Voet & Voet

Case for gout

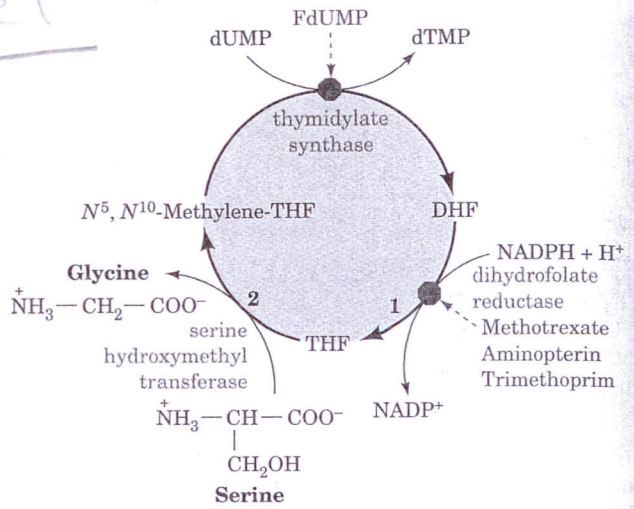


Figure 22-15. **Regeneration of N^5,N^{10}-methylenetetrahydrofolate.** The DHF product of the thymidylate synthase reaction is converted back to N^5,N^{10}-methylene-THF by the sequential actions of (1) dihydrofolate reductase and (2) serine hydroxymethyltransferase. The sites of action of some inhibitors are indicated by red octagons. Thymidylate synthase is inhibited by FdUMP, whereas dihydrofolate reductase is inhibited by the antifolates methotrexate, aminopterin, and trimethoprim (see Box 22-1).

Figure 22-16. **A ribbon diagram of human dihydrofolate reductase in complex with folate.** The helices of this monomeric enzyme are drawn in yellow, the β sheets in orange, and the other polypeptide segments in blue. [Courtesy of Jay F. Davies II and Joseph Kraut, University of California at San Diego.] See the Interactive Exercises.

Tetrahydrofolate Is Regenerated in Two Reactions

The thymidylate synthase reaction is biochemically unique in that it oxidizes THF to DHF; no other enzymatic reaction employing a THF cofactor alters this coenzyme's net oxidation state. The DHF product of the thymidylate synthase reaction is recycled back to N^5,N^{10}-methylene-THF through two sequential reactions (Fig. 22-15):

1. DHF is reduced to THF by NADPH as catalyzed by **dihydrofolate reductase (DHFR;** Fig. 22-16). Although in most organisms DHFR is a monomeric, monofunctional enzyme, in protozoa and some plants DHFR and thymidylate synthase occur on the same polypeptide chain to form a bifunctional enzyme that has been shown to channel DHF from its thymidylate synthase to its DHFR active sites.
2. Serine hydroxymethyltransferase (Section 20-4A) transfers the hydroxymethyl group of serine to THF yielding N^5,N^{10}-methylene-THF and glycine.

Inhibition of thymidylate synthase or DHFR blocks dTMP synthesis and is therefore the basis of cancer chemotherapies (see Box 22-1).

4. NUCLEOTIDE DEGRADATION

Most foodstuffs, being of cellular origin, contain nucleic acids. Dietary nucleic acids survive the acid medium of the stomach; they are degraded to their component nucleotides, mainly in the intestine, by pancreatic nucleases and intestinal phosphodiesterases. The ionic nucleotides, which cannot pass through cell membranes, are then hydrolyzed to nucleosides by a variety of group-specific nucleotidases and nonspecific phosphatases. N

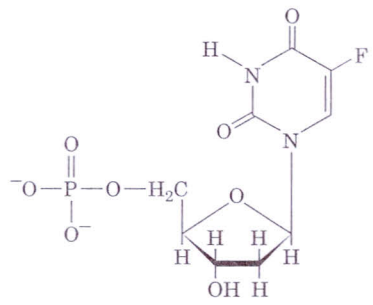
Box 22-1

BIOCHEMISTRY IN HEALTH AND DISEASE

Inhibition of Thymidylate Synthesis in Cancer Therapy

dTMP synthesis is a critical process for rapidly proliferating cells, such as cancer cells, which require a steady supply of dTMP for DNA synthesis. Interruption of dTMP synthesis can therefore kill these cells. Most normal mammalian cells, which grow slowly if at all, require less dTMP and so are less sensitive to agents that inhibit thymidylate synthase or dihydrofolate reductase (notable exceptions are the bone marrow cells that constitute the blood-forming tissue and much of the immune system, the intestinal mucosa, and hair follicles).

