SECTION 9

Nucleic Acid Metabolism

CONTACT INFORMATION

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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 proc-

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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

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- PURINE
- GOUT
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Nucleotide metabolism involves several interconnected pathways (Figure 2.8). Nucleotides can be synthesized *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 *de novo* synthesis, salvage, and degradation of nucleotides result in clinical disorders, and many drugs target these pathways.

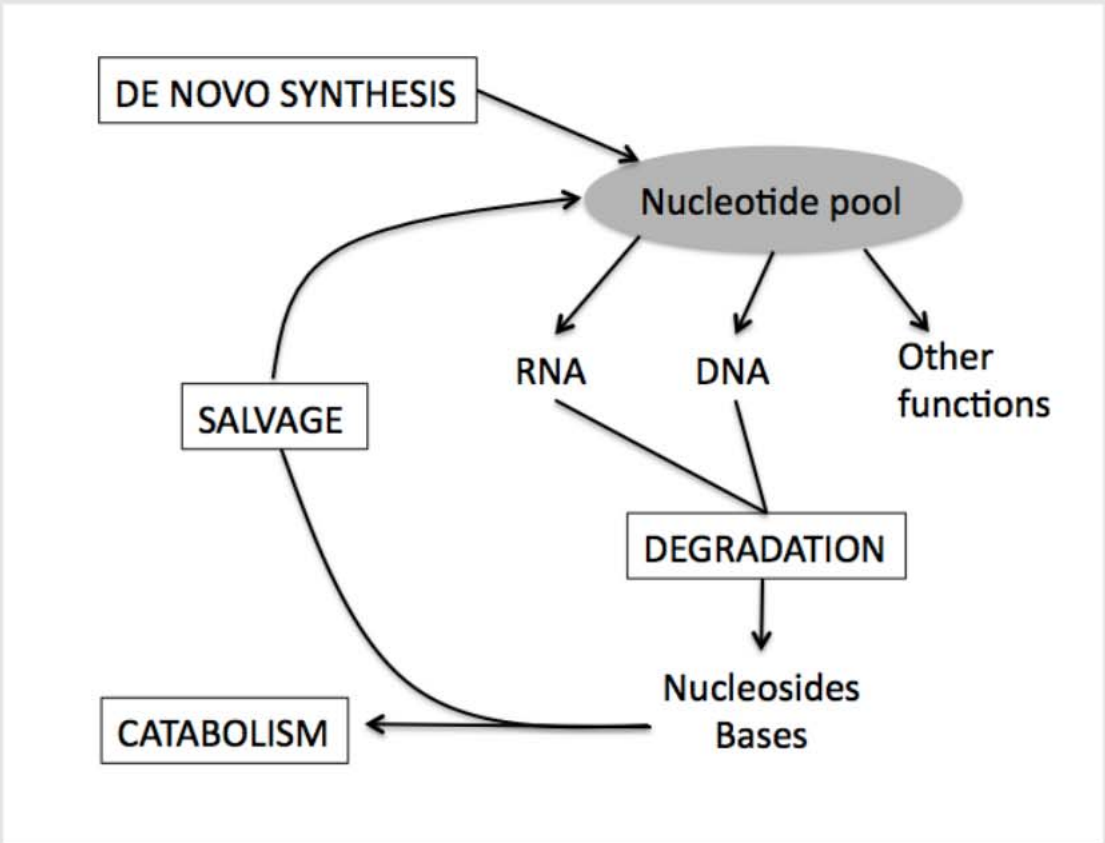

FIGURE 2.8 Overview of Nucleotide Metabolism

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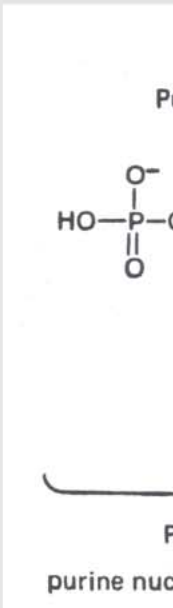

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phosphate group at the 5' position either a *nucleotide* or a *nucleoside 5'-monophosphate*. The 8 major species of nucleoside triphosphates are listed in Table 1.

