Nucleic Acid Metabolism

OBJECTIVES

• Describe the difference between a pyrimidine and a purine base, discern a nucleoside from a nucleotide, and name the sugars found in nucleotides.

• List the names of the common purine and pyrimidine bases and nucleosides.

• Explain why deficiency of glucose 6-phosphate dehydrogenase (G6PD) can result in hemolytic anemia.

• Describe the roles of vitamin B12 and the folate coenzymes in nucleotide metabolism, and name the processes that are impaired when these vitamins are deficient.

• Compare the pathways for purine and pyrimidine ribonucleotide synthesis with respect to design, entry point for PRPP, and folate requirements.

• Describe the regulation of ribonucleotide synthesis.

• Name the enzyme that catalyzes the formation of deoxyribonucleotides and describe the reaction it catalyzes.

• Describe the reaction catalyzed by thymidylate synthase and explain its role.

• Explain how purine bases and nucleosides are salvaged, and describe the clinical consequences of deficiencies in ADA and HGPRT.

• Describe how purine bases are catabolized and the relationship of purine catabolism to hyperuricemia and gout.

ANTI-OBJECTIVE

• Chemical structures, every step in any given pathway (see the key words for those you’ll be responsible for)
Some of the material in this chapter provides important foundation for content in future blocks, but will not be covered on the Immunology exam. You may wish to return to this chapter in I-3 Micro to address anti-folate antibacterial drugs and the risks of use of antimalarial drugs in individuals with G6PD deficiency. Both of these topics will come up again in M3 with regard to hematology and cancer treatment. So this is a good time to establish a basic foundation!

KEY WORDS

- Adenosine Deaminase (ADA)
- Lesch-Nyhan Syndrome
- Allopurinol
- Nucleoside
- Anemia
- Nucleotide
- Cobalamin (Vitamin B12)
- One-carbon groups
- Dihydrofolate Reductase (DHFR)
- Pentose Phosphate Pathway
- Folate
- PRPP (5-phosphoribosyl-1-pyrophosphate)
- Folic Acid

I. OVERVIEW

Nucleotides serve as building blocks for RNA and DNA. Among other important roles, nucleotides can serve as sources of energy (i.e., ATP), physiological signaling mediators (i.e., adenosine in control of coronary blood flow), secondary messengers (cAMP and cGMP), and allosteric enzyme effectors.
Nucleotide metabolism involves several interconnected pathways (Figure 2.8). Nucleotides can be synthesized \textit{de novo}, or from components “salvaged” from the degradation products of nucleic acids. When in excess, nucleotides are degraded to products that can either be consumed by other pathways or excreted. Defects in the pathways for \textit{de novo} synthesis, salvage, and degradation of nucleotides result in clinical disorders, and many drugs target these pathways.

**Figure 2.8 Overview of Nucleotide Metabolism**

**Review of Nucleotide Nomenclature**

A \textbf{nucleotide} is a compound that contains a purine or pyrimidine base, a 5-carbon sugar, and one or more phosphate groups (Figure 2.9). The common sugar (ribose or deoxyribose) and base (adenine, guanine, cytosine, uracil, or thymine) components are shown in Figure 2.10. A \textbf{nucleoside} lacks phosphate groups, and thus every nucleotide is essentially a phosphorylated nucleoside. It is equally correct to call the compound that contains the base adenine, a ribose sugar, and one

**Figure 2.9 General Structure of Purines and Pyrimidines**

The carbon atoms of the sugars are numbered 1’ through 5’. (Ignore the numbering of the bases.) Reproduced with permission from Colby, \textit{Biochemistry, a Synopsis}, Lange, 1985.
phosphate group at the 5’ position either a nucleotide or a nucleoside 5’-monophosphate. The 8 major species of nucleoside triphosphates are listed in Table 1.

