

# 41 Purine and Pyrimidine Metabolism

Purines and pyrimidines are required for synthesizing **nucleotides** and **nucleic acids**. These molecules can be synthesized either from scratch, **de novo**, or **salvaged** from existing bases. Dietary uptake of purine and pyrimidine bases is low, because most of the ingested nucleic acids are metabolized by the intestinal epithelial cells.

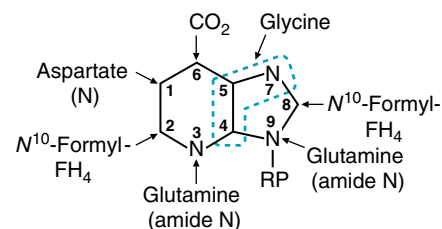
The *de novo* pathway of purine synthesis is complex, consisting of 11 steps, and requiring 6 molecules of ATP for every purine synthesized. The precursors that donate components to produce purine nucleotides include **glycine**, **ribose 5-phosphate**, **glutamine**, **aspartate**, **carbon dioxide**, and **N<sup>10</sup>-formyl FH<sub>4</sub>** (Fig. 41.1). Purines are synthesized as **ribonucleotides**, with the initial purine synthesized being **inosine monophosphate (IMP)**. Adenosine monophosphate (AMP) and guanosine monophosphate (GMP) are each derived from IMP in two-step reaction pathways.

The purine nucleotide salvage pathway allows free purine bases to be converted into nucleotides, nucleotides into nucleosides, and nucleosides into free bases. Enzymes included in this pathway are **AMP** and **adenosine deaminase**, **adenosine kinase**, **purine nucleoside phosphorylase**, **adenine phosphoribosyltransferase (APRT)**, and **hypoxanthine guanine phosphoribosyltransferase (HGPRT)**. Mutations in a number of these enzymes lead to serious diseases. Deficiencies in purine nucleoside phosphorylase and adenosine deaminase lead to **immunodeficiency disorders**. A deficiency in HGPRT leads to **Lesch-Nyhan syndrome**. The **purine nucleotide cycle**, in which aspartate carbons are converted to fumarate to replenish TCA cycle intermediates in working muscle, and the aspartate nitrogen is released as ammonia, uses components of the purine nucleotide salvage pathway.

Pyrimidine bases are first synthesized as the free base and then converted to a nucleotide. **Aspartate** and **carbamoyl phosphate** form all components of the pyrimidine ring. **Ribose 5-phosphate**, which is converted to phosphoribosyl pyrophosphate (PRPP), is required to donate the sugar phosphate to form a nucleotide. The first pyrimidine nucleotide produced is **orotate monophosphate (OMP)**. The OMP is converted to **uridine monophosphate (UMP)**, which will become the precursor for both **cytidine triphosphate (CTP)** and **deoxythymidine monophosphate (dTMP)** production.

The formation of deoxyribonucleotides requires **ribonucleotide reductase** activity, which catalyzes the reduction of ribose on nucleotide diphosphate substrates to 2'-deoxyribose. Substrates for the enzyme include adenosine diphosphate (ADP), guanosine diphosphate (GDP), cytidine diphosphate (CDP), and uridine diphosphate (UDP). Regulation of the enzyme is complex. There are two major allosteric sites. One controls the overall activity of the enzyme, whereas the other determines the substrate specificity of the enzyme. All deoxyribonucleotides are synthesized using this one enzyme.

The **regulation** of purine nucleotide biosynthesis occurs at four points in the pathway. The enzymes **PRPP synthetase**, **amidophosphoribosyl transferase**, **IMP**



**Fig. 41.1.** Origin of the atoms of the purine base. FH<sub>4</sub> = tetrahydrofolate.

*dehydrogenase, and adenylosuccinate synthetase are regulated by allosteric modifiers, as they occur at key branch points through the pathway. Pyrimidine synthesis is regulated at the first committed step, which is the synthesis of cytoplasmic carbamoyl-phosphate, by the enzyme **carbamoyl phosphate synthetase II (CPS-II)**.*

*Purines, when degraded, cannot generate energy, nor can the purine ring be substantially modified. The end product of purine ring degradation is **uric acid**, which is excreted in the urine. Uric acid has a limited solubility, and if it were to accumulate, uric acid crystals would precipitate in tissues of the body with a reduced temperature (such as the big toe). This condition of acute painful inflammation of specific soft tissues and joints is called **gout**. Pyrimidines, when degraded, however, give rise to water-soluble compounds, such as urea, carbon dioxide, and water and do not lead to a disease state if pyrimidine catabolism is increased.*




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## THE WAITING ROOM



The initial acute inflammatory process that caused **Lotta Topaigne** to experience a painful attack of gouty arthritis responded quickly to colchicine therapy (see Chapter 10). Several weeks after the inflammatory signs and symptoms in her right great toe subsided, Lotta was placed on allopurinol, a drug that reduces uric acid synthesis. Her serum uric acid level gradually fell from a pretreatment level of 9.2 mg/dL into the normal range (2.5–8.0 mg/dL). She remained free of gouty symptoms when she returned to her physician for a follow-up office visit.

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### I. PURINES AND PYRIMIDINES

As has been seen in previous chapters of this text, nucleotides serve numerous functions in different reaction pathways. For example, nucleotides are the activated precursors of DNA and RNA. Nucleotides form the structural moieties of many coenzymes (examples include NADH, FAD, and coenzyme A). Nucleotides are critical elements in energy metabolism (ATP, GTP). Nucleotide derivatives are frequently activated intermediates in many biosyntheses. For example, UDP-glucose and CDP-diacylglycerol are precursors of glycogen and phosphoglycerides, respectively. S-Adenosylmethionine carries an activated methyl group. In addition, nucleotides act as second messengers in intracellular signaling (e.g., cAMP, cGMP). Finally, nucleotides and nucleosides act as metabolic allosteric regulators. Think about all of the enzymes that have been studied that are regulated by levels of ATP, ADP, and AMP.

Dietary uptake of purine and pyrimidine bases is minimal. The diet contains nucleic acids and the exocrine pancreas secretes deoxyribonuclease and ribonuclease, along with the proteolytic and lipolytic enzymes. This enables digested nucleic acids to be converted to nucleotides. The intestinal epithelial cells contain alkaline phosphatase activity, which will convert nucleotides to nucleosides. Other enzymes within the epithelial cells tend to metabolize the nucleosides to uric acid, or to salvage them for their own needs. Approximately 5% of ingested nucleotides will make it into the circulation, either as the free base or as a nucleoside. Because of the minimal dietary uptake of these important molecules, de novo synthesis of purines and pyrimidines is required.

## II. PURINE BIOSYNTHESIS

The purine bases are produced *de novo* by pathways that use amino acids as precursors and produce nucleotides. Most *de novo* synthesis occurs in the liver (Fig. 41.2), and the nitrogenous bases and nucleosides are then transported to other tissues by red blood cells. The brain also synthesizes significant amounts of nucleotides. Because the *de novo* pathway requires six high-energy bonds per purine produced, a salvage pathway, which is used by many cell types, can convert free bases and nucleosides to nucleotides.

