METABOLISM OF NUCLEOTIDES

TEJASVI NAVADHITAMASTU

"Let our (the teacher and the taught) learning be radiant"

Let our efforts at learning be luminous and filled with joy, and endowed with the force of purpose

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E –content

Course: M.Sc.
Subject: Biochemistry; Biotechnology
Topic: Nucleotide Metabolism
Subtopic: Nucleotide Metabolism
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NUCLEOTIDES

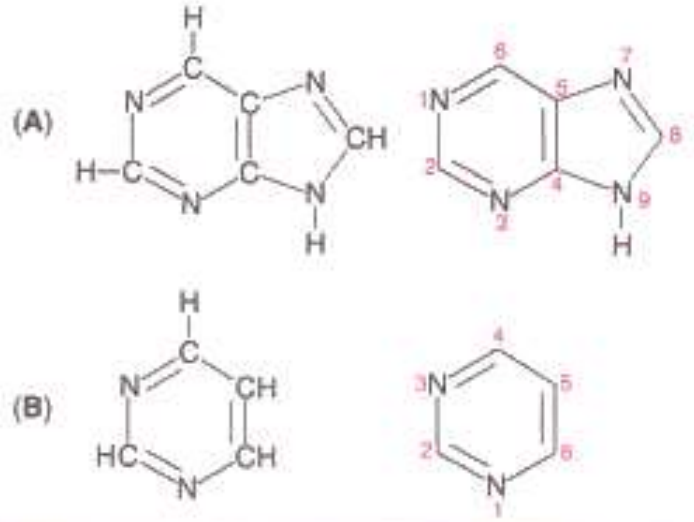


Fig. 5.1 : General structure of nitrogen bases (A) Purine (B) Pyrimidine (The positions are numbered according to the international system).

