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22: Nucleotide Metabolism

Overview



Ribonucleoside and deoxyribonucleoside phosphates (nucleotides) are essential for all cells. Without them, neither ribonucleic acid (RNA) nor deoxyribonucleic acid (DNA) can be produced, and, therefore, proteins cannot be synthesized nor can cells proliferate. Nucleotides also serve as carriers of activated intermediates in the synthesis of some carbohydrates, lipids, and conjugated proteins (e.g., uridine diphosphate [UDP]-glucose and cytidine diphosphate [CDP]-choline) and are structural components of several essential coenzymes, such as coenzyme A, flavin adenine dinucleotide (FAD[H₂]), nicotinamide adenine dinucleotide (NAD[H]), and nicotinamide adenine dinucleotide phosphate (NADP[H]). Nucleotides, such as cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), serve as second messengers in signal transduction pathways. In addition, nucleotides play an important role as energy sources in the cell. Finally, nucleotides are important regulatory compounds for many of the pathways of intermediary metabolism, inhibiting or activating key enzymes. The purine and pyrimidine bases found in nucleotides can be synthesized *de novo* or can be obtained through salvage pathways that allow the reuse of the preformed bases resulting from normal cell turnover. (Note: Few of the purines and pyrimidines supplied by diet are utilized; instead, nearly all of the nucleic acids that enter the gastrointestinal [GI] tract are degraded.)

Structure

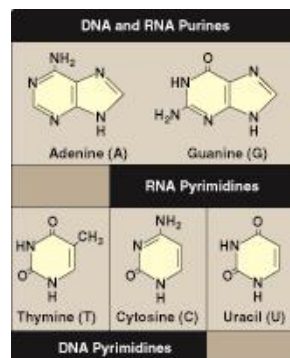


Nucleotides are composed of a nitrogenous base; a pentose monosaccharide; and one, two, or three phosphate groups. The nitrogen-containing bases belong to two families of compounds: the purines and the pyrimidines.

Purine and pyrimidine bases

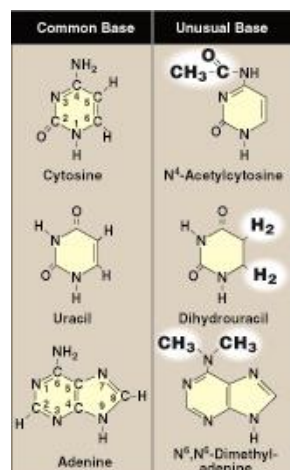
Purines are double-ringed structures, whereas pyrimidines have a single ring. Both DNA and RNA contain the same purine bases: adenine (A) and guanine (G). Both DNA and RNA contain the pyrimidine cytosine (C), but they differ in their second pyrimidine base: DNA contains thymine (T), whereas RNA contains uracil (U). T and U differ in that only T has a methyl group (Fig. 22.1). Unusual (modified) bases are occasionally found in some species of DNA (e.g., in some viral DNA) and RNA (e.g., in transfer RNA [tRNA]). Base modifications include methylation, glycosylation, acetylation, and reduction. Some examples of unusual bases are shown in Figure 22.2. (Note: The presence of an unusual base in a nucleotide sequence may aid in its recognition by specific enzymes or protect it from being degraded by nucleases.)

FIGURE 22.1



ines commonly found in DNA and RNA.

FIGURE 22.2

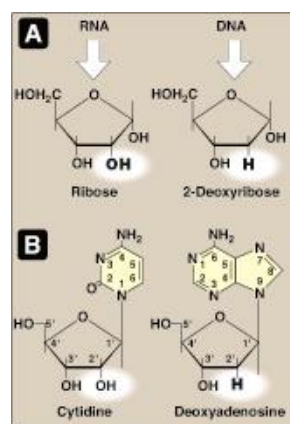


l bases.

Nucleosides

The addition of a pentose sugar to a base through an N-glycosidic bond (see p. 94) produces a nucleoside. If the sugar is ribose, a ribonucleoside is produced, and if the sugar is 2-deoxyribose, a deoxyribonucleoside is produced (Fig. 22.3A). The ribonucleosides of A, G, C, and U are named adenosine, guanosine, cytidine, and uridine, respectively. The deoxyribonucleosides of A, G, C, and T have the added prefix deoxy- (e.g., deoxyadenosine). (Note: The compound deoxythymidine is often simply called thymidine, with the deoxy-prefix being understood, because it is incorporated into DNA only.) The carbon and nitrogen atoms in the rings of the base and the sugar are numbered separately (see Fig. 22.3B). (Note: Carbons in the pentose are numbered 1' to 5'. Thus, when the 5'-carbon of a nucleoside [or nucleotide] is referred to, a carbon atom in the pentose, rather than an atom in the base, is being specified.)

FIGURE 22.3

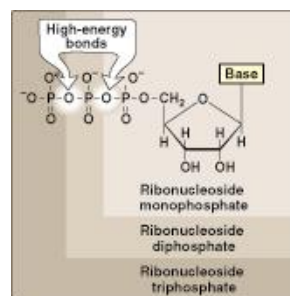


nucleic acids. **B:** Examples of the numbering systems for purine- and pyrimidine- nucleosides.

Nucleotides

The addition of one or more phosphate groups to a nucleoside produces a nucleotide. The first phosphate group is attached by an ester linkage to the 5'-OH of the pentose, forming a nucleoside 5'-phosphate or a 5'-nucleotide. The type of pentose is denoted by the prefix in the names 5'-ribonucleotide and 5'-deoxyribonucleotide. If one phosphate group is attached to the 5'-carbon of the pentose, the structure is a nucleoside monophosphate, like adenosine monophosphate (AMP, or adenylate). If a second or third phosphate is added to the nucleoside, a nucleoside diphosphate (e.g., adenosine diphosphate [ADP] or triphosphate, e.g., ATP) results (Fig. 22.4). The second and third phosphates are each connected to the nucleotide by a “high-energy bond” (a bond with a large, negative change in free energy [$-\Delta G$, see p. 78] of hydrolysis). (Note: The phosphate groups are responsible for the negative charges associated with nucleotides and cause DNA and RNA to be referred to as nucleic acids.)

FIGURE 22.4



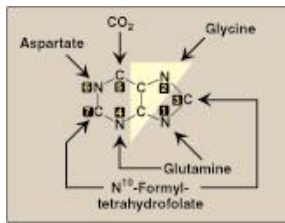
monophosphate, diphosphate, and triphosphate.

Purine Nucleotide Synthesis



The atoms of the purine ring are contributed by a number of compounds, including amino acids (aspartate, glycine, and glutamine), carbon dioxide (CO_2), and N^{10} -formyltetrahydrofolate (N^{10} -formyl-THF), as shown in Figure 22.5. The purine ring is constructed primarily in the liver by a series of reactions that add the donated carbons and nitrogens to a preformed ribose 5-phosphate. (Note: Synthesis of ribose 5-phosphate from glucose 6-phosphate by the pentose phosphate pathway is discussed on p. 162.)

