diphosphate (dGDP), and deoxyuridine diphosphate (dUDP).

For **deoxythymidine 5'-monophosphate (dTMP)** to be formed, dUDP must first be converted to deoxyuridine 5'-monophosphate (dUMP). **Thymidylate synthase** then converts dUMP to dTMP.

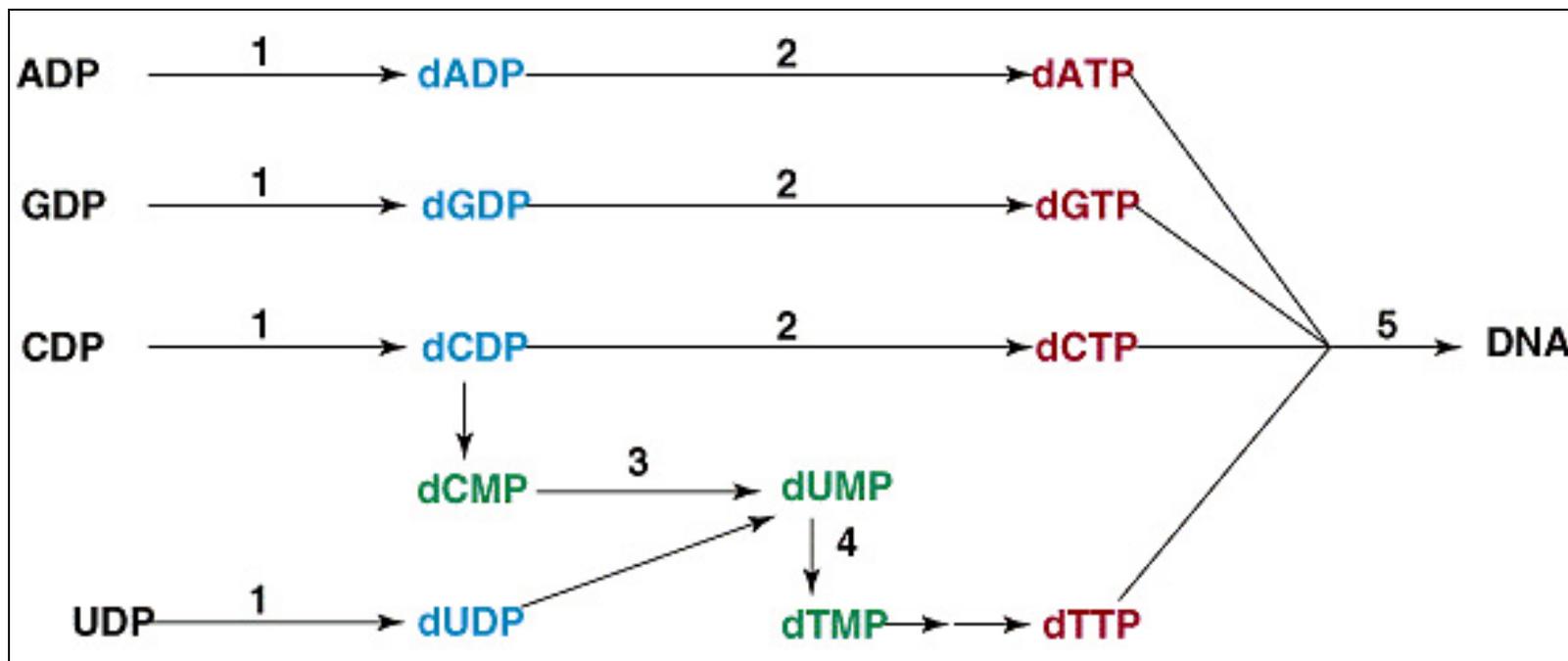
1. In this reaction, a methylene group from N^5,N^{10} -methylenetetrahydrofolate is transferred and reduced to a methyl group N^5,N^{10} -Methylenetetrahydrofo-late is then oxidized to dihydrofolate.
2. Tetrahydrofolate is regenerated by dihydrofolate reductase in a reaction that requires NADPH.

Salvage pathway for the synthesis of thymine deoxyribonucleotides

1. **Thymine phosphorylase** converts thymine to thymidine.