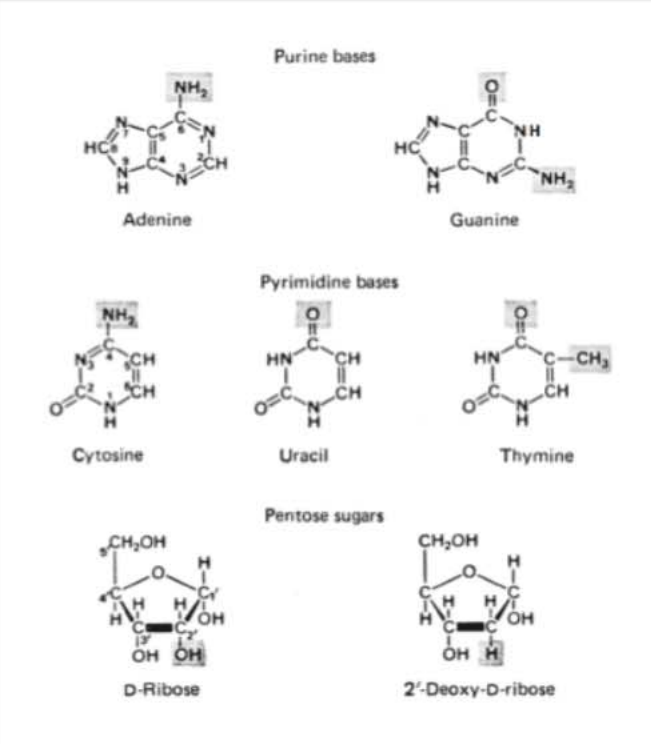

FIGURE 2.10 Component sugars and bases of the common nucleotides.

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Table 1: Common nucleoside triphosphates.

Nucleoside Triphosphates	Names of Components	
	Bases	Nucleosides
Ribonucleotides		
Adenosine triphosphate (ATP)	Adenine	Adenosine
Guanosine triphosphate (GTP)	Guanine	Guanosine
Cytidine triphosphate (CTP)	Cytosine	Cytidine
Uridine triphosphate (UTP)	Uracil	Uridine
Deoxyribonucleotides		
Deoxyadenosine triphosphate (dATP)	Adenine	Deoxyadenosine
Deoxyguanosine triphosphate (dGTP)	Guanine	Deoxyguanosine
Deoxycytidine triphosphate (dCTP)	Cytosine	Deoxycytidine
Deoxythymidine triphosphate (dTTP)	Thymine	Deoxythymidine