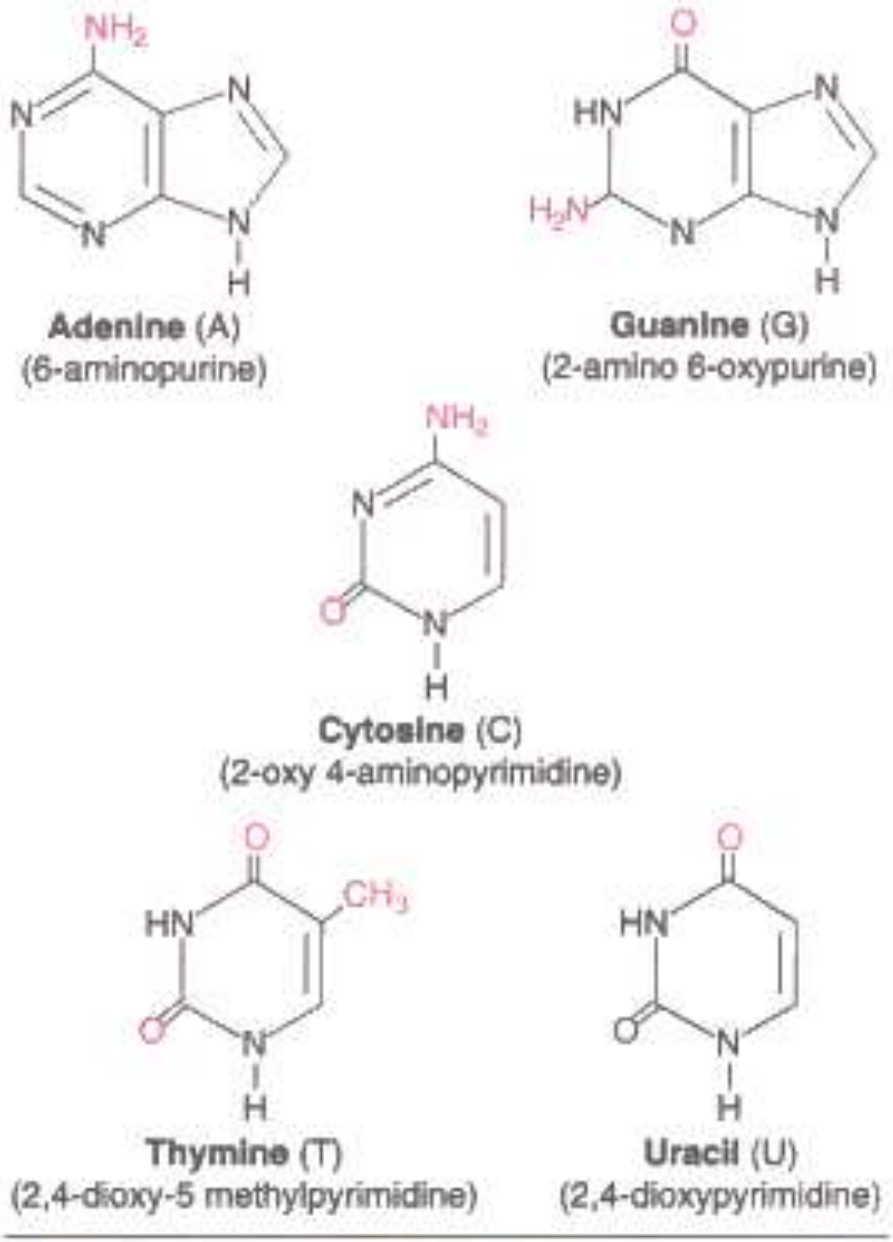


Fig. 5.2 : Structures of major purines (A, G) and pyrimidines (C, T, U) found in nucleic acids.

BIOSYNTHESIS OF PURINE RIBONUCLEOTIDES

Many compounds contribute to the purine ring of the nucleotides (***Fig.17.1***).

1. N_1 of purine is derived from amino group of aspartate.

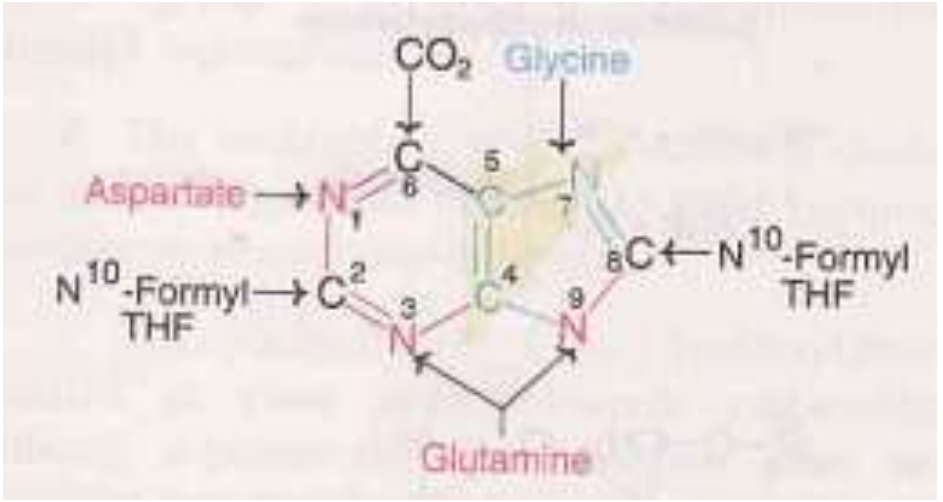
2. C_2 and C_8 arise from formate of N^{10}-formyl THF.

3. N_3 and N_9 are obtained from amide group of glutamine.

4. C_4, C_5 and N_7 are contributed by glycine.

5. C_6 directly comes from CO_2.

It should be remembered that purine bases are not synthesized as such, but they are formed as ribonucleotides. ***The purines are built upon a pre-existing ribose 5-phosphate.*** Liver is the major site for purine nucleotide synthesis. Erythrocytes, polymorphonuclear leukocytes and brain cannot produce purines.



1. Ribose 5-phosphate, produced in the hexose monophosphate shunt of carbohydrate metabolism is the starting material for purine nucleotide synthesis. It reacts with ATP to form phosphoribosyl pyrophosphate (PRPP).

2. Glutamine transfers its amide nitrogen to PRPP to replace pyrophosphate and produce 5-phosphoribosylamine. The enzyme ***PRPP glutamyl amidotransferase*** is controlled by ***feedback inhibition*** of nucleotides (IMP, AMP and GMP). This reaction is the '***committed step***' in purine nucleotide biosynthesis.

3. Phosphoribosylamine reacts with glycine in the presence of ATP to form glycinamide ribosyl 5-phosphate or glycinamide ribotide (GAR).

4. N^{10}-Formyl tetrahydrofolate donates the formyl group and the product formed is formyl-glycinamide ribosyl 5-phosphate.