### A. De Novo Synthesis of the Purine Nucleotides

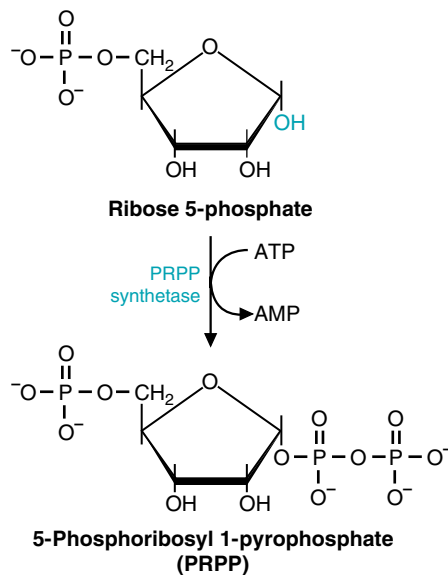
#### 1. SYNTHESIS OF IMP

As purines are built on a ribose base (see Fig. 41.2), an activated form of ribose is used to initiate the purine biosynthetic pathway. 5-Phosphoribosyl-1-pyrophosphate (PRPP) is the activated source of the ribose moiety. It is synthesized from ATP and ribose 5'-phosphate (Fig. 41.3), which is produced from glucose through the pentose phosphate pathway (see Chapter 29). The enzyme that catalyzes this reaction, PRPP synthetase, is a regulated enzyme (see section II.A.5); however, this step is not the committed step of purine biosynthesis. PRPP has many other uses, which are described as the chapter progresses.

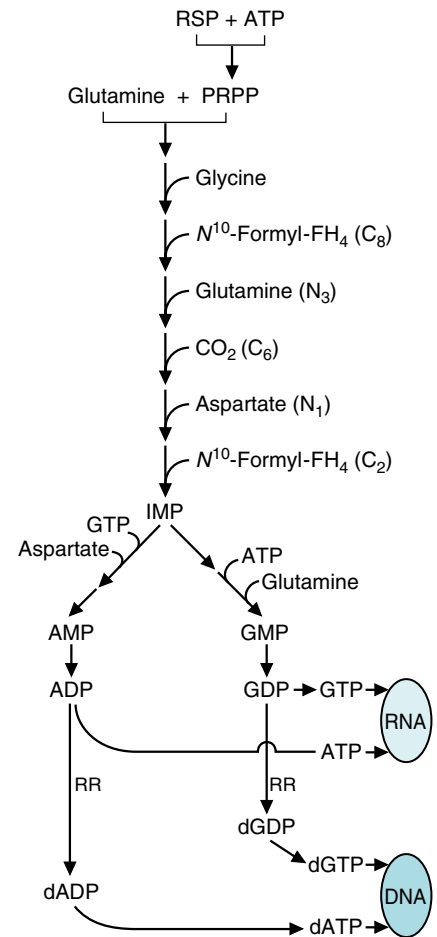
In the first committed step of the purine biosynthetic pathway, PRPP reacts with glutamine to form phosphoribosylamine (Fig. 41.4). This reaction, which produces nitrogen 9 of the purine ring, is catalyzed by glutamine phosphoribosyl amidotransferase, a highly regulated enzyme.

In the next step of the pathway, the entire glycine molecule is added to the growing precursor. Glycine provides carbons 4 and 5 and nitrogen 7 of the purine ring (Fig. 41.5).

Subsequently, carbon 8 is provided by  $N^{10}$ -formyl  $FH_4$ , nitrogen 3 by glutamine, carbon 6 by  $CO_2$ , nitrogen 1 by aspartate, and carbon 2 by formyl tetrahydrofolate (see Fig. 41.1). Note that six molecules of ATP are required (starting with ribose 5-phosphate) to synthesize the first purine nucleotide, inosine monophosphate (IMP). This nucleotide contains the base hypoxanthine joined by an *N*-glycosidic bond from nitrogen 9 of the purine ring to carbon 1 of the ribose (Fig. 41.6).



**Fig. 41.3.** Synthesis of PRPP. Ribose 5-phosphate is produced from glucose by the pentose phosphate pathway.



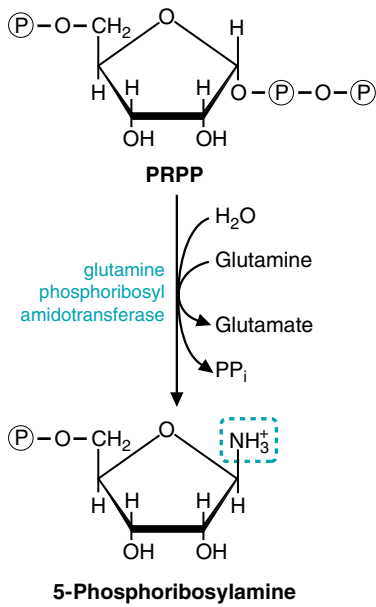
**Fig. 41.2.** Overview of purine production, starting with glutamine, ribose 5-phosphate, and ATP. The steps that require ATP are also indicated in this figure. RR = ribonucleotide reductase.  $FH_4$  = tetrahydrofolate. PRPP = 5-phosphoribosyl 1-pyrophosphate.



Cellular concentrations of PRPP and glutamine are usually below their  $K_m$  for glutamine phosphoribosyl amidotransferase. Thus, any situation which leads to an increase in their concentration can lead to an increase in *de novo* purine biosynthesis.



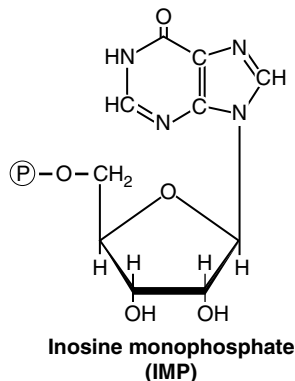
The base hypoxanthine is found in the anticodon of tRNA molecules (it is formed by the deamination of an adenine base). Hypoxanthine's role in tRNA is to allow wobble base-pairs to form, as the base hypoxanthine can base pair with adenine, cytosine, or uracil. The wobbling allows one tRNA molecule to potentially form base pairs with three different codons.



**Fig. 41.4.** The first step in purine biosynthesis. The purine base is built on the ribose moiety. The availability of the substrate PRPP is a major determinant of the rate of this reaction.



The aspartate to fumarate conversion also occurs in the urea cycle. In both cases, aspartate donates a nitrogen to the product, while the carbons of aspartate are released as fumarate.



**Fig. 41.6.** Structure of inosine monophosphate (IMP). The base is hypoxanthine.

## 2. SYNTHESIS OF AMP

IMP serves as the branchpoint from which both adenine and guanine nucleotides can be produced (see Fig. 41.2). Adenosine monophosphate (AMP) is derived from IMP in two steps (Fig. 41.7). In the first step, aspartate is added to IMP to form adenylosuccinate, a reaction similar to the one catalyzed by argininosuccinate synthetase in the urea cycle. Note how this reaction requires a high-energy bond, donated by GTP. Fumarate is then released from the adenylosuccinate by the enzyme adenylosuccinase to form AMP.

