

Nucleotide Catabolism

April 26, 2003

Bryant Miles

Ribonucleotide Reductase produces dADP, dGDP, dCDP and dUDP. These diphosphodeoxyribonucleosides are phosphorylated by ATP to generate the dNTPs. The enzyme is the same nucleoside diphosphate kinase that phosphorylates the ribonucleotides.

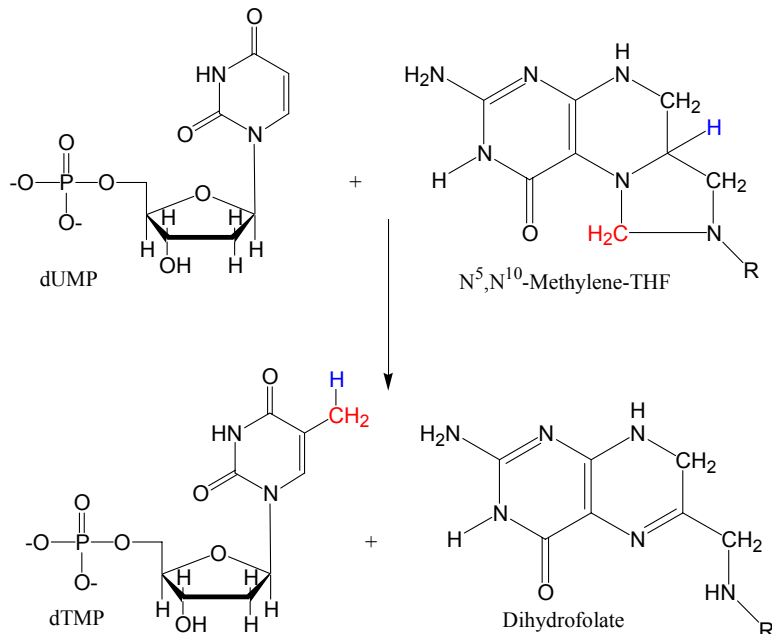


I. Thymine Biosynthesis

Ribonucleotide reductase converts CDP to dCDP and UDP to dUDP. Nucleoside diphosphate kinase phosphorylates dUDP to form dUTP. dUTP is immediately hydrolyzed into dUMP and pyrophosphate by dUTP phosphatase. This reaction is important because the concentration of dUTP must be minimized to prevent the incorporation of uracil into DNA. DNA polymerases do not discriminate between dUTP and dTTP as substrates.