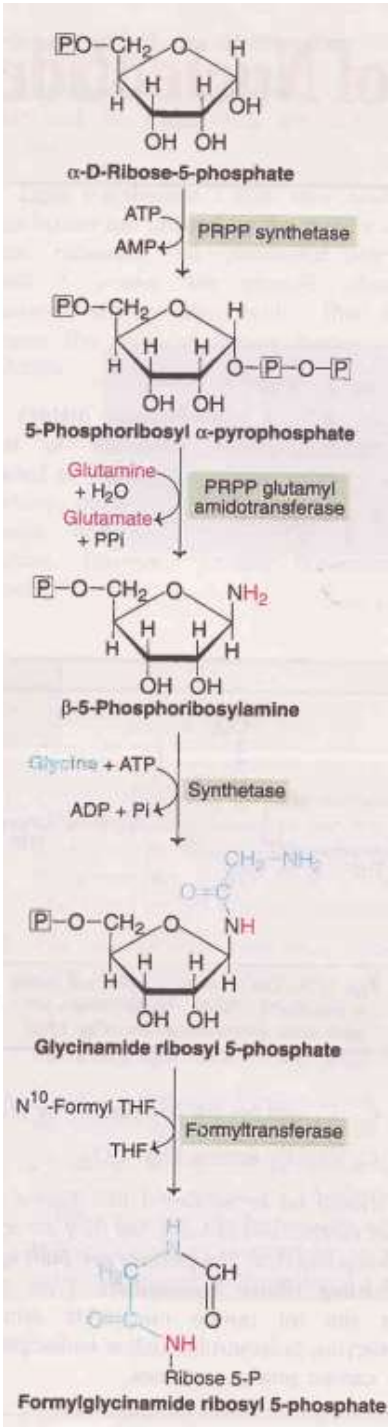
5. Glutamine transfers the second amido amino group to produce formylglycinamidine ribosyl 5-phosphate.

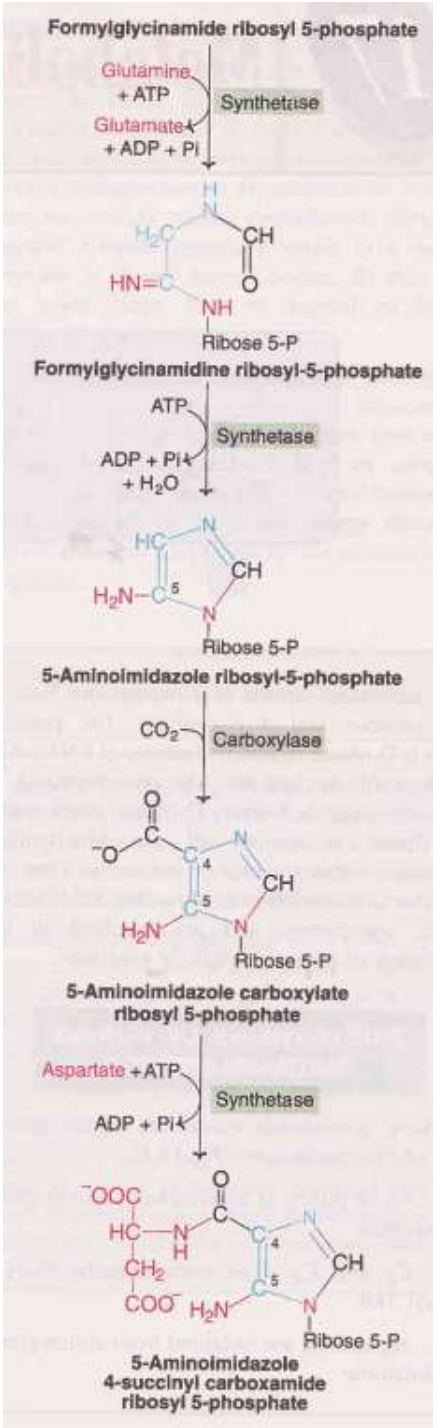
6. The imidazole ring of the purine is closed in an ATP dependent reaction to yield 5-amino-imidazole ribosyl 5-phosphate.

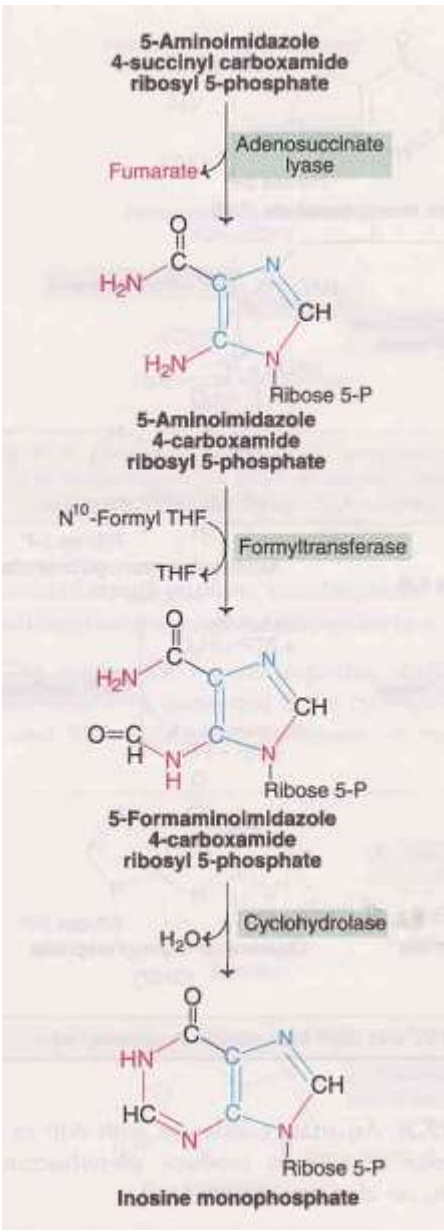
7. Incorporation of CO_2 (carboxylation) occurs to yield aminoimidazole carboxylate ribosyl 5-phosphate. This reaction ***does not require*** the vitamin ***biotin*** and/or ATP which is the case with most of the ***carboxylation*** reactions.

