Overview

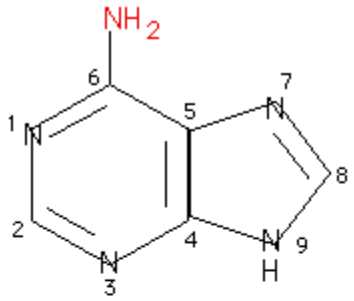
One of the important specialized pathways of a number of amino acids is the synthesis of purine and pyrimidine nucleotides. These nucleotides are important for a number of reasons. Most of them, not just ATP, are the sources of energy that drive most of our reactions. ATP is the most commonly used source but GTP is used in protein synthesis as well as a few other reactions. UTP is the source of energy for activating glucose and galactose. CTP is an energy source in lipid metabolism. AMP is part of the structure of some of the coenzymes like NAD and Coenzyme A. And, of course, the nucleotides are part of nucleic acids. Neither the bases nor the nucleotides are required dietary components. We can both synthesize them de novo and salvage and reuse those we already have.

Nitrogen Bases

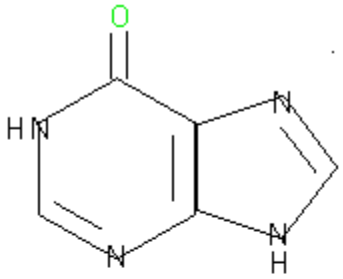
There are two kinds of nitrogen-containing bases - purines and pyrimidines. Purines consist of a six-membered and a five-membered nitrogen-containing ring, fused together. Pyridmidines have only a six-membered nitrogen-containing ring. There are 4 purines and 4 pyrimidines that are of concern to us.

Purines

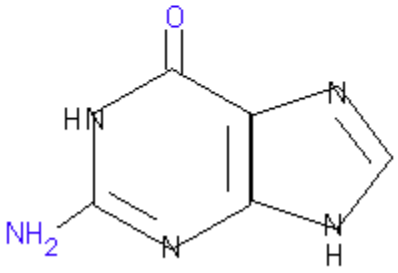
Adenine = 6-amino purine
Guanine = 2-amino-6-oxy purine
Hypoxanthine = 6-oxy purine
Xanthine = 2,6-dioxy purine



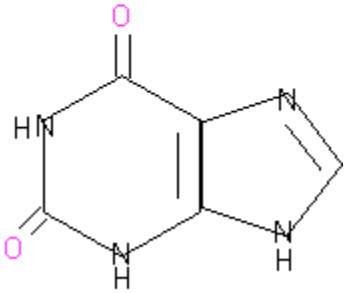
Adenine



Hypoxanthine



Guanine



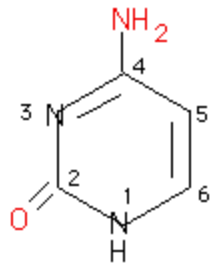
Xanthine

Adenine and guanine are found in both DNA and RNA. Hypoxanthine and xanthine are not incorporated into the nucleic acids as they are being synthesized but are important intermediates in the synthesis and degradation of the purine nucleotides.

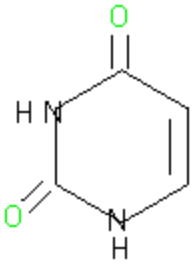
Pyrimidines

Uracil = 2,4-dioxy pyrimidine

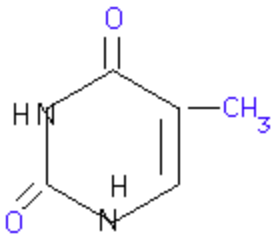
Thymine = 2,4-dioxy-5-methyl pyrimidine
Cytosine = 2-oxy-4-amino pyrimidine
Orotic acid = 2,4-dioxy-6-carboxy pyrimidine



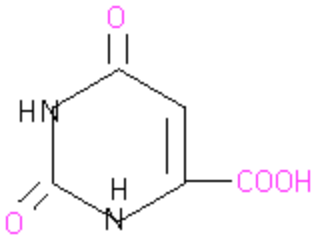
Cytosine



Uracil



Thymine



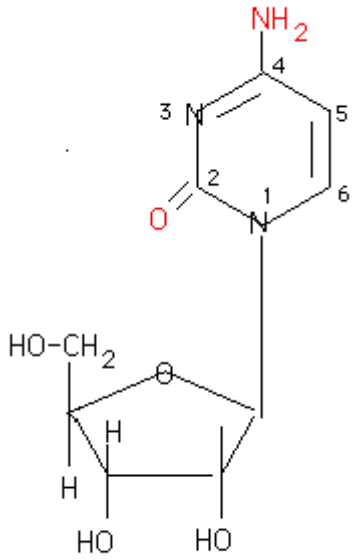
Orotic Acid

Cytosine is found in both DNA and RNA. Uracil is found only in RNA. Thymine is normally found in DNA. Sometimes tRNA will contain some thymine as well as uracil.

Nucleosides

If a sugar, either ribose or 2-deoxyribose, is added to a nitrogen base, the resulting compound is called a nucleoside. Carbon 1 of the sugar is attached to nitrogen 9 of a purine base or to nitrogen 1 of a pyrimidine base. The names of purine nucleosides end in -osine and the names of pyrimidine nucleosides end in -idine. The convention is to number the ring atoms of the base normally and to use l', etc. to distinguish the ring atoms of the sugar. Unless otherwise specificed, the sugar is assumed to be ribose. To indicate that the sugar is 2'-deoxyribose, a d- is placed before the name.

Adenosine
Guanosine
Inosine - the base in inosine is hypoxanthine
Uridine
Thymidine
Cytidine

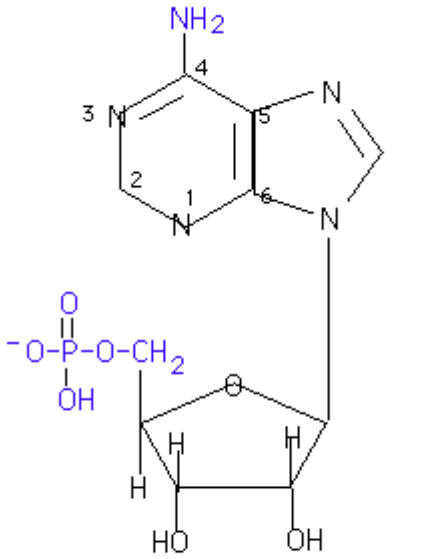


Cytidine

Nucleotides

Adding one or more phosphates to the sugar portion of a nucleoside results in a nucleotide. Generally, the phosphate is in ester linkage to carbon 5' of the sugar. If more than one phosphate is present, they are generally in acid anhydride linkages to each other. If such is the case, no position designation in the name is required. If the phosphate is in any other position, however, the position must be designated. For example, 3'-5' cAMP indicates that a phosphate is in ester linkage to both the 3' and 5' hydroxyl groups of an adenosine molecule and forms a cyclic structure. 2'-GMP would indicate that a phosphate is in ester linkage to the 2' hydroxyl group of a guanosine. Some representative names are:

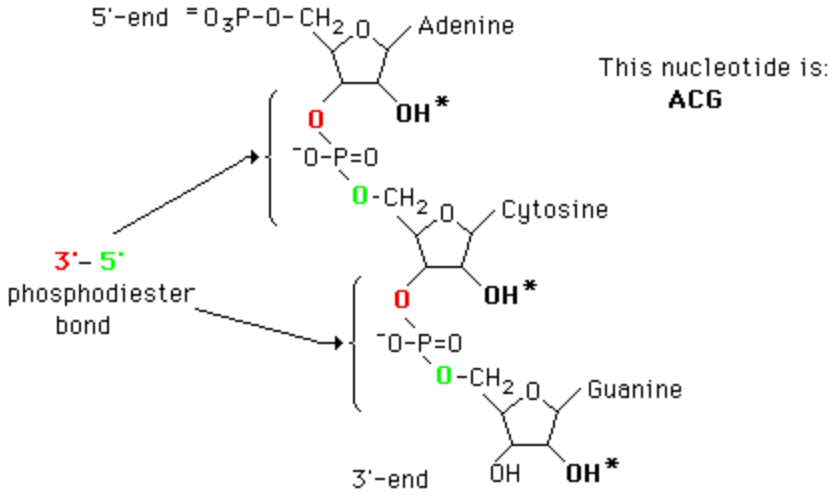
AMP = adenosine monophosphate = adenylic acid
CDP = cytidine diphosphate
dGTP = deoxy guanosine triphosphate
dTTP = deoxy thymidine triphosphate (more commonly designated TTP)
cAMP = 3'-5' cyclic adenosine monophosphate



Adenosine Monophosphate
AMP

Polynucleotides

Nucleotides are joined together by 3'-5' phosphodiester bonds to form polynucleotides. Polymerization of ribonucleotides will produce an RNA while polymerization of deoxyribonucleotides leads to DNA.



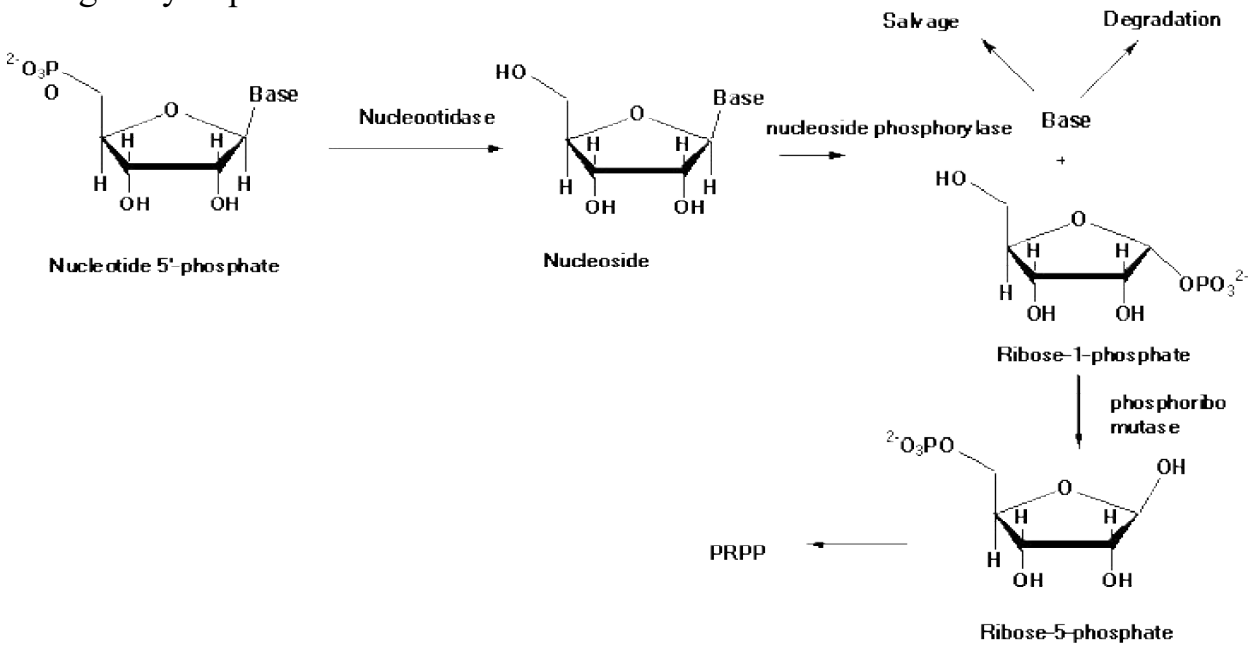
* In DNA, this atom would be H instead of OH.

Hydrolysis of Polynucleotides

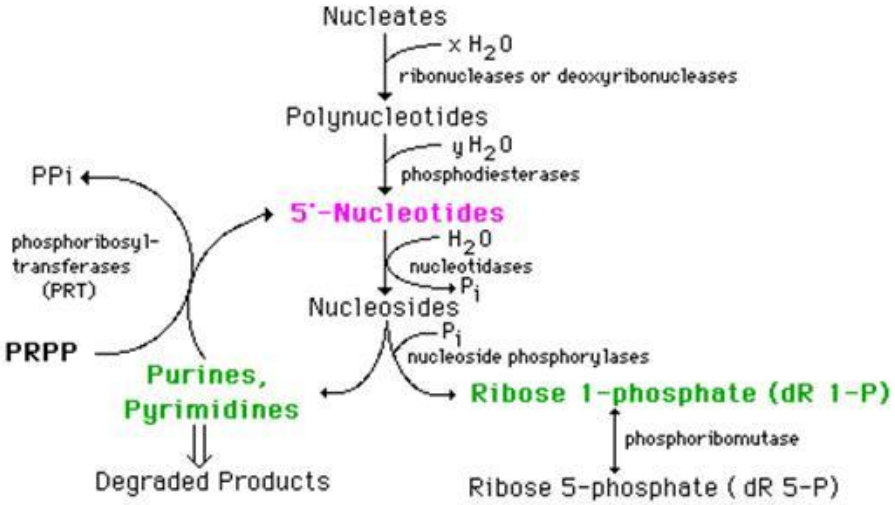
Most, but not all, nucleic acids in the cell are associated with protein. Dietary nucleoprotein is degraded by pancreatic enzymes and tissue nucleoprotein by lysosomal enzymes. After dissociation of the protein and nucleic acid, the protein is metabolized like any other protein.

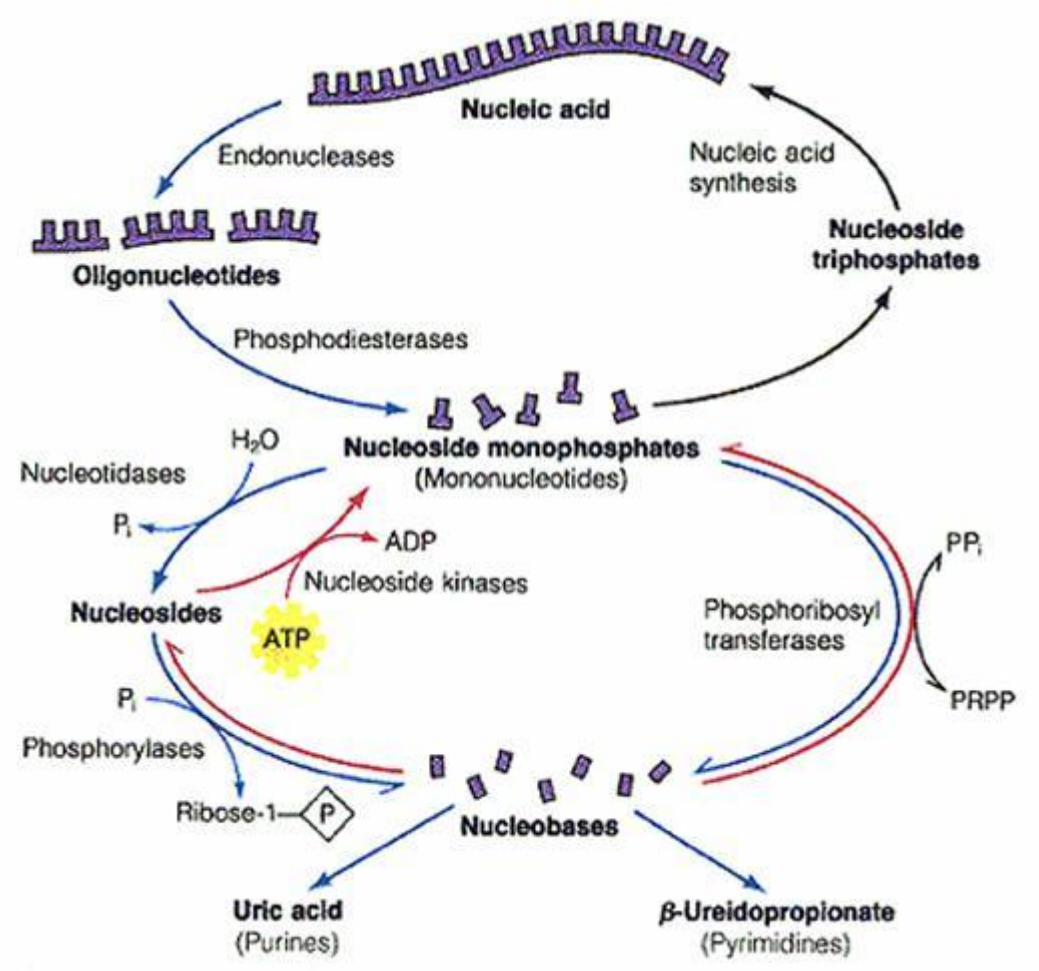
The nucleic acids are hydrolyzed randomly by nucleases to yield a mixture of polynucleotides. These are further cleaved by phosphodiesterases (exonucleases) to a mixture of the mononucleotides. The specificity of the pancreatic nucleotidases

gives the 3'-nucleotides and that of the lysosomal nucleotidases gives the biologically important 5'-nucleotides.