## 3. SYNTHESIS OF GMP

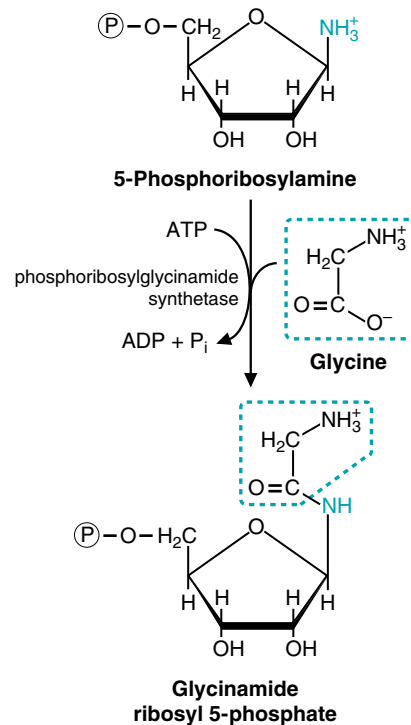
GMP is also synthesized from IMP in two steps (Fig. 41.8). In the first step, the hypoxanthine base is oxidized by IMP dehydrogenase to produce the base xanthine and the nucleotide xanthosine monophosphate (XMP). Glutamine then donates the amide nitrogen to XMP to form GMP in a reaction catalyzed by GMP synthetase. This second reaction requires energy, in the form of ATP.

## 4. PHOSPHORYLATION OF AMP AND GMP

AMP and GMP can be phosphorylated to the di- and triphosphate levels. The production of nucleoside diphosphates requires specific nucleoside monophosphate kinases, whereas the production of nucleoside triphosphates requires nucleoside diphosphate kinases, which are active with a wide range of nucleoside diphosphates. The purine nucleoside triphosphates are also used for energy-requiring processes in the cell and also as precursors for RNA synthesis (see Fig. 41.2).

## 5. REGULATION OF PURINE SYNTHESIS

Regulation of purine synthesis occurs at several sites (Fig. 41.9). Four key enzymes are regulated: PRPP synthetase, amidophosphoribosyl transferase,



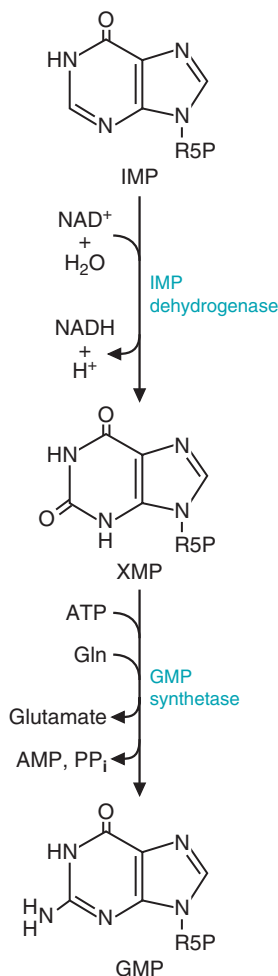
**Fig. 41.5.** Incorporation of glycine into the purine precursor. The ATP is required for the condensation of the glycine carboxylic acid group with the 1'-amino group of phosphoribosyl 1-amine.

adenylosuccinate synthetase, and IMP dehydrogenase. The first two enzymes regulate IMP synthesis; the last two regulate the production of AMP and GMP, respectively.

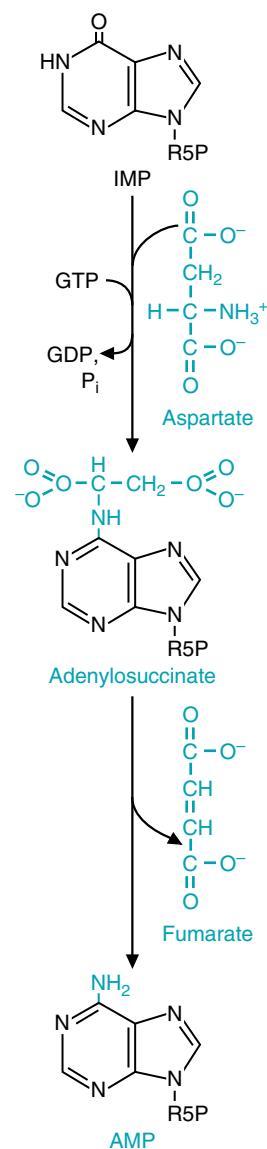
A primary site of regulation is the synthesis of PRPP. PRPP synthetase is negatively affected by GDP and, at a distinct allosteric site, by ADP. Thus, the simultaneous binding of an oxypurine (eg., GDP) and an aminopurine (eg., ADP) can occur with the result being a synergistic inhibition of the enzyme. This enzyme is not the committed step of purine biosynthesis; PRPP is also used in pyrimidine synthesis and both the purine and pyrimidine salvage pathways.

The committed step of purine synthesis is the formation of 5-phosphoribosyl 1-amine by glutamine phosphoribosyl amidotransferase. This enzyme is strongly inhibited by GMP and AMP (the end products of the purine biosynthetic pathway). The enzyme is also inhibited by the corresponding nucleoside di- and triphosphates, but under cellular conditions, these compounds probably do not play a central role in regulation. The active enzyme is a monomer of 133,000 daltons but is converted to an inactive dimer (270,000 daltons) by binding of the end products.

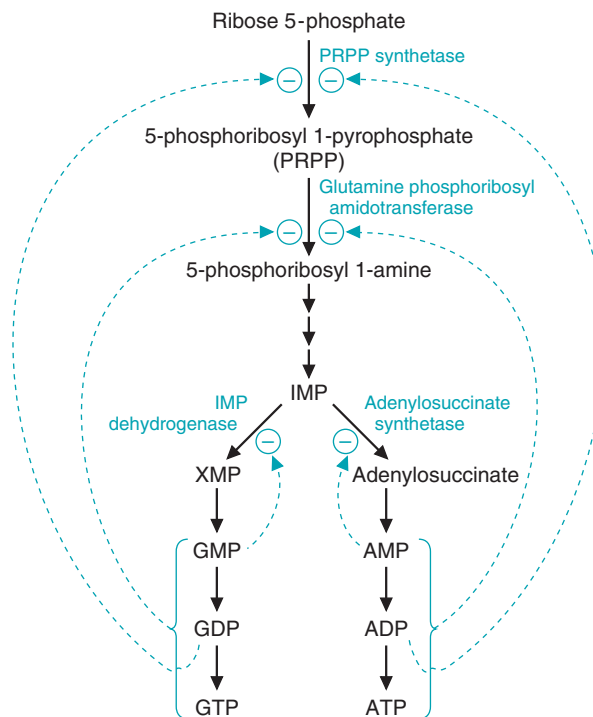
The enzymes that convert IMP to XMP and adenylosuccinate are both regulated. GMP inhibits the activity of IMP dehydrogenase, and AMP inhibits adenylosuccinate synthetase. Note that the synthesis of AMP is dependent on GTP (of which GMP is a precursor), whereas the synthesis of GMP is dependent on ATP (which is made from AMP). This serves as a type of positive regulatory mechanism to balance the pools of these precursors: when the levels of ATP are high, GMP will be



**Fig. 41.8.** The conversion of IMP to GMP. Note that ATP is required for the synthesis of GMP.



**Fig. 41.7.** The conversion of IMP to AMP. Note that GTP is required for the synthesis of AMP.



**Fig. 41.9.** The regulation of purine synthesis. PRPP synthetase has two distinct allosteric sites, one for ADP, the other for GDP. Glutamine phosphoribosyl amidotransferase contains adenine nucleotide and guanine nucleotide binding sites; the monophosphates are the most important, although the di- and tri-phosphates will also bind to and inhibit the enzyme. Adenylosuccinate synthetase is inhibited by AMP; IMP dehydrogenase is inhibited by GMP.

made; when the levels of GTP are high, AMP synthesis will take place. GMP and AMP act as negative effectors at these branch points, a classic example of feedback inhibition.