5-Fluorodeoxyuridylate (FdUMP)

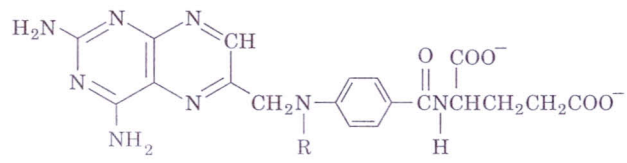


5-Fluorodeoxyuridylate (FdUMP)

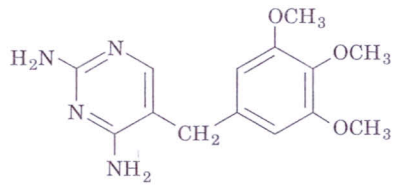
is an irreversible inhibitor of thymidylate synthase. This substance, like dUMP, binds to the enzyme (an F atom is not much larger than an H atom) and undergoes the first two steps of the normal enzymatic reaction (Fig. 22-14). In Step 3, however, the enzyme cannot abstract the F atom as F^+ (F is the most electronegative element) so that the enzyme is frozen in an enzyme–FdUMP–THF ternary covalent complex.

Enzyme inhibitors such as FdUMP, which inactivate an enzyme only after undergoing part or all of its normal catalytic reaction, are called **mechanism-based inhibitors** (alternatively, **suicide substrates** because they cause the enzyme to "commit suicide"). Because of their extremely high specificity, mechanism-based inhibitors are among the most useful therapeutic agents.

Inhibition of DHFR blocks dTMP synthesis as well as all other THF-dependent biological reactions, because the THF converted to DHF by the thymidylate synthase reaction cannot be regenerated. **Methotrexate (amethopterin)**, **aminopterin**, and **trimethoprim**



R = H **Aminopterin**
R = CH_3 **Methotrexate (amethopterin)**



Trimethoprim

are DHF analogs that competitively although nearly irreversibly bind to DHFR with an ~1000-fold greater affinity than does DHF. These **antifolates** (substances that interfere with the action of folate cofactors) are effective anticancer agents, particularly against childhood leukemias. In fact, a successful chemotherapeutic strategy is to treat a cancer victim with a lethal dose of methotrexate and some hours later "rescue" the patient (but hopefully not the cancer) by administering massive doses of 5-formyl-THF and/or thymidine. Trimethoprim, which was discovered by George Hitchings and Gertrude Elion, binds much more tightly to bacterial DHFRs than to those of mammals and is therefore a clinically useful antibacterial agent.

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cleosides may be directly absorbed by the intestinal mucosa or further degraded to free bases and ribose or ribose-1-phosphate through the action of **nucleosidases** and **nucleoside phosphorylases:**

$$\text{Nucleoside} + H_2O \xrightarrow{\text{nucleosidase}} \text{base} + \text{ribose}$$

$$\text{Nucleoside} + P_i \xrightarrow[\text{phosphorylase}]{\text{nucleoside}} \text{base} + \text{ribose-1-P}$$

Radioactive labeling experiments have demonstrated that only a small fraction of the bases of ingested nucleic acids are incorporated into tissue