![Figure 2.10](image)

**Figure 2.10** Component sugars and bases of the common nucleotides.


<table>
<thead>
<tr>
<th>Nucleoside Triphosphates</th>
<th>Names of Components</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ribonucleotides</strong></td>
<td></td>
</tr>
<tr>
<td>Adenosine triphosphate (ATP)</td>
<td>Adenine, Adenosine</td>
</tr>
<tr>
<td>Guanosine triphosphate (GTP)</td>
<td>Guanine, Guanosine</td>
</tr>
<tr>
<td>Cytidine triphosphate (CTP)</td>
<td>Cytosine, Cytidine</td>
</tr>
<tr>
<td>Uridine triphosphate (UTP)</td>
<td>Uracil, Uridine</td>
</tr>
<tr>
<td><strong>Deoxyribonucleotides</strong></td>
<td></td>
</tr>
<tr>
<td>Deoxyadenosine triphosphate (dATP)</td>
<td>Adenine, Deoxyadenosine</td>
</tr>
<tr>
<td>Deoxyguanosine triphosphate (dGTP)</td>
<td>Guanine, Deoxyguanosine</td>
</tr>
<tr>
<td>Deoxycytidine triphosphate (dCTP)</td>
<td>Cytosine, Deoxycytidine</td>
</tr>
<tr>
<td>Deoxythymidine triphosphate (dTTP)</td>
<td>Thymine, Deoxythymidine</td>
</tr>
</tbody>
</table>

Table 1: Common nucleoside triphosphates.

II. NUCLEOTIDE BIOSYNTHESIS

A. THE BIG PICTURE

Given that the goal of nucleotide synthesis is to create eight distinct types of nucleotide, each containing three modular parts (sugar, base, phosphate(s)) the process is understandably complex. As with any biochemical pathway, a metabolic map is helpful for conceptualizing the overall framework. **Figure 2.11** tracks the assembly of each type of nucleotide at the “big picture” level. While we are here, it is useful to think about the regulation of nucleotide production. The total intracellular concentration of a given type of nucleotide (for example, AMP + ADP + ATP) is tightly regulated and stays relatively constant, although there may be major changes in the individual concentrations, depending on the energy state of the cell (e.g., ATP increases while AMP decreases). The pathways for the *de novo* synthesis of purines and pyrimidines are primarily regulated by the concentrations of their own products, which inhibit further production. This form of regulation ensures an adequate supply of nucleotides while preventing their overproduction. We will spend some time deconstructing this map in lecture. A good place to dive
B. The Pentose Phosphate Pathway

1. **Overview**

Synthesis of nucleotides requires a source of ribose 5-phosphate. This compound is produced from glucose 6-phosphate via the **pentose phosphate pathway** (also called the **hexose monophosphate shunt**). In addition to producing ribose 5-phosphate, the pathway is a major source of **NADPH**, a coenzyme required for anabolism and in repair of oxidative damage. Recall that glucose 6-phosphate is formed in all cells via the first step in glycolysis and other biochemical pathways.

The pentose phosphate pathway occurs in the cytosol of cells. The pathway has what are termed oxidative and non-oxidative parts, which can operate more or less independently. The oxidative part of the pathway converts glucose 6-phosphate to ribulose 5-phosphate and produces 2 NADPH ([Figure 2.12](#)). Most of the steps in this part of the pathway are thermodynamically irreversible, thus ensuring that the cell maintains a high NADPH/NADP ratio, which is particularly important in cells that carry out NADPH-dependent processes such as fatty acid biosynthesis (liver, lactating mammary glands, adipose), steroid hormone synthesis (testes, ovaries, adrenal cortex), and reduction of glu-
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tathione (erythrocytes; a key reaction in protection from oxidative damage). Further utilization of ribulose 5-phosphate occurs via reversible non-oxidative reactions. In cells that have large needs for nucleotides, most of the ribulose 5-phosphate is converted to ribose 5-phosphate and used for nucleotide biosynthesis. In cells that need more NADPH than nucleotides, the excess ribulose 5-phosphate is converted to compounds that enter glycolysis in a series of reversible reactions (not shown).