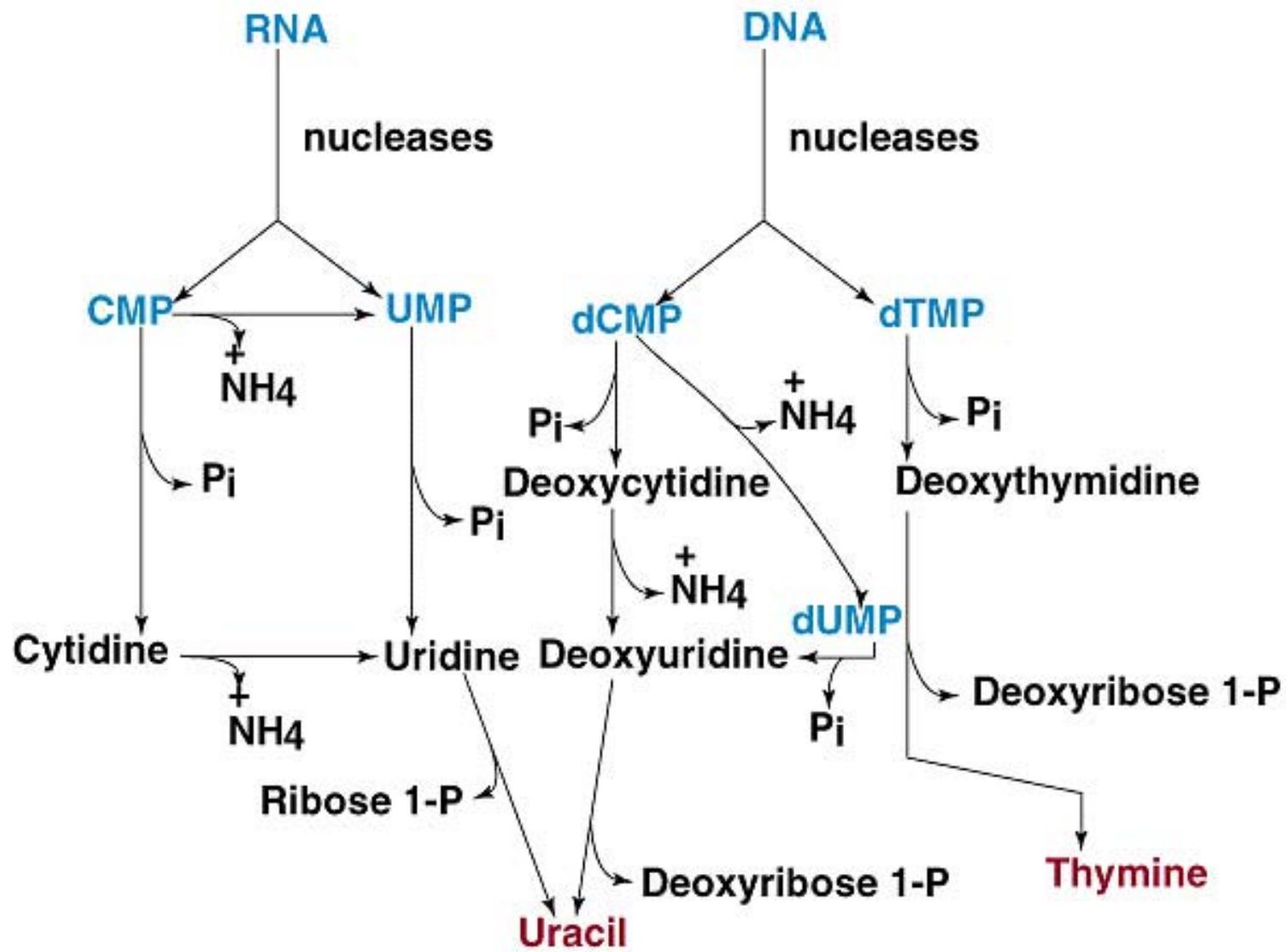
2. **Thymidine kinase** converts thymidine to dTMP.

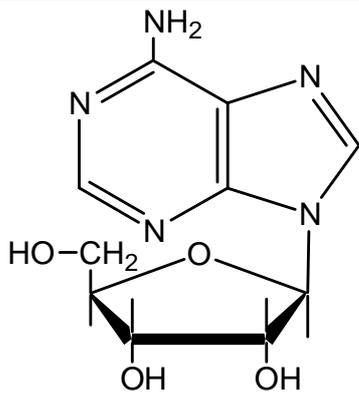


DEGRADATION OF PURINE AND PYRIMIDINE NUCLEOTIDES. A summary of the interconversions includes the degradation of the bases, nucleosides, and nucleoside mono-, di- and triphosphates. The nucleotides that result from the degradation of RNA and DNA are broken down within the cell.