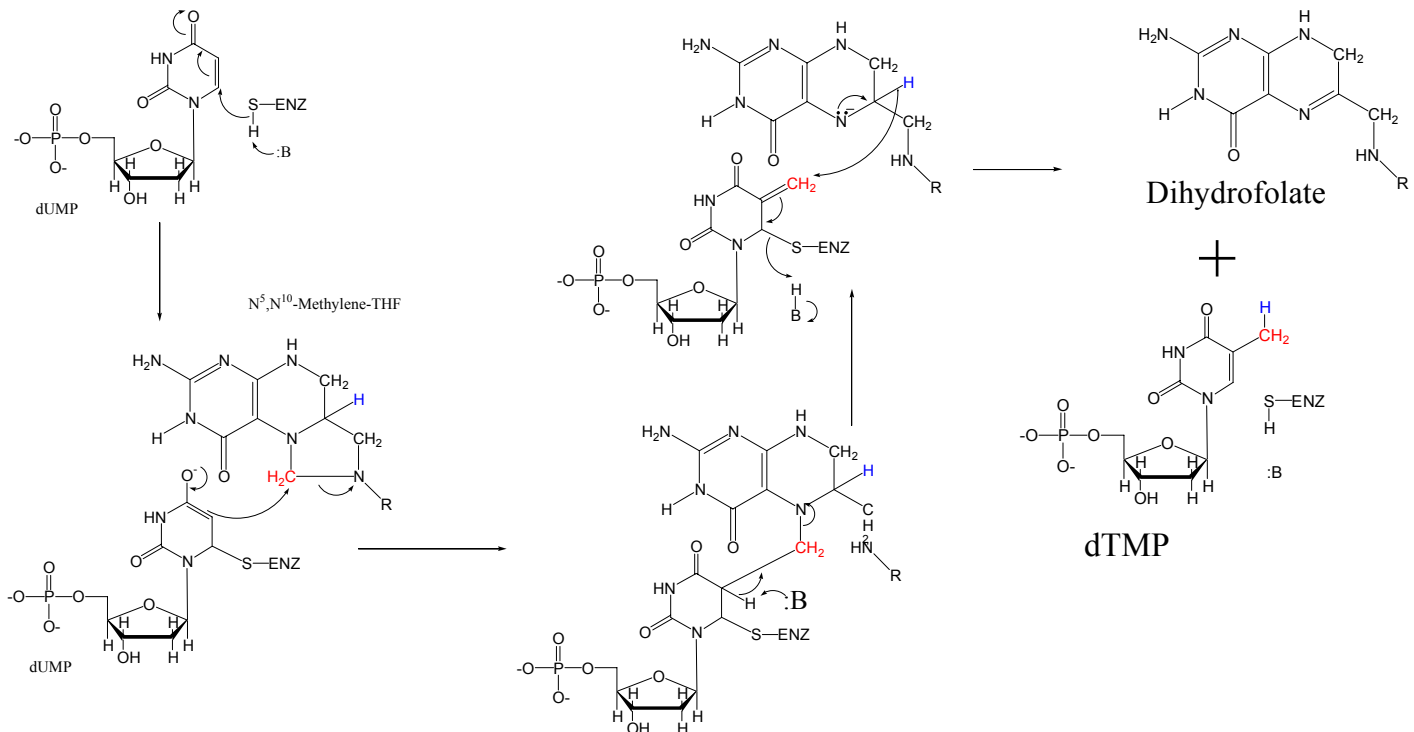


Deoxyuridine monophosphate is methylated to form deoxythymidine monophosphate by thymidylate synthase. Thymidylate synthase methylates dUMP using $\text{N}^5, \text{N}^{10}$ -methylene-THF as the methyl donor.



Note that the methylene carried by the THF has the same oxidation state as a hydroxymethyl group. This methylene is transferred to the uracil and then reduced to a methyl group. The reduction occurs by transferring a hydride from tetrahydrofolate which produces dihydrofolate. Dihydrofolate is reduced back to tetrahydrofolate by dihydrofolate reductase. Dihydrofolate reductase uses NADPH as the reductant.

This is an interesting reaction. Let's check out the mechanism.



1. Thymidylate synthase has a nucleophilic cysteine residue Cys-146 that attacks the C6 carbon of dUMP to form a covalent intermediate.
2. The attack of the cysteine generates an enolate ion that attacks the $-\text{CH}_2-$ carried by $\text{N}^5, \text{N}^{10}$ -methylene-THF to form an enzyme-dUMP-THF ternary complex.
3. An enzyme base abstracts the proton from the C5 position of dUMP to eliminate the THF cofactor.
4. A hydride is transferred from C6 of THF to the methylene group on the pyrimidine to form dTMP and dihydrofolate.

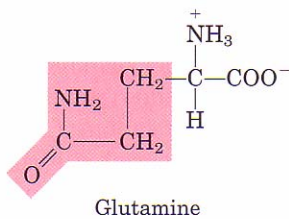
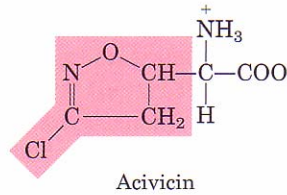
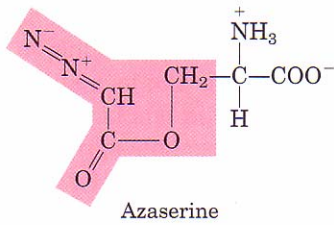
Tetrahydrofolate is regenerated from dihydrofolate by NADPH. This reduction is catalyzed by dihydrofolate reductase. The $\text{N}^5, \text{N}^{10}$ -methylene-THF is regenerated by serine hydroxymethyl transferase converting serine into glycine.