2. G6PD deficiency

Interestingly, the key disease state associated with defects in the pentose phosphate pathway arises NOT from an inability to make nucleotides, but rather deficient NADPH production. We will discuss this common disease only briefly here; you will return to it in the Microbiology portion of the course and again in M3 later in the year.

The first NADPH-producing reaction of the pentose phosphate pathway is catalyzed by glucose 6-phosphate dehydrogenase (G6PD), an enzyme produced from a gene on the X chromosome. Unlike other cells, erythrocytes lack mitochondrial pathways that produce NADPH, and rely solely on the pentose phosphate pathway for NADPH production. Mutations that reduce G6PD activity result in a decreased ability to produce NADPH, which impairs the normal red blood cell response to oxidative damage. G6PD-deficient individuals are typically asymptomatic unless exposed to conditions that increase production of reactive oxidants (e.g. hydrogen peroxide) that damage hemoglobin, membrane lipids, and other cellular components. Such oxidative damage can lead to hemolytic anemia. Conditions that can precipitate hemolytic ane-

Figure 2.12 The oxidative portion of the pentose phosphate pathway.
NADPH is produced in the first and third reactions. Reproduced with permission from Colby, Biochemistry, a Synopsis, Lange, 1985.
mia in the setting of G6PD deficiency include infection, use of certain drugs (including sulfa drugs and antimalarial drugs), and consumption of fava beans.

How is NADPH protective? Normally, hydrogen peroxide is eliminated by glutathione. Glutathione is a tripeptide made up of glutamate, cysteine and glycine (Figure 2.13). Its sulfur-containing side chain can reduce hydrogen peroxide to water, and is oxidized in the process. Glutathione reductase restores glutathione to its reduced form using NADPH as the source of reducing power. Thus mutations that decrease G6PD activity impair the red cell’s ability to detoxify hydrogen peroxide.

G6PD deficiency is very common - about 400 million people worldwide are affected. Frequencies are highest in tropical Africa, parts of the Middle East, and Southeast Asia.

C. Formation of PRPP

Let’s return now to building nucleotides. To provide the proper substrate on which to add or build a base, the 1’ carbon of the ribose 5-phosphate formed in the pentose phosphate pathway is “activated.” PRPP (for 5-phosphoribosyl-1-pyrophosphate) is the ribose derivative that results from the donation of a pyrophosphate group from ATP (Figure 2.14) in a reaction catalyzed by PRPP synthetase. PRPP is the sugar directly

**Figure 2.13** Protection of red blood cells by NADPH

NADPH protects the red blood cell from oxidative damage by maintaining glutathione in its reduced form. Reproduced with permission from Colby, *Biochemistry, a Synopsis*, Lange, 1985.

**Figure 2.14** Synthesis of PRPP by PRPP synthetase

used to produce nucleotides *de novo* and via salvage pathways. PRPP synthetase is subject to feedback inhibition by purine and pyrimidine nucleotides.

D. *De novo* Ribonucleotide Synthesis

Purine and pyrimidine nucleoside 5’-monophosphates can be synthesized *de novo* from PRPP and various carbon and nitrogen donors. The raw materials for both types of nucleotide have a common origin. However, the pathways by which they are formed are separate and distinct in organization. In the pyrimidine pathway, the ring structure of the base is assembled first and then attached to the pentose sugar PRPP. In contrast, the purine pathway starts with the pentose sugar and builds the ring structure of the base upon it.

Nucleoside 5’-monophosphates are phosphorylated to form the corresponding diphosphates by one of several nucleoside monophosphate kinases, each of which is specific for the base component of the nucleotide (see Figure 2.11 for specifics). Nucleoside diphosphates can be converted to triphosphates by a non-specific nucleoside diphosphate kinase (NDK). ATP is the phosphate donor in all of these phosphorylation reactions.