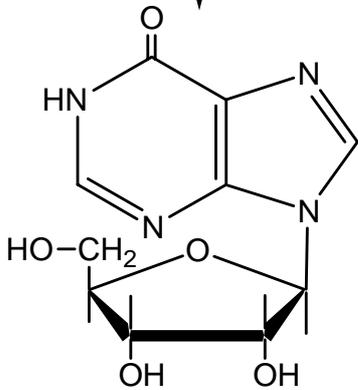
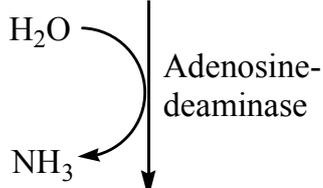
Nucleotidases remove the 5'-phosphates from purine and pyrimidine ribo- and deoxyribonucleotides, converting them to ribo- and deoxyribonucleosides.

- 1. AMP is degraded by two pathways:** It may first be deaminated to IMP, with IMP then hydrolyzed to inosine; or it may be hydrolyzed to adenosine first, with the adenosine then deaminated to inosine by **adenosine deaminase**.
- 2. Deoxy AMP (dAMP)** is hydrolyzed to deoxyadenosine, which is then deaminated to deoxyinosine by **adenosine deaminase**.
- 3. GMP and deoxyguanosine 5'-monophosphate (dGMP)** are hydrolyzed to guanosine and deoxyguanosine respectively.
- 4. Cytidine monophosphate (CMP)** may be deaminated to UMP and then hydrolyzed to uridine, or it may be hydrolyzed to cytidine and then deaminated to uridine.
- 5. dCMP** may be deaminated to dUMP and then hydrolyzed to deoxyuridine, or it may be hydrolyzed to deoxycytidine and then deaminated to deoxyuridine.
- 6. UMP** is hydrolyzed to uridine.
- 7. dTMP** is hydrolyzed to thymidine.

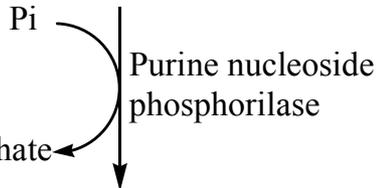




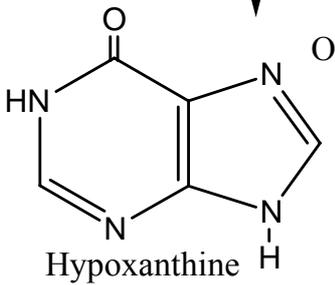
Adenosine



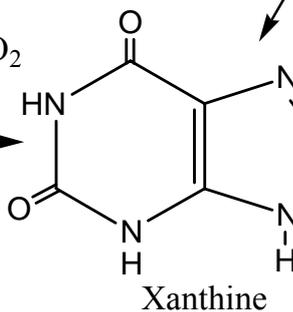
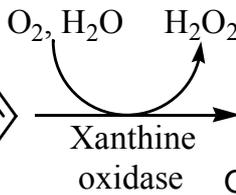
Inosine



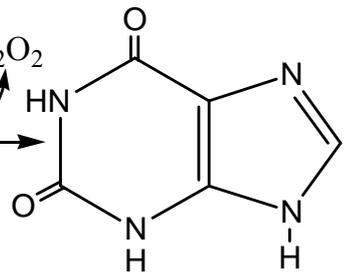
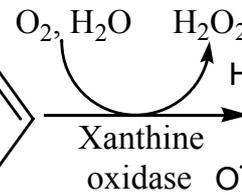
Ribose 1-phosphate



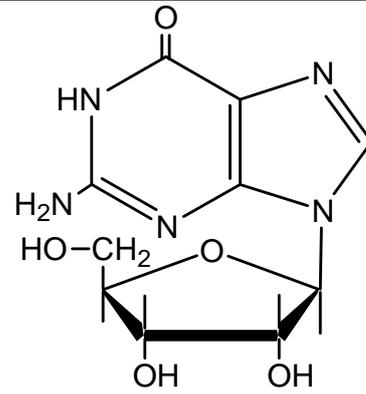
Hypoxanthine



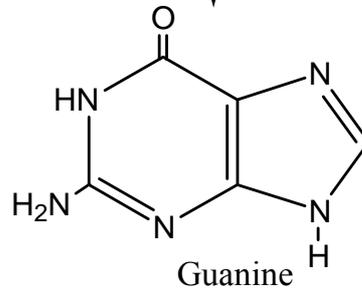
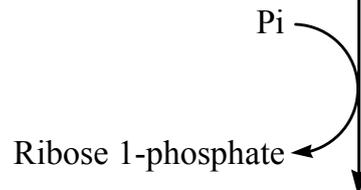
Xanthine