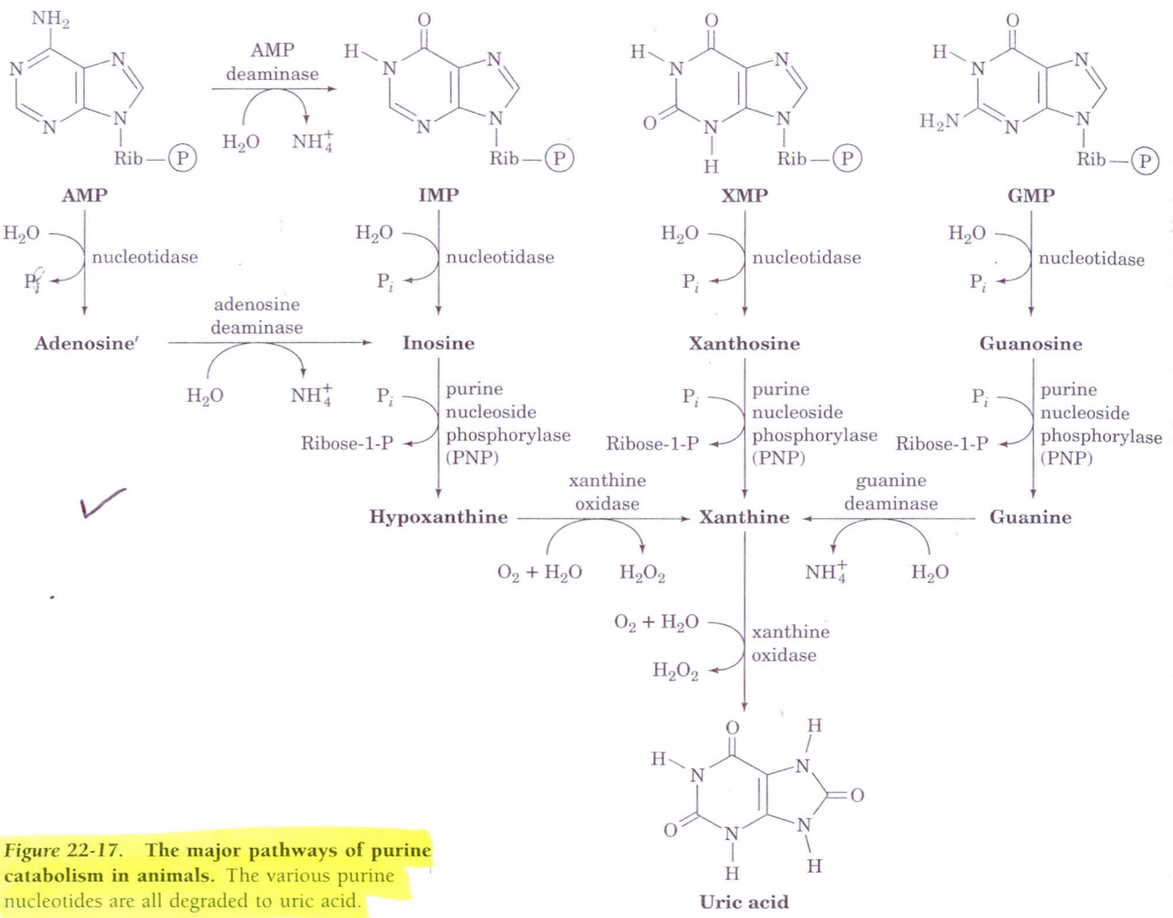


Figure 22-17. **The major pathways of purine catabolism in animals.** The various purine nucleotides are all degraded to uric acid.

nucleic acids. Evidently, *the de novo pathways of nucleotide biosynthesis largely satisfy an organism's need for nucleotides*. Consequently, ingested bases are mostly degraded and excreted. Cellular nucleic acids are also subject to degradation as part of the continual turnover of nearly all cellular components. In this section, we outline these catabolic pathways and discuss the consequences of several of their inherited defects.

A. Catabolism of Purines

The major pathways of purine nucleotide and deoxynucleotide catabolism in animals are diagrammed in Fig. 22-17. The pathways in other organisms differ somewhat, but all the pathways lead to uric acid. Of course, the pathway intermediates may be directed to purine nucleotide synthesis via salvage reactions. In addition, ribose-1-phosphate, a product of the reaction catalyzed by **purine nucleoside phosphorylase (PNP;** Fig. 22-18), is a precursor of PRPP.