Now let’s take a step back to look at how the nucleoside 5’-monophosphates are formed from PRPP and other precursors.

1. Purine Nucleotide Synthesis

a. The pathway

The pathway for the *de novo* synthesis of purine ribonucleotides is shown in abbreviated form in Figure 2.15. In the first reaction, glutamine donates its amide group to carbon 1 of PRPP, forming the first nitrogen of the purine ring. The remaining atoms of the purine ring are added stepwise. Additional nitrogen atoms are derived from glycine, glutamate, and aspartate. Carbon atoms are donated by CO2 and formyl-H4folate. Completion of the base results in a purine nucleoside monophosphate, called inosine 5’-monophosphate (IMP). IMP is the “parent” purine nucleoside monophosphate from which both adenosine monophosphate (AMP) and guanosine monophosphate (GMP) are formed.
b. **REGULATION**

Several enzymes in purine nucleotide synthesis are inhibited by purine nucleotides, ensuring that their production is matched to cellular needs. The first enzyme of the purine pathway, glutamine PRPP amidotransferase, is feedback-inhibited by a number of purine nucleotides. Intracellular concentrations of glutamine and PRPP are usually below the Km for this enzyme. Thus an increase in their concentration can lead to overproduction of purine nucleotides.

c. **CLINICAL CONNECTIONS**

A few important drugs inhibit key steps in *de novo* purine nucleotide synthesis and as a result kill rapidly dividing cells. Mercaptopurine and azathioprine (a prodrug that is converted in the body to mercaptopurine), both inhibitors of several steps in *de novo* purine synthesis, are cancer chemotherapeutic drugs that are also used as immunosuppressants in the treatment of many diseases, including rheumatoid arthritis, lupus, and IBD.

IMP dehydrogenase, an enzyme that carries out one of the steps in synthesis of GMP from IMP, is inhibited by the immunosuppressant drug **mycophenolate mofetil**, which is widely used to prevent solid
organ transplant rejection. Lymphocytes are unique in being unable to utilize salvage pathways to generate GMP. Their dependence on de novo synthesis for GMP means proliferation of these cells is selectively inhibited by this drug. Note the role of formyl-H$_4$folate, a form of reduced folate carrying a formyl group, in providing a 1-carbon unit to the synthesis of the inosine base. The contribution of folate cofactors and vitamin B12 to nucleotide metabolism and the actions of drugs on these processes will be discussed in more detail below.

2. **Pyrimidine Nucleotide Synthesis**

a. **The Pathway**

*De novo* pyrimidine biosynthesis begins with the formation of carbamoyl phosphate from the amide group of glutamine, CO$_2$, and a phosphoryl group of ATP ([Figure 2.16](#)) via the enzyme carbamoyl phosphate synthase-II (CPSII). Carbamoyl phosphate becomes part of the pyrimidine ring. The remaining atoms of the ring are added as a unit in the form of aspartate. The resulting N-carbamoyl aspartate is converted to a free pyrimidine base, orotate, by ring closure and oxidation. The base is then joined to PRPP to form a nucleoside monophosphate, orotidine.

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**Figure 2.16** *De novo* synthesis of pyrimidine nucleotides

Amino acid donors of carbons and nitrogens are shown, but should not be memorized. Modified with permission from Colby, *Biochemistry, a Synopsis*, Lange, 1985.
monophosphate (OMP). Uridine monophosphate (UMP) is derived directly from OMP by decarboxylation. UMP is phosphorylated to produce UTP. CTP arises from an amidation reaction catalyzed by CTP synthase. The synthesis of TTP is described later.

b. Regulation
CPSII catalyzes the key regulated step in pyrimidine synthesis. The enzyme is inhibited by UTP and activated by PRPP. Thus, as pyrimidine concentrations decrease (as indicated by UTP concentration), CPSII activity increases and more pyrimidines are produced. CTP synthase is inhibited by its product, CTP.