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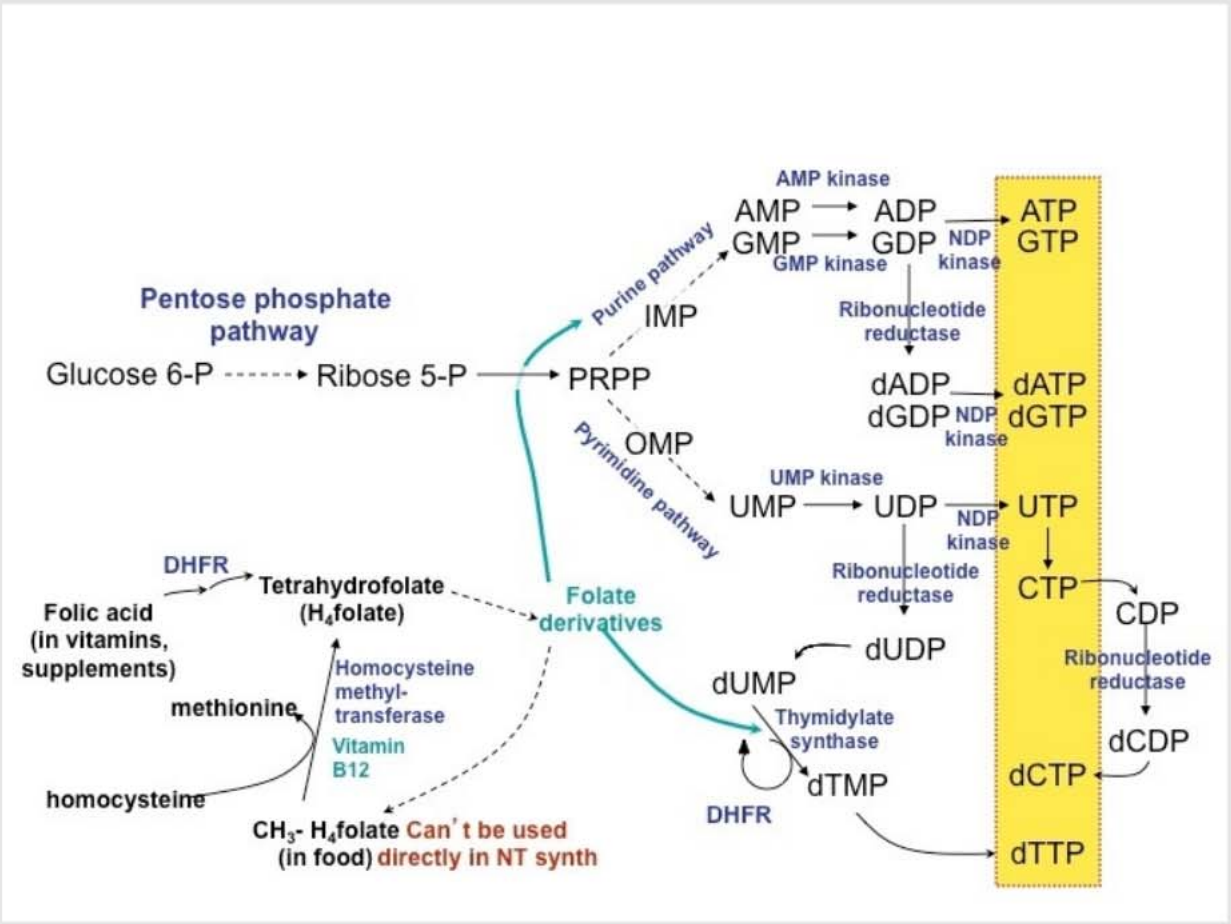

FIGURE 2.11 *De novo* nucleotide synthesis "big picture."

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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 reactons. 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).

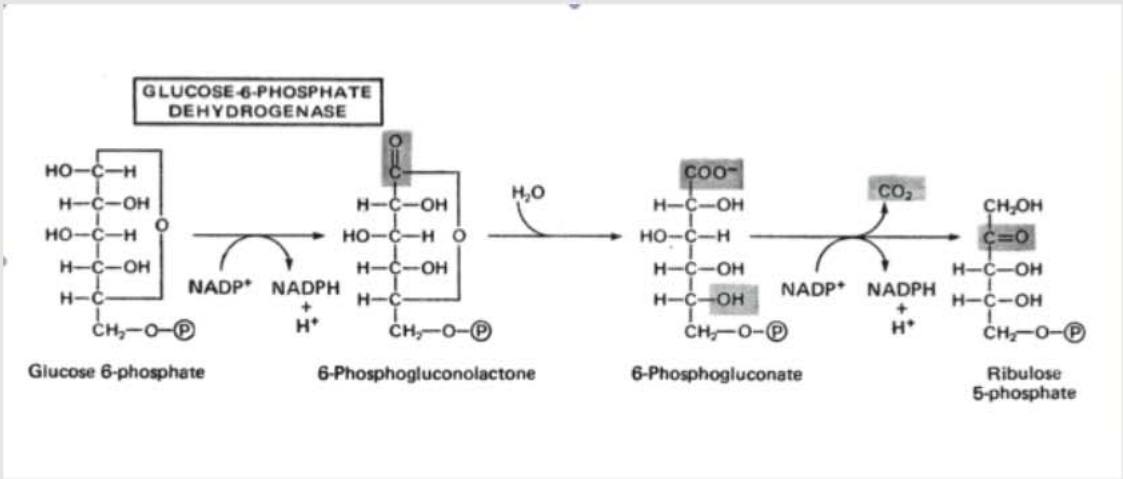

FIGURE 2.12 The oxidative portion of the pentose phosphate pathway.