Figure 22-18. **The X-ray structure of human erythrocyte purine nucleoside phosphorylase.** Each identical subunit is differently colored. The yellow subunit is shown in complex with a guanine molecule and two phosphate ions. [Courtesy of Mike Carson, University of Alabama at Birmingham; X-ray structure determined by Stephen Ealick and Charles Bugg, University of Alabama at Birmingham.] See the Interactive Exercises.

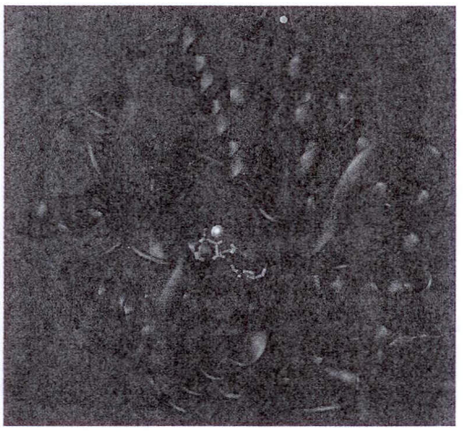


Figure 22-19. **A ribbon diagram of murine adenosine deaminase.** The enzyme is viewed approximately down the axis of its α/β barrel from the N-terminal ends of its β strands. A transition state analog, **6-hydroxyl-1,6-dihydropurine ribonucleoside (HDPR)**, is shown in skeletal form with its C, N, and O atoms green, blue, and red. The enzyme-bound Zn^{2+} ion, which is coordinated by HDPR's 6-hydroxyl group, is represented by a silver sphere. [Based on an X-ray structure by Florante Quiocho, Baylor College of Medicine.] **See the Interactive Exercises.**

Adenosine and deoxyadenosine are not degraded by mammalian PNP. Rather, adenine nucleosides and nucleotides are deaminated by **adenosine deaminase (ADA)** and **AMP deaminase** to their corresponding inosine derivatives, which can then be further degraded.

ADA is an eight-stranded α/β barrel (Fig. 22-19) with its active site in a pocket at the C-terminal end of the β barrel, as in all known α/β barrel enzymes. A catalytically essential zinc ion is bound in the deepest part of the active site pocket. Mutations that affect the active site of ADA cause immune system disorders (see Box 22-2).

The Purine Nucleotide Cycle

The deamination of AMP to IMP, when combined with the synthesis of AMP from IMP (Fig. 22-3, *left*), has the net effect of deaminating aspar-

Box 22-2

BIOCHEMISTRY IN HEALTH AND DISEASE

Severe Combined Immunodeficiency Disease

Abnormalities in purine nucleoside metabolism arising from rare genetic defects in adenosine deaminase selectively kill lymphocytes (a type of white blood cell). Since lymphocytes mediate much of the immune response, ADA deficiency results in **severe combined immunodeficiency disease (SCID)**. Without special protective measures, this disease is invariably fatal in infancy because of overwhelming infection. The mutations in all eight known ADA variants obtained from SCID patients appear to structurally perturb the active site of ADA.

Biochemical considerations provide a plausible explanation of SCID's etiology (causes). In the absence of active ADA, deoxyadenosine is phosphorylated to yield levels of dATP that are 50-fold greater than normal. This high concentration of dATP inhibits ribonucleotide reductase (Section 22-3A), thereby preventing the synthesis of the other dNTPs, choking off DNA synthesis and thus cell proliferation. The tissue-specific effect of ADA deficiency on the immune system can be explained by the observation that lymphoid tissue is particularly active in deoxyadenosine phosphorylation.