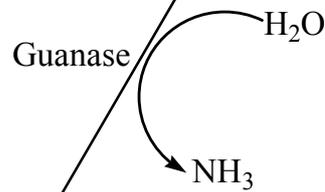
Uric acid



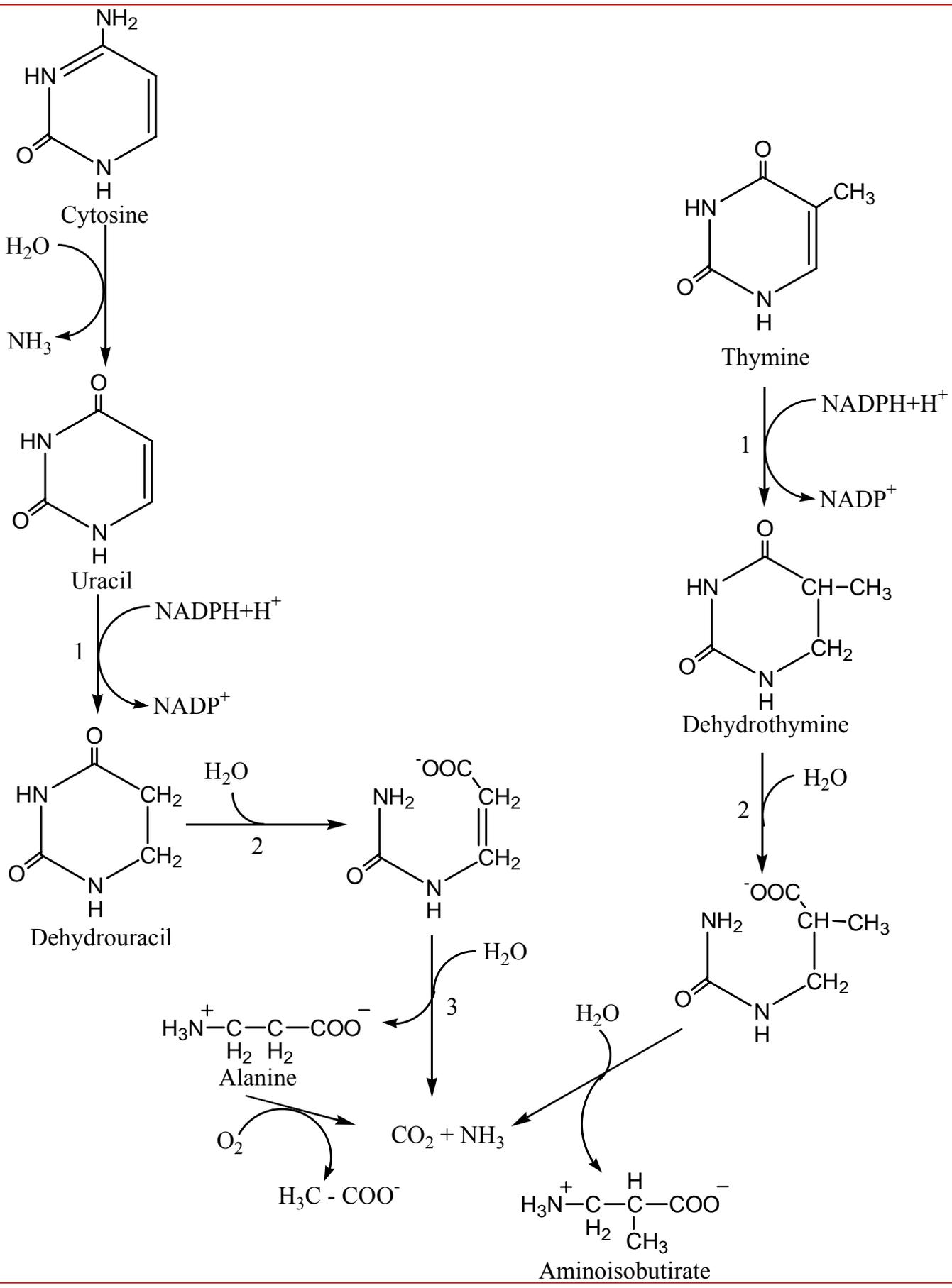
Guanosine



Guanine



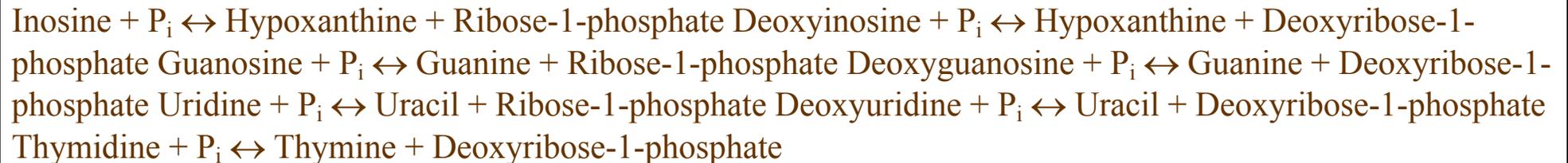
Catabolism of purine nucleotides up to uric acid.