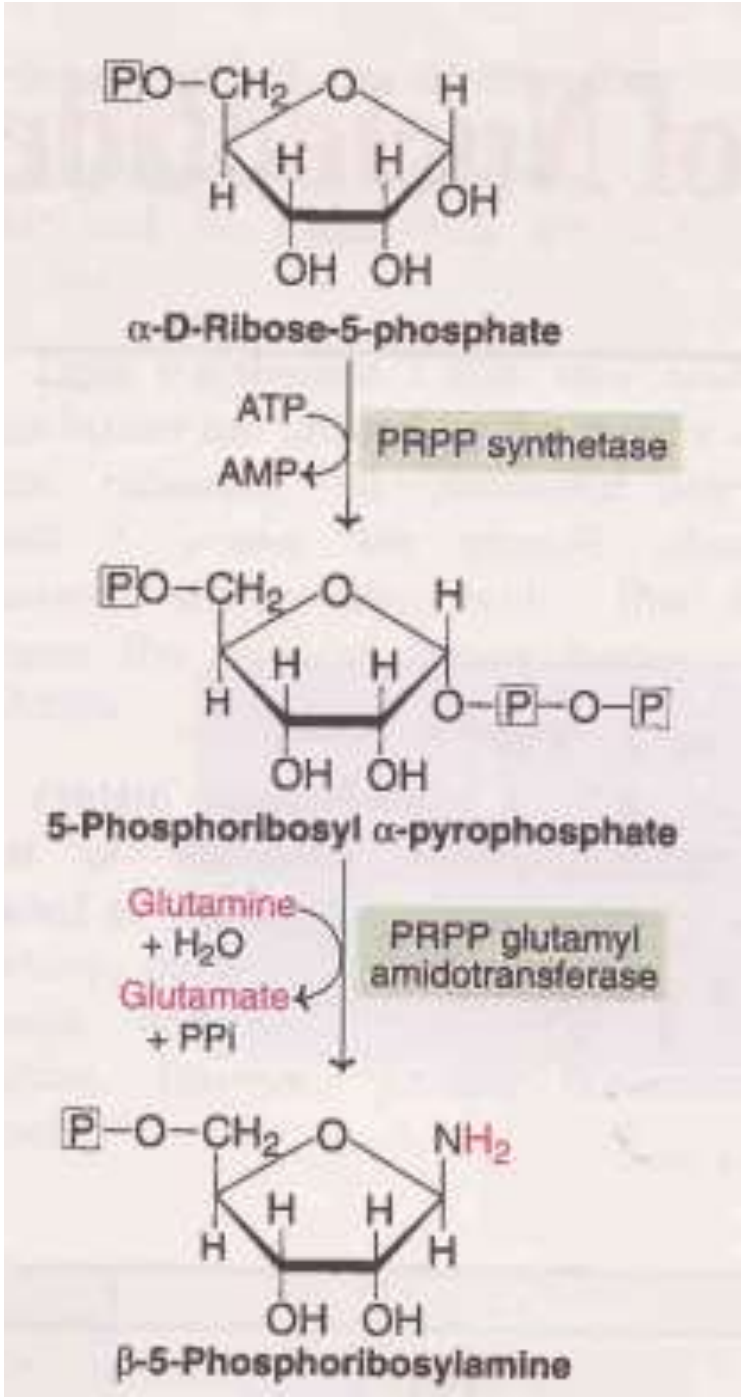
8. Aspartate condenses with the product in reaction 7 to form aminoimidazole 4-succinyl carboxamide ribosyl 5-phosphate.