c. Clinical Connections
A few important clinical connections are worth mentioning. First, do you remember that carbamoyl phosphate is an intermediate in another key metabolic pathway? Carbamoyl phosphate is utilized as a substrate in the urea cycle by the enzyme ornithine transcarbamoylase. Inherited deficiency of this enzyme (the most common urea cycle defect) is associated with hyperammonemia and associated problems, but is also marked by elevated blood and urinary orotate, because excess carba-
moyl phosphate shunts into pyrimidine nucleotide metabolism. Another inherited defect in pyrimidine nucleotide synthesis, orotic aciduria, arises from deficiency of enzymes that convert orotate to OMP and UMP. In addition to urinary excretion of orotate, this disease is associated with poor growth and megaloblastic anemia. Also importantly, a step in synthesis of UMP from orotic acid is inhibited by the antirheumatic drug leflunomide.

E. Deoxyribonucleotide Biosynthesis

1. Ribonucleotide Reductase
Deoxyribonucleotides needed for DNA synthesis are formed from ribonucleotides by the reduction of the sugar ring at the 2’ position. A single enzyme, ribonucleotide reductase, catalyzes the conversion of each of the ribonucleoside diphosphates to the corresponding deoxyribonucleoside diphosphates (Figure 2.17). NADPH donates the reducing equivalents used in this reaction. Nucleoside diphosphate kinase converts the products of ribonucleotide reductase to their corresponding triphosphate, generating the nucleotides needed for DNA synthesis, with the exception of dTTP (discussed below).
Regulation of ribonucleotide reductase activity is effected mainly through an allosteric site, to which ATP binds and activates the enzyme, and dATP binds and inhibits the enzyme. A chemotherapeutic drug, hydroxyurea, acts by inhibiting ribonucleotide reductase and reducing the dNTP pool available to rapidly dividing cells.

2. PRODUCTION OF dTTP

Thymine-containing nucleotides must be generated from uracil-containing nucleotides. dUMP is the substrate for thymidylate synthase, which methylates uracil, forming dTMP (Figure 2.18). The one-carbon group, donated by methylene-H₄folate, is transferred to dUMP and simultaneously reduced. In this process, H₄folate is oxidized to H₂folate (dihydrofolate). Dihydrofolate must be reduced back to H₄folate by dihydrofolate reductase before it can again serve as a one-carbon carrier.
F. FOLATE AND VITAMIN B12 IN DE NOVO NUCLEOTIDE SYNTHESIS

1. ROLES AND METABOLISM

Having seen folate cofactors utilized as 1-carbon carriers in two parts of nucleotide biosynthesis, now is a good time to delve more deeply into this water-soluble vitamin’s metabolism, along with that of another important water-soluble vitamin, vitamin B12 (cobalamin), which plays a key role in the formation of active folate.

The generic term “folate” refers to a group of compounds that include folic acid in their structures (Figure 2.19). The biologically active form of folate is a reduced derivative of folic acid, tetrahydrofolate (H₄folate). Polyglutamation (addition of glutamate residues to the existing glutamate in the structure of folic acid) is required for retention and utilization of folate intracellularly, but only monoglutamated folates can be transported across cell membranes. These are important considerations in the pharmacokinetic parameters of drugs that are folate analogues.

The type of “folate” contained in vitamin and dietary supplements is folic acid. When folic acid is consumed, it is reduced to H₄folate and polyglutamated inside cells. The enzyme responsible for reducing folic acid is dihydrofolate reductase (DHFR), the same enzyme needed to reduce dihydrofolate in dTTP synthesis (Figure 2.20). Although vitamin supplements contain folic acid, the unsupplemented human diet contains very little folic acid. Folates are synthesized in bacteria and in higher plants, and found in green leafy vegetables, fruits, and legumes. Most of the naturally occurring dietary folate consists of polyglutamated N5- methyl-H₄folate, a methylated H₄folate derivative (see Figure 2.20).
Nucleotide synthesis enzymes cannot directly utilize methyl-H$_4$folate as a 1-carbon carrier. Therefore dietary folate cannot be used for nucleotide biosynthesis until its methyl group has been removed. This step is carried out by an enzyme called homocysteine methyltransferase, which transfers the methyl group to the amino acid homocysteine, generating methionine (Figure 2.21).