II. Anti-Tumor Reagents.

Cancer cells are rapidly proliferating cells. Normal mammalian cells grow and proliferate slowly. Because cancer cells are rapidly growing and proliferating they rapidly consume nucleotides for the synthesis of RNA and DNA. The result is cancer cells are more susceptible to inhibitors of nucleotide synthesis than normal cells. When studying pyrimidine and purine nucleotide biosynthesis, you soon realize that glutamine is the major source of nitrogen for nucleotide biosynthesis. These enzymes that use glutamine as the nitrogen source all contain a homologous glutamine amidotransferase domain. Let's list these enzymes.

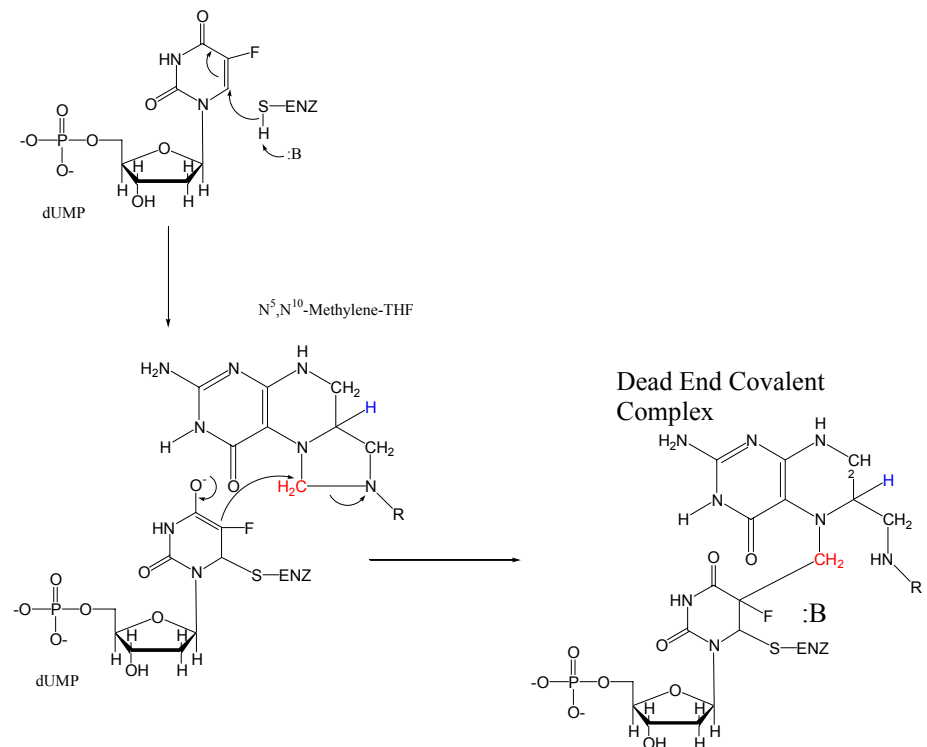
1. CPSII
2. CTP synthetase
3. Amidophosphoribosyl transferase
4. FGAM synthetase
5. GMP synthetase

Rapidly proliferating cancer cells are voracious consumers of glutamine. They require glutamine for pyrimidine and purine synthesis. Inhibitors of these glutamine amidotransferases have potential as chemotherapeutic agents. Shown to the left are 2 potent inhibitors of glutamine amido transferases, azaserine and acivicin. Both these inhibitors irreversibly inactivate the GAT subunits.