## B. Purine Salvage Pathways

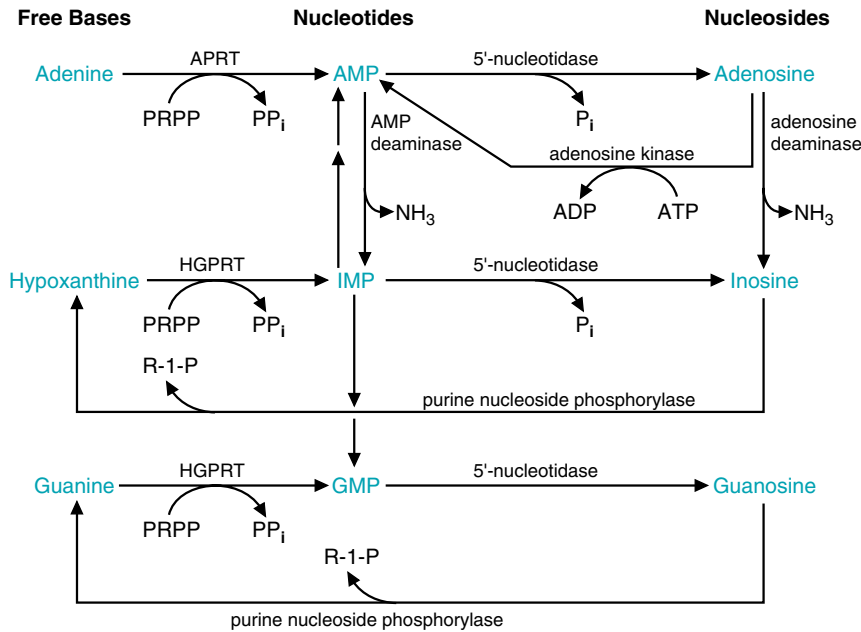
Most of the *de novo* synthesis of the bases of nucleotides occurs in the liver, and to some extent in the brain, neutrophils, and other cells of the immune system. Within the liver, nucleotides can be converted to nucleosides or free bases, which can be transported to other tissues via the red blood cell in the circulation. In addition, the small amounts of dietary bases or nucleosides that are absorbed also enter cells in this form. Thus, most cells can salvage these bases to generate nucleotides for RNA and DNA synthesis. For certain cell types, such as the lymphocytes, the salvage of bases is the major form of nucleotide generation.

The overall picture of salvage is shown in Figure 41.10. The pathways allow free bases, nucleosides, and nucleotides to be easily interconverted. The major enzymes required are purine nucleoside phosphorylase, phosphoribosyl transferases, and deaminases.

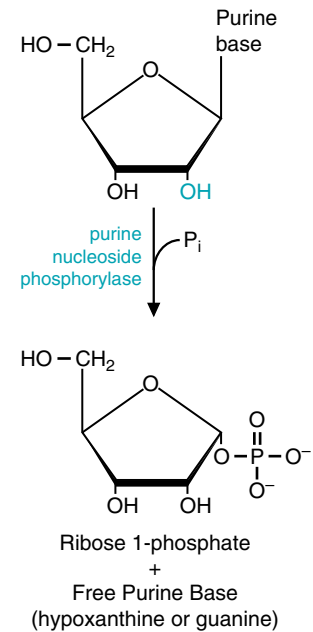
Purine nucleoside phosphorylase catalyzes a phosphorolysis reaction of the N-glycosidic bond that attaches the base to the sugar moiety in the nucleosides guanosine and inosine (Fig. 41.11). Thus, guanosine and inosine are converted to guanine and hypoxanthine, respectively, along with ribose 1-phosphate. The ribose 1-phosphate can be isomerized to ribose 5-phosphate, and the free bases then salvaged or degraded, depending on cellular needs.



A deficiency in purine nucleoside phosphorylase activity leads to an immune disorder in which T-cell immunity is compromised. B-cell immunity, conversely, may be only slightly compromised or even normal. Children lacking this activity have recurrent infections, and more than half display neurologic complications. Symptoms of the disorder first appear at between 6 months and 4 years of age.



**Fig. 41.10.** Salvage of bases. The purine bases hypoxanthine and guanine react with PRPP to form the nucleotides inosine and guanosine monophosphate, respectively. The enzyme that catalyzes the reaction is hypoxanthine-guanine phosphoribosyltransferase (HGPRT). Adenine forms AMP in a reaction catalyzed by adenine phosphoribosyltransferase (APRT). Nucleotides are converted to nucleosides by 5'-nucleotidase. Free bases are generated from nucleosides by purine nucleoside phosphorylase. Deamination of the base adenine occurs with AMP and adenosine deaminase. Of the purines, only adenosine can be directly phosphorylated back to a nucleotide, by adenosine kinase.



**Fig. 41.11.** The purine nucleoside phosphorylase reaction, converting guanosine or inosine to ribose 1-phosphate plus the free bases guanine or hypoxanthine.

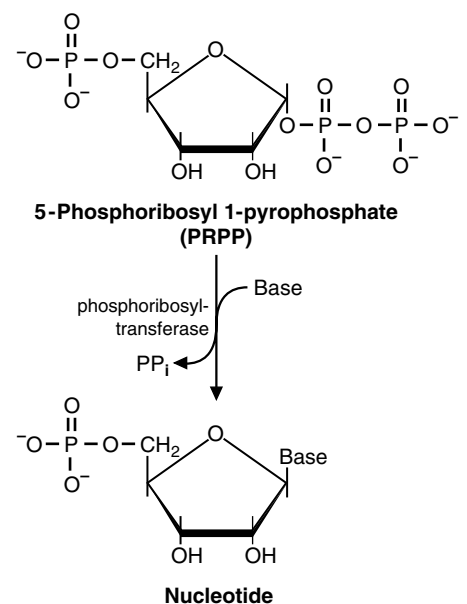
The phosphoribosyl transferase enzymes catalyze the addition of a ribose 5-phosphate group from PRPP to a free base, generating a nucleotide and pyrophosphate (Fig. 41.12). Two enzymes do this: adenine phosphoribosyl transferase (APRT) and hypoxanthine-guanine phosphoribosyl transferase (HGPRT). The reactions they catalyze are the same, differing only in their substrate specificity.

Adenosine and AMP can be deaminated by adenosine deaminase and AMP deaminase, respectively, to form inosine and IMP (see Fig. 41.10). Adenosine is also the only nucleoside to be directly phosphorylated to a nucleotide by adenosine kinase. Guanosine and inosine must be converted to free bases by purine nucleoside phosphorylase before they can be converted to nucleotides by HGPRT.

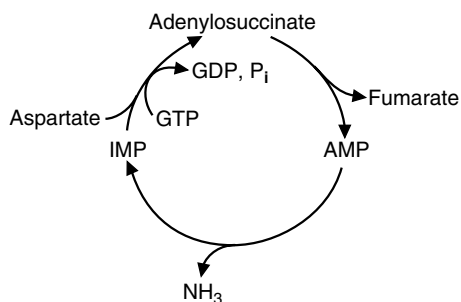
A portion of the salvage pathway that is important in muscle is the purine nucleotide cycle (Fig. 41.13). The net effect of these reactions is the deamination of aspartate to fumarate (as AMP is synthesized from IMP and then deaminated back to IMP by AMP deaminase). Under conditions in which the muscle must generate energy, the fumarate derived from the purine nucleotide cycle is used anaplerotically to replenish TCA cycle intermediates and to allow the cycle to operate at a high speed. Deficiencies in enzymes of this cycle lead to muscle fatigue during exercise.