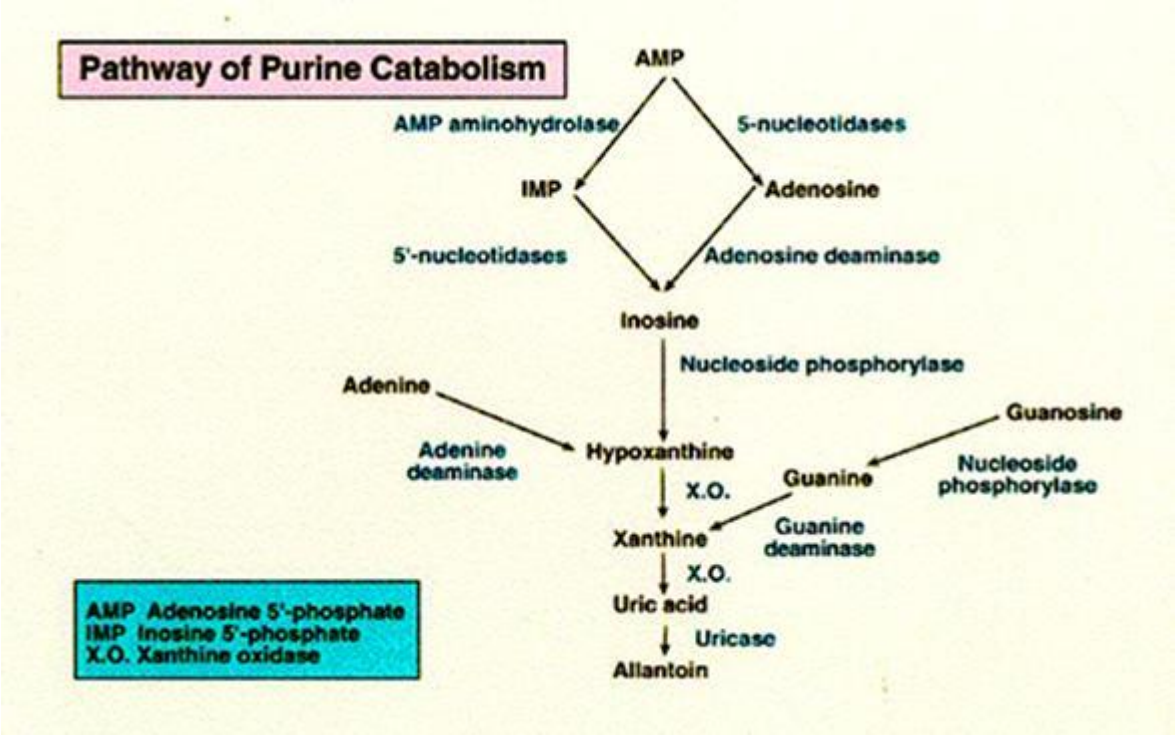


The nucleotides are hydrolyzed by nucleotidases to give the nucleosides and P_i. This is probably the end product in the intestine with the nucleosides being the primary form absorbed. In at least some tissues, the nucleosides undergo phosphorolysis with nucleoside phosphorylases to yield the base and ribose 1-P (or deoxyribose 1-P). Since R 1-P and R 5-P are in equilibrium, the sugar phosphate can either be reincorporated into nucleotides or metabolized via the Hexose Monophosphate Pathway. The purine and pyrimidine bases released are either degraded or salvaged for reincorporation into nucleotides. There is significant turnover of all kinds of RNA as well as the nucleotide pool. DNA doesn't turnover but portions of the molecule are excised as part of a repair process.





Purine and pyrimidines from tissue turnover which are not salvaged are catabolized and excreted. Little dietary purine is used and that which is absorbed is largely catabolized as well. Catabolism of purines and pyrimidines occurs in a less useful fashion than did the catabolism of amino acids in that we do not derive any significant amount of energy from the catabolism of purines and pyrimidines. Pyrimidine catabolism, however, does produce beta-alanine, and the endproduct of purine catabolism, which is uric acid in man, may serve as a scavenger of reactive oxygen species.



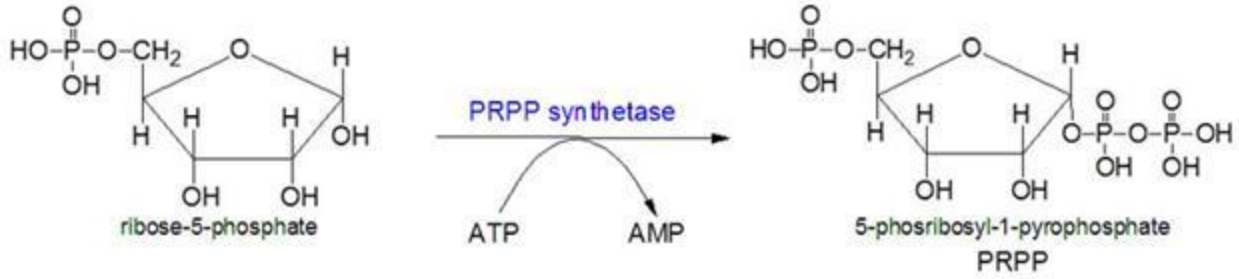
The metabolic requirements for the nucleotides and their cognate bases can be met by both dietary intake or synthesis *de novo* from low molecular weight precursors. Indeed, the ability to salvage nucleotides from sources within the body alleviates any nutritional requirement for nucleotides, thus the purine and pyrimidine bases are not required in the diet. The salvage pathways are a major source of nucleotides for synthesis of DNA, RNA and enzyme co-factors.

Extracellular hydrolysis of ingested nucleic acids occurs through the concerted actions of endonucleases, phosphodiesterases and nucleoside phosphorylases. Endonucleases degrade DNA and RNA at internal sites leading to the production of oligonucleotides. Oligonucleotides are further digested by phosphodiesterases that act from the ends inward yielding free nucleosides. The bases are hydrolyzed from nucleosides by the action of phosphorylases that yield ribose-1-phosphate and free bases. If the nucleosides and/or bases are not re-utilized the purine bases are further degraded to uric acid and the pyrimidines to β-aminoiosobutyrate, NH_3 and CO_2.

Purine and pyrimidine bases which are not degraded are recycled - *i.e.* reincorporated into nucleotides. This recycling, however, is not sufficient to meet total body requirements and so some *de novo* synthesis is essential. There are definite tissue differences in the ability to carry out *de novo* synthesis. *De novo* synthesis of purines is most active in liver. Non-hepatic tissues generally have limited or even no *de novo* synthesis. Pyrimidine synthesis occurs in a variety of tissues. For purines, especially, non-hepatic tissues rely heavily on preformed bases - those salvaged from their own intracellular turnover supplemented by bases synthesized in the liver and delivered to tissues via the blood.

"Salvage" of purines is reasonable in most cells because xanthine oxidase, the key enzyme in taking the purines all of the way to uric acid, is significantly active only in liver and intestine. The bases generated by turnover in non-hepatic tissues are not readily degraded to uric acid in those tissues and, therefore, are available for

salvage. The liver probably does less salvage but is very active in *de novo* synthesis - not so much for itself but to help supply the peripheral tissues. *De novo* synthesis of both purine and pyrimidine nucleotides occurs from readily available components.

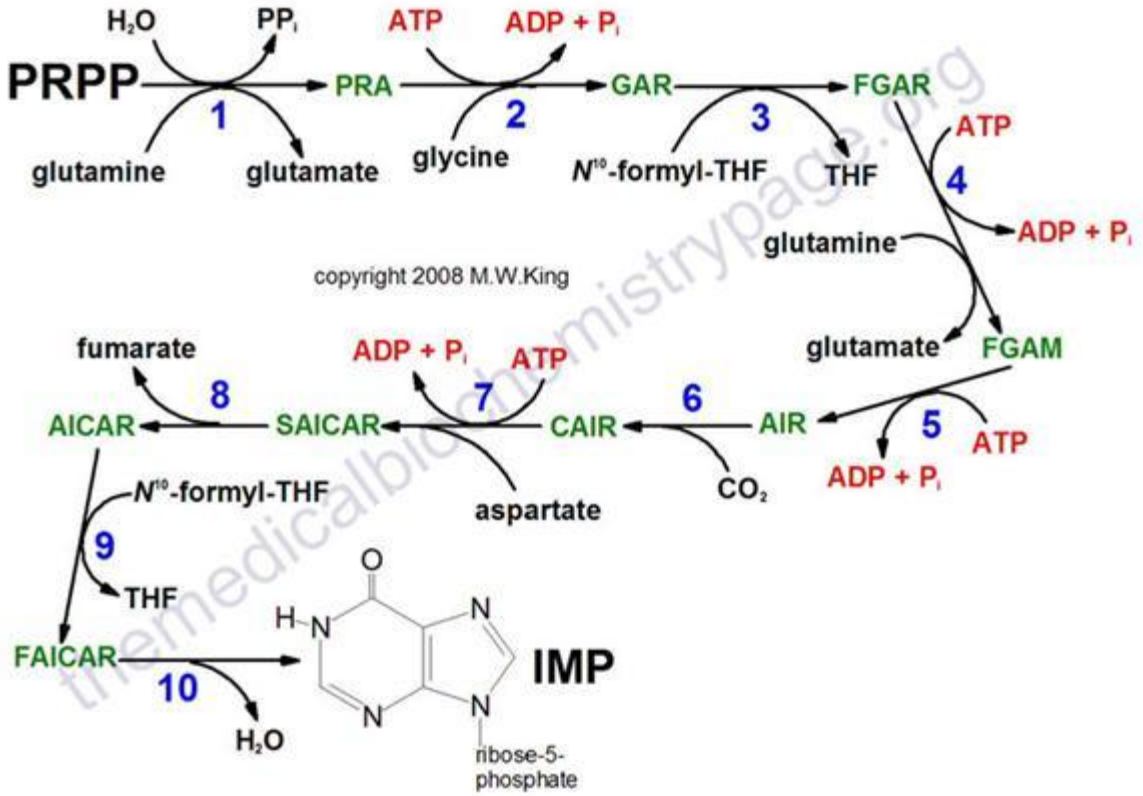


Phosphoribosyl pyrophosphate (PRPP) is important in both, and in these pathways the structure of ribose is retained in the product nucleotide, in contrast to its fate in the tryptophan and histidine biosynthetic pathways discussed earlier. An amino acid is an important precursor in each type of pathway: glycine for purines and aspartate for pyrimidines. Glutamine again is the most important source of amino groups — in five different steps in the de novo pathways. Aspartate is also used as the source of an amino group in the purine pathways, in two steps. Two other features deserve mention. First, there is evidence, especially in the de novo purine pathway, that the enzymes are present as large, multienzyme complexes in the cell, a recurring theme in our discussion of metabolism. Second, the cellular pools of nucleotides (other than ATP) are quite small, perhaps 1% or less of the amounts required to synthesize the cell's DNA.

Therefore, cells must continue to synthesize nucleotides during nucleic acid synthesis, and in some cases nucleotide synthesis may limit the rates of DNA replication and transcription. Because of the importance of these processes in dividing cells, agents that inhibit nucleotide synthesis have become particularly important to modern medicine. We examine here the biosynthetic pathways of purine and pyrimidine nucleotides and their regulation, the formation of the deoxynucleotides, and the degradation of purines and pyrimidines to uric acid and urea. We end with a discussion of chemotherapeutic agentsthat affect nucleotide synthesis.

De Novo Synthesis of Purine Nucleotides

The two parent purine nucleotides of nucleic acids are adenosine-monophosphate (AMP; adenylate) and guanosine-monophosphate (GMP; guanylate), containing the purine bases adenine and guanine. Figure shows the origin of the carbon and nitrogen atoms of the purine ring system, as determined by John Buchanan using isotopic tracer experiments in birds. The detailed pathway of purine biosynthesis was worked out primarily by Buchanan and G. Robert Greenberg in the 1950s.

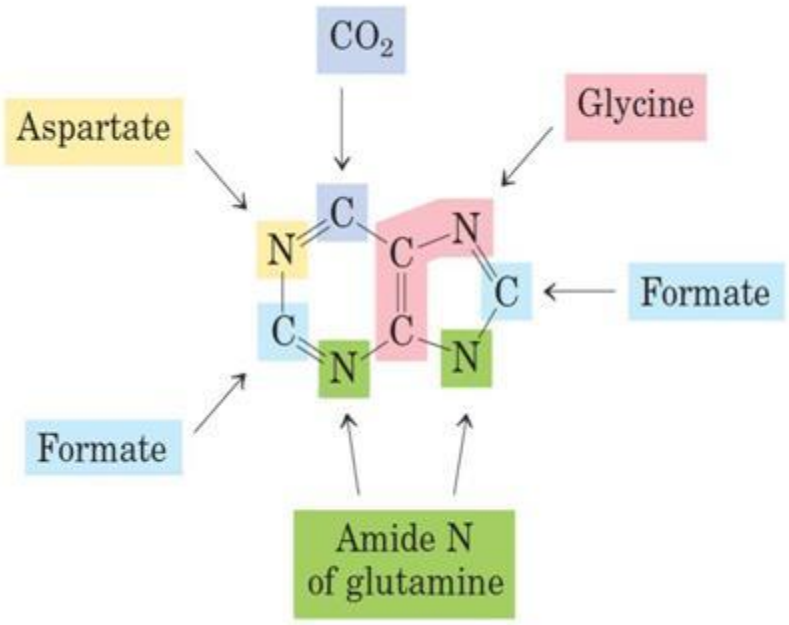


Synthesis of the first fully formed purine nucleotide, inosine monophosphate, IMP begins with 5-phospho-α-ribosyl-1-pyrophosphate, PRPP. Through a series of reactions utilizing ATP, tetrahydrofolate (THF) derivatives, glutamine, glycine and aspartate this pathway yields IMP. The rate limiting reaction is catalyzed by glutamine PRPP amidotransferase, enzyme indicated by 1 in the Figure. The structure of the nucleobase of IMP (hypoxanthine) is shown. Place mouse over the green intermediate names to see structures.