Catabolism of pyrimidine nucleotides.

Nucleoside phosphorylases catalyze the phosphorolysis of nucleosides to **free bases and ribose-1 -phosphate or deoxyribose-1 -phosphate**.

1. In phosphorolysis, a bond is cleaved by the addition of inorganic phosphate (P_i) across it:



2. Nucleoside phosphorylases readily catalyze the **reverse reaction**, the conversion of a free base to a nucleoside. The reverse reaction is important in salvage pathways, especially of uracil and thymine.

The final product of purine degradation is uric acid, which is produced via the following pathway.

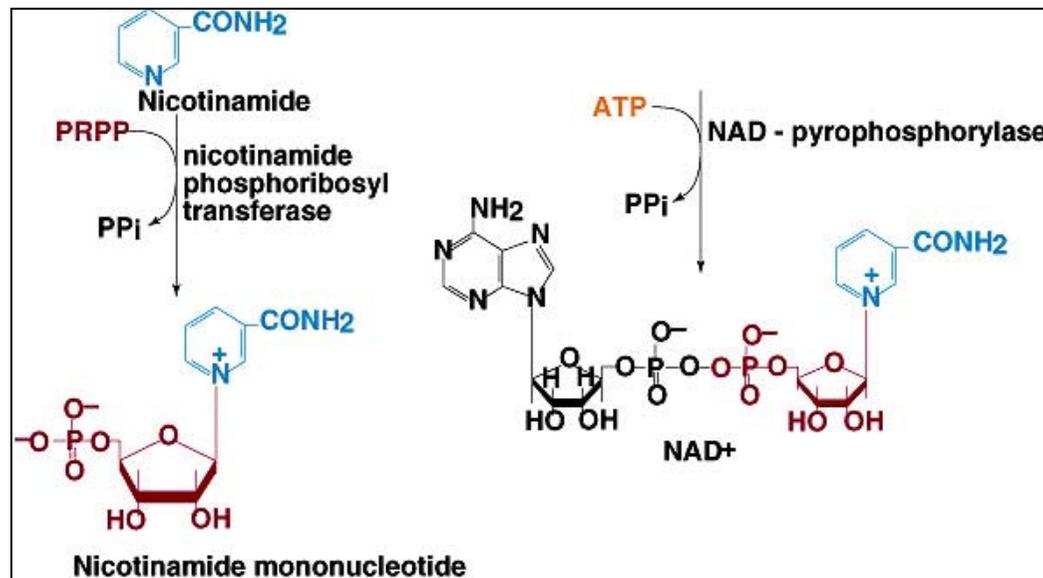
- 1. Hypoxanthine**, from the breakdown of AMP, is oxidized to xanthine by the enzyme **xanthine oxidase**.
- 2. Guanine**, from the breakdown of GMP, is deaminated to xanthine.
- 3. Xanthine** is oxidized to uric acid by **xanthine oxidase**.
 - a. Oxygen (O_2)** is required, and **hydrogen peroxide (H_2O_2)** is generated in the oxidations by xanthine oxidase.
 - b. Xanthine oxidase** contains **molybdenum**, which is why this element is required in trace amounts in humans.
This enzyme also contains **iron and sulfur**.

The final products of pyrimidine degradation are p-alanine (from uracil) and β -aminoisobutyrate (from thymine).