Lesch-Nyhan syndrome is caused by a defective hypoxanthine-guanine phosphoribosyltransferase (HGPRT) (see Fig. 41.12). In this condition, purine bases cannot be salvaged. Instead, they are degraded, forming excessive amounts of uric acid. Individuals with this syndrome suffer from mental retardation. They are also prone to chewing off their fingers and performing other acts of self-mutilation.



**Fig. 41.12.** The phosphoribosyltransferase reaction. APRT uses the free base adenine; HGPRT can use either hypoxanthine or guanine as a substrate.



**Fig. 41.13.** The purine nucleotide cycle. Using a combination of biosynthetic and salvage enzymes, the net effect is the conversion of aspartate to fumarate plus ammonia, with the fumarate playing an anaplerotic role in the muscle.



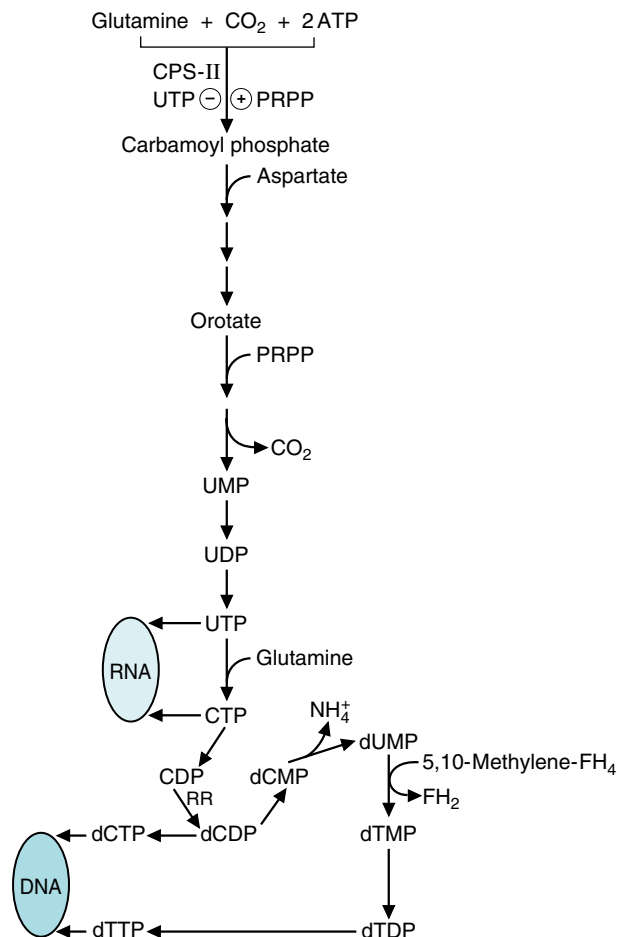
In bacteria, aspartate transcarbamoylase is the regulated step of pyrimidine production. This is a very complex enzyme and was a model system for understanding how allosteric enzymes were regulated. In humans, however, this enzyme is not regulated.

### III. SYNTHESIS OF THE PYRIMIDINE NUCLEOTIDES

#### A. De Novo Pathways

In the synthesis of the pyrimidine nucleotides, the base is synthesized first, and then it is attached to the ribose 5'-phosphate moiety (Fig. 41.14). The origin of the atoms of the ring (aspartate and carbamoyl-phosphate, which is derived from carbon dioxide and glutamine) is shown in Fig. 41.15. In the initial reaction of the pathway, glutamine combines with bicarbonate and ATP to form carbamoyl phosphate. This reaction is analogous to the first reaction of the urea cycle, except that it uses glutamine as the source of the nitrogen (rather than ammonia) and it occurs in the cytosol (rather than in mitochondria). The reaction is catalyzed by carbamoyl phosphate synthetase II, which is the regulated step of the pathway. The analogous reaction in urea synthesis is catalyzed by carbamoyl phosphate synthetase I, which is activated by *N*-acetylglutamate. The similarities and differences between these two carbamoyl phosphate synthetase enzymes is described in Table 41.1.

In the next step of pyrimidine biosynthesis, the entire aspartate molecule adds to carbamoyl phosphate in a reaction catalyzed by aspartate transcarbamoylase. The molecule subsequently closes to produce a ring (catalyzed by dihydroorotase), which is oxidized to form orotic acid (or its anion, orotate) through the actions of dihydroorotate dehydrogenase. The enzyme orotate phosphoribosyl transferase catalyzes the transfer of ribose 5-phosphate from PRPP to orotate, producing orotidine 5'-phosphate, which is decarboxylated by orotidylic acid dehydrogenase to form



**Fig. 41.14.** Synthesis of the pyrimidine bases. CPSII = carbamoyl phosphate synthetase II. RR = ribonucleotide reductase;  $\oplus$  = stimulated by;  $\ominus$  = inhibited by;  $\text{FH}_2$  and  $\text{FH}_4$  = forms of folate.



**Table 41.1. Comparison of Carbamoyl Phosphate Synthetases (CPSI and CPSII)**

	CPS-I	CPS-II
Pathway	Urea cycle	Pyrimidine biosynthesis
Source of nitrogen	NH <sub>4</sub> <sup>+</sup>	Glutamine
Location	Mitochondria	Cytosol
Activator	<i>N</i> -Acetylglutamate	PRPP
Inhibitor	–	UTP

uridine monophosphate (UMP) (Fig. 41.16). In mammals, the first three enzymes of the pathway (carbamoyl phosphate synthetase II, aspartate transcarbamoylase, and dihydroorotase) are located on the same polypeptide, designated as CAD. The last two enzymes of the pathway are similarly located on a polypeptide known as UMP synthase (the orotate phosphoribosyl transferase and orotidylic acid dehydrogenase activities).

UMP is phosphorylated to UTP. An amino group, derived from the amide of glutamine, is added to carbon 4 to produce CTP by the enzyme CTP synthetase (this reaction cannot occur at the nucleotide monophosphate level). UTP and CTP are precursors for the synthesis of RNA (see Fig. 41.14). The synthesis of thymidine triphosphate (TTP) will be described in section IV.

## B. Salvage of Pyrimidine Bases

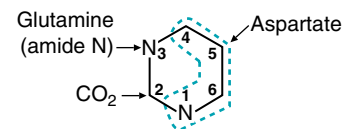
Pyrimidine bases are normally salvaged by a two-step route. First, a relatively non-specific pyrimidine nucleoside phosphorylase converts the pyrimidine bases to their respective nucleosides (Fig. 41.17). Notice that the preferred direction for this reaction is the reverse phosphorylase reaction, in which phosphate is being released and is not being used as a nucleophile to release the pyrimidine base from the nucleoside. The more specific nucleoside kinases then react with the nucleosides, forming nucleotides (Table 41.2). As with purines, further phosphorylation is carried out by increasingly more specific kinases. The nucleoside phosphorylase–nucleoside kinase route for synthesis of pyrimidine nucleoside monophosphates is relatively inefficient for salvage of pyrimidine bases because of the very low concentration of the bases in plasma and tissues.