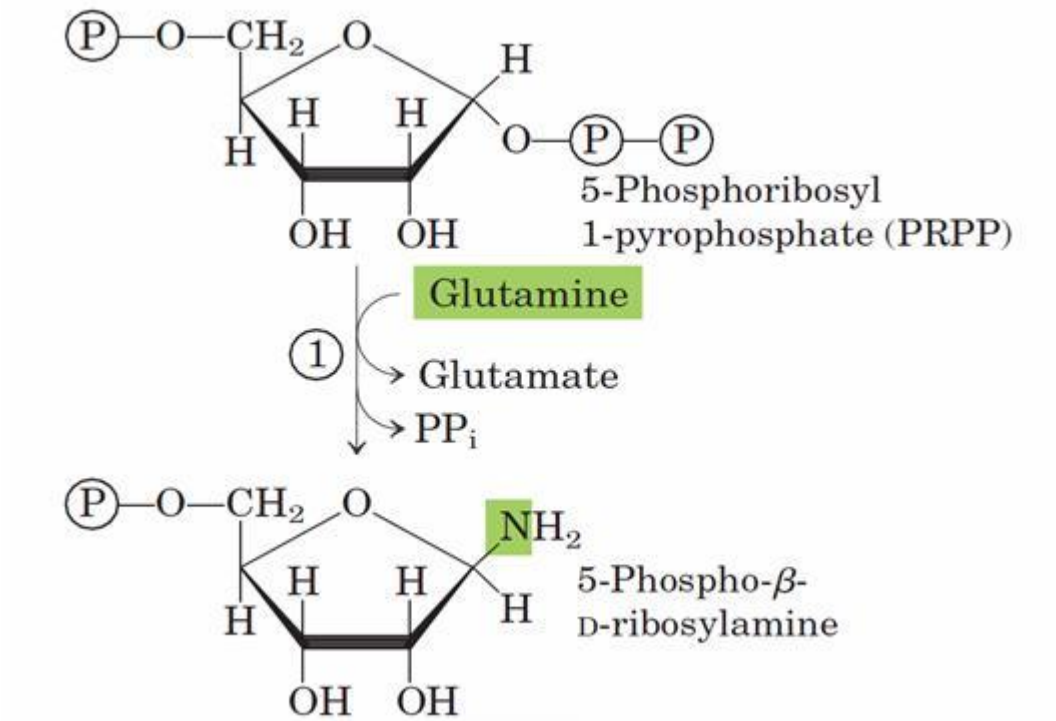
Enzyme names:

1. glutamine phosphoribosylpyrophosphate amidotransferase
2. glycinamide ribotide synthase
3. glycinamide ribotide transformylase
4. formylglycinamide synthase
5. aminoimidazole ribotide synthase
6. aminoimidazole ribotide carboxylase
7. succinylaminoimidazolecarboxamide ribotide synthase
8. adenylosuccinate lyase
9. aminoimidazole carboxamide ribotide transformylase
10. IMP cyclohydrolase

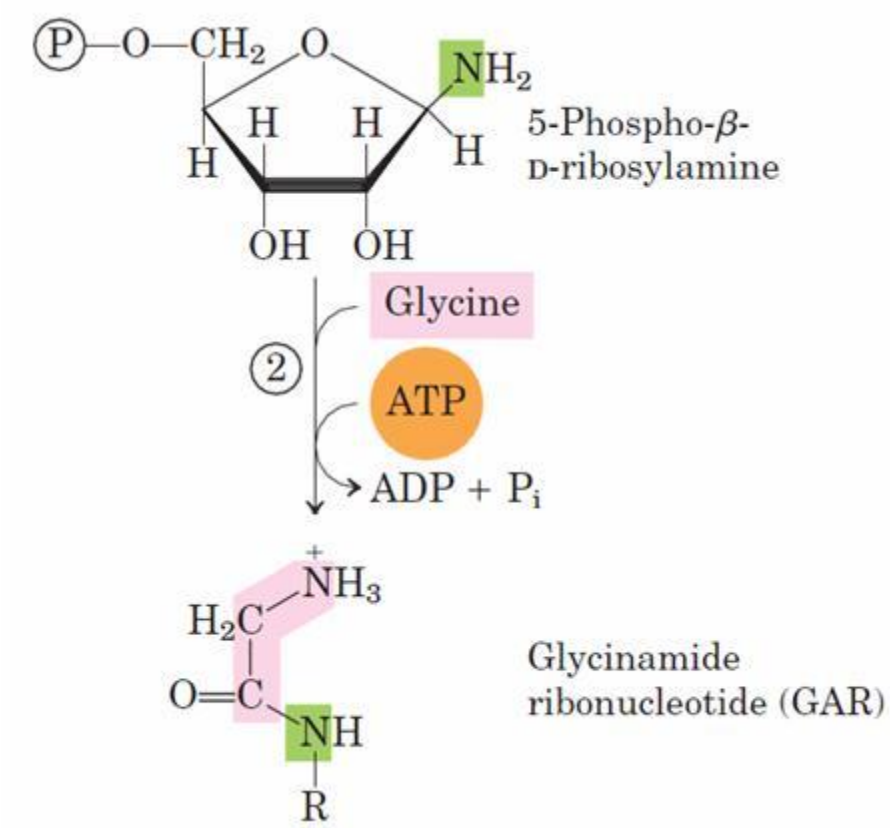
In the first committed step of the pathway, an amino group donated by glutamine is attached at C-1 of PRPP.



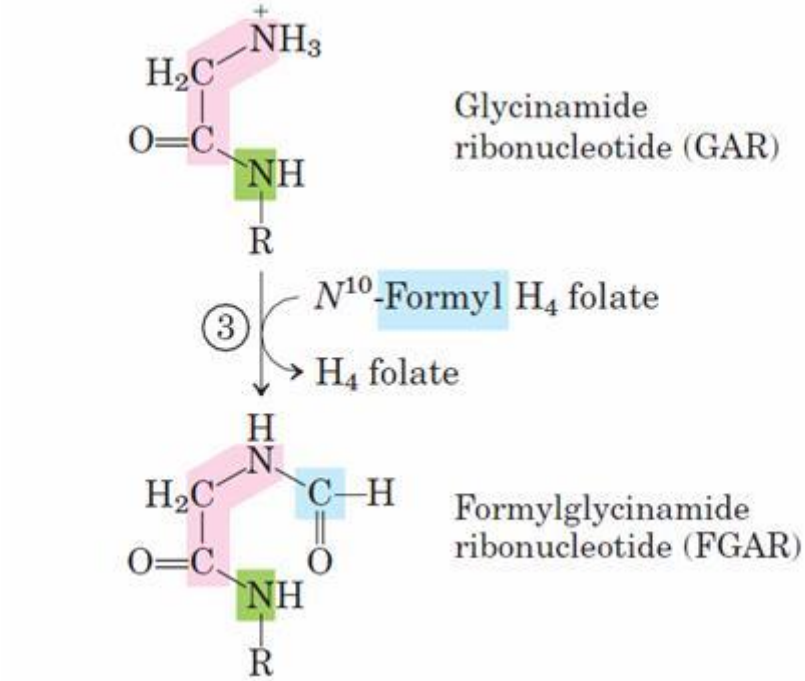
The resulting 5-phosphoribosylamine is highly unstable, with a half-life of 30 seconds at pH 7.5. The purine ring is subsequently built up on this structure. The pathway described here is identical in all organisms, with the exception of one step that differs in higher eukaryotes as noted below.



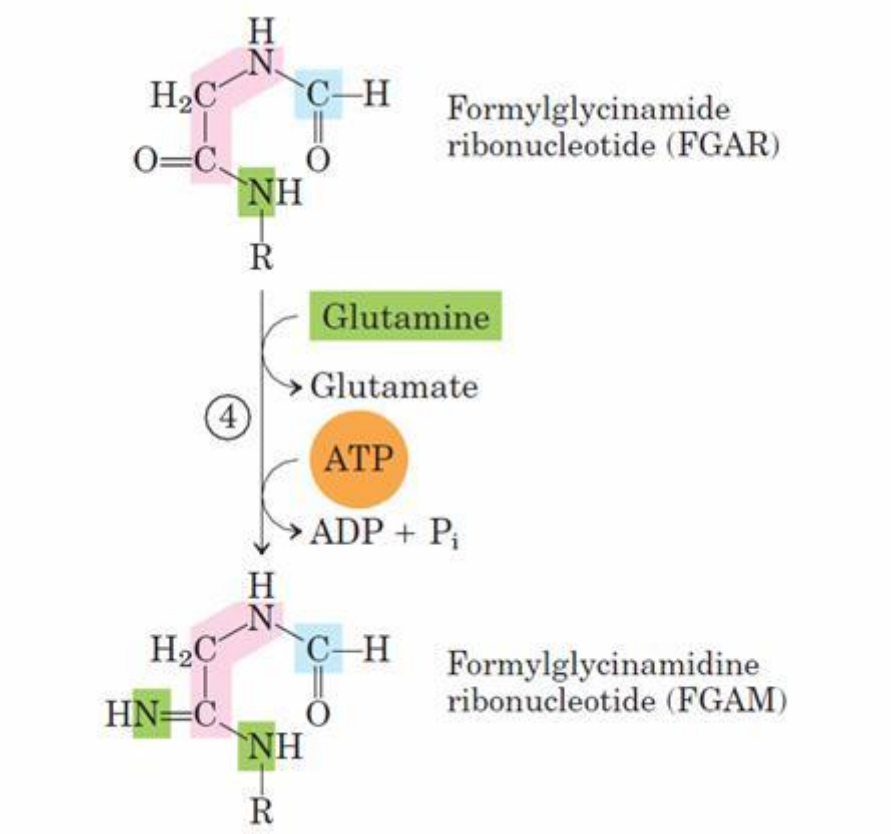
The second step is the addition of three atoms from glycine (step 2). An ATP is consumed to activate the glycine carboxyl group (in the form of an acyl phosphate) for this condensation reaction:



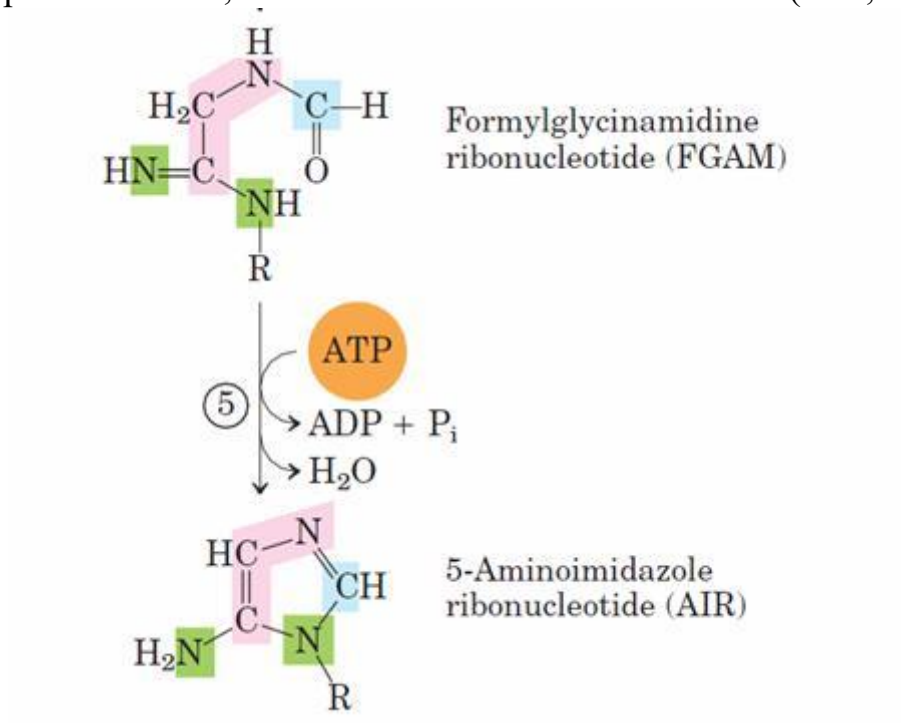
The added glycine amino group is then formylated by *N*10- formyltetrahydrofolate (step 3):



A nitrogen is contributed by glutamine (step 4):

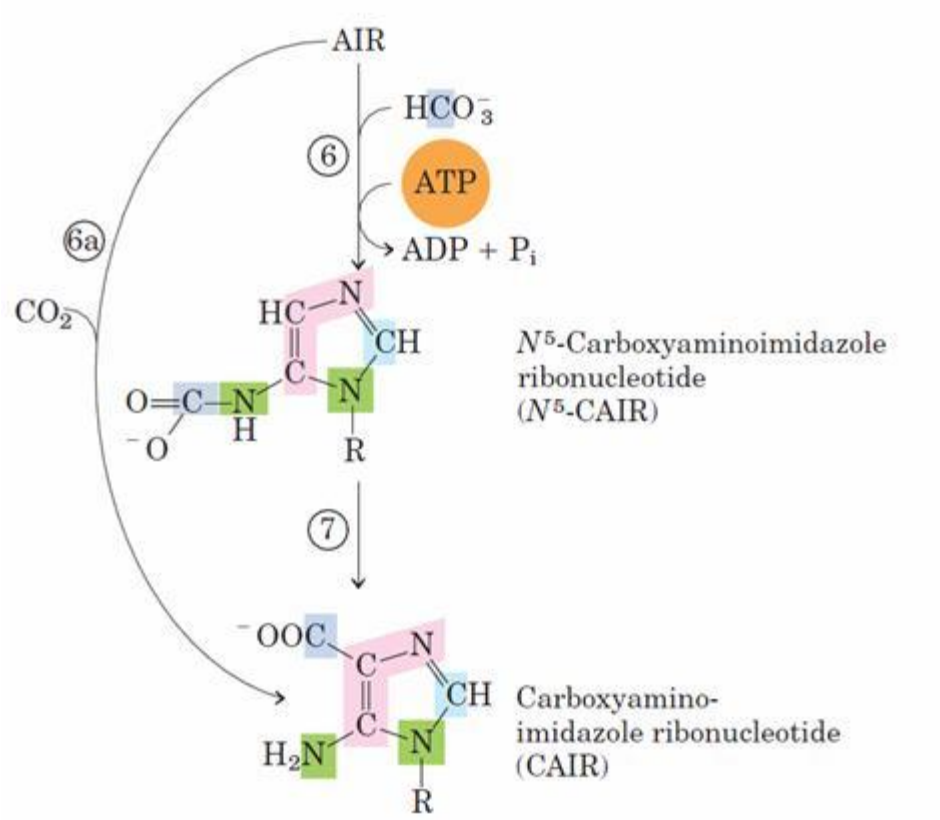


Before dehydration and ring closure yield the five-membered imidazole ring of the purine nucleus, as 5-aminoimidazole ribonucleotide (AIR; step 5).

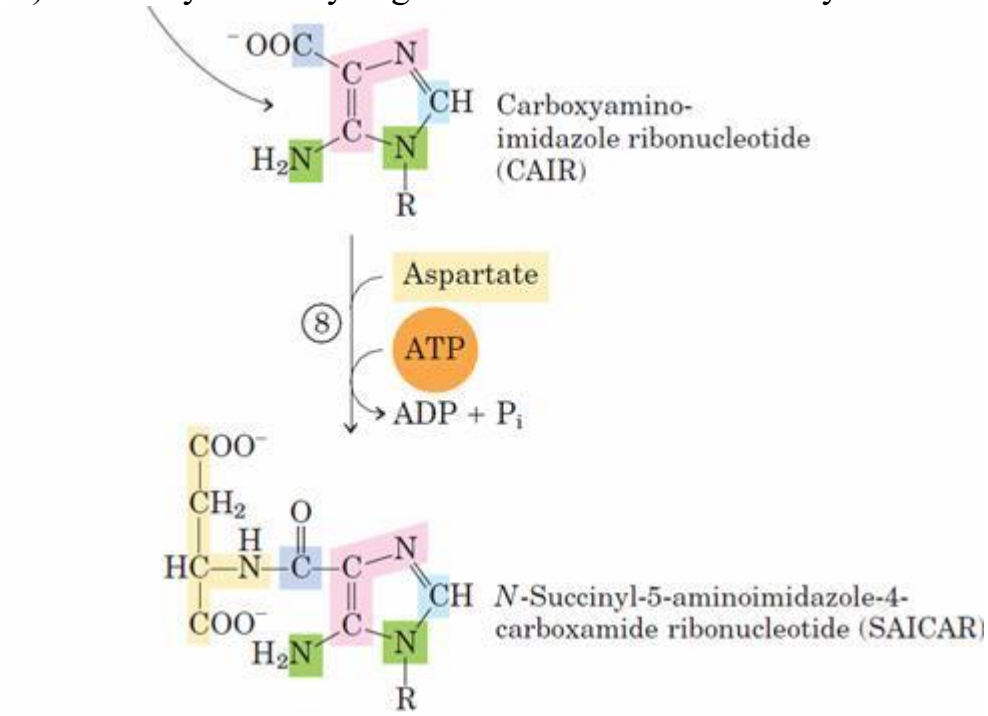


At this point, three of the six atoms needed for the second ring in the purine structure are in place. To complete the process, a carboxyl group is first added (step 6). This carboxylation is unusual in that it does not require biotin, but instead uses the bicarbonate generally present in aqueous solutions. A rearrangement

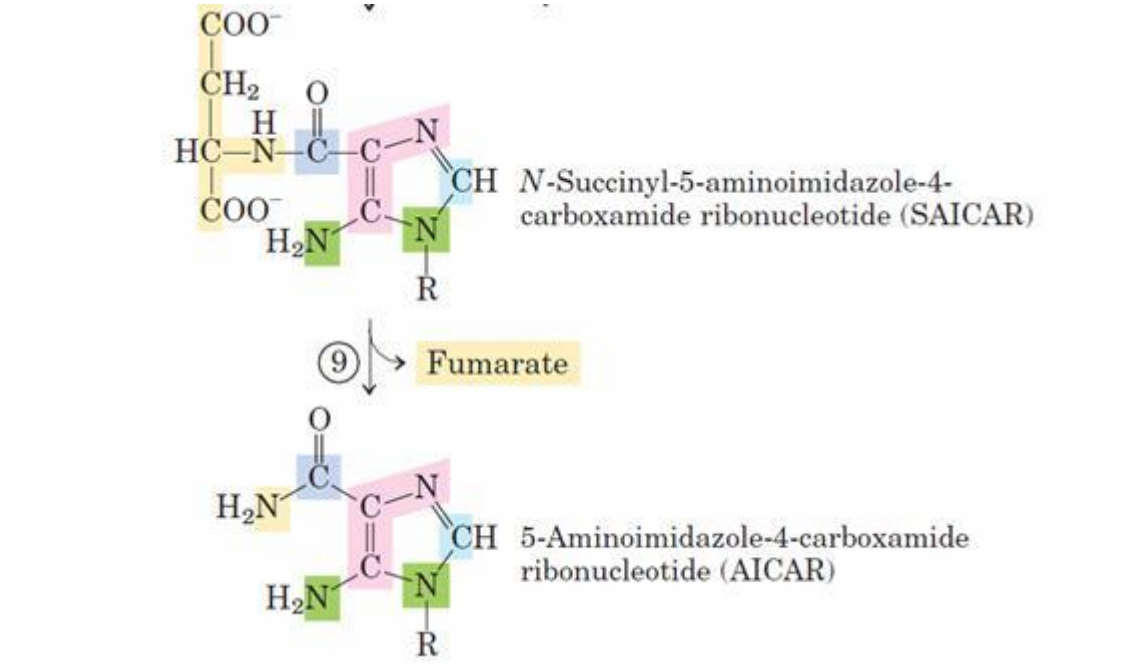
transfers the carboxylate from the exocyclic amino group to position 4 of the imidazole ring (step 7).