FIGURE 22.5



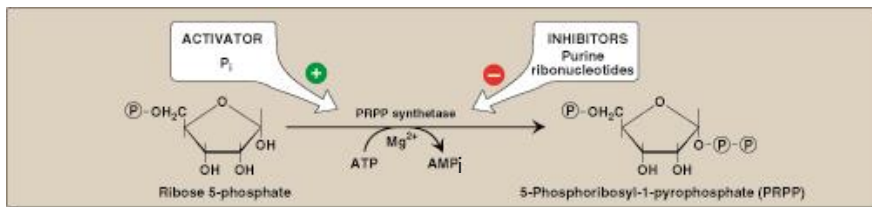
individual atoms in the purine ring.

atoms are added is shown by the numbers in the black boxes (see Fig. 22.7). $\text{CO}_2 =$

5-Phosphoribosyl-1-pyrophosphate synthesis

5-Phosphoribosyl-1-pyrophosphate (PRPP) is an activated pentose that participates in the synthesis and salvage of purines and pyrimidines. Synthesis of PRPP from ATP and ribose 5-phosphate is catalyzed by PRPP synthetase (Fig. 22.6). This X-linked enzyme is activated by inorganic phosphate and inhibited by purine nucleotides (end product inhibition). (Note: Because the sugar moiety of PRPP is ribose, ribonucleotides are the end products of *de novo* purine synthesis. When deoxyribonucleotides are required for DNA synthesis, the ribose sugar moiety is reduced [see p. 330].)

FIGURE 22.6



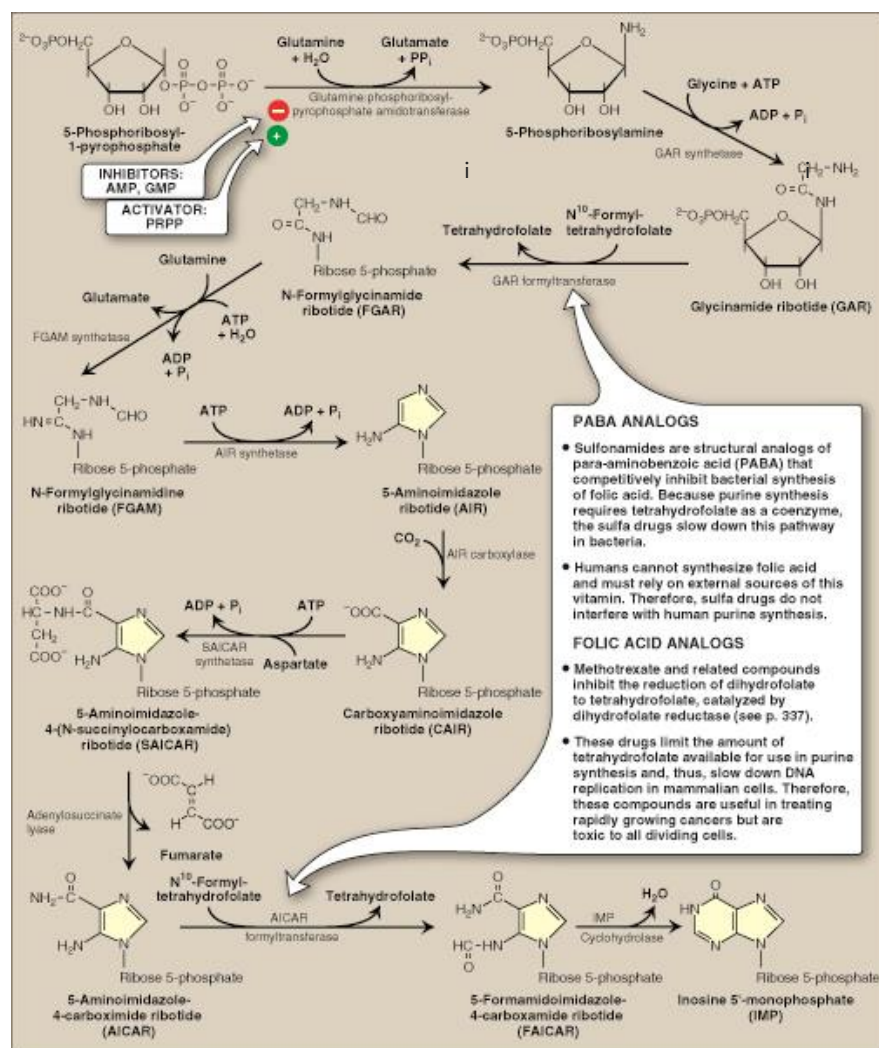
reaction.

PRPP is used in other pathways such as adenosine monophosphate; $\text{Mg} =$

5-Phosphoribosylamine synthesis

Synthesis of 5-phosphoribosylamine from PRPP and glutamine is shown in Figure 22.7. The amide group of glutamine replaces the pyrophosphate group attached to carbon 1 of PRPP. This is the committed step in purine nucleotide biosynthesis. The enzyme that catalyzes the reaction, glutamine:phosphoribosylpyrophosphate amidotransferase (GPAT), is inhibited by the purine 5'-nucleotides AMP and guanosine monophosphate (GMP, or guanylate), the end products of the pathway. The rate of the reaction is also controlled by the intracellular concentration of PRPP. (Note: The concentration of PRPP is normally far below the Michaelis constant [K_m] for the GPAT. Therefore, any small change in the PRPP concentration causes a proportional change in rate of the reaction [see p. 63].)

FIGURE 22.7



ery effect of some structural analogs.

monophosphate; PRPP = 5-phosphoribosyl-1-pyrophosphate; CO₂ = carbon dioxide.

Inosine monophosphate synthesis

The next nine steps in purine nucleotide biosynthesis leading to the synthesis of inosine monophosphate ([IMP] whose base is hypoxanthine) are illustrated in Figure 22.7. IMP is the parent purine nucleotide for AMP and GMP. Four steps in this pathway require ATP as an energy source, and two steps in the pathway require N¹⁰-formyl-THF as a one-carbon donor (see p. 296). (Note: Hypoxanthine is found in tRNA [see Fig. 32.9 on p. 503].)

Synthetic inhibitors

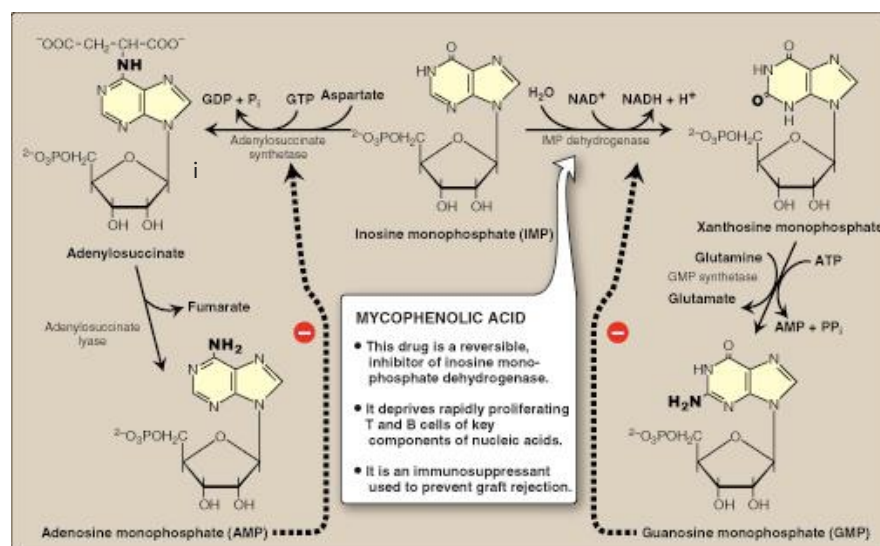
Some synthetic inhibitors of purine synthesis (e.g., the sulfonamides) are designed to inhibit the growth of rapidly dividing microorganisms without interfering with human cell functions (see Fig. 22.7). Other purine synthesis inhibitors, such as structural analogs of folic acid (e.g., methotrexate), are used pharmacologically to control the spread of cancer by interfering with the synthesis of nucleotides and, therefore, of DNA and RNA (see Fig. 22.7).