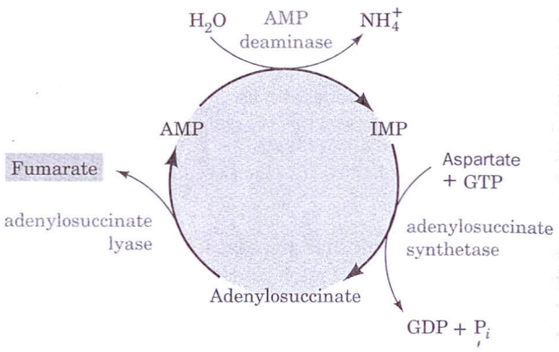
Immune system function in SCID patients can be boosted to a limited extent by injecting normal ADA to which several molecules of the biologically inert **polyethylene glycol (PEG)**

$$HO{-}(CH_2{-}CH_2O)_n{-}H$$

Polyethylene glycol

have been covalently linked. Without this modification, the liver clears ADA from the circulation within minutes, whereas PEG–ADA remains in the blood for 1–2 weeks. Evidently, the PEG masks the ADA from the receptors that otherwise filter it from the blood. Nevertheless, a more efficient treatment for ADA deficiency may be gene therapy (Section 3-5D). In this approach, lymphocytes are extracted from the blood of an ADA-deficient child and grown in the laboratory. Genetic engineering techniques are used to insert a normal ADA gene into the cells, which are then returned to the child. In one case, ADA-producing cells have persisted in the child for several years.

Other immune system defects result from a deficiency of purine nucleoside phosphorylase. This enzyme deficiency kills the so-called *T* lymphocytes but not the *B* lymphocytes and therefore causes an immunodeficiency syndrome of lesser severity than SCID (the *T* and *B* lymphocytes mediate different aspects of the immune response). This observation suggests that the selective inhibition of PNP may suppress the excess *T* lymphocyte activity associated with such autoimmune diseases as rheumatoid arthritis, psoriasis, and insulin-dependent diabetes and impede the growth of cancers such as *T* cell lymphomas and leukemias.



Net: H_2O + Aspartate + GTP → NH_4^+ + GDP + P_i + fumarate

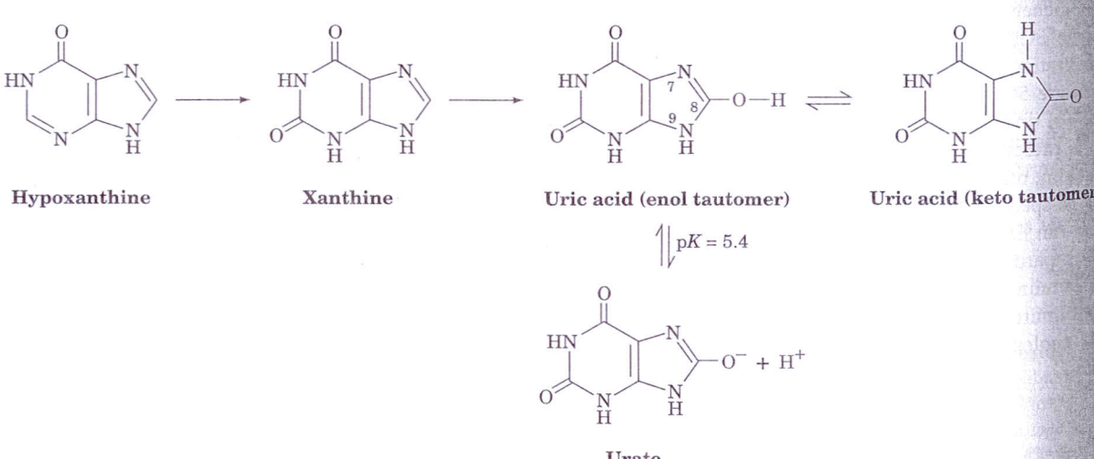
Figure 22-20. **The purine nucleotide cycle.** This pathway functions in muscle to prime the citric acid cycle by generating fumarate.

tate to yield fumarate (Fig. 22-20). John Lowenstein demonstrated that this **purine nucleotide cycle** has an important metabolic role in skeletal muscle. An increase in muscle activity requires an increase in the activity of the citric acid cycle. This process usually occurs through the generation of additional citric acid cycle intermediates (Section 16-5B). Muscles, however, lack most of the enzymes that catalyze these anaplerotic (filling up) reactions in other tissues. Instead, muscle replenishes its citric acid cycle intermediates with fumarate generated in the purine nucleotide cycle.