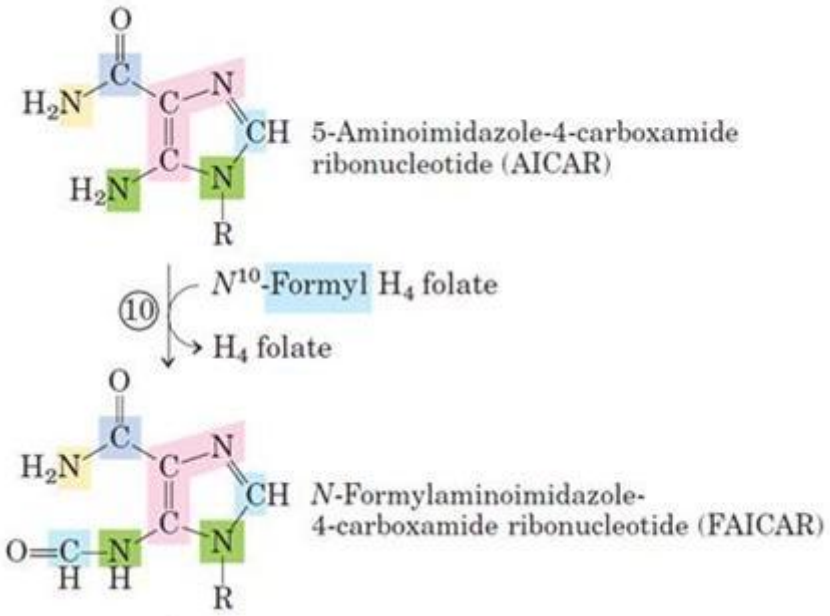
Steps 6 and 7 are found only in bacteria and fungi. In higher eukaryotes, including humans, the 5-aminoimidazole ribonucleotide product of step 5 is carboxylated directly to carboxyaminoimidazole ribonucleotide in one step instead of two (step 6a). The enzyme catalyzing this reaction is AIR carboxylase.



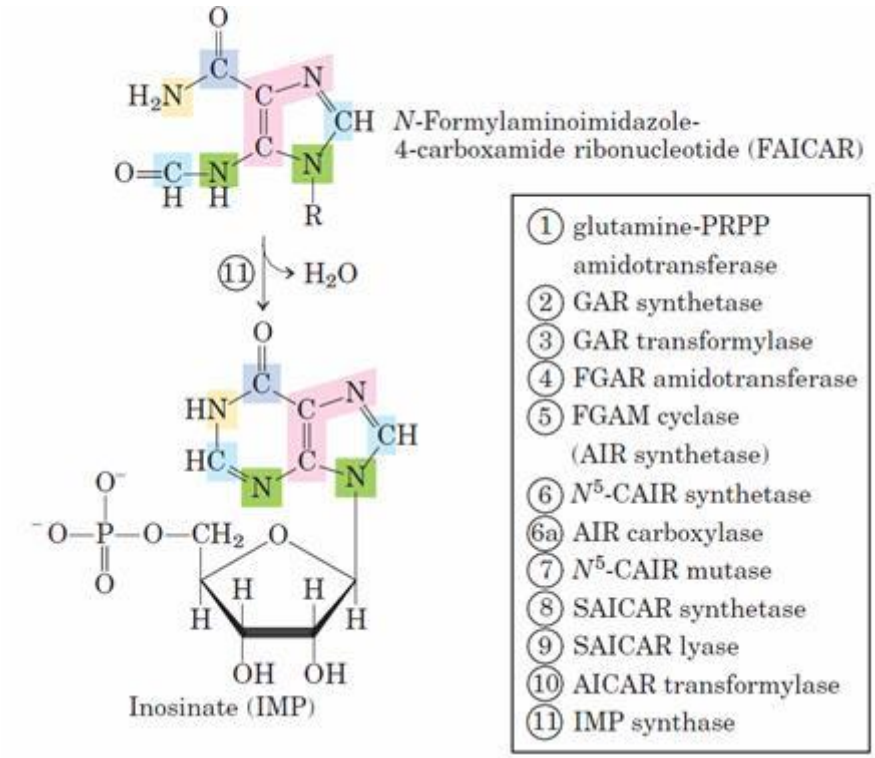
Aspartate now donates its amino group in two steps (8 and 9): formation of an amide bond, followed by elimination of the carbon skeleton of aspartate (as fumarate).



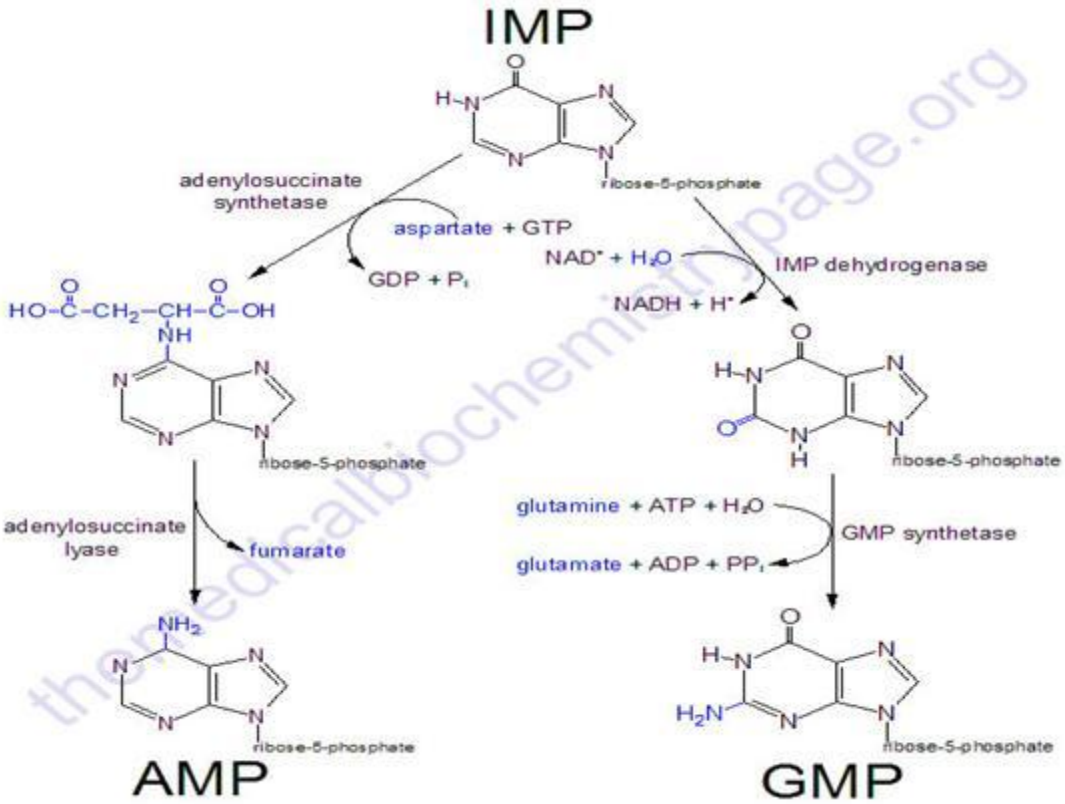
Recall that aspartate plays an analogous role in two steps of the urea cycle. The final carbon is contributed by *N*10-formyltetrahydrofolate (step 10),



and a second ring closure takes place to yield the second fused ring of the purine nucleus (step 11).

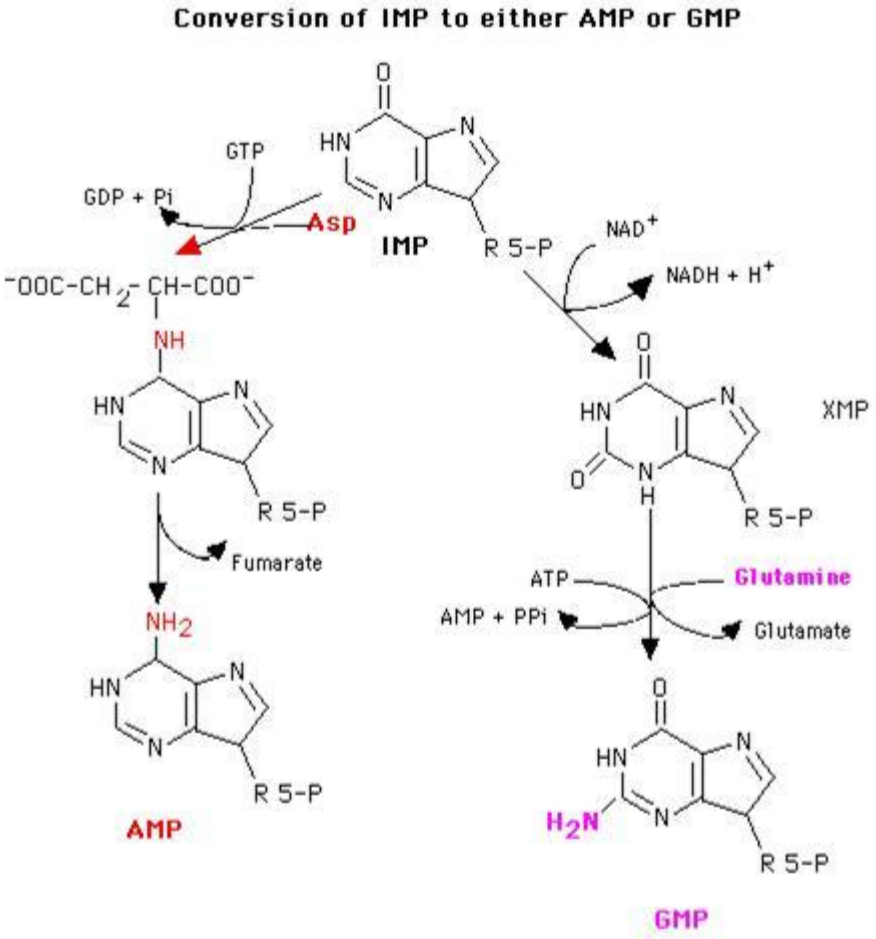


The first intermediate with a complete purine ring is inosinate (IMP).

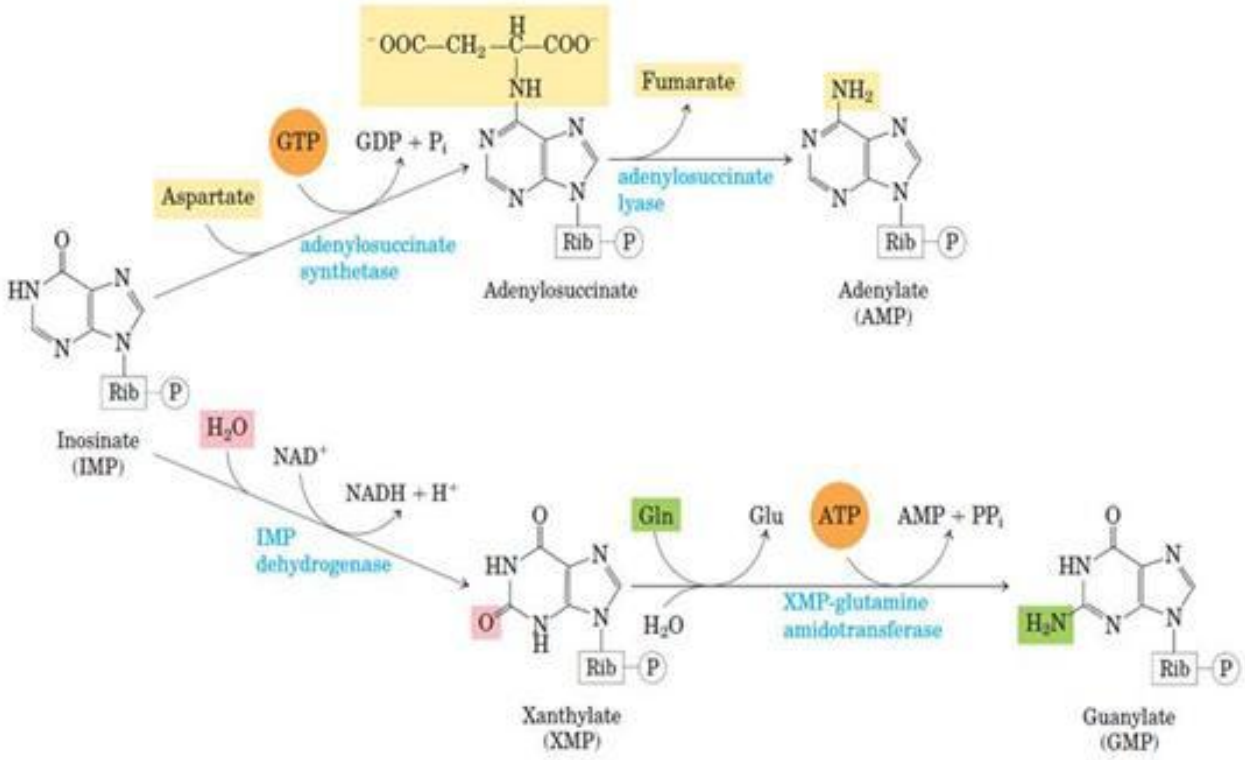


As in the tryptophan and histidine biosynthetic pathways, the enzymes of IMP synthesis appear to be organized as large, multienzyme complexes in the cell. Once again, evidence comes from the existence of single polypeptides with several functions, some catalyzing nonsequential steps in the pathway. In eukaryotic cells ranging from yeast to fruit flies to chickens, steps 1 , 3 , and 5 are catalyzed by a multifunctional protein. An additional multifunctional protein catalyzes steps 10

and 11. In humans, a multifunctional enzyme combines the activities of AIR carboxylase and SAICAR synthetase (steps 6a and 8).



In bacteria, these activities are found on separate proteins, but a large noncovalent complex may exist in these cells. The channeling of reaction intermediates from one enzyme to the next permitted by these complexes is probably especially important for unstable intermediates such as 5-phosphoribosylamine.
Conversion of inosinate to adenylate requires the insertion of an amino group derived from aspartate; this takes place in two reactions similar to those used to introduce N-1 of the purine ring, (steps 8 and 9). A crucial difference is that GTP rather than ATP is the source of the high-energy phosphate in synthesizing adenylosuccinate.