Inhibitors of human purine synthesis are extremely toxic to tissues, especially to developing structures such as those in a fetus, or to cell types that normally replicate rapidly, including those of bone marrow, skin, GI tract, immune system, or hair follicles. As a result, individuals taking such anticancer drugs can experience adverse effects, including anemia, scaly skin, GI tract disturbance, immunodeficiency, and hair loss.

Adenosine and guanosine monophosphate synthesis

The conversion of IMP to either AMP or GMP uses a two-step, energy- and nitrogen-requiring pathway (Fig. 22.8). (Note: AMP synthesis requires guanosine triphosphate [GTP] as an energy source and aspartate as a nitrogen source, whereas GMP synthesis requires ATP and glutamine.) Also, the first reaction in each pathway is inhibited by the end product of that pathway. This provides a mechanism for diverting IMP to the synthesis of the purine present in lesser amounts. If both AMP and GMP are present in adequate amounts, the *de novo* pathway of purine nucleotide synthesis is inhibited at the GPAT step.

FIGURE 22.8



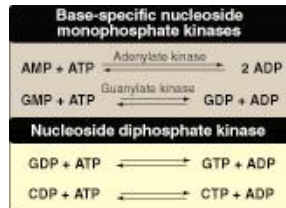
te) showing feedback inhibition. e di- and triphosphates; P_i = inorganic

Mycophenolic acid is a reversible inhibitor of IMP dehydrogenase, the enzyme used to generate GMP. Proliferating T and B lymphocytes are highly susceptible to low levels of this key purine nucleotide, so mycophenolic acid is an effective immunosuppressant agent to prevent organ transplant rejection (kidney, heart, and liver), as well as to treat certain immune disorders such as lupus or Crohn disease.

Nucleoside di- and triphosphate synthesis

Nucleoside diphosphates are synthesized from the corresponding nucleoside monophosphates by base-specific nucleoside monophosphate kinases (Fig. 22.9). (Note: These kinases do not discriminate between ribose and deoxyribose in the substrate.) ATP is generally the source of the transferred phosphate because it is present in higher concentrations than the other nucleoside triphosphates. Adenylate kinase is particularly active in the liver and in muscle, where the turnover of energy from ATP is high. Its function is to maintain equilibrium among the adenine nucleotides (AMP, ADP, and ATP). Nucleoside diphosphates and triphosphates are interconverted by nucleoside diphosphate kinase, an enzyme that, unlike the monophosphate kinases, has broad substrate specificity.

FIGURE 22.9



Base-specific nucleoside monophosphates to di- and triphosphates.

Adenine mono- and diphosphates; GMP, GDP, and GTP = guanosine mono-, di-, and triphosphates; CDP and CTP = cytidine di- and triphosphates.

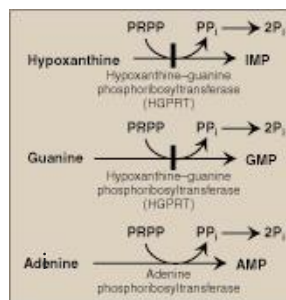
Purine salvage pathway

Purines that result from the normal turnover of cellular nucleic acids, or the small amount that is obtained from the diet and not degraded, can be converted to nucleoside triphosphates and used by the body. This is referred to as the salvage pathway for purines. (Note: Salvage is particularly important in the brain.)

Purine base salvage to nucleotides

Two enzymes are involved: adenine phosphoribosyltransferase (APRT) and X-linked hypoxanthine–guanine phosphoribosyltransferase (HGPRT). Both use PRPP as the source of the ribose 5-phosphate group (Fig. 22.10). The release of pyrophosphate and its subsequent hydrolysis by pyrophosphatase makes these reactions irreversible. (Note: Adenosine is the only purine nucleoside to be salvaged. It is phosphorylated to AMP by adenosine kinase.)

FIGURE 22.10



Purine nucleotide synthesis.

Complete deficiency of HGPRT results in Lesch–Nyhan syndrome. Partial deficiencies of HGPRT (as the amount of functional enzyme increases, the severity of the symptoms decreases.) IMP, GMP, and AMP = inosine, guanosine, and adenosine monophosphates; PRPP = 5-phosphoribosyl-1- pyrophosphate;

Lesch–Nyhan syndrome

This is a rare, X-linked recessive disorder associated with a virtually complete deficiency of HGPRT. The deficiency results in an inability to salvage hypoxanthine or G, from which excessive amounts of uric acid, the end product of purine degradation, are then produced (see p. 331). In addition, the lack of this salvage pathway causes increased PRPP levels and decreased IMP and GMP levels. As a result, GPAT (the regulated step in purine synthesis) has excess substrate and decreased inhibitors available, and *de novo* purine synthesis is increased. The combination of decreased purine reutilization and increased purine synthesis results in increased degradation of purines and the production of large amounts of uric acid, making HGPRT deficiency an inherited cause of hyperuricemia. In patients with Lesch–Nyhan syndrome, the hyperuricemia frequently results in the formation of uric acid stones in the kidneys (urolithiasis) and the deposition of urate crystals in the joints (gouty arthritis) and soft tissues. In addition, the syndrome is characterized by motor dysfunction, cognitive deficits, and behavioral disturbances that include self-mutilation (e.g., biting of lips and fingers), as shown in Figure 22.11.

FIGURE 22.11



of a patient with Lesch–Nyhan syndrome.

Deoxyribonucleotide Synthesis



The nucleotides described thus far all contain ribose (ribonucleotides). DNA synthesis, however, requires 2'-deoxyribonucleotides, which are produced from ribonucleoside diphosphates by the enzyme ribonucleotide reductase during the S-phase of the cell cycle (see p. 471). (Note: The same enzyme acts on pyrimidine ribonucleotides.)

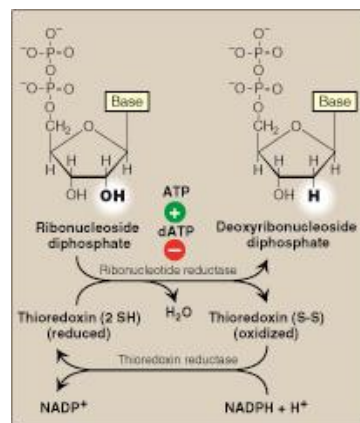
Ribonucleotide reductase

Ribonucleotide reductase (ribonucleoside diphosphate reductase) is a dimer composed of two nonidentical subunits, R1 (or, α) and the smaller R2 (or, β), and is specific for the reduction of purine nucleoside diphosphates (ADP and GDP) and pyrimidine nucleoside diphosphates (CDP and UDP) to their deoxy forms (dADP, dGDP, dCDP, and dUDP). The immediate donors of the hydrogen atoms needed for the reduction of the 2'-hydroxyl group are two sulfhydryl (–SH) groups on the enzyme itself (R1 subunit), which form a disulfide bond during the reaction (see p. 19). (Note: R2 contains the stable tyrosyl radical required for catalysis at R1.)