The importance of the purine nucleotide cycle in muscle metabolism is indicated by the observation that the activities of the three enzymes involved are all severalfold higher in muscle than in other tissues. In fact, individuals with an inherited deficiency in muscle AMP deaminase **(myoadenylate deaminase deficiency)** are easily fatigued and usually suffer from cramps after exercise.

Xanthine Oxidase Is a Mini-Electron-Transport System

Xanthine oxidase converts hypoxanthine (the base of IMP) to xanthine, and xanthine to uric acid (Fig. 22-17, *bottom*). The reaction product is an enol (which has a pK of 5.4; hence the name uric *acid*). The enol tautomerizes to the more stable keto form.



In mammals, xanthine oxidase occurs almost exclusively in the liver and the small intestinal mucosa. It is a dimeric protein of identical 130-kD subunits, each of which contains an entire "zoo" of electron-transfer agents: an FAD, a Mo complex that cycles between its Mo(VI) and Mo(IV) oxidation states, and two different Fe–S clusters. The final electron acceptor is O_2, which is converted to H_2O_2, a potentially harmful oxidizing agent (Section 17-5B) that is subsequently converted to H_2O and O_2 by catalase.

B. *Fate of Uric Acid*

In humans and other primates, the final product of purine degradation is uric acid, which is excreted in the urine. The same is true of birds, terrestrial reptiles, and many insects, but these organisms, which do not excrete urea, also catabolize their excess amino acid nitrogen to uric acid via purine biosynthesis. This complicated system of nitrogen excretion has a straightforward function: *It conserves water.* Uric acid is only sparingly soluble in water, so its excretion as a paste of uric acid crystals is accompanied by very little water. In contrast, the excretion of an equivalent amount of the much more water-soluble urea osmotically sequesters a significant amount of water.

In all other organisms, uric acid is further processed before excretion (Fig. 22-21). Mammals other than primates oxidize it to their excretory product, **allantoin,** in a reaction catalyzed by the Cu-containing enzyme **urate oxidase.** A further degradation product, **allantoic acid,** is excreted by teleost (bony) fish. Cartilaginous fish and amphibia further degrade allantoic acid to urea prior to excretion. Finally, marine invertebrates decompose urea to NH_4^+.

Gout Is Caused by an Excess of Uric Acid

Gout is a disease characterized by elevated levels of uric acid in body fluids. Its most common manifestation is excruciatingly painful arthritic joint inflammation of sudden onset, most often of the big toe (Fig. 22-22), caused by deposition of nearly insoluble crystals of sodium urate. Sodium urate and/or uric acid may also precipitate in the kidneys and ureters as stones, resulting in renal damage and urinary tract obstruction.

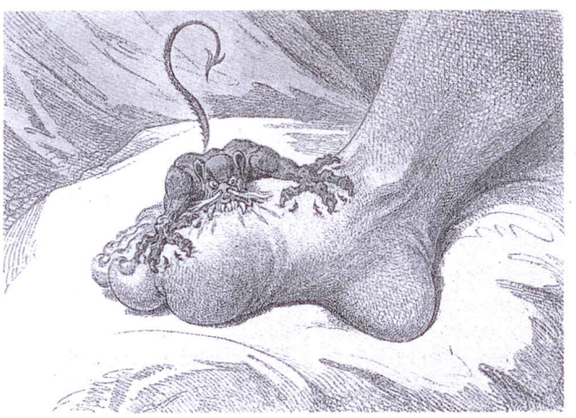


Figure 22-22. *The Gout*, a cartoon by James Gilroy (1799). [Courtesy of Yale University Medical Historical Library.]