![Figure 2.20](image1.png) **Figure 2.20** Dihydrofolate reductase converts folic acid to H$_4$folate in two steps. Reproduced with permission from Colby, *Biochemistry, a Synopsis*. Lange, 1985.

![Figure 2.21](image2.png) **Figure 2.21** The reaction catalyzed by homocysteine methyltransferase

The dashed line indicates that only a portion of the structure of methyl-H$_4$folate is shown. Reproduced with permission from Colby, *Biochemistry, a Synopsis*. Lange, 1985.
The resulting $\text{H}_4\text{folate}$ can then pick up a formyl (HCO) or methylene (CH2) group and take part in nucleotide synthesis. Homocysteine methyltransferase requires a vitamin B12 derivative (methylcobalamin) as its coenzyme. In individuals who lack homocysteine methyltransferase or its coenzyme, dietary folate is trapped as methyl- $\text{H}_4\text{folate}$, and nucleotide synthesis is impaired. Because methyl-$\text{H}_4\text{folate}$ is a poor substrate for the enzyme that attaches glutamate residues, the folate is not retained by cells and is excreted from the body.

$\text{H}_4\text{folate}$ picks up the one-carbon groups needed for nucleotide synthesis from several sources, and the formyl, methenyl, and methylene derivatives can be interconverted by freely reversible reactions (Figure 2.22). Recall that the formyl derivative is required for purine synthesis, while the methylene derivative is needed for synthesis of thymine. Methylene-$\text{H}_4\text{folate}$ can be reduced to methyl-$\text{H}_4\text{folate}$, via an irreversible reaction. This step removes folate from the pool that can participate in nucleotide synthesis. The folate can return to the active pool only by transferring its methyl group to homocysteine.

2. **Clinical Connections**

a. **Folate and/or B12 Deficiencies**

Folate is found in all tissues of the body. Tissues with the highest requirement for folate and vitamin B12 are those with the highest turnover of cells, which are hematopoietic cells and gastrointestinal epithelial cells. One consequence of either folate deficiency or vitamin B12 deficiency is the development of anemia due to impaired nucleotide synthesis. This particular form of anemia is megaloblastic (megalo = large, blast = immature stage in cellular development), meaning that large, immature red blood cells are found in

![Figure 2.22 The big picture of folate metabolism](image-url)
circulation. This anemia will be discussed further in the M3 block.

In addition to anemia, vitamin B12 deficiency causes neurologic disturbances, including peripheral neuropathy. Unlike other cells in the body, cells in the nervous system are dependent on B12 for generation of methionine, the direct precursor to an important methyl donor called S-adenosylmethionine. The neurological problems seen in B12 deficiency are believed to be caused by hypomethylation within the nervous system.

Major sources of vitamin B12 are meat, eggs, dairy products, fish, and seafood. As you recall from M&N, absorption of B12 is a complex process. It will not be reviewed here, except to say that B12 must be bound to intrinsic factor (IF) in order to be absorbed in the distal ileum. It has been estimated that 10 – 15% of people over the age of 60 are vitamin B12 deficient. Among the causes are decreased gastric acidity, autoimmune destruction of the parietal cells of the stomach, and autoantibodies against intrinsic factor. Failure to produce intrinsic factor due to autoimmune destruction of intrinsic factor or the parietal cells of the stomach leads to a pernicious anemia, a form of megaloblastic anemia.