Reduced enzyme regeneration

In order for ribonucleotide reductase to continue to produce deoxyribonucleotides at R1, the disulfide bond created during the production of the 2'-deoxy carbon must be reduced. The source of the reducing equivalents is thioredoxin, a protein coenzyme of ribonucleotide reductase. Thioredoxin contains two cysteine residues separated by two amino acids in the peptide chain. The two –SH groups of thioredoxin donate their hydrogen atoms to ribonucleotide reductase, forming a disulfide bond in the process (Fig. 22.12).

FIGURE 22.12



ribonucleotides to deoxyribonucleotides.

ATP = adenosine triphosphate; dATP = deoxyadenosine triphosphate.

Reduced thioredoxin regeneration

Thioredoxin must be converted back to its reduced form in order to continue performing its function. The reducing equivalents are provided by NADPH + H⁺, and the reaction is catalyzed by thioredoxin reductase, a selenoprotein (see p. 297).

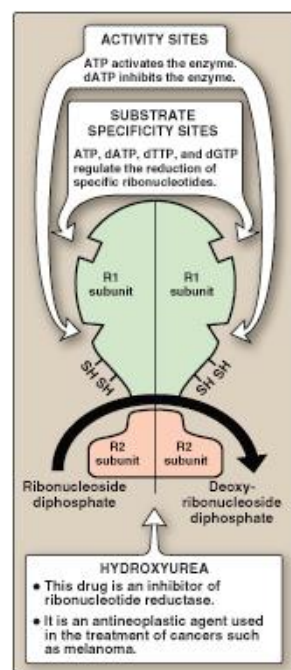
Deoxyribonucleotide synthesis regulation

Ribonucleotide reductase is responsible for maintaining a balanced supply of the deoxyribonucleotides required for DNA synthesis. Consequently, the regulation of the enzyme is complex. In addition to the catalytic site, R1 contains two distinct allosteric sites involved in regulating enzymic activity (Fig. 22.13).

Activity sites

The binding of deoxyadenosine triphosphate (dATP) to allosteric sites (known as activity sites) on R1 inhibits the overall catalytic activity of the enzyme and, therefore, prevents the reduction of any of the four nucleoside diphosphates. This effectively prevents DNA synthesis and explains the toxicity of increased levels of dATP seen in conditions such as adenosine deaminase (ADA) deficiency (see p. 334). In contrast, ATP bound to these sites activates the enzyme.

FIGURE 22.13



cleotide reductase.

is also referred to as α and the R2 as β .) dATP, dTTP, and dGTP = deoxyadenosine, deoxythymidine, and deoxyguanosine triphosphates.

Substrate specificity sites

The binding of nucleoside triphosphates to additional allosteric sites (known as substrate specificity sites) on R1 regulates substrate specificity, causing an increase in the conversion of different species of ribonucleotides to deoxyribonucleotides as they are required for DNA synthesis. For example, deoxythymidine triphosphate binding at the specificity site causes a conformational change that allows reduction of GDP to dGDP at the catalytic site when ATP is at the activity site.

CLINICAL APPLICATION 22.1

Hydroxyurea

The drug hydroxyurea (hydroxycarbamide) inhibits ribonucleotide reductase, thereby inhibiting the generation of substrates for DNA synthesis. The drug is an antineoplastic agent and is used in the treatment of cancers such as melanoma. Hydroxyurea is also used in the treatment of sickle cell anemia (see p. 38). However, the increase in fetal hemoglobin seen with hydroxyurea is because of changes in gene expression and not to ribonucleotide reductase inhibition.

Purine Nucleotide Degradation

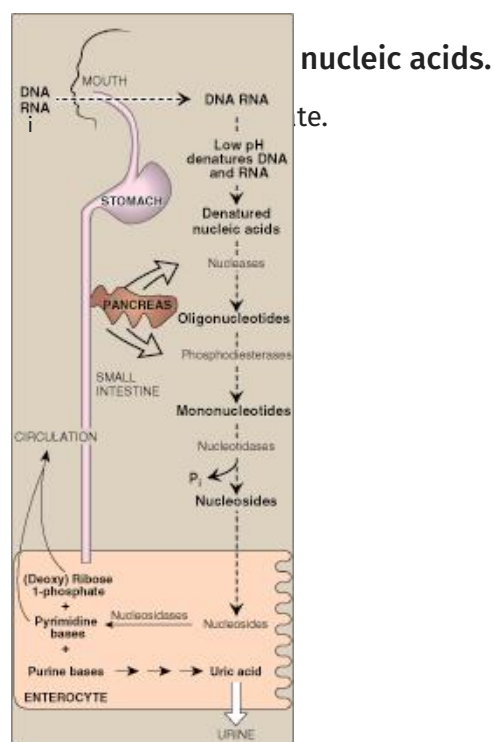


Degradation of dietary nucleic acids occurs in the small intestine, where pancreatic nucleases hydrolyze them to nucleotides. The nucleotides are sequentially degraded by intestinal enzymes to nucleosides, phosphorylated sugars, and free bases. Uric acid is the end product of intestinal purine degradation. (Note: Purine nucleotides from *de novo* synthesis are degraded in the liver primarily. The free bases are sent out from the liver and salvaged by peripheral tissues.)

Degradation in the small intestine

Ribonucleases and deoxyribonucleases, secreted by the pancreas, hydrolyze dietary RNA and DNA to oligonucleotides that are further hydrolyzed by pancreatic phosphodiesterases, producing a mixture of 3'- and 5'-mononucleotides. At the intestinal mucosal surface, nucleotidases remove the phosphate groups hydrolytically, releasing nucleosides that are taken into enterocytes by sodium-dependent transporters and degraded by nucleosidases (nucleoside phosphorylases) to free bases plus (deoxy) ribose 1-phosphate. Dietary purine bases are not used to any appreciable extent for the synthesis of tissue nucleic acids. Instead, they are degraded to uric acid in the enterocytes. Most of the uric acid enters the blood and is eventually excreted in the urine. A summary of this pathway is shown in [Figure 22.14](#). (Note: Mammals other than primates express urate oxidase [uricase], which cleaves the purine ring, generating allantoin. Modified recombinant urate oxidase is now used clinically to lower urate levels.)