Pyrimidine phosphorylase can use all of the pyrimidines but has a preference for uracil and is sometimes called uridine phosphorylase. The phosphorylase uses cytosine fairly well but has a very, very low affinity for thymine; therefore, a ribonucleoside containing thymine is almost never made in vivo. A second phosphorylase, thymine phosphorylase, has a much higher affinity for thymine and adds a deoxyribose residue (see Fig. 41.17).

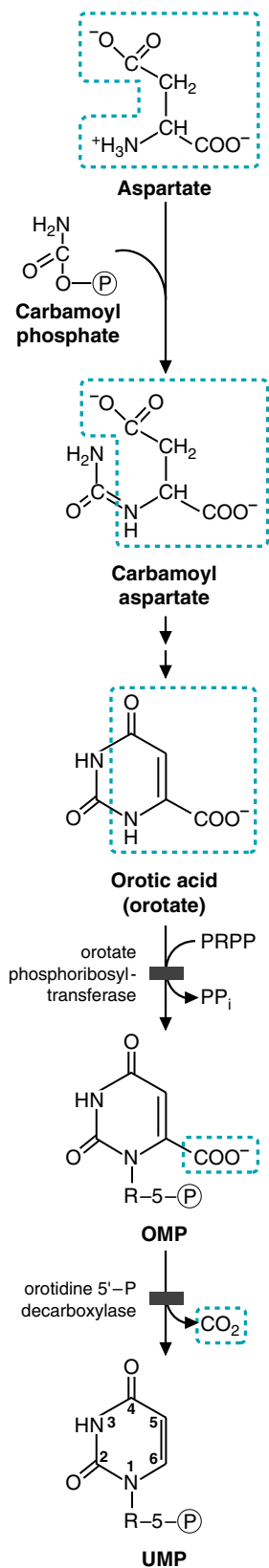
Of the various ribonucleosides and deoxyribonucleoside kinases, one that merits special mention is *thymidine kinase* (TK). This enzyme is allosterically inhibited by dTTP. Activity of thymidine kinase in a given cell is closely related to the proliferative state of that cell. During the cell cycle, the activity of TK rises dramatically as cells enter S phase, and in general rapidly dividing cells have high levels of this enzyme. Radiolabeled thymidine is widely used for isotopic labeling of DNA, for example, in radioautographic investigations or to estimate rates of intracellular DNA synthesis.

**Table 41.2. Salvage Reactions for Conversion of Pyrimidine Nucleosides to Nucleotides.**

Enzyme	Reaction
Uridine-cytidine kinase	Uridine + ATP → UMP + ADP Cytidine + ATP → CMP + ADP
Deoxythymidine kinase	deoxythymidine + ATP → dTMP + ADP
Deoxycytidine kinase	Deoxycytidine + ATP → dCMP + ADP

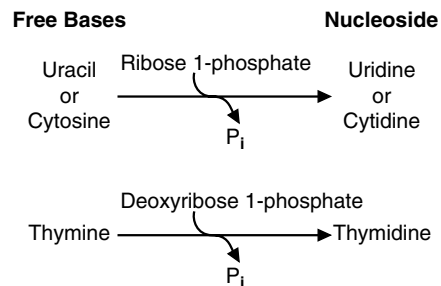
**Fig. 41.15.** The origin of the bases in the pyrimidine ring.

In hereditary orotic aciduria, orotic acid is excreted in the urine because the enzymes that convert it to uridine monophosphate, orotate phosphoribosyltransferase and orotidine 5'-phosphate decarboxylase, are defective (see Fig. 41.16). Pyrimidines cannot be synthesized, and, therefore, normal growth does not occur. Oral administration of uridine is used to treat this condition. Uridine, which is converted to UMP, bypasses the metabolic block and provides the body with a source of pyrimidines, as both CTP and dTMP can be produced from UMP.



■ Block in hereditary orotic aciduria

**Fig. 41.16.** Conversion of carbamoyl phosphate and aspartate to UMP. The defective enzymes in hereditary orotic aciduria are indicated (■).



**Fig. 41.17.** Salvage reactions for pyrimidine nucleoside production. Thymine phosphorylase uses deoxyribose 1-phosphate as a substrate, such that ribothymidine is rarely formed.

### C. Regulation of De Novo Pyrimidine Synthesis

The regulated step of pyrimidine synthesis in humans is carbamoyl phosphate synthetase II. The enzyme is inhibited by UTP and activated by PRPP (see Fig. 41.14). Thus, as pyrimidines decrease in concentration (as indicated by UTP levels), CPS-II is activated and pyrimidines are synthesized. The activity is also regulated by the cell cycle. As cells approach S-phase, CPS-II becomes more sensitive to PRPP activation and less sensitive to UTP inhibition. At the end of S-phase, the inhibition by UTP is more pronounced, and the activation by PRPP is reduced. These changes in the allosteric properties of CPS-II are related to its phosphorylation state. Phosphorylation of the enzyme at a specific site by a MAP kinase leads to a more easily activated enzyme. Phosphorylation at a second site by the cAMP-dependent protein kinase leads to a more easily inhibited enzyme.

### IV. THE PRODUCTION OF DEOXYRIBONUCLEOTIDES

For DNA synthesis to occur, the ribose moiety must be reduced to deoxyribose (Fig. 41.18). This reduction occurs at the dinucleotide level and is catalyzed by ribonucleotide reductase, which requires the protein thioredoxin. The deoxyribonucleoside diphosphates can be phosphorylated to the triphosphate level and used as precursors for DNA synthesis (see Figs. 41.2 and 41.14).

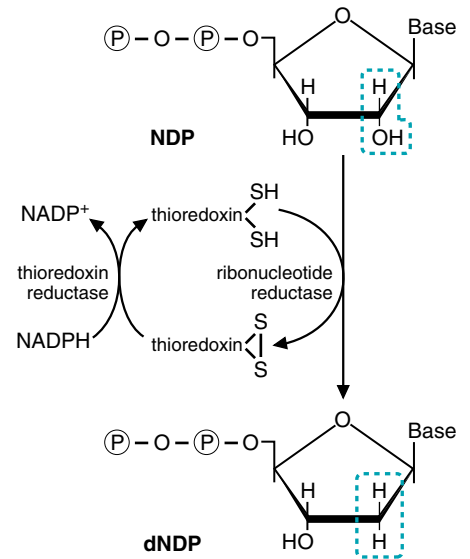
The regulation of ribonucleotide reductase is quite complex. The enzyme contains two allosteric sites, one controlling the activity of the enzyme and the other controlling the substrate specificity of the enzyme. ATP bound to the activity site activates the enzyme; dATP bound to this site inhibits the enzyme. Substrate specificity is more complex. ATP bound to the substrate site activates the reduction of pyrimidines (CDP and UDP), to form dCDP and dUDP. The dUDP is not used for DNA synthesis; rather, it is used to produce dTMP (see below). Once dTMP is produced, it is phosphorylated to dTTP, which then binds to the substrate site and induces the reduction of GDP. As dGTP accumulates, it replaces dTTP in the substrate site and allows ADP to be reduced to dADP. This leads to the accumulation of dATP, which will inhibit the overall activity of the enzyme. These allosteric changes are summarized in Table 41.3.

dUDP can be dephosphorylated to form dUMP, or, alternatively, dCMP can be deaminated to form dUMP. Methylene tetrahydrofolate transfers a methyl group to dUMP to form dTMP (see Figure 40.5). Phosphorylation reactions produce dTTP, a precursor for DNA synthesis and a regulator of ribonucleotide reductase.