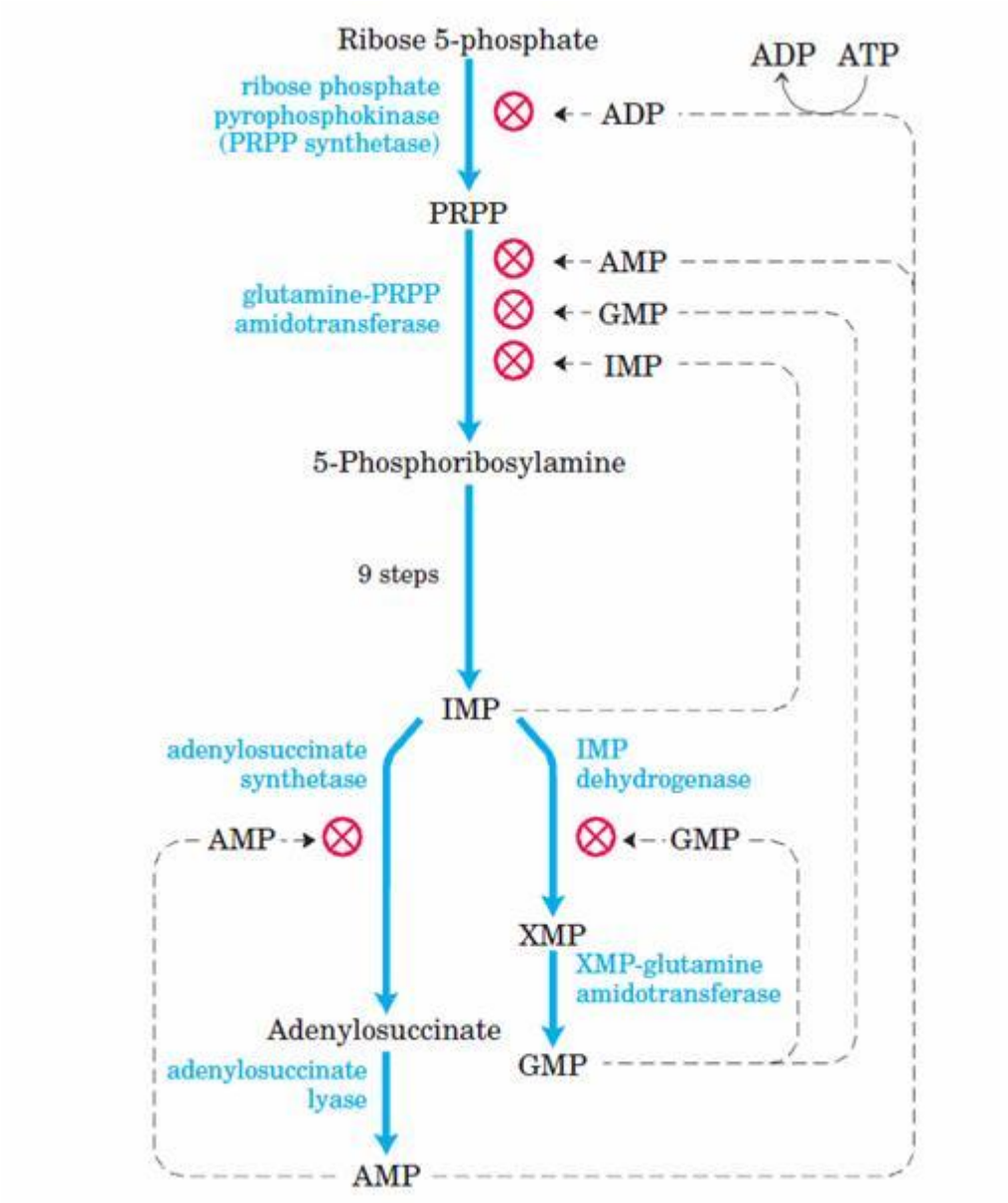
Guanylate is formed by the NAD-requiring oxidation of inosinate at C-2, followed by addition of an amino group derived from glutamine. ATP is cleaved to AMP and PPi in the final step.

REGULATION OF PURINE NUCLEOTIDE BIOSYNTHESIS

Three major feedback mechanisms cooperate in regulating the overall rate of de novo purine nucleotide synthesis and the relative rates of formation of the two end products, adenylate and guanylate. The first mechanism is exerted on the first reaction that is unique to purine synthesis — transfer of an amino group to PRPP to form 5-phosphoribosylamine. This reaction is catalyzed by the allosteric enzyme glutamine-PRPP amidotransferase, which is inhibited by the end products IMP, AMP, and GMP. AMP and GMP act synergistically in this concerted inhibition. Thus, whenever either AMP or GMP accumulates to excess, the first step in its biosynthesis from PRPP is partially inhibited.

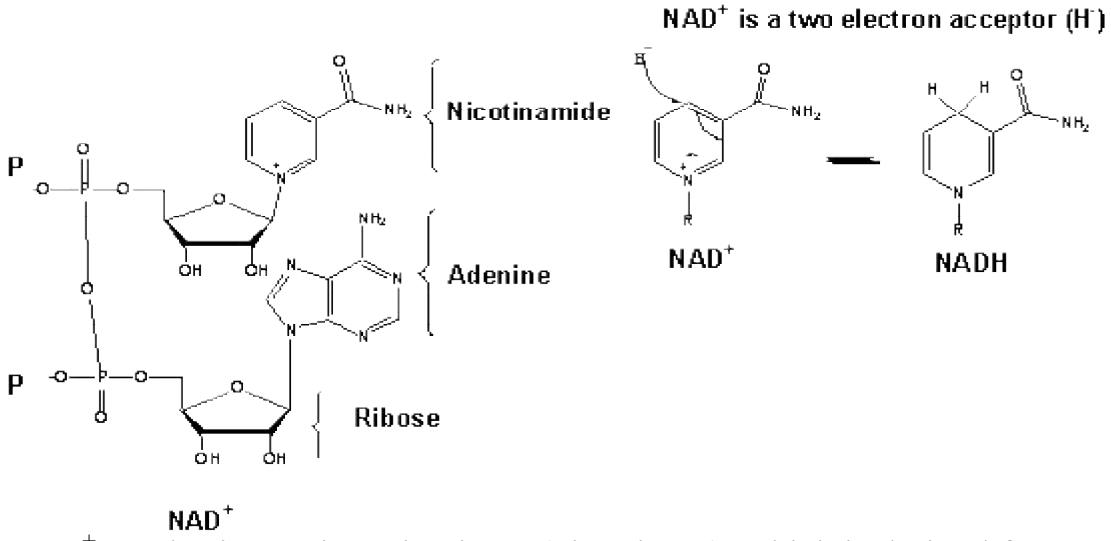
In the second control mechanism, exerted at a later stage, an excess of GMP in the cell inhibits formation of xanthylate from inosinate by IMP dehydrogenase, without affecting the formation of AMP. Conversely, an accumulation of adenylate inhibits formation of adenylosuccinate by adenylosuccinate synthetase, without affecting the biosynthesis of GMP. In the third mechanism, GTP is required in the conversion of IMP to AMP (step 1), whereas ATP is required for conversion of IMP to GMP (step 4), a reciprocal arrangement that tends to balance the synthesis of the two ribonucleotides.

The final control mechanism is the inhibition of PRPP synthesis by the allosteric regulation of ribose phosphate pyrophosphokinase. This enzyme is inhibited by ADP and GDP, in addition to metabolites from other pathways of which PRPP is a starting point.

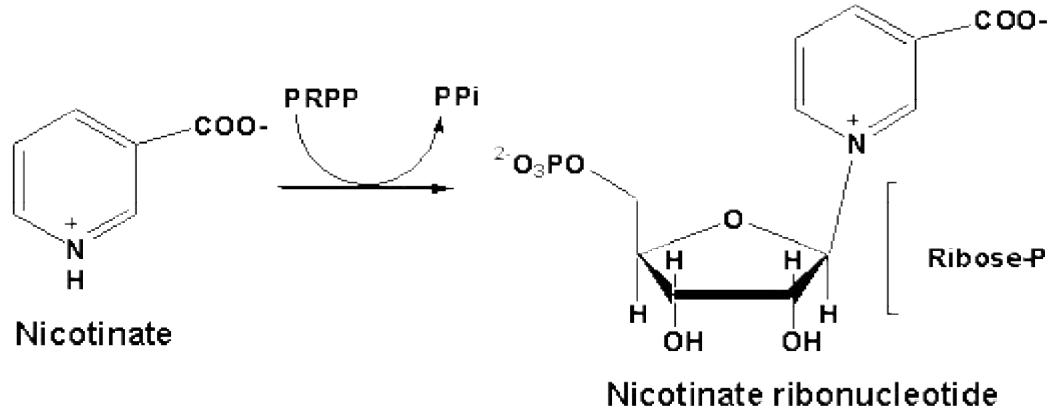


Biosynthesis of NAD^+

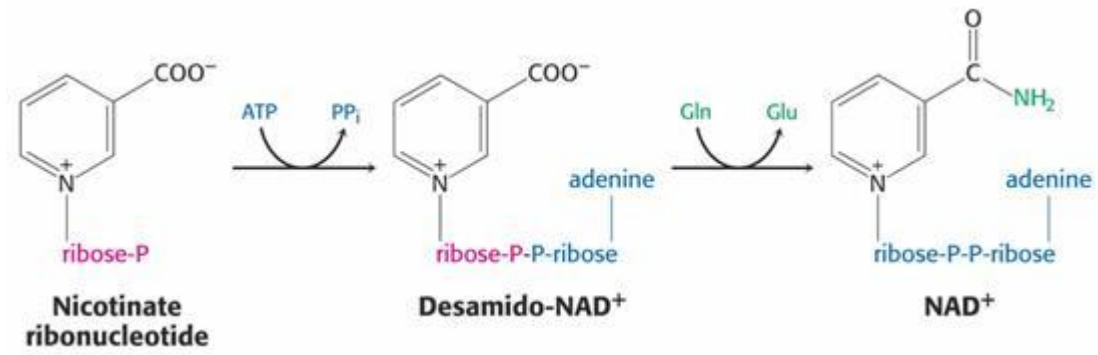
Nicotinamide adenine dinucleotide (NAD^+) and its phosphorylated analog, $NADP^+$, are important coenzymes that participate in a number of biological processes involving electron transfer. NAD^+ contains an AMP moiety as part of the molecule:



NAD^+ synthesis requires nicotinate (vitamin B_6), which is derived from tryptophan. In the first step, nicotinate ribonucleotide is formed from nicotinate and PRPP:



In the following steps, an AMP moiety is transferred from ATP to nicotinate ribonucleotide to form desamido-NAD^+. Finally, the carboxyl group of desamido-NAD is converted to amide using glutamine as an ammonia donor:



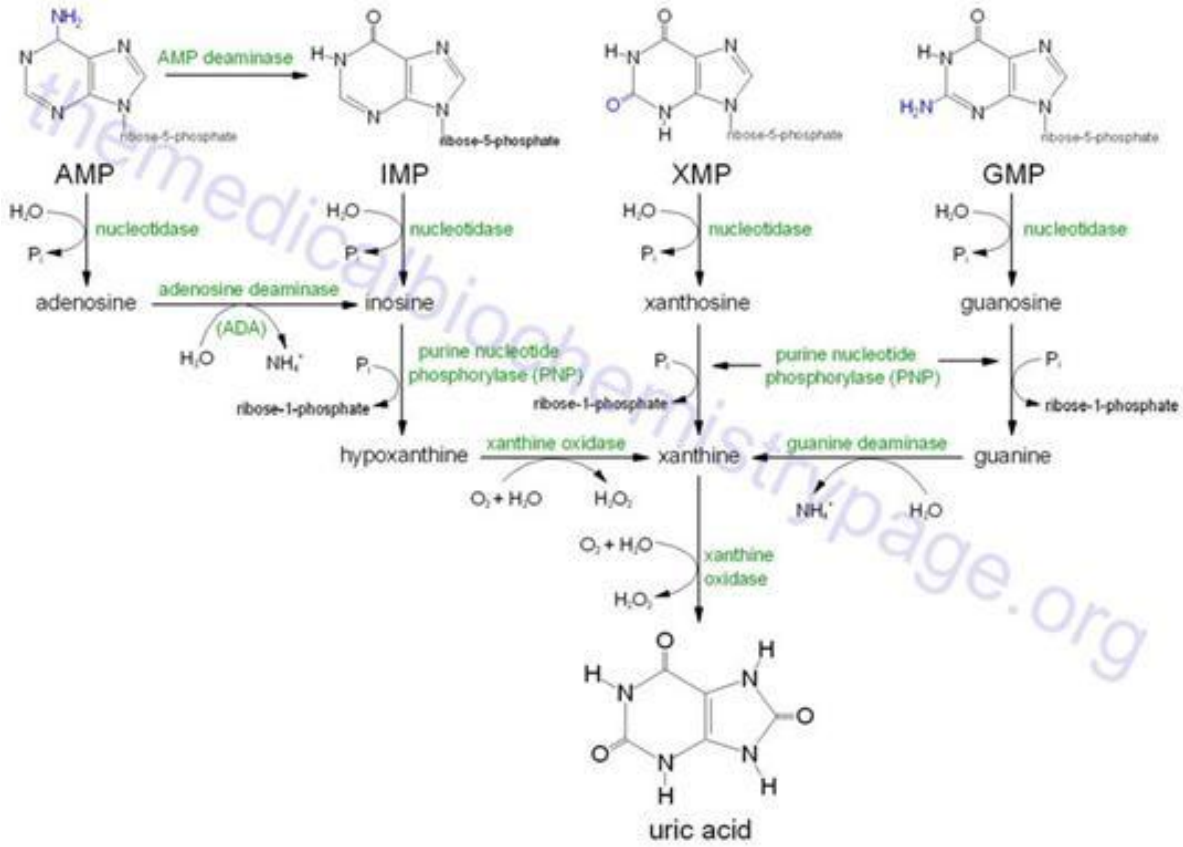
NADP is obtained by phosphorylation of the 2'-OH of the adenine ribose by ATP in the presence of NAD^+ kinase.

The essential rate limiting steps in purine biosynthesis occur at the first two steps of the pathway. The synthesis of PRPP by PRPP synthetase is feed-back inhibited by purine-5'-nucleotides (predominantly AMP and GMP). Combinatorial effects of those two nucleotides are greatest, e.g., inhibition is maximal when the correct concentration of both adenine and guanine nucleotides is achieved.

The amidotransferase reaction catalyzed by PRPP amidotransferase is also feed-back inhibited allosterically by binding ATP, ADP and AMP at one inhibitory site and GTP, GDP and GMP at another. Conversely the activity of the enzyme is stimulated by PRPP.
Additionally, purine biosynthesis is regulated in the branch pathways from IMP to AMP and GMP. The accumulation of excess ATP leads to accelerated synthesis of GMP, and excess GTP leads to accelerated synthesis of AMP.

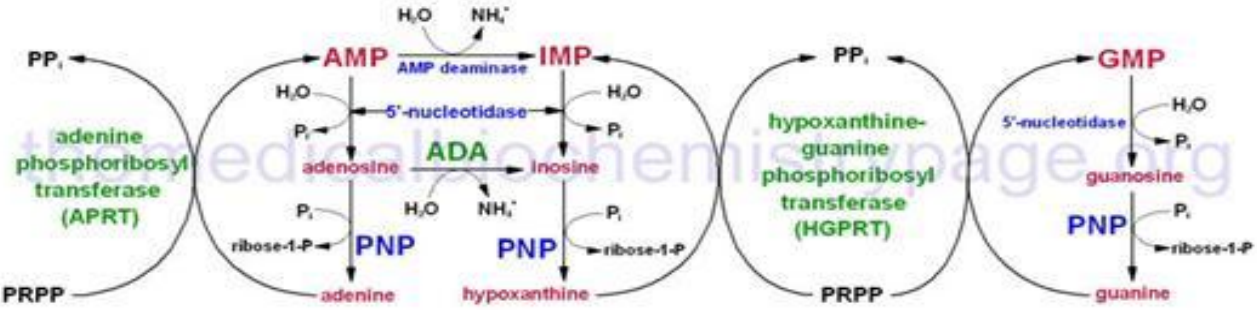
Catabolism and Salvage of Purine Nucleotides

Catabolism of the purine nucleotides leads ultimately to the production of uric acid which is insoluble and is excreted in the urine as sodium urate crystals.