NADPH is produced in the first and third reactions. Reproduced with permission from Colby, *Biochemistry, a Synopsis*, Lange, 1985.

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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

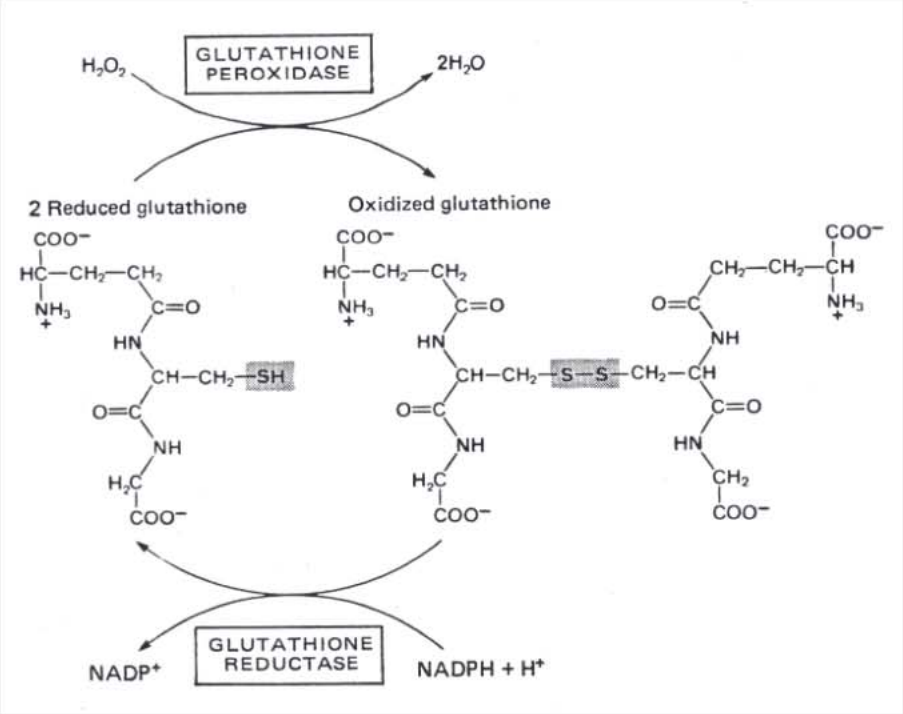

FIGURE 2.13 Protection of red blood cells by NADPH

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.

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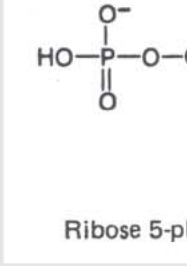

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used to produce nucleotides *de novo* and via salvage pathways. PRPP synthetase is subject to feedback inhibition by purine and pyrimidine nucleotides.

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.