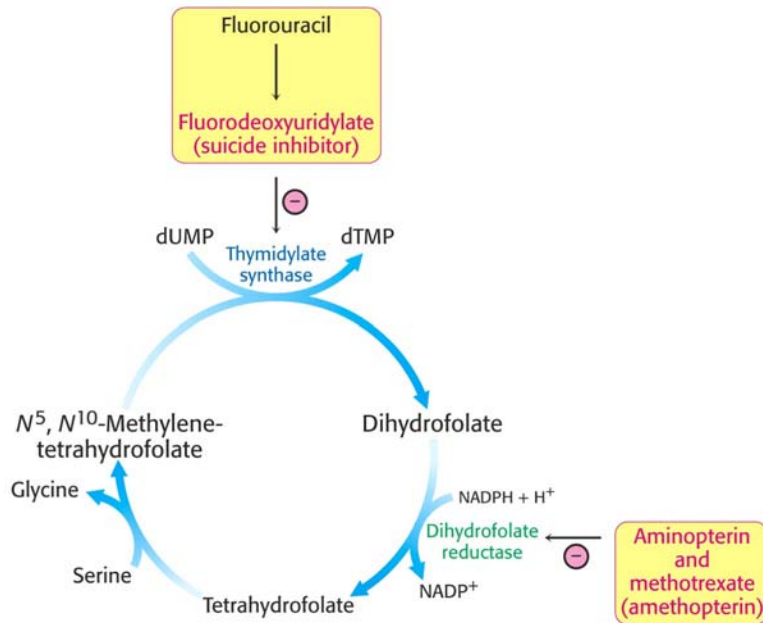
The other targets for pharmaceutical reagents are thymidylate synthase and dihydrofolate reductase. One inhibitor of thymidylate synthase is fluorouracil. Fluorouracil itself does not inhibit anything, but in cells fluorouracil is a substrate for pyrimidine phosphoribosyltransferase (the pyrimidine salvage enzyme) which condenses fluorouracil with PRPP to form fluorouracil monophosphate. Fluorouracil monophosphate is phosphorylated by nucleoside monophosphate kinase to form fluorouracil diphosphate which is a substrate for ribonucleotide reductase to produce 2'-deoxyfluorouracil diphosphate which is then phosphorylated by nucleoside diphosphate kinase and hydrolyzed by UTP phosphorylase to form 2'-deoxyfluorouracil monophosphate which is a potent irreversible inhibitor of thymidylate synthase. This is yet another example of a suicide inhibitor.

The mechanism for the irreversible inhibition is shown below.



Note that there is no proton for the general base to abstract. The formation of the covalent enzyme-dFUMP-THF ternary complex is irreversible.

Methotrexate and Aminopterin are potent inhibitors of dihydrofolate reductase.

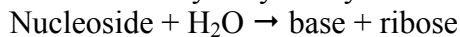


III. Dietary Nucleic Acids

Nucleic acids are ubiquitous biomolecules. A significant amount of nucleic acids are ingested in our diets. Nucleic acids are digested in our intestinal tract to nucleotides by various nucleases and phosphodiesterases. Nucleotides are then dephosphorylated by nonspecific phosphatases.



The nucleosides are hydrolyzed by nucleosidases to release the bases.