FIGURE 22.14

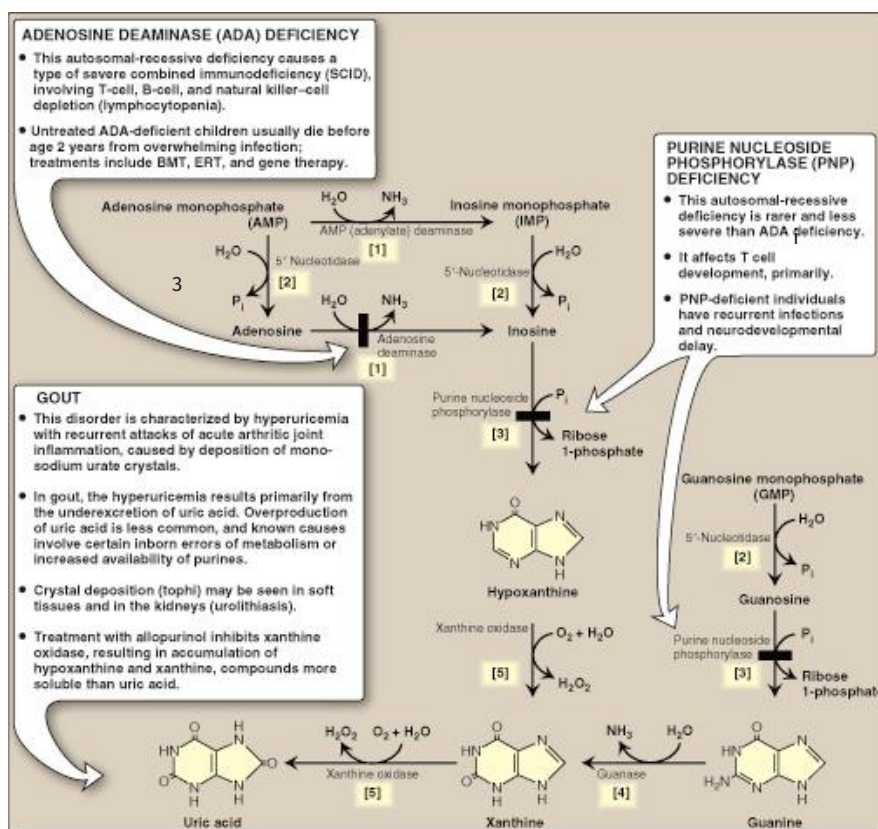


Uric acid formation

A summary of the steps in the production of uric acid and the genetic diseases associated with deficiencies of specific degradative enzymes are shown in [Figure 22.15](#). (Note: The bracketed numbers refer to specific reactions in the figure.)

1. An amino group is removed from AMP to produce IMP by AMP (adenylate) deaminase or from adenosine to produce inosine (hypoxanthine-ribose) by ADA.
2. IMP and GMP are converted into their respective nucleoside forms, inosine and guanosine, by the action of 5'-nucleotidase.
3. Purine nucleoside phosphorylase converts inosine and guanosine into their respective purine bases, hypoxanthine and G. (Note: A mutase interconverts ribose 1- and ribose 5-phosphate.)
4. G is deaminated to form xanthine.
5. Hypoxanthine is oxidized by molybdenum-containing xanthine oxidase (XO) to xanthine, which is further oxidized by XO to uric acid, the final product of human purine degradation. Uric acid is excreted primarily in the urine.

FIGURE 22.15



g some of the genetic diseases
 citations in the text.) BMT = bone
 organic phosphate; H₂O₂ = hydrogen

Diseases associated with purine degradation

Gout

Gout is a disorder initiated by high levels of uric acid (the end product of purine catabolism) in blood (hyperuricemia), as a result of either the overproduction or underexcretion of uric acid. The hyperuricemia can lead to the deposition of monosodium urate (MSU) crystals in the joints and an inflammatory response to the crystals, causing first acute and then progressing to chronic gouty arthritis. Nodular masses of MSU crystals (tophi) may be deposited in the soft tissues, resulting in chronic tophaceous gout (Fig. 22.16). Formation of uric acid stones in the kidney (urolithiasis) may also be seen. (Note: Hyperuricemia is not sufficient to cause gout, but gout is always preceded by hyperuricemia. Hyperuricemia is typically asymptomatic but may be indicative of comorbid conditions such as hypertension.) The definitive diagnosis of gout requires aspiration and examination of synovial fluid (Fig. 22.17) from an affected joint (or material from a tophus) using polarized light microscopy to confirm the presence of needle-shaped MSU crystals (Fig. 22.18).

Uric acid underexcretion

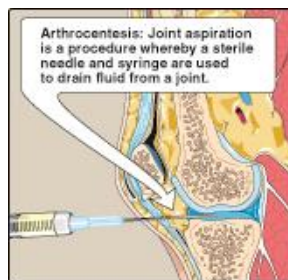
In >90% of individuals with hyperuricemia, the cause is underexcretion of uric acid. Underexcretion can be primary, because of as-yet-unidentified inherent excretory defects, or secondary to known disease processes that affect how the kidney handles urate (e.g., in lactic acidosis, lactate increases renal urate reabsorption, thereby decreasing its excretion) and to environmental factors such as the use of drugs (e.g., thiazide diuretics) or exposure to lead (saturnine gout).

FIGURE 22.16



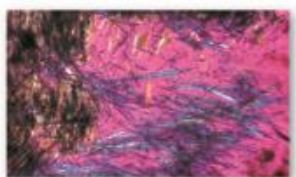
From Rubin E, Reiser HM. Principles of Rubin's Pathology. 7th ed. Wolters Kluwer; 2019, Figure 22-43D.

FIGURE 22.17



Arthrocentesis can help to define causes of joint swelling and arthritis, such as infection, autoimmune disease.

FIGURE 22.18



Identified by the presence of negatively birefringent monosodium urate crystals in synovial fluid examined by polarized light microscopy.

Often associated with polymorphonuclear leukocytes.

From Rubin E, Reiser HM. Principles of Rubin's Pathology. 7th ed. Wolters Kluwer; 2019, Figure 22-43D.

Uric acid overproduction

A less common cause of hyperuricemia is from the overproduction of uric acid. Primary hyperuricemia is, for the most part, idiopathic (having no known cause). However, several identified mutations in the gene for X-linked PRPP synthetase result in the enzyme having an increased maximal velocity ($[V_{max}]$, see p. 61) for the production of PRPP, a lower K_m (see p. 63) for ribose 5-phosphate, or a decreased sensitivity to purine nucleotides, its allosteric inhibitors (see p. 66). In each case, increased availability of PRPP increases purine production, resulting in elevated levels of plasma uric acid. Lesch–Nyhan syndrome (see p. 329) also causes hyperuricemia as a result of the decreased salvage of hypoxanthine and G and the subsequent increased availability of PRPP. Secondary hyperuricemia is typically the consequence of increased availability of purines (e.g., in patients with myeloproliferative disorders or who are undergoing chemotherapy and so have a high rate of cell turnover). Hyperuricemia can also be the result of seemingly unrelated metabolic diseases, such as von Gierke disease (see [Fig. 11.8](#) on p. 141) or hereditary fructose intolerance (see p. 152).

A diet rich in meat, seafood (particularly shellfish), and ethanol is associated with increased risk of gout, whereas a diet rich in low-fat dairy products is associated with a decreased risk.