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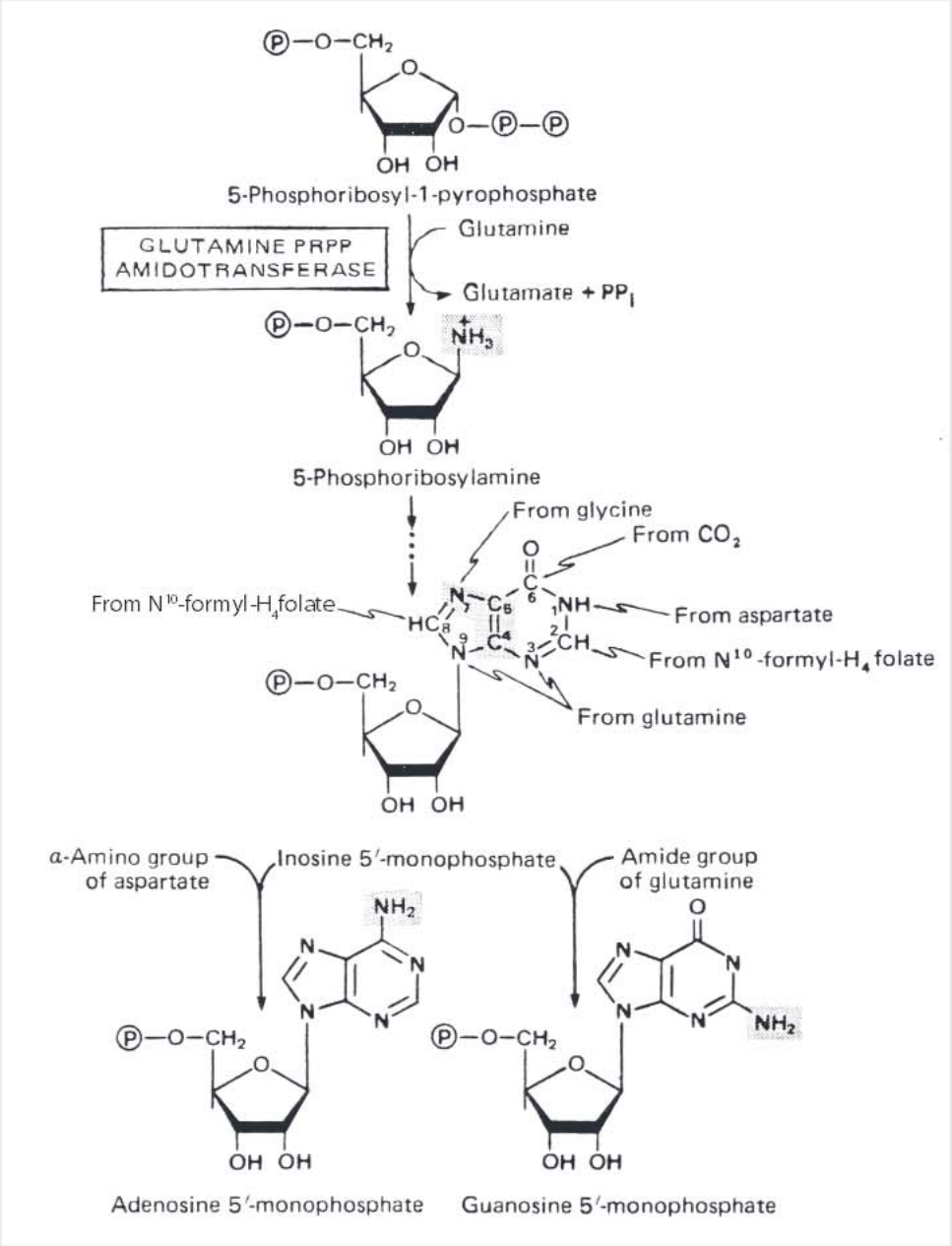

FIGURE 2.15 *De novo* synthesis of purine nucleotides.

The origins of some of the carbons (C) and nitrogens (N) in the purine bases are shown, but should not be memorized. The dashed lines indicate that several intervening steps are not shown. Reproduced with permission from Colby, *Biochemistry, a Synopsis*, Lange, 1985.

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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_4folate**, 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, CO2, 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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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 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-

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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.