b. Cytotoxic anticancer agents used as immunosuppressants

Wing to their central role in nucleotide metabolism, the folate cofactors are necessary for the growth of all known organisms. Drugs that block the formation of H4folate (“antifolates”, amongst the class of drugs known as antimetabolites) are effective in the treatment of bacterial infections, some forms of cancer, and as immunosuppressants. You will learn more about the antibacterial drugs later in I3, and the chemotherapeutic agents in M3. For now, know that methotrexate is an inhibitor of DHFR used to treat rheumatoid and inflammatory bowel diseases (at low doses) and cancers (at higher doses). By interfering with the folic acid cycle and reduction of folic acid, this drug kills rapidly dividing cells and thus prevents clonal expansion of B and T lymphocytes.
III. NUCLEOTIDE CATABOLISM AND SALVAGE

A. OVERVIEW

Nucleotide turnover occurs continuously in cells. Breakdown of DNA and RNA releases nucleoside 5’-monophosphates, which can be hydrolyzed by 5’-nucleotidases to yield nucleosides. Although both purine and pyrimidine nucleosides can be degraded to waste products that are excreted, the catabolic pathways have branch points in most cells at which the components of nucleotides can be salvaged (Figure 2.23). Having shared catabolism and salvage pathways saves metabolic energy while preventing nucleotide pools from reaching toxic levels.

Unless the flow of nucleotides into the shared salvage/catabolism pathway is greater than usual, normally more components are salvaged than are catabolized. (Intestinal epithelial cells are exceptions to this rule, and completely catabolize the components of dietary nucleotides rather than salvage them.) This makes sense because although nucleotide synthesis is energetically costly, the complete breakdown yields very little energy.

As with many metabolites, the liver is a “way station” for nucleotides. The liver is a major site for both de novo nucleotide synthesis and degradation. Excess nucleosides and bases are supplied by hepatocytes to other tissues, such as the brain and muscle, which utilize salvage pathways to generate needed nucleotides. (Note that nucleosides and bases cross membranes but nucleotides do not.) The salvage pathways also make it possible for nucleotides and their components released by cells undergoing apoptosis to be re-used. Many cell types contain 5’-nucleotidases on the outer surface of
the plasma membrane and can therefore convert extra-cellular nucleotides to nucleosides, which can be taken up using Na+/nucleoside symporters.

Pyrimidine catabolism and salvage pathways are rarely associated with disease. Derangements of purine salvage/catabolism are more common. Purine salvage pathways are also important clinically for metabolism of certain drugs.

B. Purine Salvage

1. The Pathway

The purine salvage pathway is shown in Figure 2.24. Of the purine ribonucleosides, only adenosine can be phosphorylated directly to form a nucleotide, by adenosine kinase. Ribonucleosides can also proceed further down the catabolic pathway. Adenosine deaminase (ADA) turns adenosine into inosine by removing the amino group of the base. The next enzyme of the pathway, purine nucleoside phosphorylase (PNP), acts on both inosine and guanosine. PNP cleaves the bonds between the bases and the sugars, releasing hypoxanthine and guanine. These bases can be salvaged by hypoxanthine guanine phosphoribosyl transferase (HGPRT), which reattaches phosphorylated ribose using PRPP as a substrate, thereby producing inosine monophosphate and guanosine monophosphate. Interestingly, lymphocytes lack the ability to produce GMP via salvage (see earlier section that addresses mycophenolate mofetil).

2. Genetic Defects in Purine Salvage

a. SCID

Severe Combined Immuno deficiency Syndrome due to ADA/PNP deficiency Individuals who lack either adenosine deaminase or purine nucleoside phosphorylase fail to develop normal immune systems and usually die of infection early in child-
hood. Approximately 20% of patients with autosomal recessive Severe Combined Immunodeficiency Disease (SCID) have mutations in their ADA genes.