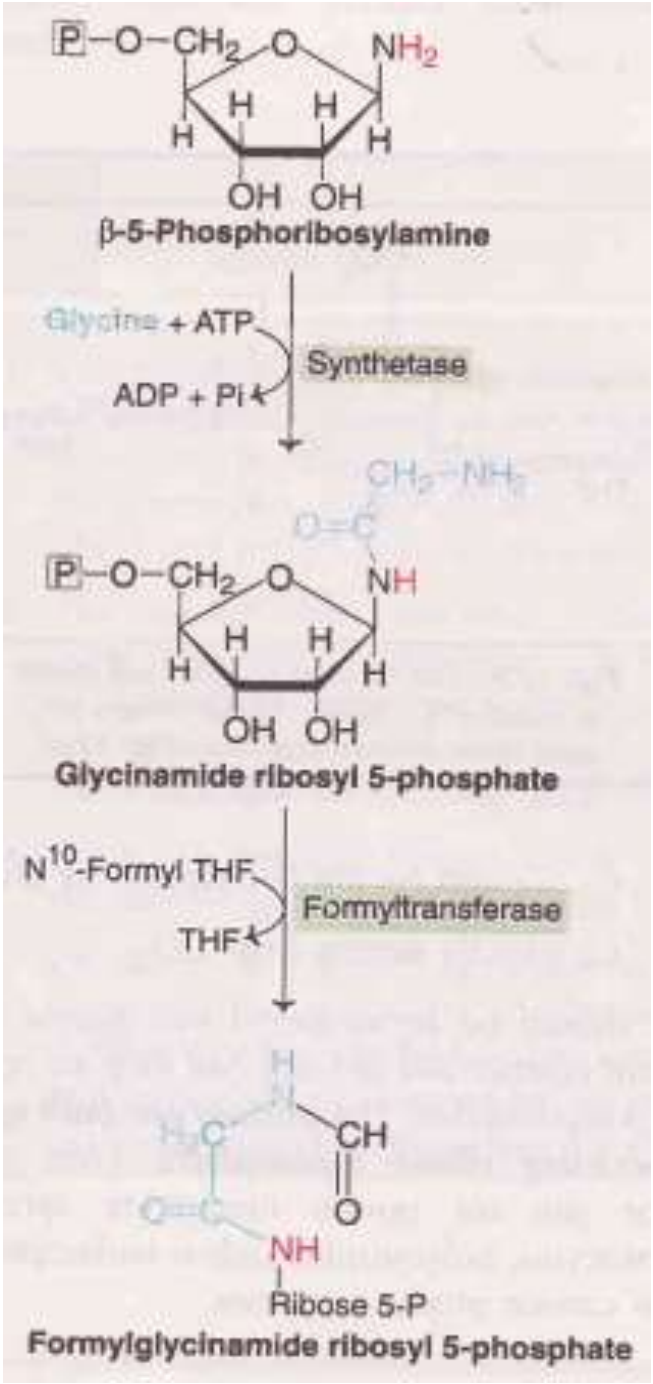
9. Adenosuccinate lyase cleaves off fumarate and only the amino group of aspartate is retained to yield aminoimidazole 4-carboxamide ribosyl 5-phosphate.