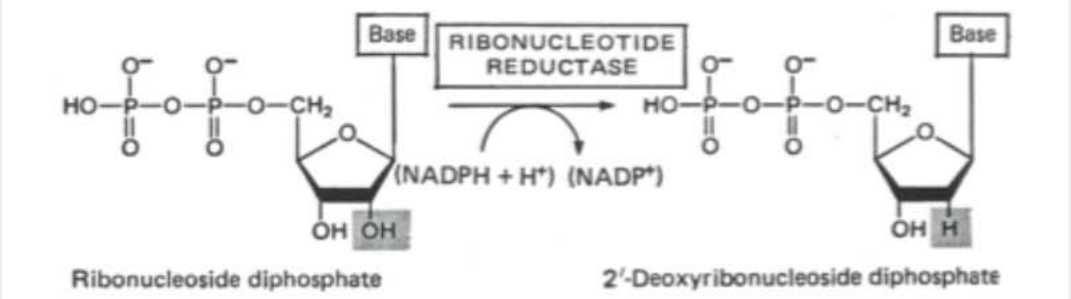


FIGURE 2.17 Synthesis of the Deoxyribonucleotides

Synthesis of the deoxyribonucleotides is catalyzed by ribonucleotide reductase. Reproduced with permission from Colby, *Biochemistry, a Synopsis*, Lange, 1985.

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-H_4folate**, is

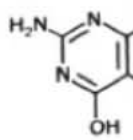
F. FOLATE AND VITAMIN B12 IN DE NOVO NUCLEOTIDE SYNTHESIS

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** (H_4folate). 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 con-



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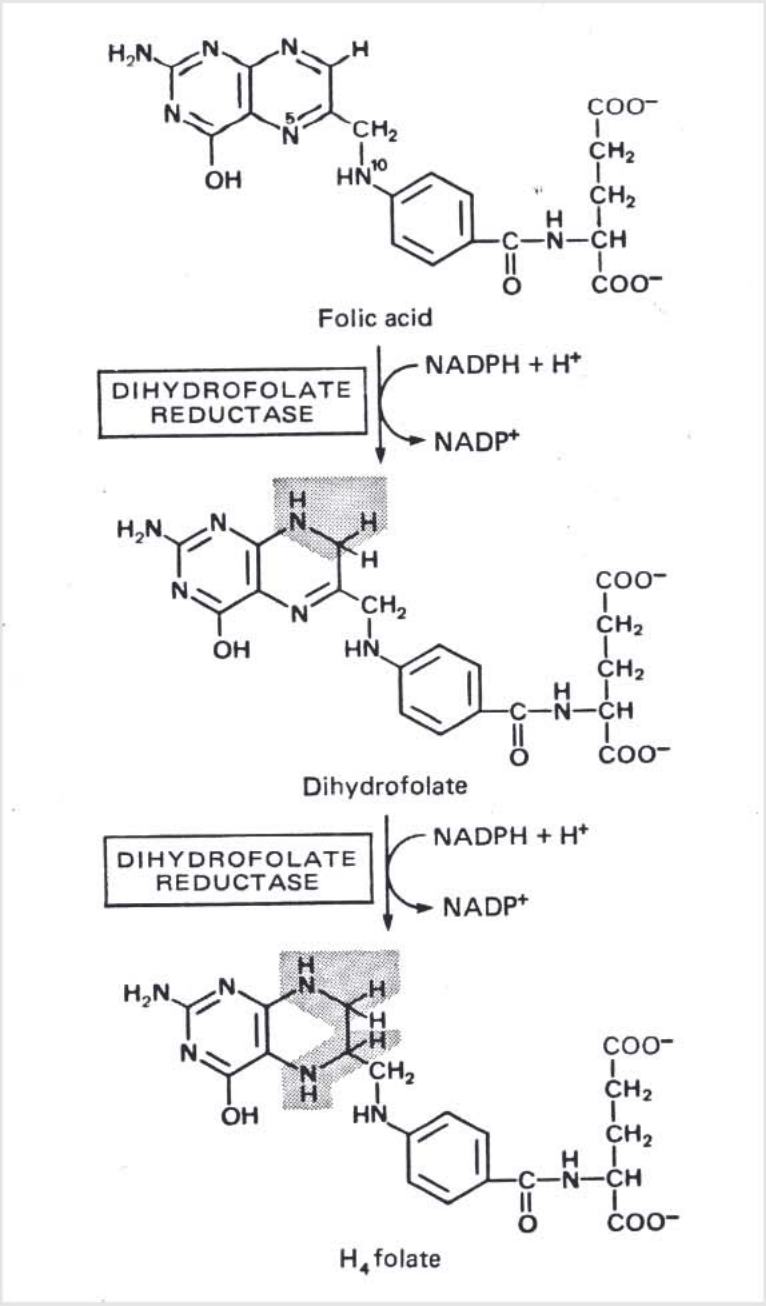

FIGURE 2.20 Dihydrofolate reductase converts folic acid to H_4folate in two steps.