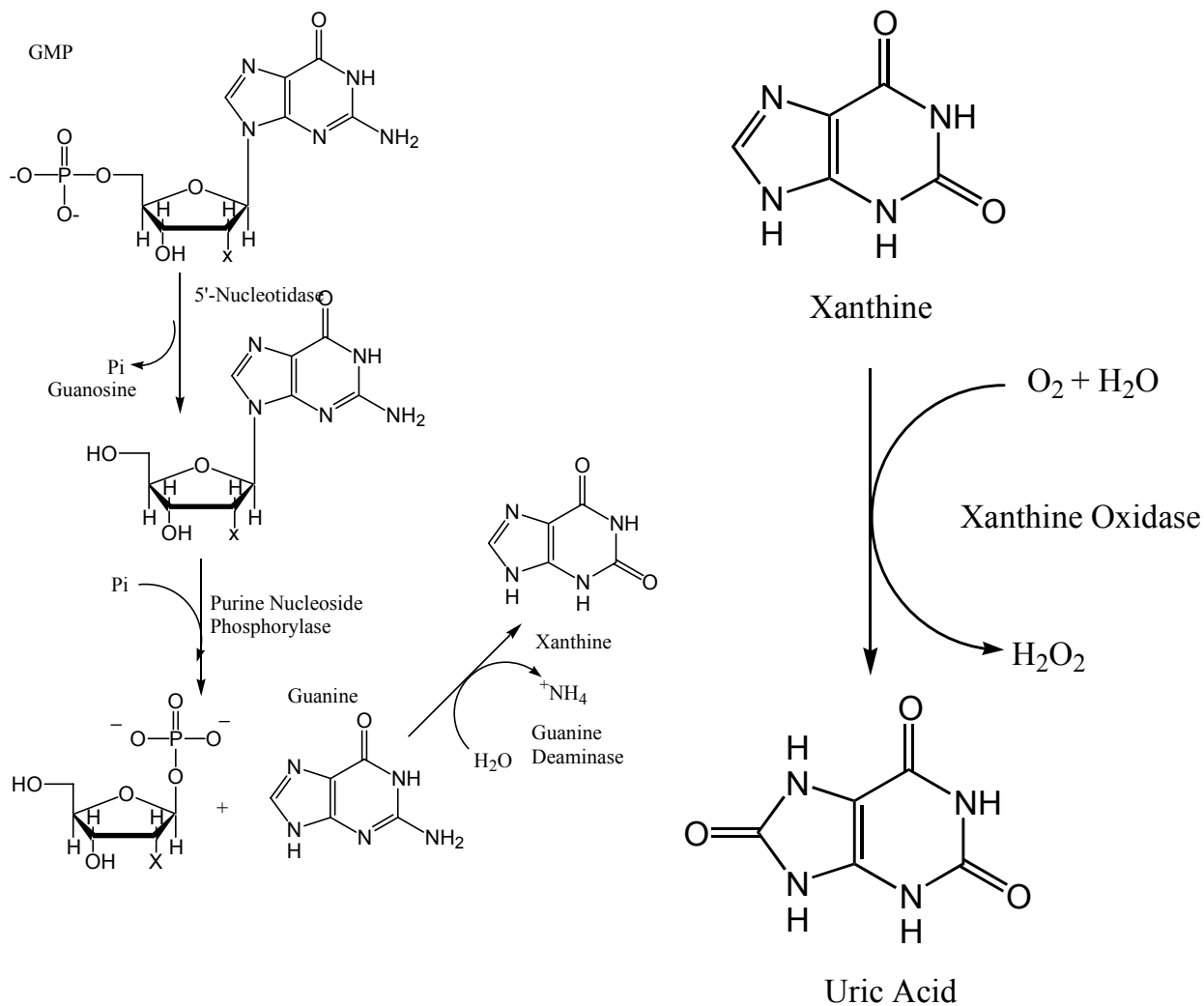
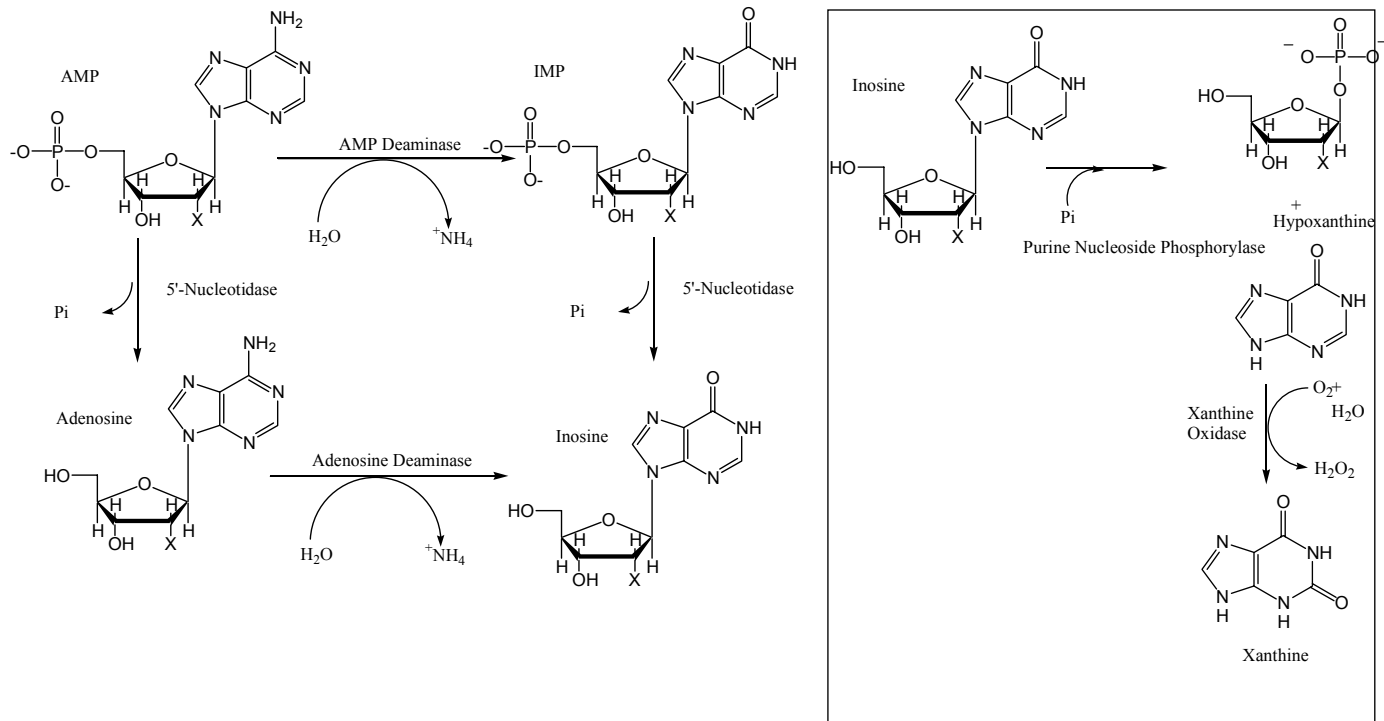
The ribose liberated in this reaction can be catabolized for energy. And is the only portion of the nucleotide that can be used as a source of metabolic energy.