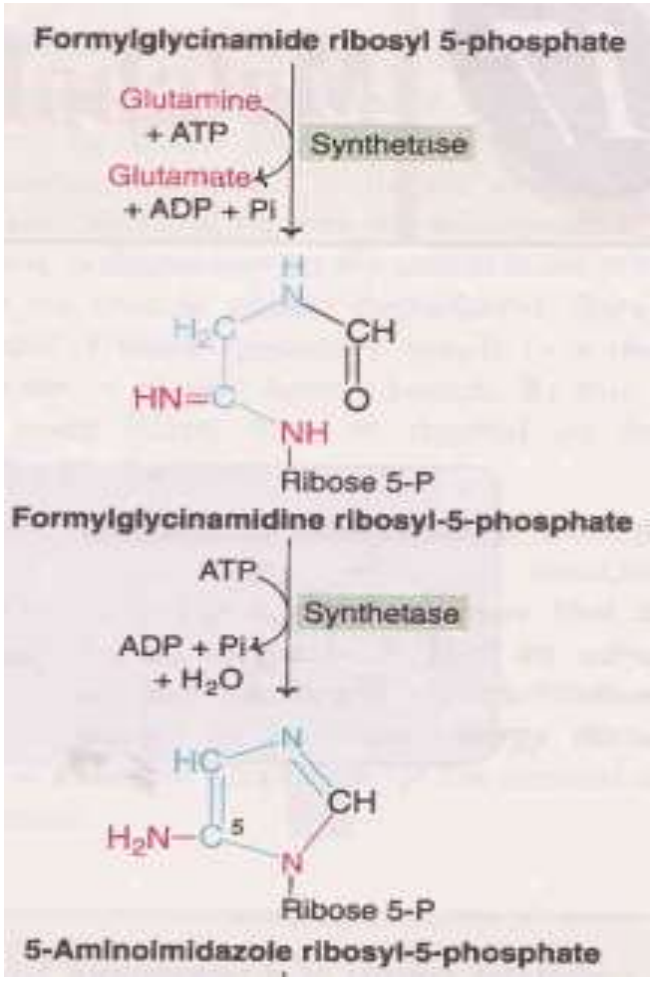


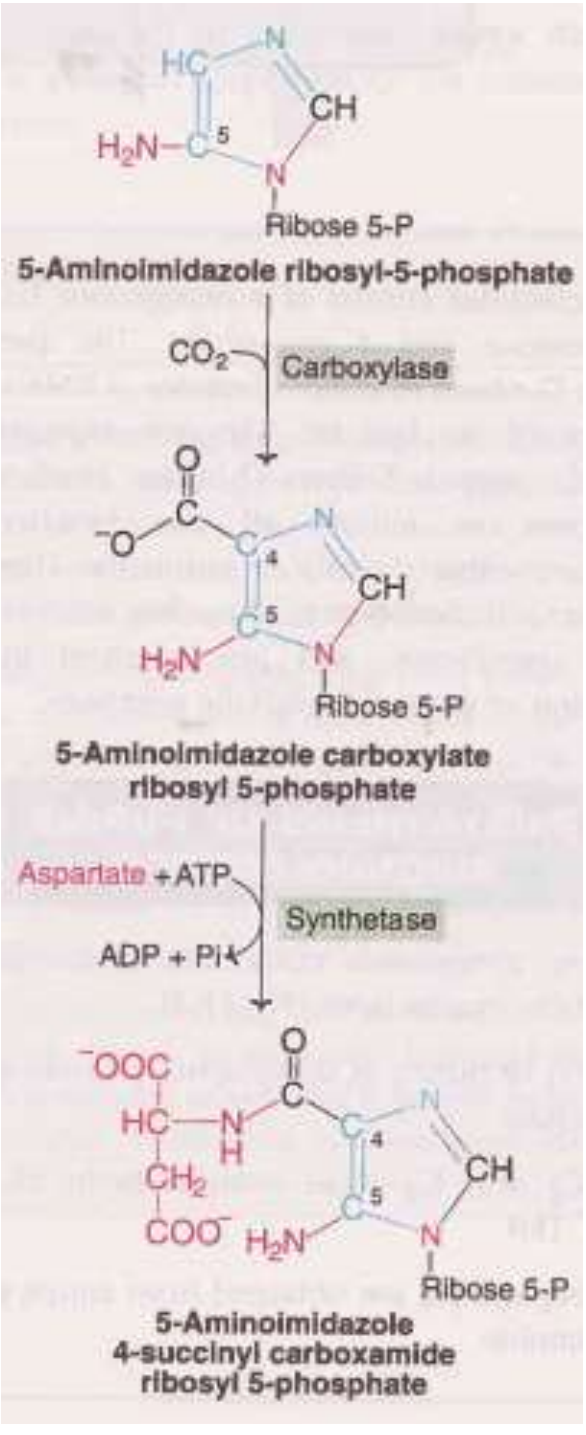


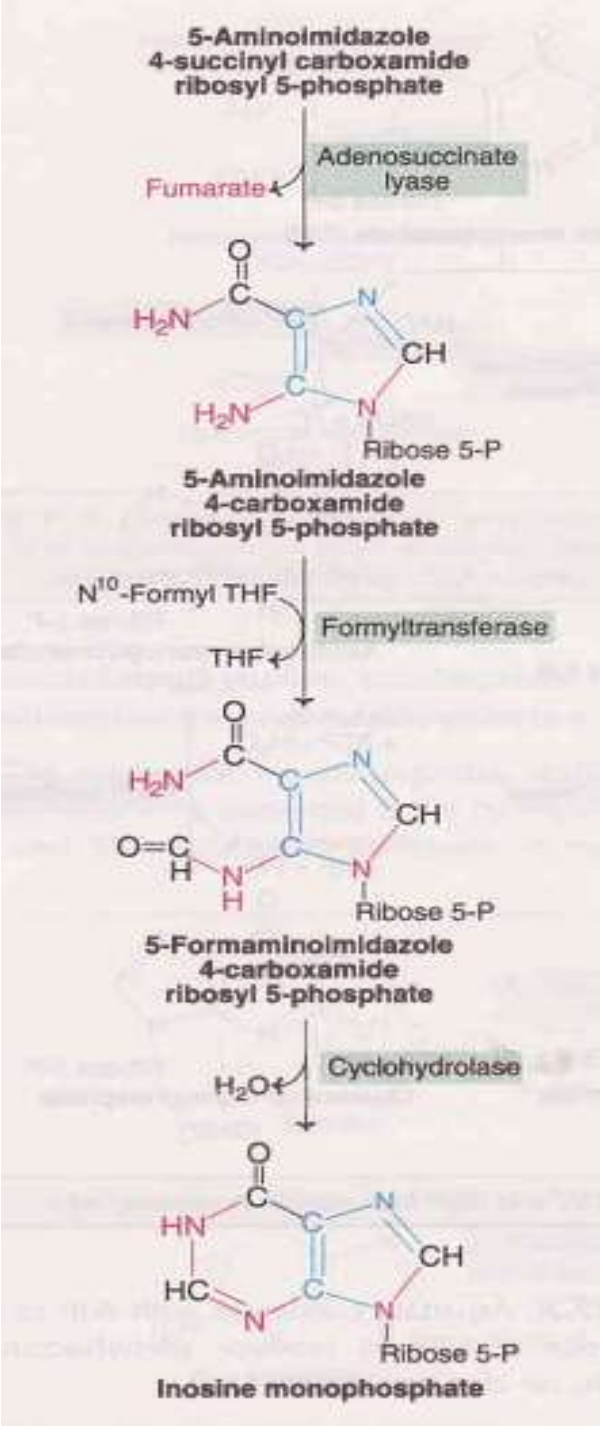



α-D-Ribose-5-phosphate
ATP
AMP
PRPP synthetase
5-Phosphoribosyl α-pyrophosphate
Glutamine
+ H2O
Glutamate
+ PPi
PRPP glutamyl amidotransferase
β-5-Phosphoribosylamine


β-5-Phosphoribosylamine
Glycine + ATP
ADP + Pi
Synthetase
Glycinamide ribosyl 5-phosphate
N10-Formyl THF
THF
Formyltransferase
Ribose 5-P
Formylglycinamide ribosyl 5-phosphate


Formylglycinamide ribosyl 5-phosphate
Glutamine
+ ATP
Glutamate
+ ADP + Pi
Synthetase
Ribose 5-P
Formylglycinamidine ribosyl-5-phosphate
ATP
ADP + Pi
+ H2O
Synthetase
Ribose 5-P
5-Aminoimidazole ribosyl-5-phosphate


Ribose 5-P
5-Aminoimidazole ribosyl-5-phosphate
CO2
Carboxylase
Ribose 5-P
5-Aminoimidazole carboxylate ribosyl 5-phosphate
Aspartate + ATP
ADP + Pi
Synthetase
Ribose 5-P
5-Aminoimidazole 4-succinyl carboxamide ribosyl 5-phosphate


5-Aminoimidazole 4-succinyl carboxamide ribosyl 5-phosphate
Fumarate
Adenosuccinate lyase
Ribose 5-P
5-Aminoimidazole 4-carboxamide ribosyl 5-phosphate
N10-Formyl THF
THF
Formyltransferase
Ribose 5-P
5-Formaminoimidazole 4-carboxamide ribosyl 5-phosphate
H2O
Cyclohydrolase
Ribose 5-P
Inosine monophosphate

Synthesis of AMP and GMP from IMP

Inosine monophosphate is the immediate precursor for the formation of AMP and GMP (*Fig.17.3*). Aspartate condenses with IMP in the presence of GTP to produce adenylsuccinate which, on cleavage, forms AMP.

For the synthesis of GMP, IMP undergoes NAD^+ dependent dehydrogenation to form xanthosine monophosphate (XMP). Glutamine then transfers amide nitrogen to XMP to produce GMP.