Treatment

Acute attacks of gout are treated with anti-inflammatory agents. Colchicine, steroidal drugs such as prednisone, and nonsteroidal drugs such as indomethacin are used. (Note: Colchicine prevents formation of microtubules, thereby decreasing the movement of neutrophils into the affected area. Like the other anti-inflammatory drugs, it has no effect on uric acid levels.) Long-term therapeutic strategies for gout involve lowering the uric acid level below its saturation point (6.5 mg/dl), thereby preventing the deposition of MSU crystals. Uricosuric agents, such as probenecid or sulfinpyrazone, that increase renal excretion of uric acid, are used in patients who are underexcretors of uric acid. Allopurinol, a structural analog of hypoxanthine, inhibits uric acid synthesis and is used in patients who are overproducers of uric acid. Allopurinol is oxidized to oxypurinol, a long-lived inhibitor of XO. This results in an accumulation of hypoxanthine and xanthine (see [Fig. 22.15](#)), compounds more soluble than uric acid and, therefore, less likely to initiate an inflammatory response. In patients with normal levels of HGPRT, the hypoxanthine can be salvaged, reducing the levels of PRPP and, therefore, *de novo* purine synthesis. Febuxostat, a nonpurine inhibitor of XO, is also available. (Note: Uric acid levels in the blood normally are close to the saturation point. One reason for this may be the strong antioxidant effects of uric acid.)

Adenosine deaminase deficiency

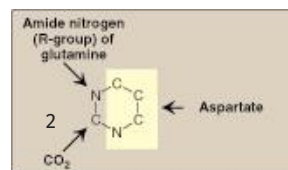
ADA is expressed in a variety of tissues, but, in humans, lymphocytes have the highest activity of this cytoplasmic enzyme. A deficiency of ADA results in an accumulation of adenosine, which is converted to its ribonucleotide or deoxyribonucleotide forms by cellular kinases. As dATP levels rise, ribonucleotide reductase is inhibited, thereby preventing the production of all deoxyribose-containing nucleotides (see p. 330). Consequently, cells cannot make DNA and divide. (Note: The dATP and adenosine that accumulate in ADA deficiency lead to developmental arrest and apoptosis of lymphocytes.) In its most severe form, this autosomal-recessive disorder causes a type of severe combined immunodeficiency disease (SCID), involving a decrease in T cells, B cells, and natural killer cells. ADA deficiency accounts for ~14% of cases of SCID in the United States. Treatments include bone marrow transplantation, enzyme replacement therapy, and gene therapy (see p. 552). Without appropriate treatment, children with this disorder usually die from infection by age 2 years. (Note: Purine nucleoside phosphorylase deficiency results in a less severe immunodeficiency primarily involving T cells.)

Pyrimidine Synthesis and Degradation



Unlike the synthesis of the purine ring, which is constructed on a pre-existing ribose 5-phosphate, the pyrimidine ring is synthesized before being attached to ribose 5-phosphate, which is donated by PRPP. The sources of the atoms in the pyrimidine ring are glutamine, CO_2 , and aspartate (Fig. 22.19).

FIGURE 22.19



Individual atoms in the pyrimidine ring.

Carbamoyl phosphate synthesis

The regulated step of this pathway in mammalian cells is the synthesis of carbamoyl phosphate from glutamine and CO_2 , catalyzed by carbamoyl phosphate synthetase (CPS) II. CPS II is inhibited by uridine triphosphate (UTP, the end product of this pathway, which can be converted into the other pyrimidine nucleotides) and is activated by PRPP. (Note: Carbamoyl phosphate, synthesized by CPS I, is also a precursor of urea [see p. 281]. Defects in ornithine transcarbamylase (OTC) of the urea cycle promote pyrimidine synthesis because of increased availability of carbamoyl phosphate. A comparison of the two enzymes is presented in Figure 22.20.)

FIGURE 22.20

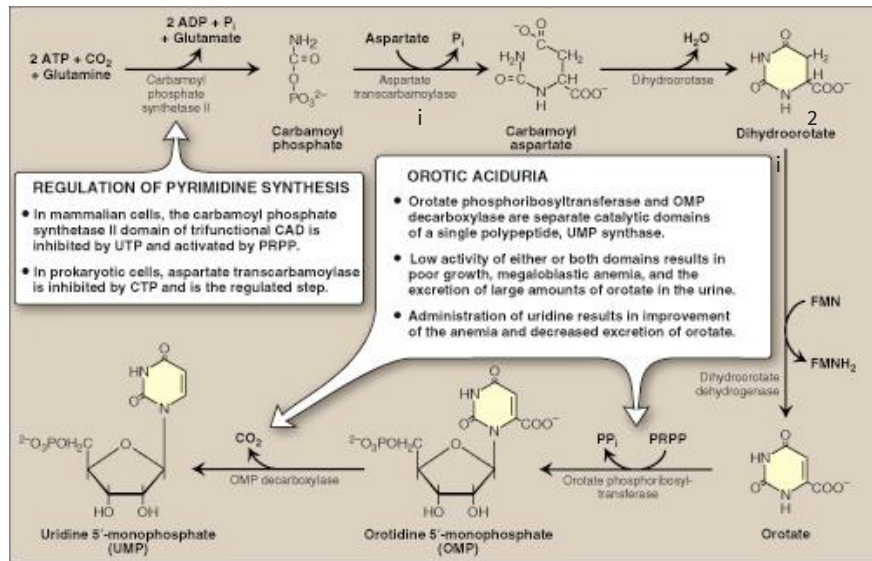
Variable	CPS I	CPS II
Cellular location	Mitochondria	Cytosol
Pathway involved	Urea cycle	Pyrimidine synthesis
Source of nitrogen	Ammonia	γ -Amide group of glutamine
Regulators	Activator: N-acetylglutamate	Activator: PRPP Inhibitor: UTP

Differences between carbamoyl phosphate synthetase (CPS) I and II.
 Carbamoyl phosphate synthetase I (CPS I) is involved in the urea cycle, while CPS II is involved in pyrimidine synthesis. CPS I uses ammonia as the source of nitrogen, while CPS II uses the γ -amide group of glutamine. CPS I is activated by N-acetylglutamate, while CPS II is activated by PRPP and inhibited by UTP.

Orotic acid synthesis

The second step in pyrimidine synthesis is the formation of carbamoylaspartate, catalyzed by aspartate transcarbamoylase. The pyrimidine ring is then closed by dihydroorotase. The resulting dihydroorotate is oxidized to produce orotic acid (orotate), as shown in Figure 22.21. Flavin mononucleotide (FMN) is reduced in this reaction.

FIGURE 22.21



Flavin mononucleotide; CTP = cytidine triphosphate.