### V. DEGRADATION OF PURINE AND PYRIMIDINE BASES

#### A. Purine Bases

The degradation of the purine nucleotides (AMP and GMP) occurs mainly in the liver (Fig. 41.19). Salvage enzymes are used for most of these reactions. AMP is first deaminated to produce IMP (AMP deaminase). Then IMP and GMP are dephosphorylated (5'-nucleotidase), and the ribose is cleaved from the base by purine nucleoside phosphorylase. Hypoxanthine, the base produced by cleavage of IMP, is converted by xanthine oxidase to xanthine, and guanine is deaminated by



**Fig. 41.18.** Reduction of ribose to deoxyribose. Reduction occurs at the nucleoside diphosphate level. A ribonucleoside diphosphate (NDP) is converted to a deoxyribonucleoside diphosphate (dNDP). Thioredoxin is oxidized to a disulfide, which must be reduced for the reaction to continue producing dNDP. N = a nitrogenous base.



When ornithine transcarbamoylase is deficient (urea cycle disorder), excess carbamoyl phosphate from the mitochondria leaks into the cytoplasm. The elevated levels of cytoplasmic carbamoyl phosphate lead to pyrimidine production, as the regulated step of the pathway, the reaction catalyzed by carbamoyl synthetase II, is being bypassed. Thus, orotic aciduria results.



Gout is caused by excessive uric acid levels in the blood and tissues. To determine whether a person with gout has developed this problem because of overproduction of purine nucleotides or because of a decreased ability to excrete uric acid, an oral dose of an  $^{15}\text{N}$ -labeled amino acid is sometimes used. Which amino acid would be most appropriate to use for this purpose?

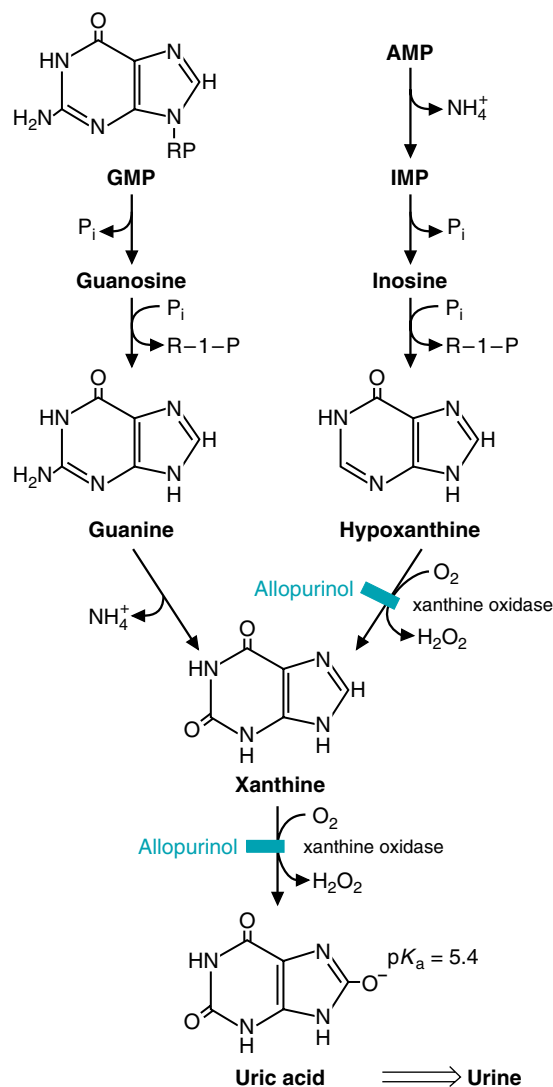
**Table 41.3. Effectors of Ribonucleotide Reductase Activity**

Preferred Substrate	Effector Bound to Overall Activity Site	Effector Bound to Substrate Specificity Site
None	dATP	Any nucleotide
CDP	ATP	ATP or dATP
UDP	ATP	ATP or dATP
ADP	ATP	dGTP
GDP	ATP	dTTP

**A:** The entire glycine molecule is incorporated into the precursor of the purine nucleotides. The nitrogen of this glycine also appears in uric acid, the product of purine degradation.  $^{15}\text{N}$ -labeled glycine could be used, therefore, to determine whether purines are being over-produced.

Uric acid has a  $\text{pK}_a$  of 5.4. It is ionized in the body to form urate. Urate is not very soluble in an aqueous environment. The quantity in normal human blood is very close to the solubility constant.

Normally, as cells die, their purine nucleotides are degraded to hypoxanthine and xanthine, which are converted to uric acid by xanthine oxidase (see Fig. 41.15). Allopurinol (a structural analog of hypoxanthine) is a substrate for xanthine oxidase. It is converted to oxypurinol (also called alloxanthine), which remains tightly bound to the enzyme, preventing further catalytic activity (see Fig. 8.19). Thus, allopurinol is a suicide inhibitor. It reduces the production of uric acid and hence its concentration in the blood and tissues (e.g., the synovial lining of the joints in **Lotta Topaigne's** great toe). Xanthine and hypoxanthine accumulate, and urate levels decrease. Overall, the amount of purine being degraded is spread over three products rather than appearing in only one. Therefore, none of the compounds exceeds its solubility constant, precipitation does not occur, and the symptoms of gout gradually subside.



**Fig. 41.19.** Degradation of the purine bases. The reactions inhibited by allopurinol are indicated. A second form of xanthine oxidase exists that uses  $\text{NAD}^+$  instead of  $\text{O}_2$  as the electron acceptor.

the enzyme guanase to produce xanthine. The pathways for the degradation of adenine and guanine merge at this point. Xanthine is converted by xanthine oxidase to uric acid, which is excreted in the urine. Xanthine oxidase is a molybdenum-requiring enzyme that uses molecular oxygen and produces hydrogen peroxide ( $\text{H}_2\text{O}_2$ ). Another form of xanthine oxidase exists that uses  $\text{NAD}^+$  as the electron acceptor (see Chapter 24).

Note how little energy is derived from the degradation of the purine ring. Thus, it is to the cell's advantage to recycle and salvage the ring, because it costs energy to produce and not much is obtained in return.

## B. Pyrimidine Bases

The pyrimidine nucleotides are dephosphorylated, and the nucleosides are cleaved to produce ribose 1-phosphate and the free pyrimidine bases cytosine, uracil, and thymine. Cytosine is deaminated, forming uracil, which is converted to  $\text{CO}_2$ ,  $\text{NH}_4^+$ ,

and  $\beta$ -alanine. Thymine is converted to  $\text{CO}_2$ ,  $\text{NH}_4^+$ , and  $\beta$ -aminoisobutyrate (Fig. 41.20). These products of pyrimidine degradation are excreted in the urine or converted to  $\text{CO}_2$ ,  $\text{H}_2\text{O}$ , and  $\text{NH}_4^+$  (which forms urea). They do not cause any problems for the body, in contrast to urate, which is produced from the purines and can precipitate, causing gout. As with the purine degradation pathway, little energy can be generated by pyrimidine degradation.