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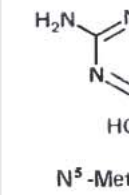

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The resulting H_4folate can then pick up a formyl (HCO) or methylene (CH2) group and take part in nucleotide synthesis. Homocysteine methyltransferase requires a vitamin B12 derivative (methylcobalamin) as its coenzyme. In individuals who lack homocysteine methyltransferase or its coenzyme, dietary folate is trapped as methyl- H_4folate, and nucleotide synthesis is impaired. Because methyl-H4folate is a poor substrate for the enzyme that attaches glutamate residues, the folate is not retained by cells and is excreted from the body.

H_4folate 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-H_4folate can be reduced to methyl-H_4folate, 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*

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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

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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 com-

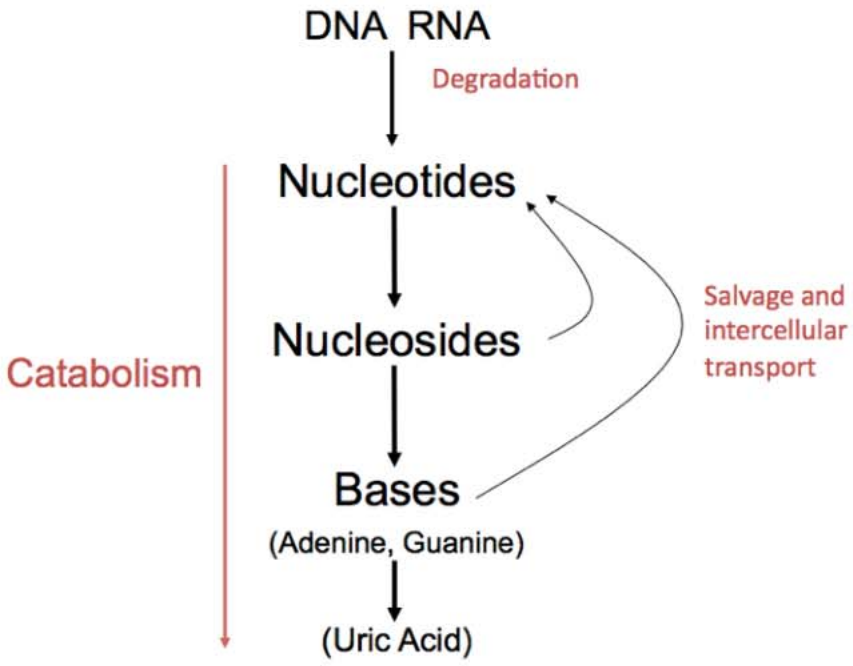

FIGURE 2.23 Overview of nucleotide salvage.

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the plasma membrane and can therefore convert extracellular 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