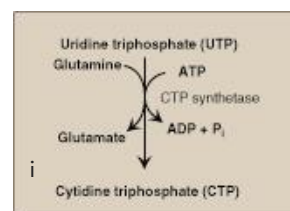
Pyrimidine nucleotide synthesis

The completed pyrimidine ring is converted to the nucleotide orotidine monophosphate (OMP) in the second stage of pyrimidine nucleotide synthesis (see Fig. 22.21). As seen with the purines, PRPP is the ribose 5-phosphate donor. The enzyme orotate phosphoribosyltransferase produces OMP and releases pyrophosphate, thereby making the reaction biologically irreversible. (Note: Both purine and pyrimidine synthesis require glutamine, aspartic acid, and PRPP as essential precursors.) OMP (orotidylate) is decarboxylated to uridine monophosphate (UMP) by orotidylate decarboxylase. The phosphoribosyltransferase and decarboxylase activities are separate catalytic domains of a single polypeptide called UMP synthase. Hereditary orotic aciduria (a very rare disorder) may be caused by a deficiency of one or both activities of this bifunctional enzyme, resulting in orotic acid in the urine (see Fig. 22.21). Since the first reaction of pyrimidine biosynthesis is feedback inhibited by UTP, hereditary orotic aciduria and its associated anemia is treated with uridine. Recall that a deficiency of OTC in the urea cycle would present with elevated urinary levels of orotate (see p. 535). This is because the carbamoyl phosphate substrate of OTC is funneled instead into pyrimidine synthesis. UMP is sequentially phosphorylated to UDP and UTP. (Note: The UDP is a substrate for ribonucleotide reductase, which generates dUDP. The dUDP is phosphorylated to dUTP, which is rapidly hydrolyzed to dUMP by UTP diphosphatase [dUTPase]. Thus, dUTPase plays an important role in reducing availability of dUTP as a substrate for DNA synthesis, thereby preventing erroneous incorporation of U into DNA.)

Cytidine triphosphate synthesis

Cytidine triphosphate (CTP) is produced by amination of UTP by CTP synthetase (Fig. 22.22), with glutamine providing the nitrogen. Some of this CTP is dephosphorylated to CDP, which is a substrate for ribonucleotide reductase. The dCDP product can be phosphorylated to dCTP for DNA synthesis or dephosphorylated to dCMP that is deaminated to dUMP.

FIGURE 22.22



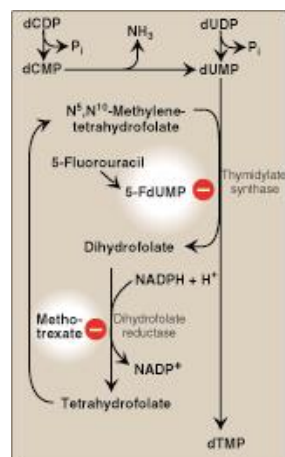
from UTP.

For RNA synthesis, is converted to dCTP for DNA synthesis.) ADP = adenosine diphosphate; P_i = inorganic phosphate.

Deoxythymidine monophosphate synthesis

dUMP is converted to deoxythymidine monophosphate (dTMP) by thymidylate synthase, which uses N^5,N^{10} -methylene-THF as the source of the methyl group (see p. 296). This is an unusual reaction in that THF contributes not only a one-carbon unit but also two hydrogen atoms from the pteridine ring, resulting in the oxidation of THF to dihydrofolate ([DHF], Fig. 22.23). Inhibitors of thymidylate synthase include T analogs such as 5-fluorouracil, which serve as antitumor agents. 5-Fluorouracil is metabolically converted to 5-fluorodeoxyuridine monophosphate (5-FdUMP), which becomes permanently bound to the inactivated thymidylate synthase, making the drug a suicide inhibitor (see p. 64). DHF can be reduced to THF by DHF reductase (see Fig. 28.2, p. 424), an enzyme that is inhibited by folate analogs such as methotrexate. By decreasing the supply of THF, these drugs not only inhibit purine synthesis (see Fig. 22.7), but, by preventing methylation of dUMP to dTMP, they also decrease the availability of this essential component of DNA. DNA synthesis is inhibited and cell growth slowed. Thus, these drugs are used to treat cancer. (Note: Acyclovir [a purine analog] and 3'-azido-3'-deoxythymidine [AZT, a pyrimidine analog] are used to treat infections of herpes simplex virus and human immunodeficiency virus, respectively. Each inhibits the viral DNA polymerase.)

FIGURE 22.23



from dUMP, illustrating sites of action of antineoplastic drugs.

Pyrimidine salvage and degradation

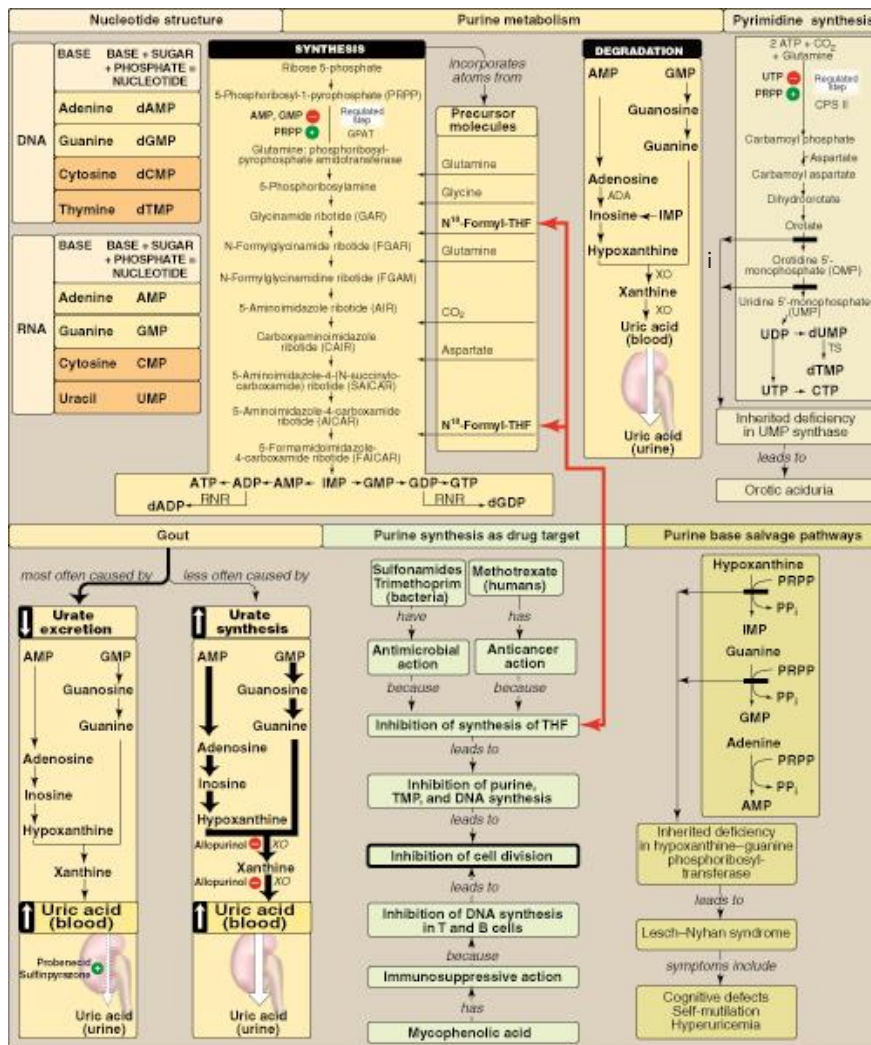
Unlike the purine ring, which is not cleaved in humans and is excreted as poorly soluble uric acid, the pyrimidine ring is opened and degraded to highly soluble products, β -alanine (from the degradation of CMP and UMP) and β -aminoisobutyrate (from TMP degradation), with the production of ammonia and CO_2 . Pyrimidine bases can be salvaged to nucleosides, which are phosphorylated to nucleotides. However, their high solubility makes pyrimidine salvage less significant clinically than purine salvage. (Note: The salvage of pyrimidine nucleosides is the basis for using uridine in the treatment of hereditary orotic aciduria [see p. 336].)