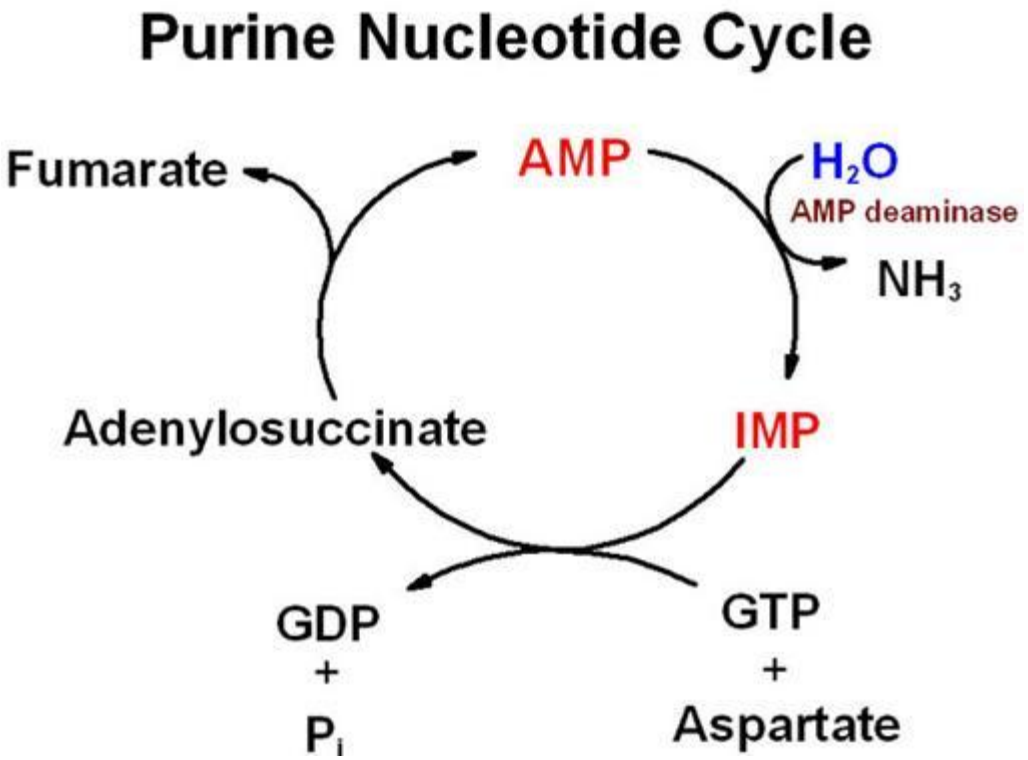
The synthesis of nucleotides from the purine bases and purine nucleosides takes place in a series of steps known as the salvage pathways. The free purine bases, adenine, guanine, and hypoxanthine, can be reconverted to their corresponding nucleotides by phosphoribosylation. Two key transferase enzymes are involved in the salvage of purines: adenosine phosphoribosyltransferase (APRT), which catalyzes the following reaction:
adenine + PRPP <----> AMP + PP_i
and hypoxanthine-guanine phosphoribosyltransferase (HGPRT), which catalyzes the following reactions:
hypoxanthine + PRPP <----> IMP + PP_i
guanine + PRPP <----> GMP + PP_i

A critically important enzyme of purine salvage in rapidly dividing cells is adenosine deaminase (ADA) which catalyzes the deamination of adenosine to inosine. Deficiency in ADA results in the disorder called severe combined immunodeficiency, SCID (and briefly outlined below).



Salvage pathways for purine nucleotides

Purine nucleotide phosphorylases (PNPs) can also contribute to the salvage of the bases through a reversal of the catabolism pathways. However, this pathway is less significant than those catalyzed by the phosphoribosyltransferases.
The synthesis of AMP from IMP and the salvage of IMP via AMP catabolism have the net effect of deaminating aspartate to fumarate. This process has been termed the purine nucleotide cycle (see diagram below). This cycle is very important in muscle cells. Increases in muscle activity create a demand for an increase in the TCA cycle, in order to generate more NADH for the production of ATP. However, muscle lacks most of the enzymes of the major anapleurotic reactions. Muscle replenishes TCA-cycle intermediates in the form of fumarate generated by the purine nucleotide cycle.



The purine nucleotide cycle serves an important function within exercising muscle. The generation of fumarate provides skeletal muscle with its' only source of anapleurotic substrate for the TCA cycle. In order for continued operation of the cycle during exercise, muscle protein must be utilized to supply the amino nitrogen for the generation of aspartate. The generation of asparate occurs by the standard transamination reactions that interconvert amino acids with α-ketoglutarate to form glutamate and glutamate with oxaloacetate to form aspartate. Myoadenylate deaminase is the muscle-specific isoenzyme of AMP deaminase, and deficiencies in myoadenylate deaminase lead to post-exercise fatigue, cramping and myalgias.

Clinical Significances of Purine Metabolism

Clinical problems associated with nucleotide metabolism in humans are predominantly the result of abnormal catabolism of the purines. The clinical consequences of abnormal purine metabolism range from mild to severe and even fatal disorders. Clinical manifestations of abnormal purine catabolism arise from the insolubility of the degradation by product, uric acid.

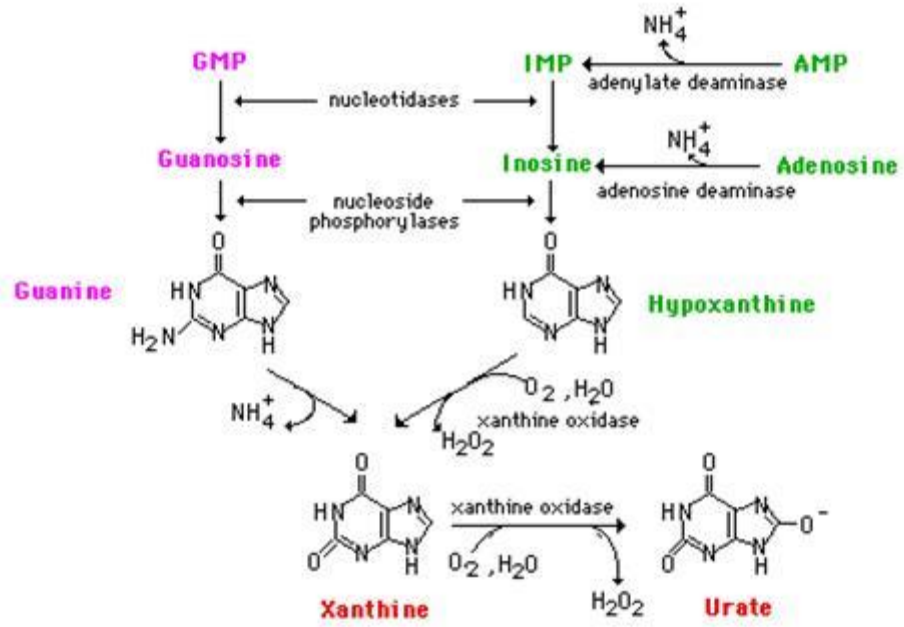
Nucleotides to Bases

Guanine nucleotides are hydrolyzed to the nucleoside guanosine which undergoes phosphorolysis to guanine and ribose 1-P. Man's intracellular nucleotidases are not very active toward AMP, however. Rather, AMP is deaminated by the enzyme adenylate (AMP) deaminase to IMP. In the catobilsm of purine nucleotides, IMP is further degraded by hydrolysis with nucleotidase to inosine and then phosphorolysis to hypoxanthine.

Adenosine does occur but usually arises from S-Adenosylmethionine during the course of transmethylation reactions. Adenosine is deaminated to inosine by an adenosine deaminase. Deficiencies in either adenosine deaminase or in the purine nucleoside phosphorylase lead to two different immunodeficiency diseases by mechanisms that are not clearly understood. With adenosine deaminase deficiency, both T and B-cell immunity is affected. The phosphorylase deficiency affects the T cells but B cells are normal. In September, 1990, a 4 year old girl was treated for adenosine deaminase deficiency by genetically engineering her cells to incorporate the gene. The treatment,so far, seems to be successful.

Whether or not methylated purines are catabolized depends upon the location of the methyl group. If the methyl is on an $-NH_2$, it is removed along with the $-NH_2$ and the core is metabolized in the usual fashion. If the methyl is on a ring nitrogen, the compound is excreted unchanged in the urine.

Bases to Uric Acid



Both adenine and guanine nucleotides converge at the common intermediate xanthine. Hypoxanthine, representing the original adenine, is oxidized to xanthine by the enzyme xanthine oxidase. Guanine is deaminated, with the amino group released as ammonia, to xanthine. If this process is occurring in tissues other than liver, most of the ammonia will be transported to the liver as glutamine for ultimate excretion as urea.
Xanthine, like hypoxanthine, is oxidized by oxygen and xanthine oxidase with the production of hydrogen peroxide. In man, the urate is excreted and the hydrogen peroxide is degraded by catalase. Xanthine oxidase is present in significant concentration only in liver and intestine. The pathway to the nucleosides, possibly to the free bases, is present in many tissues.

URIC ACID

- Uric acid is the end product of purine metabolism.
- Hyperuricaemia is associated with a tendency to form crystals of monosodium urate causing:
- Clinical gout (due to the deposition of monosodium urate crystals in the cartilage, synovium and synovial fluid of joints),
- Renal calculi
- Tophi (accretions of sodium urate in soft tissues)
- Acute urate nephropathy (due to sudden increases in urate production leading to widespread crystallisation in the renal tubules).

URIC ACID METABOLISM:
- Sources of purines in humans:

- Diet
- Degradation of endogenous nucleotides
- De novo synthesis (energy requiring process).
- Purines are degraded to uric acid.
- Urate is excreted via 2 routes:
- 1/3: Secretion into the gut, and subsequent degradation by bacterial uricase to CO_2 and NH_3.
- 2/3: Renal excretion:
- Urate is filtered at the glomeruli.
- Proximal tubular reabsorption of 99% of filtered load.
- More distal part of proximal tubules: secretion (also some reabsorption, but less than secretion).
- Net excretion of 10% of filtered load.
- Body urate pool (and plasma concentration) depends on the relative rates of urate formation and urate excretion.
-
- De novo synthesis leads to the formation of IMP (inosine monophosphate), which can be converted to AMP (adenosine monophosphate) and GMP (guanosine monophosphate) (NUCLEOTIDES: purine base + sugar + PO_4).
- Nucleotide degradation involves the formation of the respective nucleosides (inosine, adenosine and guanosine) (NUCLEOSIDES: purine base + sugar), these are subsequently metabolised to the respective purine bases (hypoxanthine, adenine and guanine) (PURINE BASES).
- Hypoxanthine and guanine can be metabolised directly to xanthine, but AMP/adenosine have to be converted to IMP/inosine first.
- Xanthine is metabolised to uric acid by the enzyme xanthine oxidase, also responsible for conversion of hypoxanthine to xanthine.
- Because de novo synthesis is an energy requiring process, excretion of uric acid results in net energy loss. However, salvage pathways exist to convert purines back to their parent nucleotides and are therefore energy saving – accomplished by the following enzymes:
- For guanine and hypoxanthine: HGPRT (hypoxanthine-guanine phosphoribosyl transferase).
- For adenine: APRT (adenine phosphoribosyl transferase).

GOUT

- Gout is a group of metabolic diseases associated with hyperuricaemia and deposition of crystals of monosodium urate in tissues.
- Prevalence: 3/1000, males affected more than females (8-10:1).
- Presentation usually occurs in males over 30 years of age and females after the menopause.
- There are 4 stages in the development of the disorder:

1. Asymptomatic hyperuricaemia:

- Hyperuricaemia is usually present for many years before the onset of symptoms.
- NB: Only 1 in 20 subjects with hyperuricaemia will eventually develop clinical gout.

2. Acute gouty arthritis:

- Classical presentation is acute inflammation of the metatarsophalangeal joint of the big toe (70%), and the first attack is usually monoarticular (affects only 1 joint).
- Other joints that may be involved are the ankle, knee, wrist, elbow, and small joints of hands and feet.

3. Intercritical gout:

- Some patients may have only 1 attack, whilst others have recurrent attacks at shorter intervals.
- Between attacks the patient is usually asymptomatic except for hyperuricaemia.

4. Chronic tophaceous gout:

- This follows recurrent attacks and is characterised by the development of tophi (swellings containing uric acid crystals) in the periarticular tissue.
- Other sites include the helix of the ear, bursae and tendons.
- Complications of hyperuricaemia:
- Urolithiasis (kidney stones):
- 10% of gouty patients develop urate stones and 10% of all renal calculi are due to urate.
- Renal failure:
- Acute renal failure due to obstructive uropathy (urate crystals) may occur during cytotoxic treatment of malignancy (allopurinol cover should be used), and has also been described in gouty subjects after severe exercise.
- Progressive chronic renal insufficiency is an important cause of morbidity and mortality in untreated chronic tophaceous gout.
- Associated conditions:
- Alcoholism
- Dysmetabolic syndrome (Insulin resistance syndrome)(syndrome X): Obesity, characteristic dyslipidaemia (increased triglycerides, decreased HDL cholesterol, small dense LDL), hypertension, impaired glucose tolerance, prothrombotic state.

- Diagnosis:
- The laboratory evaluation of hyperuricaemia is discussed below. It is important to recognise that:
- Hyperuricaemia is not synonymous with gout (1 in 20 develop gout)
- Gout can be precipitated by a sudden change (either increase or decrease) in urate concentration.
- An acute gout attack may be associated with a normal plasma urate level (due to a fall in urate level as seen with a change in diet, decrease in alcohol consumption), although hyperuricaemia will be demonstrated at some stage.

- Diagnosis is therefore usually made on clinical grounds.
- Definitive diagnosis: Examination of synovial fluid under polarizing light microscope for monosodium urate crystals (needle shaped and strongly negatively birefringent).
- Therapeutic agents used in gout and hyperuricaemia:
- Three groups of drugs are available:

Allopurinol (structural analogue of hypoxanthine), and its major metabolite, oxypurinol, inhibit the enzyme xanthine oxidase, producing a decrease in the plasma and urinary concentrations of urate (hypoxanthine does not accumulate if the salvage pathway is intact).
Initial treatment with allopurinol should be covered with an anti-inflammatory agent, because an acute attack of gout can be precipitated when the initial dose is given (sudden decrease in urate can cause mobilisation from body pools).

Two severe disorders, both quite well described, are associated with defects in purine metabolism: **Lesch-Nyhan syndrome and severe combined immunodeficiency disease (SCID).** Lesch-Nyhan syndrome results from the loss of a functional HGPRT gene. The disorder is inherited as a sex-linked trait, with the HGPRT gene on the X chromosome (Xq26-q27.2). Patients with this defect exhibit not only severe symptoms of gout but also a severe malfunction of the nervous system. In the most serious cases, patients resort to self-mutilation. Death usually occurs before patients reach their 20th year.
Lesch-Nyhan syndrome (LNS) is a disorder related to defects in the activity of the purine nucleotide salvage enzyme, hypoxanthine-guanine phosphoribosyltransferase (HGPRT). HGPRT catalyzes the following two interconversions:
hypoxanthine + PRPP <---> IMP + PP_i
guanine + PRPP <---> GMP + PP_i