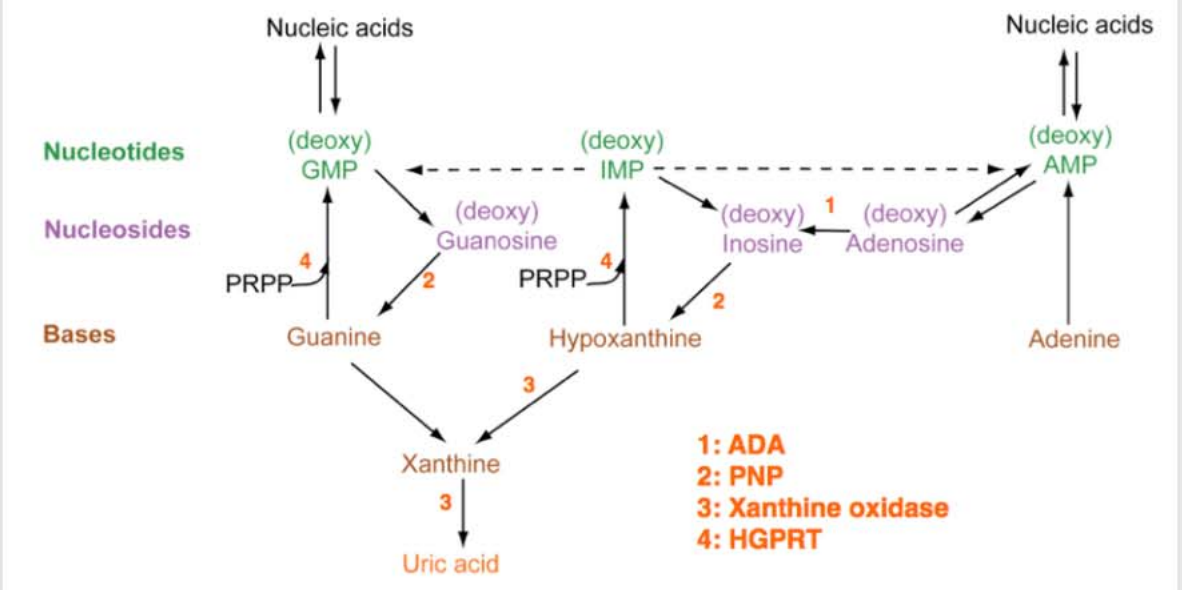

FIGURE 2.24 Overview of purine nucleoside and base salvage, and purine base catabolism

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

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.

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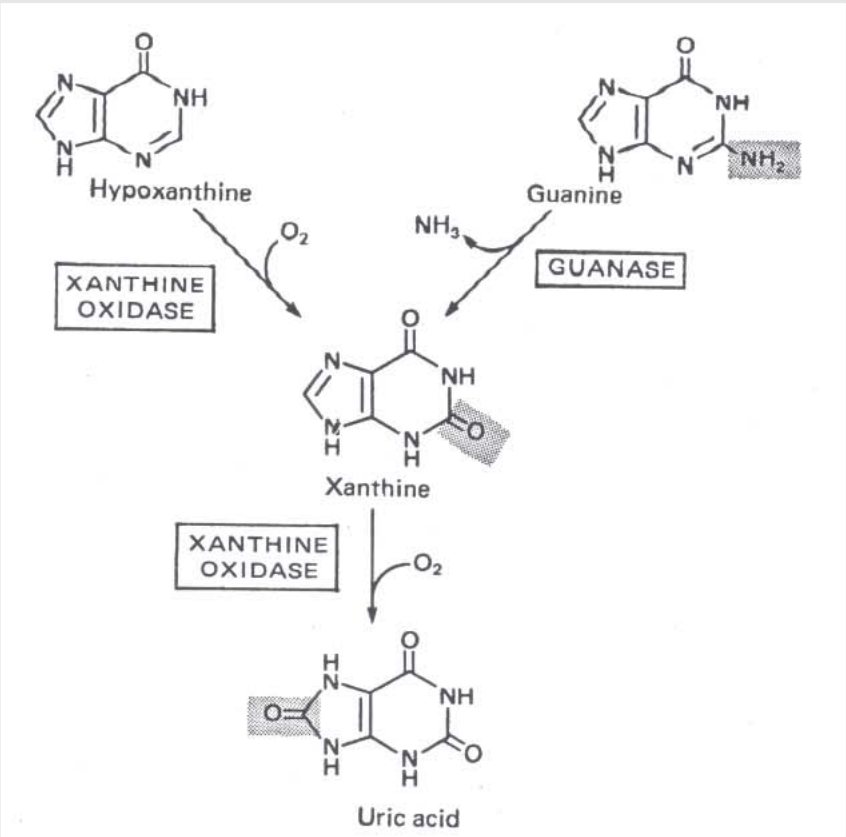

FIGURE 2.25 Uric acid is the end product of purine catabolism.

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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.