Chapter Summary



- **Nucleotides** are composed of a **nitrogenous base (A, G, C, U, and T)**; a **pentose sugar**; and one, two, or three **phosphate groups (Fig. 22.24)**.

FIGURE 22.24



ate amidotransferase; ADA = adenosine deaminase; AMP = adenosine monophosphate; dAMP = deoxyadenosine monophosphate; dGMP = deoxyguanosine monophosphate; dCMP = deoxycytidine monophosphate; dTMP = deoxythymidine monophosphate; PRPP = 5-phosphoribosyl-1-

- A and G are **purines**, and C, U, and T are **pyrimidines**.
- If the sugar is **ribose**, the nucleotide is a **ribonucleoside phosphate** (e.g., AMP), and it can have several functions in the cell, including being a component of **RNA**. If the sugar is **deoxyribose**, the nucleotide is a **deoxyribonucleoside phosphate** (e.g., deoxyAMP) and will be found almost exclusively as a component of **DNA**.
- The **committed step in purine synthesis** uses **PRPP** (an activated pentose that provides the **ribose 5-phosphate** for *de novo* purine and pyrimidine synthesis and salvage) and nitrogen from **glutamine** to produce phosphoribosylamine. The enzyme is **GPAT** and is inhibited by AMP and GMP (the end products of the pathway) and activated by PRPP.
- Purine nucleotides can also be produced from preformed purine bases by using **salvage reactions** catalyzed by **APRT** and **HGPRT**. A near-total deficiency of HGPRT causes **Lesch–Nyhan syndrome**, a severe, inherited form of hyperuricemia accompanied by compulsive self-mutilation.

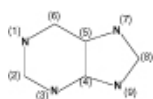
- All deoxyribonucleotides are synthesized from ribonucleotides by the enzyme **ribonucleotide reductase**. This enzyme is highly regulated (e.g., it is strongly inhibited by **dATP**, a compound that is overproduced in bone marrow cells in individuals with **ADA deficiency**). ADA deficiency causes **SCID**.
- The end product of purine degradation is **uric acid**, a compound of low solubility whose overproduction or undersecretion causes **hyperuricemia** that, if accompanied by the deposition of **MSU crystals** in joints and soft tissues and an inflammatory response to those crystals, results in **gout**.
- The first step in **pyrimidine synthesis**, the production of carbamoyl phosphate by **CPS II**, is the **regulated** step in this pathway (it is inhibited by **UTP** and activated by PRPP). The UTP produced by this pathway can be converted to CTP.
- **Deoxyuridine monophosphate** can be converted to dTMP by **thymidylate synthase**, an enzyme targeted by anticancer drugs such as **5-fluorouracil**.
- The regeneration of **tetrahydrofolate** from DHF produced in the thymidylate synthase reaction requires **dihydrofolate reductase**, an enzyme targeted by the drug **methotrexate**.

Study Questions



Choose the **ONE** best answer.

22.1. Azaserine, a drug with research applications, inhibits glutamine-dependent enzymes. Incorporation of which of the ring nitrogens (N) in the generic purine structure shown would most likely be affected by azaserine?



- A. 1
- B. 3
- C. 7
- D. 9

Correct answer = D. The N at position 9 is supplied by glutamine in the first step of purine *de novo* synthesis, and its incorporation would be affected by azaserine. The N at position 1 is supplied by aspartate and at position 7 by glycine. The N at position 3 is also supplied by glutamine, but azaserine would have inhibited purine synthesis prior to this step.

22.2. A 42-year-old male undergoing radiation therapy for prostate cancer develops severe pain in the metatarsal phalangeal joint of his right big toe. Monosodium urate crystals are detected by polarized light microscopy in fluid obtained from this joint by arthrocentesis. This patient's pain is directly caused by the overproduction of the end product of which of the following metabolic pathways?

- A. *De novo* pyrimidine biosynthesis
- B. Pyrimidine degradation
- C. *De novo* purine biosynthesis
- D. Purine salvage
- E. Purine degradation

Correct answer = E. The patient's pain is caused by gout, resulting from an inflammatory response to the crystallization of excess urate (as monosodium urate) in his joints. Radiation therapy caused cell death, with degradation of nucleic acids and their constituent purines. Uric acid, the end product of purine degradation, is a relatively insoluble compound that can cause gout (and kidney stones). Pyrimidine metabolism is not associated with uric acid production. Overproduction of purines can indirectly result in hyperuricemia. Purine salvage decreases uric acid production.

22.3. Which one of the following enzymes of nucleotide metabolism is correctly paired with its pharmacologic inhibitor?

- A. Dihydrofolate reductase—methotrexate
- B. Inosine monophosphate dehydrogenase—hydroxyurea
- C. Ribonucleotide reductase—5-fluorouracil
- D. Thymidylate synthase—allopurinol
- E. Xanthine oxidase—probenecid

Correct answer = A. Methotrexate interferes with folate metabolism by acting as a competitive inhibitor of the enzyme dihydrofolate reductase. This starves cells for tetrahydrofolate and makes them unable to synthesize purines and thymidine monophosphate. Inosine monophosphate dehydrogenase is inhibited by mycophenolic acid. Ribonucleotide reductase is inhibited by hydroxyurea. Thymidylate synthase is inhibited by 5-fluorouracil. Xanthine oxidase is inhibited by allopurinol. Probenecid increases renal excretion of urate but does not inhibit its production.

22.4. A 1-year-old female patient is lethargic, weak, and anemic. Her height and weight are low for her age. Her urine contains an elevated level of orotic acid. Activity of uridine monophosphate synthase is low.

Administration of which of the following is most likely to alleviate her symptoms?

- A. Adenine
- B. Guanine
- C. Hypoxanthine
- D. Thymidine
- E. Uridine

Correct answer = E. The elevated excretion of orotic acid and low activity of uridine monophosphate (UMP) synthase indicate that the patient has orotic aciduria, a very rare genetic disorder affecting *de novo* pyrimidine synthesis. Deficiencies in one or both catalytic domains of UMP synthase leave the patient unable to synthesize pyrimidines. Uridine, a pyrimidine nucleoside, is a useful treatment because it bypasses the missing activities and can be salvaged to UMP, which can be converted to all the other pyrimidines. Although thymidine is a pyrimidine nucleoside, it cannot be converted to other pyrimidines. Hypoxanthine, guanine, and adenine are all purine bases and cannot be converted to pyrimidines.

22.5. What laboratory test would help in distinguishing an orotic aciduria caused by ornithine transcarbamylase deficiency from that caused by uridine monophosphate synthase deficiency?

In both orotic aciduria and ornithine transcarbamylase deficiency, there are elevated urinary orotate levels. Blood ammonia level would be expected to be elevated in ornithine transcarbamylase deficiency that affects the urea cycle, but not in uridine monophosphate synthase deficiency.