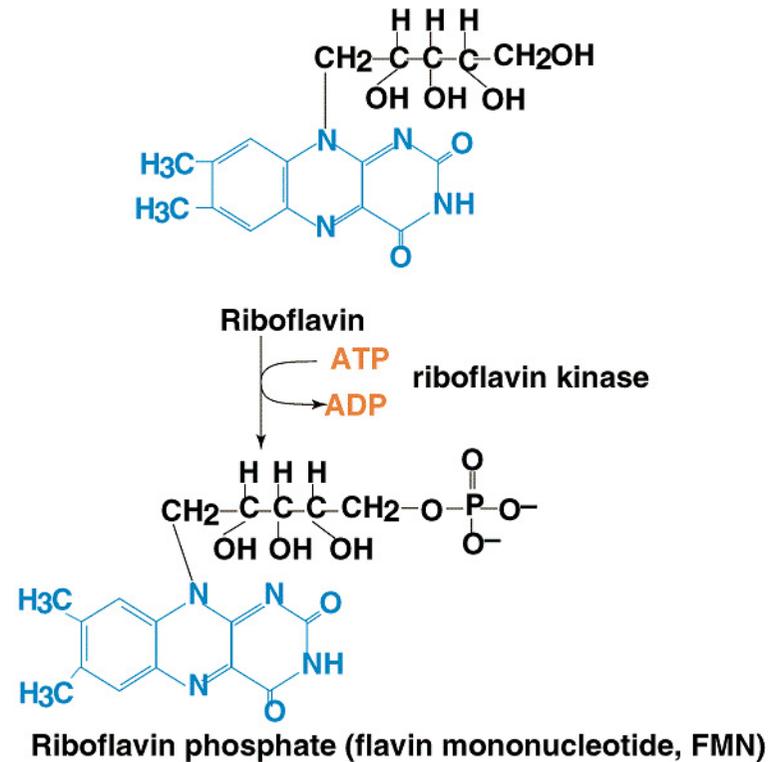
BIOSYNTHESIS OF NUCLEOTIDE COENZYMES

Nicotinamide adenine dinucleotide (NAD⁺), a major electron acceptor in oxidative phosphorylation, is synthesized through several routes. Tryptophan, nicotinate (niacin), or nicotinamide may serve as precursors.

Oxidized nicotinamide adenine dinucleotide phosphate (NADP⁺) is synthesized from NAD⁺. NADPH, the reduced form of NADP⁺, is the major electron donor in biosyntheses. The 2'-hydroxyl of the adenosine ribose is phosphorylated.



Flavin adenine dinucleotide (FAD), also a major electron acceptor in oxidative phosphorylation, is synthesized from riboflavin.



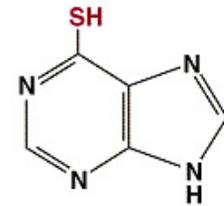
Coenzyme A (CoA), the major carrier of acyl groups in metabolism, is synthesized from pantothenate.

INHIBITORS OF PURINE AND PYRIMIDINE METABOLISM. The turnover of nucleotides in neoplastic tissues is often high. Several compounds that inhibit nucleotide synthesis are useful therapeutically in treating neoplasms.

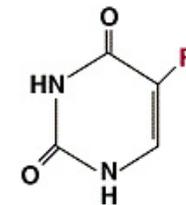
5-Fluorouracil is an analog of uracil. It is often used clinically to treat many cancers including those of the gastrointestinal tract and breast.

- 1. 5-Fluorouridine 5'-monophosphate (F-UMP).** 5-Fluorouracil undergoes conversion to the nucleoside 5'-monophosphate F-UMP in cells.
 - a. Formation.** Fluorouracil may react directly with PRPP to form F-UMP, or it may first be converted to fluorouridine and then phosphorylated to F-UMP.
 - b. Phosphorylation.** F-UMP may be phosphorylated to the nucleoside triphosphate and be incorporated into RNA, where it may have some inhibitory effect on transcription.
- 2. F-UMP can be phosphorylated to the nucleoside diphosphate.** In this form, it is reduced to the deoxyribonucleotide.
 - a. F-dUMP.** The reduced nucleoside diphosphate is dephosphorylated to 5-fluorodeoxyuridine 5'-monophosphate (F-dUMP), which is the **critical form for antineoplastic activity**,
 - b. Interaction.** F-dUMP interacts with **thymidylate synthase and N^5,N^{10} methylenetetrahydrofolate**. This complex resembles the transition state formed during the conversion of dUMP to dTMP.

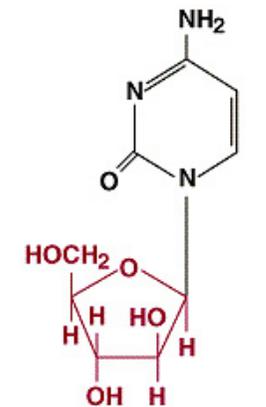
Inhibition. The transfer of a methylene group to the pyrimidine ring is blocked by the fluorine atom. Therefore, thymidylate synthase is trapped in a complex with F-dUMP, and synthesis of dTMP is inhibited.