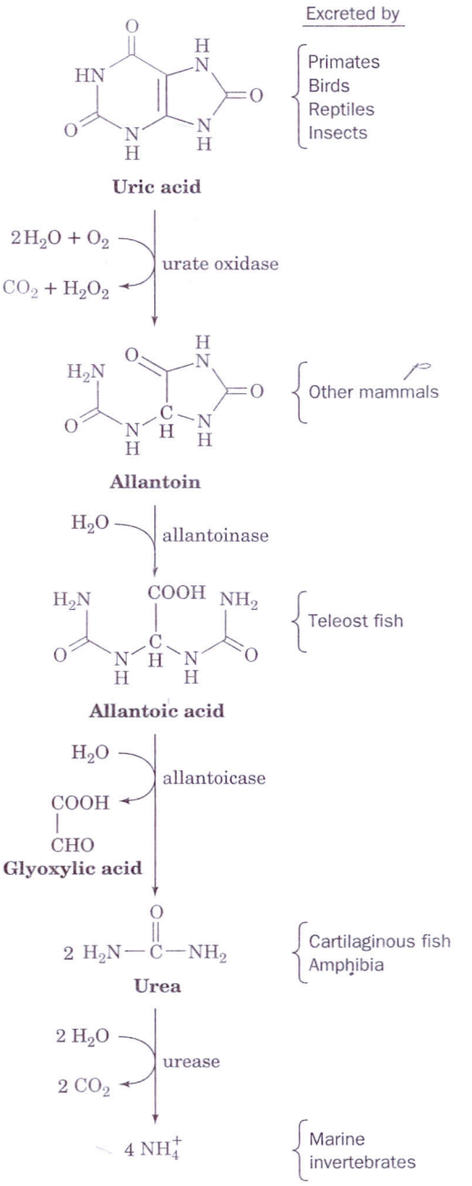
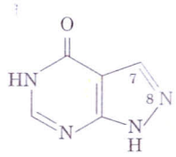


Figure 22-21. The degradation of uric acid to ammonia. The process is arrested at different stages in the indicated species, and the resulting nitrogen-containing product is excreted.

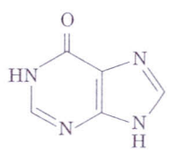
Gout, which affects ~3 per 1000 persons, predominantly males, has been traditionally, although inaccurately, associated with overindulgent eating and drinking. The probable origin of this association is that in previous centuries, when wine was often contaminated with lead during its manufacture and storage, heavy drinking resulted in chronic lead poisoning that, among other things, decreases the kidney's ability to excrete uric acid.

The most prevalent cause of gout is impaired uric acid excretion (although usually for reasons other than lead poisoning). Gout may also result from a number of metabolic insufficiencies, most of which are not well characterized. One well-understood cause is HGPRT deficiency (Lesch–Nyhan syndrome in severe cases), which leads to excessive uric acid production through PRPP accumulation (Section 22-1D).

Gout can be treated by administering the xanthine oxidase inhibitor **allopurinol,** a hypoxanthine analog with interchanged N7 and C8 positions.

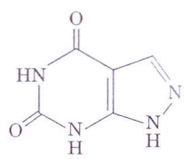


Allopurinol



Hypoxanthine

Xanthine oxidase hydroxylates allopurinol, as it does hypoxanthine, yielding **alloxanthine,**



Alloxanthine

which remains tightly bound to the reduced form of the enzyme, thereby inactivating it. Allopurinol consequently alleviates the symptoms of gout by decreasing the rate of uric acid production while increasing the levels of the more soluble hypoxanthine and xanthine. Although allopurinol controls the gouty symptoms of Lesch–Nyhan syndrome, it has no effect on its neurological symptoms.

C. Catabolism of Pyrimidines

Animal cells degrade pyrimidine nucleotides to their component bases (Fig. 22-23, *top*). These reactions, like those of purine nucleotides, occur through dephosphorylation, deamination, and glycosidic bond cleavages. The resulting uracil and thymine are then broken down in the liver through reduction (Fig. 22-23, *middle*) rather than by oxidation as occurs in purine catabolism. The end products of pyrimidine catabolism, **β-alanine** and **β-aminoisobutyrate,** are amino acids and are metabolized as such. They are converted, through transamination and activation reactions, to malonyl-CoA and methylmalonyl-CoA (Fig. 22-23, *bottom left*). Malonyl-CoA is a precursor of fatty acid synthesis (Fig. 19-22), and methylmalonyl-CoA is converted to the citric acid cycle intermediate succinyl-CoA (Fig. 19-13). Thus, *to a limited extent, catabolism of pyrimidine nucleotides contributes to the energy metabolism of the cell.*