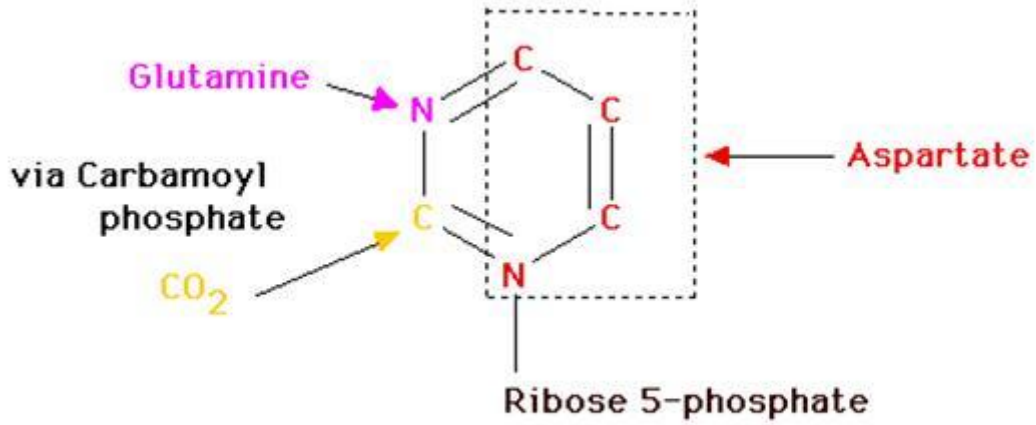
There are three over-lapping clinical syndromes associated with deficiencies in HGPRT activity. Individuals that have less than 1.5% residual enzyme activity exhibit debilitating neurologic disability, behavioral abnormalities that include impulsive and self-mutilating behaviors and varying degrees of cognitive disability in addition to overproduction of uric acid. This most severe of the three clinical syndromes is Lesch-Nyhan syndrome. Patients 1.5% to 8% of residual enzyme activity exhibit neurologic disability that ranges from clumsiness to debilitating pyramidal (CNS neurons involved in voluntary motor movement) and extrapyramidal motor dysfunction in addition to overproduction of uric acid. In cases where at least 8% of normal HGPRT activity is present, patients exhibit overproduction of uric acid and associated hyperuricemia, renal lithiasis (kidney stones) and gout. The latter circumstance (partial deficiency with at least 8% enzyme activity) is associated with Kelley-Steegmiller syndrome.
Lesch-Nyhan syndrome is inherited as an X-linked recessive disorder with an incidence of approximately 1:380,000. Since it is an X-linked disease it is found

almost exclusively in males although affected females have been identified albeit very rarely.
The HGPRT gene (symbol = HPRT) is located on the X chromosome (Xq26-q27.2) spanning 44 kbp and composed of 9 exons. In addition, 4 pseudogenes have been found. Over 270 different mutations in the HPRT gene have been identified in LNS patients. Alterations to the gene include single base insertions and deletions, large deletions, amino acid substitutions and stop codon mutations.
Clinical Features of Lesch-Nyhan Syndrome
The characteristic clinical features of the Lesch-Nyhan syndrome are mental retardation, spastic cerebral palsy, choreoathetosis, uric acid urinary stones, and self-destructive biting of fingers and lips. The overal clinical features of LNS can be divided into three broad categories. These include uric acid overproduction and its associated consequences (e.g. gouty arthritis and renal lithiasis), neurobehavioral dysfunction indicative of central nervous system involvement, and growth retardation. As indicated (and as expected from uric acid overproduction) LNS patients manifest with many of the symptoms of classic gout and will not be covered here.
All patients with Lesch-Nyhan syndrome manifest with profound motor dysfunction that is recognizable within the first 3 to 9 months of life. Infants fail to develop the ability to hold up their heads or to sit unaided. Further motor development will be delayed and the onset of pyramidal and extrapyramidal signs become evident by 1 to 2 years of age. In LNS patients there are three major signs of pyramidal dysfunction: spasticity, hyperreflexia and the extensor plantar reflex (also known as the Babinski reflex: the great toe flexes toward the top of the foot and the other toes fan out after the sole of the foot has been firmly stroked). Extrapyramidal dysfunction in LNS patients is primarily evident as dystonia (sustained muscle contractions causing twisting and repetitive movements or abnormal postures) although many patients also exhibit choreoatheosis (involuntary movement disorder in association with slow continuous writhing particularly of the hands and feet).
Most commonly associated with Lesch-Nyhan syndrome is the behavioral dysfunction manifest with impulsivity and self-mutilation particularly of the lips, fingers and tongue. LNS patients will often strike out at people around them, spit on people and use foul language. These symptoms are analogous to the uncontrollable compulsions associated with Tourette syndrome. The self-injury behavior is clearly an involuntary action as most LNS patients will learn to call out for help when they feel the compulsive behavior overtaking them, or they will sit on their hands or wear socks or gloves to limit the self-injurious behavior. Although the precise cause of the self-mutilating behavior in LNS patients is not clearly understood it is most likely that it is a form of obsessive-compulsive disorder.
SCID is most often (90%) caused by a deficiency in the enzyme adenosine deaminase (ADA). This is the enzyme responsible for converting adenosine to inosine in the catabolism of the purines. This deficiency selectively leads to a destruction of B and T lymphocytes, the cells that mount immune responses. In the

absence of ADA, deoxyadenosine is phosphorylated to yield levels of dATP that are 50-fold higher than normal. The levels are especially high in lymphocytes, which have abundant amounts of the salvage enzymes, including nucleoside kinases. High concentrations of dATP inhibit ribonucleotide reductase (see below), thereby preventing other dNTPs from being produced. The net effect is to inhibit DNA synthesis. Since lymphocytes must be able to proliferate dramatically in response to antigenic challenge, the inability to synthesize DNA seriously impairs the immune responses, and the disease is usually fatal in infancy unless special protective measures are taken. A less severe immunodeficiency results when there is a lack of purine nucleoside phosphorylase (PNP), another purine-degradative enzyme.

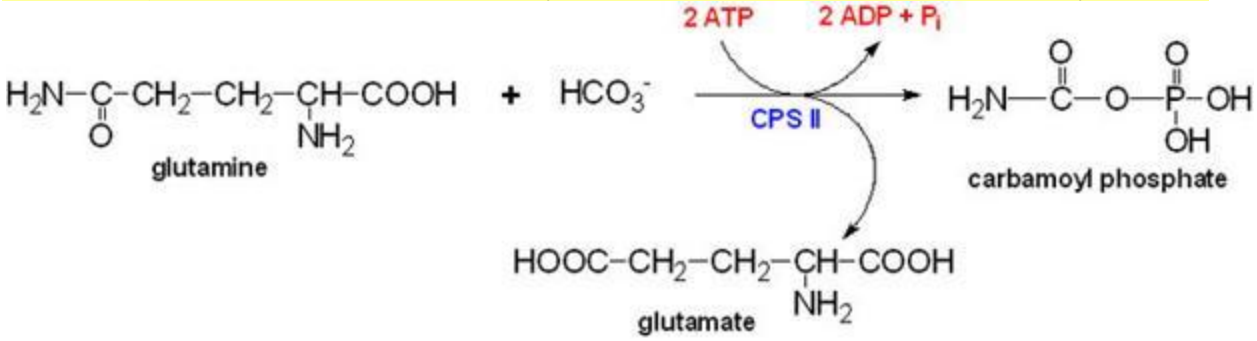
De Novo Synthesis of Pyrimidine Nucleotides

Since pyrimidine molecules are simpler than purines, so is their synthesis simpler but is still from readily available components. Glutamine's amide nitrogen and carbon dioxide provide atoms 2 and 3 or the pyrimidine ring. They do so, however, after first being converted to carbamoyl phosphate. The other four atoms of the ring are supplied by aspartate. As is true with purine nucleotides, the sugar phosphate portion of the molecule is supplied by PRPP.

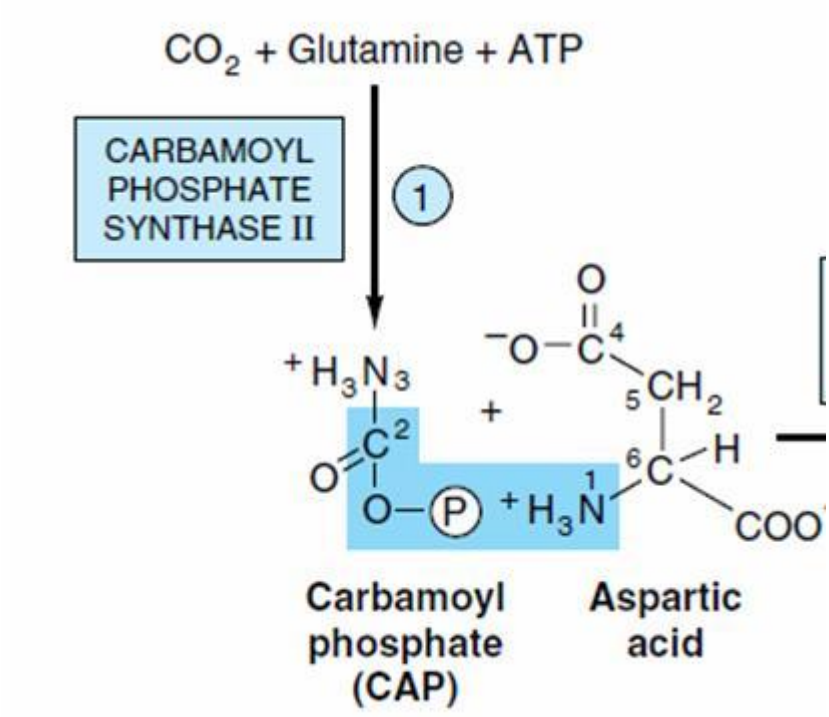


Generic pyrimidine base

Pyrimidine synthesis begins with carbamoyl phosphate synthesized in the cytosol of those tissues capable of making pyrimidines (highest in spleen, thymus, GItract and testes). This uses a different enzyme than the one involved in urea synthesis.



Carbamoyl phosphate synthetase II (CPS II) prefers glutamine to free ammonia and has no requirement for N-Acetylglutamate.



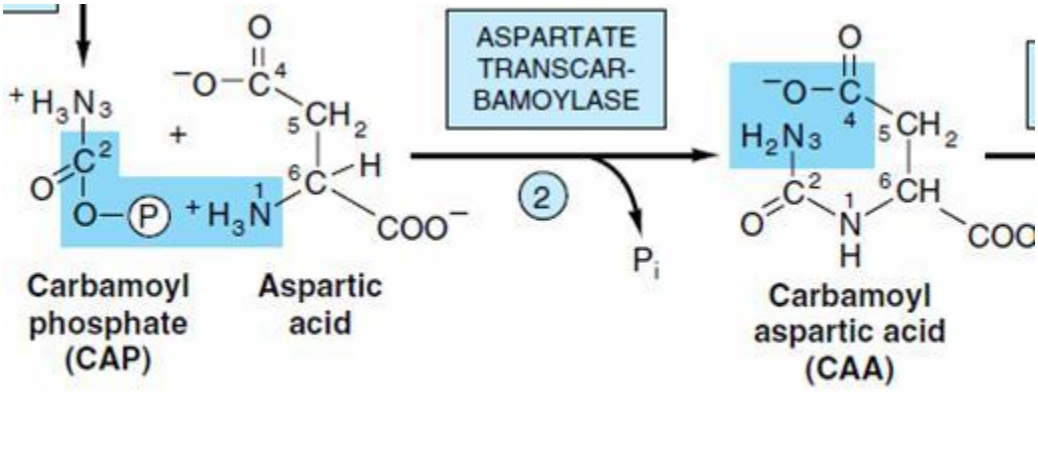
Synthesis of UMP from carbamoyl phosphate. Carbamoyl phosphate utilized in pyrimidine nucleotide synthesis differs from that synthesized in the urea cycle; it is synthesized from glutamine instead of ammonia and is synthesized in the cytosol. The reaction is catalyzed by carbamoyl phosphate synthetase II (CPS-II). Subsequently carbamoyl phosphate is incorporated into the pyrimidine nucleotide biosynthesis pathway through the action of aspartate transcarbamoylase, ATCase (enzyme #1) which is the rate limiting step in pyrimidine biosynthesis. Following completion of UMP synthesis it can be phosphorylated to UTP and utilized as a substrate for CTP synthase for the synthesis of CTP. Uridine nucleotides are also the precursors for *de novo* synthesis of the thymine nucleotides. Place mouse over green intermediate names to see structure.

Enzyme names:

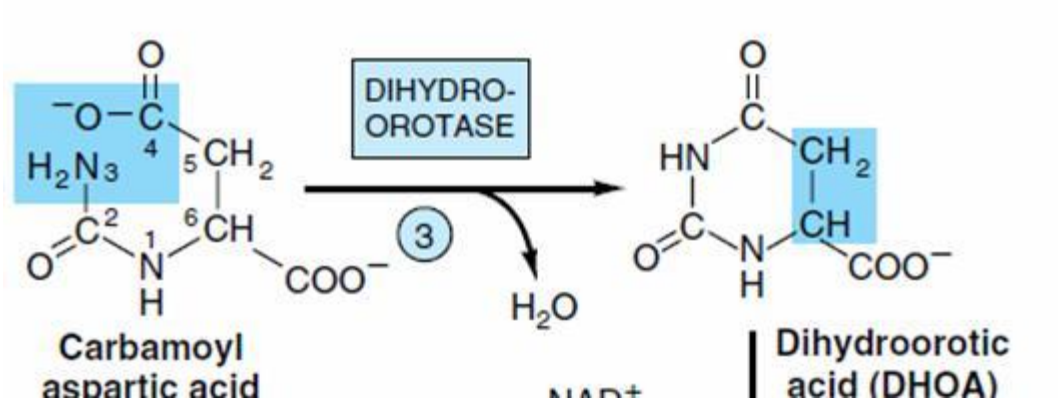
1. aspartate transcarbamoylase, ATCase
2. carbamoyl aspartate dehydratase
3. dihydroorotate dehydrogenase
4. orotate phosphoribosyltransferase
5. orotidine-5'-phosphate carboxylase

Formation of Orotic Acid

Carbamoyl phosphate condenses with aspartate in the presence of aspartate transcarbamylase to yield N-carbamylaspartate which is then converted to dihydroorotate.

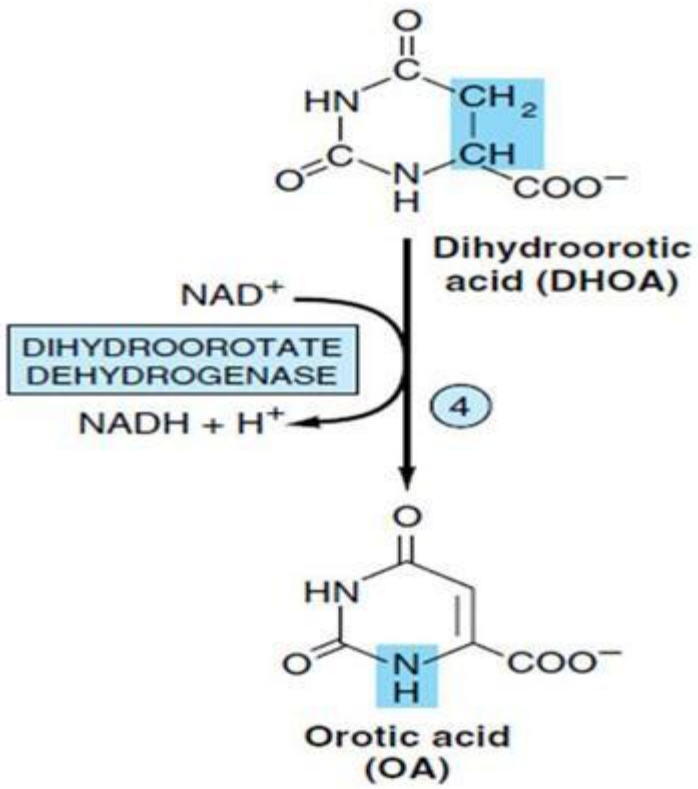


In man, CPSII, asp-transcarbamylase, and dihydroorotase activities are part of a multifunctional protein.
Oxidation of the ring by a complex, poorly understood enzyme produces the free pyrimidine, orotic acid. This enzyme is located on the outer face of the inner mitochondrial membrane, in contrast to the other enzymes which are cytosolic. Note the contrast with purine synthesis in which a nucleotide is formed first while pyrimidines are first synthesized as the free base.

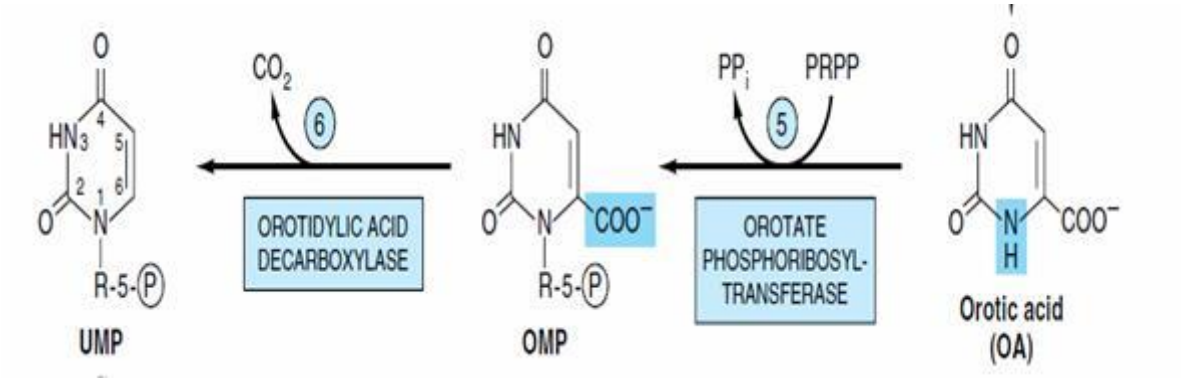


Formation of the Nucleotides

Orotic acid is converted to its nucleotide with PRPP. OMP is then converted sequentially - not in a branched pathway - to the other pyrimidine nucleotides.



Decarboxylation of OMP gives UMP. O-PRT and OMP decarboxylase are also a multifunctional protein. After conversion of UMP to the triphosphate, the amide of glutamine is added, at the expense of ATP, to yield CTP.



Control

The control of pyrimidine nucleotide synthesis in man is exerted primarily at the level of cytoplasmic CPS II. UTP inhibits the enzyme, competitively with ATP. PRPP activates it. Other secondary sites of control also exist (e.g. OMP decarboxylase is inhibited by UMP and CMP). These are probably not very important under normal circumstances.