Very little of the nucleic acids ingested in our diets become incorporated into the nucleic acids in our cells. Most of the ingested bases are excreted.

The salvage pathways are to continuously recycle cellular constituents not incorporated dietary bases.

IV. Purine Nucleotide Catabolism

The purine nucleotides are catabolized by the following pathways. GMP is converted into guanosine by 5'-nucleotidase. Next guanosine is converted into guanine and ribose-1-phosphate by purine nucleoside phosphorylase. Guanine is then converted into xanthine by guanine deaminase. AMP has two degradation pathways. One pathway converts AMP into IMP by AMP deaminase. IMP is dephosphorylated by 5'-nucleotidase to form inosine. Inosine is broken down into ribose-1-phosphate and hypoxanthine by purine nucleoside phosphorylase. The major pathway begins with dephosphorylating AMP by 5'-nucleotidase to form adenosine which is then deaminated by adenosine deaminase to form inosine. Inosine is converted into ribose-1-phosphate and hypoxanthine as previously described. Hypoxanthine is converted into xanthine by xanthine oxidase. Xanthine oxidase then oxidizes xanthine to produce uric acid. Animals such as us excrete uric acid.

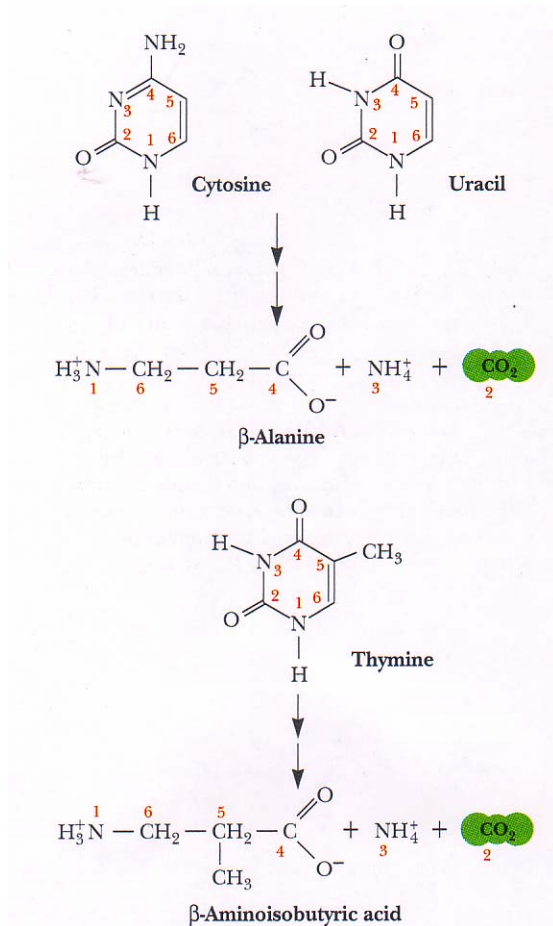


Adenosine Deaminase

A genetic deficiency of adenosine deaminase causes **severe combined immunodeficiency syndrome (SCID)**. Lack of adenosine deaminase activity causes an inability of B and T lymphocytes to proliferate and produce antibodies. Adenosine deaminase activity is also impaired in other diseases such as AIDS, anemia, lymphomas and leukemias. Adenosine deaminase deficiency is another target for gene therapy. Gene therapy is the attempt to repair a genetic deficiency by the introduction of a function gene. So far Gene therapy has experienced lots of set backs. A loss or lack of adenosine deaminase activity causes deoxyadenosine do be converted into dAMP. dAMP is then converted in to dATP. The result is high concentrations of dATP. dATP is a potent inhibitor of deoxynucleotide biosynthesis. Remember that dATP binds to the activity site of ribonucleotide reductase and turns the enzyme off.