## CLINICAL COMMENTS



Hyperuricemia in **Lotta Topaigne's** case arose as a consequence of overproduction of uric acid. Treatment with allopurinol not only inhibits xanthine oxidase, lowering the formation of uric acid with an increase in the excretion of hypoxanthine and xanthine, but also decreases the overall synthesis of purine nucleotides. Hypoxanthine and xanthine produced by purine degradation are salvaged (i.e., converted to nucleotides) by a process that requires the consumption of PRPP. PRPP is a substrate for the glutamine phosphoribosyl amidotransferase reaction that initiates purine biosynthesis. Because the normal cellular levels of PRPP and glutamine are below the  $K_m$  of the enzyme, changes in the level of either substrate can accelerate or reduce the rate of the reaction. Therefore, decreased levels of PRPP cause decreased synthesis of purine nucleotides.

## BIOCHEMICAL COMMENTS



A deficiency in adenosine deaminase activity leads to severe combined immunodeficiency disease, or SCID. In the severe form of combined immunodeficiency, both T cells (which provide cell-based immunity, see Chapter 44) and B-cells (which produce antibodies) are deficient, leaving the individual without a functional immune system. Children born with this disorder lack a thymus gland and suffer from many opportunistic infections because of the lack of a functional immune system. Death results if the child is not placed in a sterile environment. Administration of polyethylene glycol–modified adenosine deaminase has been successful in treating the disorder, and the ADA gene was the first to be used in gene therapy in treating the disorder. The question that remains, however, is that even though all cells of the body are lacking ADA activity, why are the immune cells specifically targeted for destruction?

The specific immune disorder is not caused by any defect in purine salvage pathways, as children with Lesch-Nyhan syndrome have a functional immune system, although there are other major problems in those children. This suggests that perhaps the accumulation of precursors to ADA lead to toxic effects. Three hypotheses have been proposed and are outlined below.

In the absence of ADA activity, both adenosine and deoxyadenosine will accumulate. When deoxyadenosine accumulates, adenosine kinase can convert it to dAMP. Other kinases will allow dATP to then accumulate within the lymphocyte. Why specifically the lymphocyte? The other cells of the body are secreting the deoxyadenosine they cannot use, and it is accumulating in the circulation. As the lymphocytes are present in the circulation, they tend to accumulate this compound more so than cells not constantly present within the blood. As dATP accumulates, ribonucleotide reductase becomes inhibited, and the cells can no longer produce deoxyribonucleotides for DNA synthesis. Thus, when cells are supposed to grow and differentiate in response to cytokines, they cannot, and they die.

A second hypothesis suggests that the accumulation of deoxyadenosine in lymphocytes leads to an inhibition of S-adenosylhomocysteine hydrolase, the enzyme that converts S-adenosylhomocysteine to homocysteine and adenosine. This leads

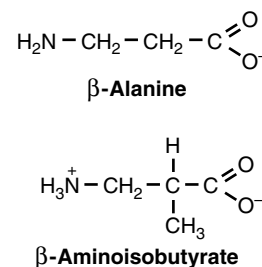


Fig. 41.20. Water-soluble end products of pyrimidine degradation.



Once nucleotide biosynthesis and salvage was understood at the pathway level, it was quickly realized that one way to inhibit cell proliferation would be to block purine or pyrimidine synthesis. Thus, drugs were developed that would interfere with a cell's ability to generate precursors for DNA synthesis, thereby inhibiting cell growth. This is particularly important for cancer cells, which have lost their normal growth regulatory properties. Such drugs have been introduced previously with a number of different patients. **Colin Tuma** was treated with 5-fluorouracil, which inhibits thymidylate synthase (dUMP to TMP synthesis). **Arlyn Foma** was treated with methotrexate for his leukemia; methotrexate inhibits dihydrofolate reductase, thereby blocking the regeneration of tetrahydrofolate and de novo purine synthesis and thymidine synthesis. **Mannie Weitzels** was treated with hydroxyurea to block ribonucleotide reductase activity, with the goal of inhibiting DNA synthesis in the leukemic cells. Development of these drugs would not have been possible without an understanding of the biochemistry of purine and pyrimidine salvage and synthesis. Such drugs also affect rapidly dividing normal cells, which brings about a number of the side effects of chemotherapeutic regimens.

**Table 41.4. Gene Disorders in Purine and Pyrimidine Metabolism**

Disease	Gene defect	Metabolite that accumulates	Clinical symptoms
Gout Severe combined immunodeficiency disease (SCID)	Multiple causes Adenosine deaminase (purine salvage pathway)	Uric acid Deoxyadenosine and derivatives thereof	Painful joints Loss of immune system, including no T or B cells
Immunodeficiency disease	Purine nucleoside phosphorylase	Purine nucleosides	Partial loss of immune system; no T cells but B cells are present
Lesch-Nyhan syndrome	Hypoxanthine-guanine phosphoribosyltransferase	Purines, uric acid	Mental retardation, self-mutilation
Hereditary orotic aciduria	UMP synthase	Orotic acid	Growth retardation

to hypo-methylation in the cell and an accumulation of S-adenosylhomocysteine. S-adenosylhomocysteine accumulation has been linked to the triggering of apoptosis.

The third hypothesis suggested is that elevated adenosine levels lead to inappropriate activation of adenosine receptors. Adenosine is also a signaling molecule, and stimulation of the adenosine receptors results in activation of protein kinase A and elevated cAMP levels in thymocytes. Elevated levels of cAMP in these cells triggers both apoptosis and developmental arrest of the cell.

Although it is still not clear which potential mechanism best explains the arrested development of immune cells, it is clear that elevated levels of adenosine and deoxyadenosine are toxic. The biochemical disorders of purine and pyrimidine metabolism discussed in this chapter are summarized in Table 41.4.

### Suggested References

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## REVIEW QUESTIONS—CHAPTER 41

- Similarities between carbamoyl phosphate synthetase I and carbamoyl phosphate synthetase II include which ONE of the following?
  - Carbon source
  - Intracellular location
  - Nitrogen source
  - Regulation by N-acetyl glutamate
  - Regulation by UMP

2. Gout can result from a reduction in activity of which one of the following enzymes?
- (A) Glutamine phosphoribosyl amidotransferase
  - (B) Glucose 6-phosphatase
  - (C) Glucose 6-phosphate dehydrogenase
  - (D) PRPP synthetase
  - (E) Purine nucleoside phosphorylase
3. Lesch-Nyhan syndrome is due to an inability to catalyze which of the following reactions?
- (A) Adenine to AMP
  - (B) Adenosine to AMP
  - (C) Guanine to GMP
  - (D) Guanosine to GMP
  - (E) Thymine to TMP
  - (F) Thymidine to TMP
4. Allopurinol can be used to treat gout because of its ability to inhibit which one of the following reactions?
- (A) AMP to XMP
  - (B) Xanthine to uric acid
  - (C) Inosine to hypoxanthine
  - (D) IMP to XMP
  - (E) XMP to GMP
5. The regulation of ribonucleotide reductase is quite complex. Assuming that an enzyme deficiency leads to highly elevated levels of dGTP, what effect would you predict on the reduction of ribonucleotides to deoxyribonucleotides under these conditions?
- (A) Elevated levels of dCDP will be produced.
  - (B) The formation of dADP will be favored.
  - (C) AMP would begin to be reduced.
  - (D) Reduced thioredoxin would become rate-limiting, thereby reducing the activity of ribonucleotide reductase.
  - (E) Deoxy-GTP would bind to the overall activity site and inhibit the functioning of the enzyme.