6-Mercaptopurine



5-Fluorouracil

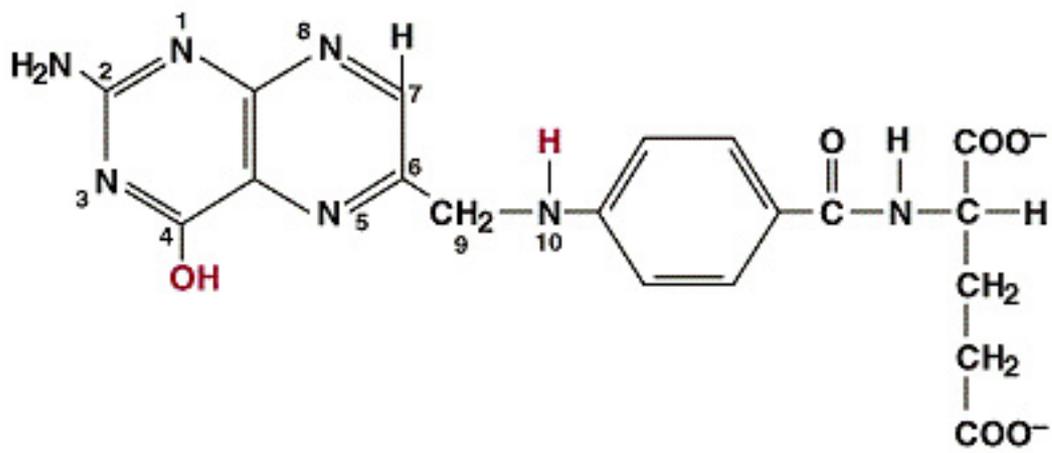


Cytosine arabinoside

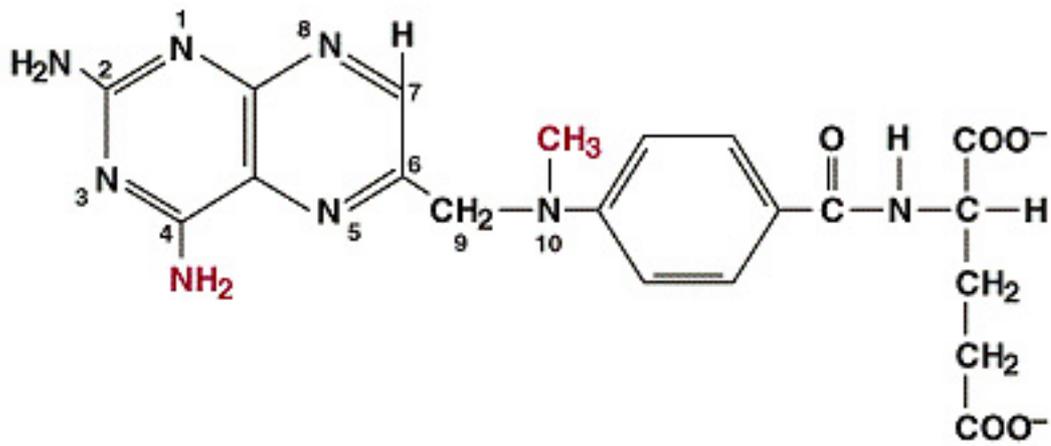
Methotrexate is an analog of folic acid that **inhibits dihydrofolate reductase**. Therefore, the regeneration of tetrahydrofolate is blocked, and synthesis of dTMP is inhibited. It is clinically used to treat many cancers including leukemias, lymphomas, and breast cancer.

6-Mercaptopurine and thioguanine are purine analogs that are used clinically in the treatment of some leukemias.

1. Both mercaptopurine and thioguanine are converted to ribonucleotides by HGPRT.
 - a. The mercaptopurine ribonucleotide **6-thiolMP** inhibits the conversion of IMP to AMP and GMP.
 - b. The thioguanine ribonucleotide **6-thioGMP** inhibits the conversion of IMP to GMP.
2. **Feedback inhibition.** Both 6-thiolMP and 6-thioGMP cause feedback inhibition of the controlling step of purine synthesis, the reaction of PRPP with glutamine.