In bacteria, aspartate transcarbamylase is the control enzyme. There is only one carbamoyl phosphate synthetase in bacteria since they do not have mitochondria. Carbamoyl phosphate, thus, participates in a branched pathway in these organisms that leads to either pyrimidine nucleotides or arginine.

Orotic aciduria refers to an excessive excretion of orotic acid in urine. It causes a characteristic form of anemia and may be associated with mental and physical retardation.
In addition to the characteristic excessive orotic acid in the urine, patients typically have megaloblastic anemia which cannot be cured by administration of vitamin B_{12} or folic acid.
It also can cause inhibition of RNA and DNA synthesis and failure to thrive. This can lead to mental and physical retardation.
Its hereditary form, an autosomal recessive disorder, can be caused by a deficiency in the enzyme UMPS, a bifunctional protein that includes the enzyme activities of orotate phosphoribosyltransferase and orotidine 5'-phosphate decarboxylase.

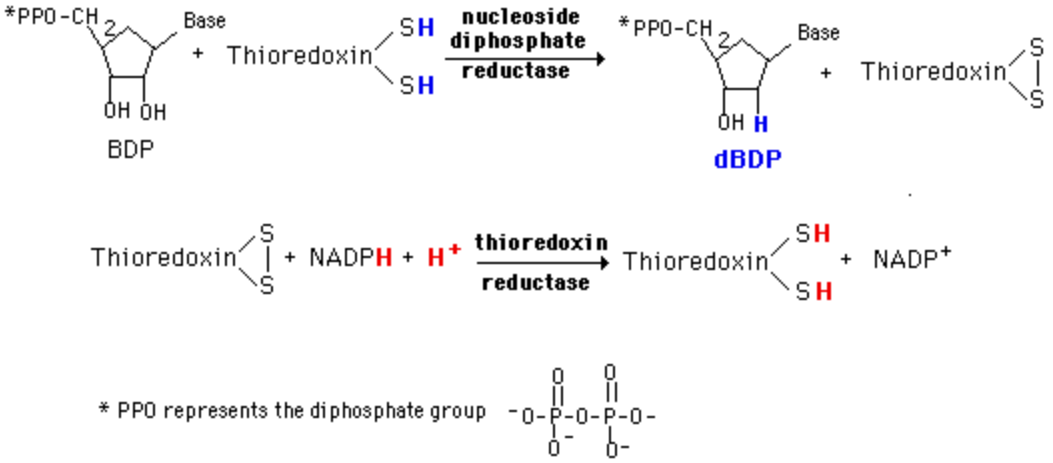
It can also arise secondary to blockage of the urea cycle, particularly in ornithine transcarbamylase deficiency (or OTC deficiency). You can distinguish this increase in orotic acid secondary to OTC deficiency from hereditary orotic aciduria (seen above) by looking at blood ammonia levels and the BUN. In OTC deficiency, because the urea cycle backs up, you will see hyperammonemia and a decreased BUN.
Administration of cytidine monophosphate and uridine monophosphate reduces urinary orotic acid and the anemia.
Administration of uridine, which is converted to UMP, will bypass the metabolic block and provide the body with a source of pyrimidine.

Pyrimidine Catabolism

In contrast to purines, pyrimidines undergo ring cleavage and the usual end products of catabolism are beta-amino acids plus ammonia and carbon dioxide. Pyrimidines from nucleic acids or the energy pool are acted upon by nucleotidases and pyrimidine nucleoside phosphorylase to yield the free bases. The 4-amino group of both cytosine and 5-methyl cytosine is released as ammonia.

Formation of Deoxyribonucleotides

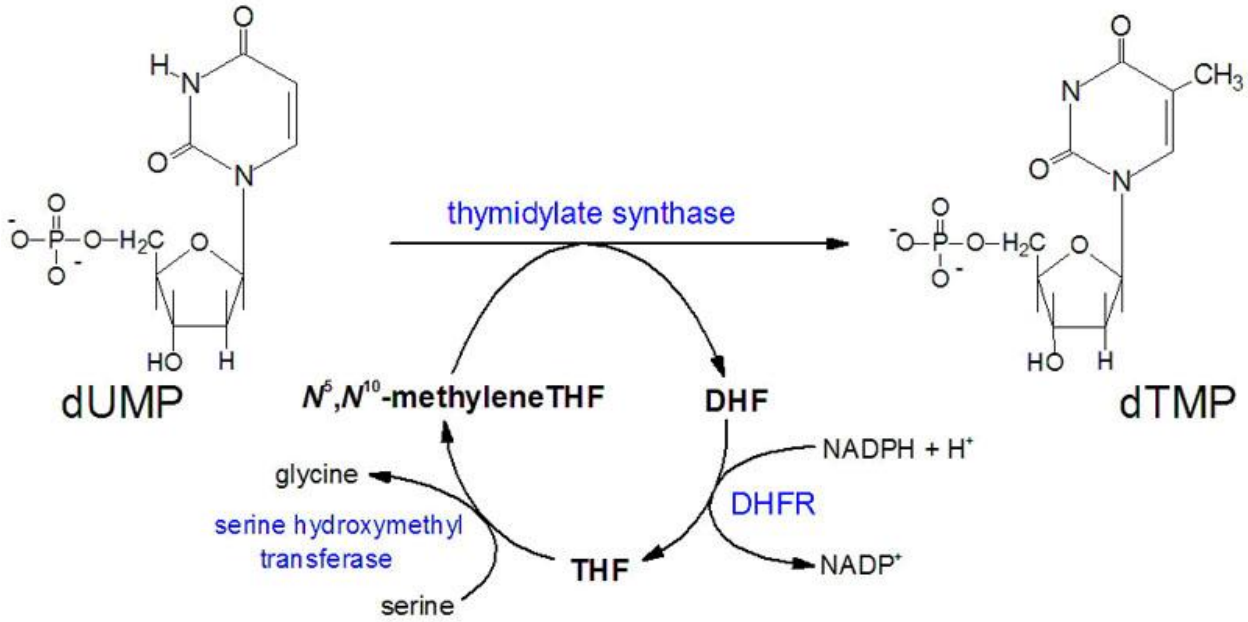
De novo synthesis and most of the salvage pathways involve the ribonucleotides. (Exception is the small amount of salvage of thymine indicated above.) Deoxyribonucleotides for DNA synthesis are formed from the ribonucleotide diphosphates (in mammals and *E. coli*).
A base diphosphate (BDP) is reduced at the 2' position of the ribose portion using the protein, thioredoxin and the enzyme nucleoside diphosphate reductase. Thioredoxin has two sulfhydryl groups which are oxidized to a disulfide bond during the process. In order to restore the thioredoxin to its reduced for so that it can be reused, thioredoxin reductase and NADPH are required.



This system is very tightly controlled by a variety of allosteric effectors. dATP is a general inhibitor for all substrates and ATP an activator. Each substrate then has a specific positive effector (a BTP or dBTP). The result is a maintenance of an appropriate balance of the deoxynucleotides for DNA synthesis.

Synthesis of the Thymine Nucleotides

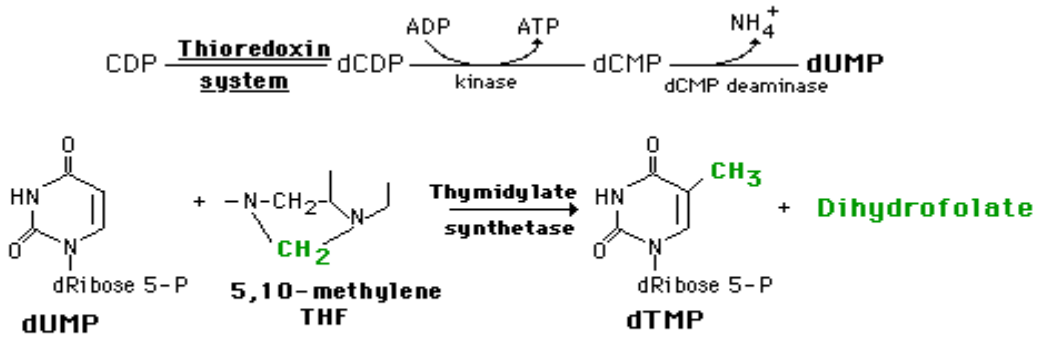
The *de novo* pathway to dTTP synthesis first requires the use of dUMP from the metabolism of either UDP or CDP. The dUMP is converted to dTMP by the action of thymidylate synthase. The methyl group (recall that thymine is 5-methyl uracil) is donated by N^5,N^{10}-methylene THF, similarly to the donation of methyl groups during the biosynthesis of the purines. The unique property of the action of thymidylate synthase is that the THF is converted to dihydrofolate (DHF), the only such reaction yielding DHF from THF. In order for the thymidylate synthase reaction to continue, THF must be regenerated from DHF. This is accomplished through the action of dihydrofolate reductase (DHFR). THF is then converted to N^5,N^{10}-THF via the action of serine hydroxymethyl transferase. The crucial role of DHFR in thymidine nucleotide biosynthesis makes it an ideal target for chemotherapeutic agents (see below).



Synthesis of dTMP from dUMP

The salvage pathway to dTTP synthesis involves the enzyme thymidine kinase which can use either thymidine or deoxyuridine as substrate:
thymidine + ATP <----> TMP + ADP
deoxyuridine + ATP <----> dUMP + ADP
The activity of thymidine kinase (one of the various deoxyribonucleotide kinases) is unique in that it fluctuates with the cell cycle, rising to peak activity during the phase of DNA synthesis; it is inhibited by dTTP.

DNA synthesis also requires dTMP (dTTP). This is not synthesized in the *de novo* pathway and salvage is not adequate to maintain the necessary amount. dTMP is generated from dUMP using the folate-dependent one-carbon pool. Since the nucleoside diphosphate reductase is not very active toward UDP, CDP is reduced to dCDP which is converted to dCMP. This is then deaminated to form dUMP. In the presence of 5,10-Methylene tetrahydrofolate and the enzyme thymidylate synthetase, the carbon group is both transferred to the pyrimidine ring and further reduced to a methyl group. The other product is dihydrofolate which is subsequently reduced to the tetrahydrofolate by dihydrofolate reductase.



Dihydrofolate must be subsequently reduced to the tetrahydro form.

Clinical Relevance of Tetrahydrofolate

Tetrahydrofolate (THF) is regenerated from the dihydrofolate (DHF) product of the thymidylate synthase reaction by the action of dihydrofolate reductase (DHFR), an enzyme that requires NADPH. Cells that are unable to regenerate THF suffer defective DNA synthesis and eventual death. For this reason, as well as the fact that dTTP is utilized only in DNA, it is therapeutically possible to target rapidly proliferating cells over non-proliferating cells through the inhibition of thymidylate synthase. Many anti-cancer drugs act directly to inhibit thymidylate synthase, or indirectly, by inhibiting DHFR.

The class of molecules used to inhibit thymidylate synthase is called the suicide substrates because they irreversibly inhibit the enzyme. Molecules of this class include 5-fluorouracil and 5-fluorodeoxyuridine. Both are converted within cells to 5-fluorodeoxyuridylate, FdUMP. It is this drug metabolite that inhibits thymidylate synthase. Many DHFR inhibitors have been synthesized, including methotrexate, aminopterin, and trimethoprim. Each of these is an analog of folic acid.

Regulation of Pyrimidine Biosynthesis

The regulation of pyrimidine synthesis occurs mainly at the first step which is catalyzed by aspartate transcarbamoylase, ATCase. Inhibited by CTP and activated by ATP, ATCase is a multifunctional protein in mammalian cells. It is capable of catalyzing the formation of carbamoyl phosphate, carbamoyl aspartate, and dihydroorotate. The carbamoyl synthetase activity of this complex is termed carbamoyl phosphate synthetase II (CPS-II) as opposed to CPS-I, which is involved in the urea cycle.

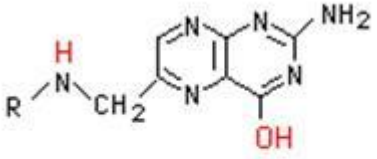
ATCase, and therefore the activity of CPS-II, is localized to the cytoplasm and prefers glutamine as a substrate. CPS-I of the urea cycle is localized in the mitochondria and utilizes ammonia. The CPS-II domain is activated by ATP and inhibited by UDP, UTP, dUTP, and CTP.

The role of glycine in ATCase regulation is to act as a competitive inhibitor of the glutamine binding site. As in the regulation of purine synthesis, ATP levels also regulate pyrimidine biosynthesis at the level of PRPP formation. An increase in the level of PRPP results in an activation of pyrimidine synthesis.

There is also regulation of OMP decarboxylase: this enzyme is competitively inhibited by UMP and, to a lesser degree, by CMP. Finally, CTP synthase is feedback-inhibited by CTP and activated by GTP.

Chemotherapeutic Agents

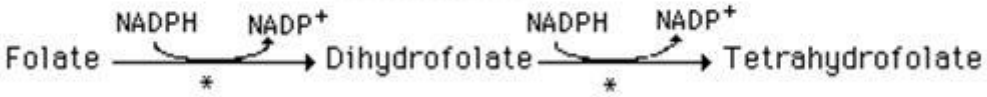
Thymidylate synthetase is particularly sensitive to availability of the folate one-carbon pool. Some of the cancer chemotherapeutic agents interfere with this process as well as with the steps in purine nucleotide synthesis involving the pool. Cancer chemotherapeutic agents like methotrexate (4-amino, 10-methyl folic acid) and aminopterin (4-amino, folic acid) are structural analogs of folic acid and inhibit dihydrofolate reductase. This interferes with maintenance of the folate pool and thus of *de novo* synthesis of purine nucleotides and of dTMP synthesis. Such agents are highly toxic and administered under careful control.



Folate

Aminopterin

Methotrexate



* Aminopterin and Methotrexate are inhibitors of dihydrofolate reductase

Catabolism and Salvage of Pyrimidine Nucleotides

Catabolism of the pyrimidine nucleotides leads ultimately to β-alanine (when CMP and UMP are degraded) or β-aminoisobutyrate (when dTMP is degraded) and NH_3 and CO_2. The β-alanine and β-aminoisobutyrate serve as $-NH_2$ donors in transamination of α-ketoglutarate to glutamate. A subsequent reaction converts the products to malonyl-CoA (which can be diverted to fatty acid synthesis) or methylmalonyl-CoA (which is converted to succinyl-CoA and can be shunted to the TCA cycle).

The salvage of pyrimidine bases has less clinical significance than that of the purines, owing to the solubility of the by-products of pyrimidine catabolism. However, as indicated above, the salvage pathway to thymidine nucleotide synthesis is especially important in the preparation for cell division. Uracil can be salvaged to form UMP through the concerted action of uridine phosphorylase and uridine kinase, as indicated:

uracil + ribose-1-phosphate <----> uridine + P_i

uridine + ATP ----> UMP + ADP
Deoxyuridine is also a substrate for uridine phosphorylase. Formation of dTMP, by salvage of dTMP requires thymine phosphorylase and the previously encountered thymidine kinase:
thymine + deoxyribose-1-phosphate <----> thymidine + P_i
thymidine + ATP ----> dTMP + ADP
The salvage of deoxycytidine is catalyzed by deoxycytidine kinase:
deoxycytidine + ATP <----> dCMP + ADP
Deoxyadenosine and deoxyguanosine are also substrates for deoxycytidine kinase, although the K_m for these substrates is much higher than for deoxycytidine.
The major function of the pyrimidine nucleoside kinases is to maintain a cellular balance between the level of pyrimidine nucleosides and pyrimidine nucleoside monophosphates. However, since the overall cellular and plasma concentrations of the pyrimidine nucleosides, as well as those of ribose-1-phosphate, are low, the salvage of pyrimidines by these kinases is relatively inefficient.

Clinical Significances of Pyrimidine Metabolism

Because the products of pyrimidine catabolism are soluble, few disorders result from excess levels of their synthesis or catabolism. Two inherited disorders affecting pyrimidine biosynthesis are the result of deficiencies in the bifunctional enzyme catalyzing the last two steps of UMP synthesis, orotate phosphoribosyl transferase and OMP decarboxylase. These deficiencies result in orotic aciduria that causes retarded growth, and severe anemia caused by hypochromic erythrocytes and megaloblastic bone marrow. Leukopenia is also common in orotic acidurias. The disorders can be treated with uridine and/or cytidine, which leads to increased UMP production via the action of nucleoside kinases. The UMP then inhibits CPS-II, thus attenuating orotic acid production.