V. Pymidine Nucleoside Catabolism

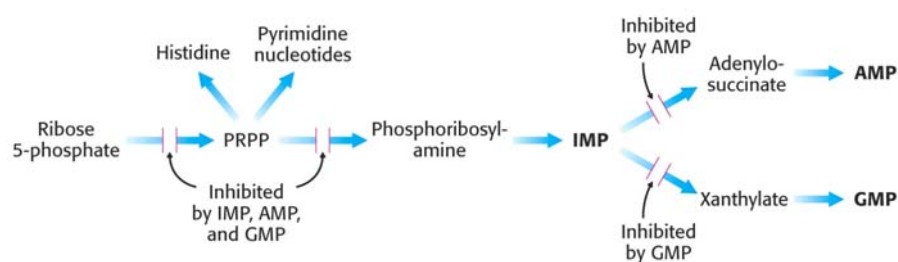
Pyrimidines are also catabolized as shown.



β -Alanine is then deaminated by a transaminase to form Malonic semialdehyde which is then oxidized and converted into malonyl-CoA. Malonyl-CoA as a precursor for fatty acid biosynthesis.

β -Aminoisobutyrate is also deaminated by a transaminase to form Methylmalonic semialdehyde which is then oxidized and converted into methylmalonyl-CoA which is converted into succinyl-CoA by methylmalonyl mutase.

VI. Regulation of Nucleotide Biosynthesis



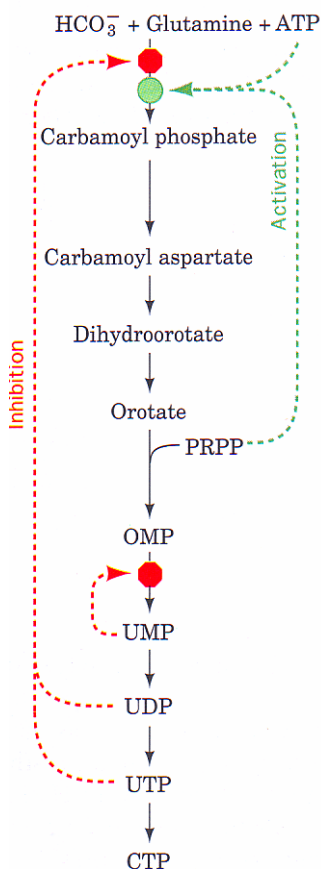
The regulation of purine biosynthesis is shown to the left.

The synthesis of PRPP is inhibited by AMP, IMP and GMP.

PRPP is a branch point for the synthesis of pyrimidines. The first committed step for purine biosynthesis is the formation of phosphoribosylamine. A reaction catalyzed by amidophosphoribosyl transferase. This enzyme is allosterically inactivated by IMP, AMP and GMP. This enzyme is activated by high concentrations of PRPP (feed forward activation). IMP is the branch point between AMP and GMP. High concentrations of AMP inhibit adenylosuccinate synthetase. High concentrations of GMP inhibit IMP dehydrogenase. The reaction catalyzed by adenylosuccinate synthetase activates IMP by the transfer of the γ -phosphate of GTP. The reaction catalyzed by GMP synthetase uses ATP to activate XMP. Thus relative high concentrations of ATP increase the rate of GMP synthesis, and relative high concentrations of GTP increase the rate of AMP synthesis.

Regulation of Pyrimidine Synthesis

(b) Animal pyrimidine biosynthesis



In animals pyrimidine biosynthesis is regulated by the activity of CPS II. UDP and UTP are allosteric inactivators of CPS II. High concentrations of ATP activate CPS II as do high concentrations of PRPP.

Orotidine-5'-monophosphate Decarboxylase is inhibited by UMP.