Folic acid



Methotrexate

DISEASES ASSOCIATED WITH DEFECTS OF NUCLEOTIDE METABOLISM

Gout

1. **Cause.** Gout is caused by the precipitation of sodium urate crystals in the joints and kidneys. Sodium urate crystals precipitate because the serum levels of urate exceed its solubility limit. Elevated uric acid levels may be due to one of several disorders.
 - a. In some patients, **PRPP synthetase** is **abnormal** and is not responsive to feedback inhibition by purine nucleoside diphosphates.
 - b. A **partial deficiency of HGPRT** leads to increased cellular levels of PRPP, which leads to increased de novo synthesis of purines. This partial deficiency does not cause any of the neurologic symptoms of Lesch-Nyhan syndrome.
2. **Effect.** Inflammation and erosion of the joints occur when leukocytes engulf the deposited crystals and consequently rupture, releasing lysosomal enzymes. Sodium urate crystals in the urinary tract impair renal function.
3. **Treatment**
 - a. **Allopurinol**, which blocks the production of uric acid, is an important drug in the treatment of gout.
 - (1) Allopurinol is oxidized by xanthine oxidase to **oxypurinol**.
 - (2) Oxypurinol binds tightly to xanthine oxidase, inhibiting its ability to oxidize xanthine or hypoxanthine. This is an example of **suicide inhibition**.
 - (3) HGPRT salvages the hypoxanthine whose levels increase upon inhibition of uric acid formation. This leads to a decrease in PRPP levels and thus to a decrease in de novo purine synthesis.
 - b. **Colchicine** is an anti-inflammatory drug that may be used to treat an acute gout attack. It inhibits leukocyte movement by affecting microtubule formation.

In glycogen storage disease type I (von Gierke's disease), a deficiency in glucose-6-phosphatase activity leads to an increase in cellular levels of ribose phosphates, which increases the levels of PRPP. The elevated levels of PRPP result in increased de novo synthesis of purines.

Lesch-Nyhan syndrome

Cause. Lesch-Nyhan syndrome is a hereditary X-linked recessive condition that is due to a severe or complete **deficiency of HGPRT activity.**

c. Increased synthesis of purines. Because there is little or no HGPRT activity in people affected with Lesch-Nyhan syndrome, hypoxanthine and guanine are not salvaged. Also, the intracellular levels of PRPP increase, whereas those of IMP and GMP decrease. This leads to increased de novo synthesis of purines.

d. Several forms of HGPRT deficiency have been identified in Lesch-Nyhan syndrome.

(1) In one form, patients have normal levels of HGPRT protein, but the enzyme is inactive.

(2) Some patients have an enzyme that is apparently unstable; its activity is higher in young red blood cells than in old.

The symptoms include hyperuricemia, gout, urinary tract stones, and the neurologic symptoms of mental retardation, spasticity, and self-mutilation. The basis for the neurologic symptoms is unknown. However, brain cells normally have much higher levels of purine salvage enzymes than other cells and may normally use salvage pathways to a greater extent.

Treatment with **allopurinol** reduces the uric acid formation but does not alleviate the neurologic symptoms. Also, because there is little or no HGPRT activity, PRPP levels and de novo purine synthesis do not decrease with allopurinol treatment. No single therapy has been found to be universally effective in treating all forms of Lesch-Nyhan syndrome.

Immunodeficiency disorders

1. Adenosine deaminase (ADA) deficiency

a. Cause. Deoxyadenosine and adenosine levels are high in people with an ADA deficiency because these nucleosides are not degraded to deoxyinosine and inosine. Lymphocytes are particularly sensitive to high levels of deoxyadenosine and adenosine. One suggested mechanism is that the increased levels of deoxyadenosine and adenosine lead to an accumulation of deoxy ATP (dATP). **High levels of dATP inhibit ribonucleotide reductase, which inhibits DNA synthesis** and accordingly the proliferation of lymphocytes.

b. Effect. T-cell and B-cell functions are defective. ADA deficiency is associated with **severe combined immunodeficiency (SCID)**.

(1) **Symptoms** of SCID appear before 6 months of age and include recurrent or chronic infections and failure to thrive (growth retardation). SCID is usually fatal, often by 18 months of age.

(2) **Treatment.** Bone marrow transplantation and enzyme replacement have been used to treat SCID. Gene therapy is an experimental treatment.

2. Purine nucleoside phosphorylase deficiency

a. Cause. In the cells of people with this deficiency, deoxy GTP (dGTP) accumulates. Like dATP, dGTP inhibits ribonucleotide reductase, but not to the same degree.

b. Effect. Purine nucleoside phosphorylase deficiency is associated with **impaired T-cell function**; B-cell function may be unaffected. The reason why primarily the function of T cells is affected is unclear.

(1) **Symptoms** of T-cell deficiency include recurrent or chronic infections.

(2) **Treatment.** No effective treatment exists. Affected people should not be exposed to infectious diseases.