Because ADA deficiency is much more common than PNP, we will focus on ADA for the remainder of this section. ADA is present in all cell types but is most abundant in lymphoid tissues, brain, and the GI tract. It is important to note that deoxyribonucleotides are substrates for salvage reactions, though they are not necessarily shown in the figures here. In ADA deficiency, the problem does not lie with an inability to generate enough AMP or dAMP via salvage to meet the cell’s needs. In contrast, current thinking is that accumulation of toxic levels of nucleotides and their metabolites result in lymphocyte death. In ADA deficiency, adenosine and deoxyadenosine levels are significantly elevated in plasma and urine. The most striking hallmark of ADA deficiency is massive accumulation of dATP in lymphocytes, which results from uptake of excessive intermediates from the blood and is hypothesized to be explained by preferential “trapping” of these phosphorylated compounds. Several models have been put forward to explain why immune cells are uniquely sensitive to deficiencies in ADA, but no definitive conclusion has been reached. Regardless, all pathophysiologic mechanisms in ADA deficiency result from the presence of increased concentrations of substrates of ADA. Interestingly, toxic metabolites that accumulate on account of the enzyme defect derive primarily from dying cells, and clinical histories are consistent with each infection resulting in sequentially more serious diminution of immune cells and function.

Hematopoietic bone marrow/stem cell transplantation is the therapy of choice for ADA deficiency. PEG-ADA enzyme replacement therapy is also an efficacious treatment. Purified ADA enzyme modified with polyethylene-glycol (to protect the enzyme from antibody-mediated destruction) is delivered via intramuscular injection. The enzyme need not be taken up into cells to reduce levels of toxic metabolites, bypassing a challenge in traditional gene therapy regimens. Enzyme-replacement therapy prolongs life, restores normal growth and development, and improves protective T cell immunity in most patients, but is ex-
pensive and requires life-long adherence. ADA-deficient SCID was the first disorder for which human gene therapy was developed, which is still a treatment under active investigation.

b. Lesch-Nyhan Syndrome

Mutations in the X-linked HGPRT gene that abolish enzyme activity result in an inability to salvage hypoxanthine or guanine. PRPP levels increase, while IMP and GMP levels decrease, alleviating inhibition of the purine synthesis pathway. Individuals with complete HGPRT deficiency develop Lesch-Nyhan Syndrome (LNS). This remarkable disorder is characterized by choreoathetosis (a movement disorder), spasticity, variable mental retardation, uric acid overproduction and gout (see below), and, most strikingly, self-mutilation (chewing off fingers and biting cheeks and lips). Treatment for LNS is symptomatic. Gout can be treated as described below. There is no standard efficacious treatment for the neurological symptoms of LNS; response to drugs is generally poor. Arm restraints and removal of teeth are usually the only way to prevent self-inflicted wounds.

C. Purine Catabolism — Uric Acid Production

A portion of the hypoxanthine and guanine produced by the purine salvage pathway is degraded as shown in Figure 2.25. Through the activities of guanase and xanthine oxidase, both guanine and hypoxanthine are converted to xanthine. Xanthine is further metabolized by xanthine oxidase to uric acid, the end product of purine catabolism. Uric acid is only sparingly soluble and is excreted in the urine, diluted with large amounts of water. If the plasma level of uric acid becomes unusually high (hyperuricemia), it may precipitate in the joints and connective tissues in the form of sodium urate crystals. Upon ingesting the crystals, macrophages initiate an inflammatory response leading to the syndrome known as gout.

In the majority of patients gout is due to underexcretion of uric acid, for as-yet-unidentified reasons. Underexcretion can also occur secondary to other disease processes or ingestion of drugs that affect urate excretion by the kidney. Less commonly, gout can arise due to overproduction of uric acid, for example, in situations of high cell turnover (i.e. myoprolif-
erative disorders, treatment of cancer with chemotherapeutic agents). Various genetic defects result in overproduction of purine catabolites, including mutations in PRPP synthetase (e.g. an elevated Vmax, increased affinity for substrate, or resistance to feedback inhibition), and Lesch-Nyhan syndrome.

Treatment of gout will be discussed in the lecture on anti-inflammatory and immunosuppressive drugs.