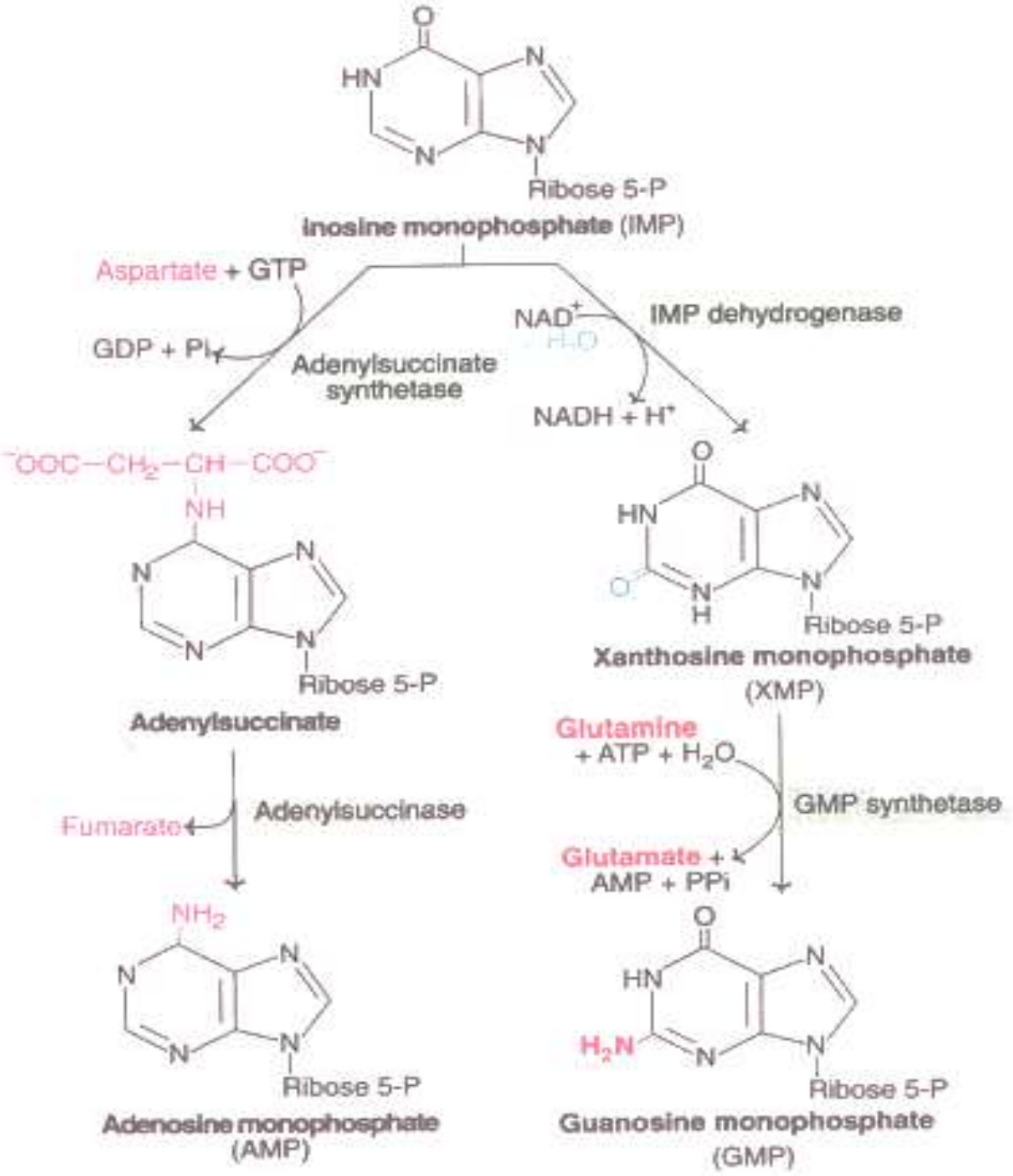
6-Mercaptopurine is an inhibitor of the synthesis of AMP and GMP. It acts on the enzyme adenylsuccinase (of AMP pathway) and IMP dehydrogenase (of GMP pathway).

Regulation of purine nucleotide biosynthesis

The purine nucleotide synthesis is well coordinated to meet the cellular demands. The intracellular concentration of ***PRPP*** regulates purine synthesis to a large extent. This, in turn, is dependent on the availability of ribose 5-phosphate and the enzyme PRPP synthetase.

PRPP glutamyl amidotransferase is controlled by a ***feedback mechanism*** by purine nucleotides. That is, if AMP and GMP are available in adequate amounts to meet the cellular requirements, their synthesis is turned off at the ***amidotransferase*** reaction.

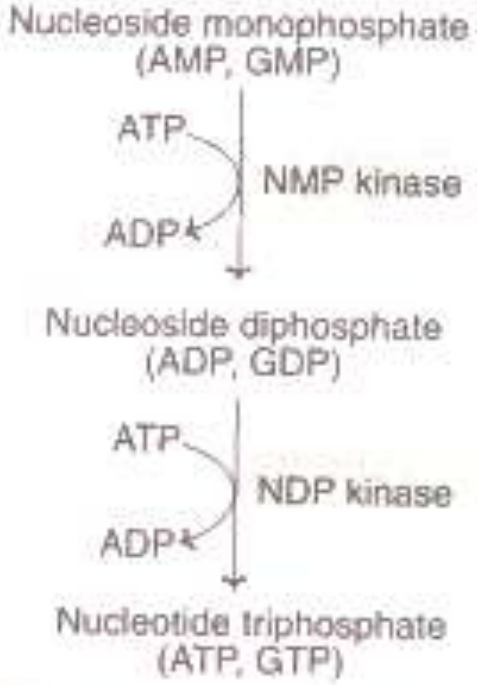
Another important stage of regulation is in the conversion of IMP to AMP and GMP. AMP inhibits adenylsuccinate synthetase while GMP inhibits IMP dehydrogenase. Thus, AMP and GMP control their respective synthesis from IMP by a feedback mechanism.



Inhibitors of purine synthesis

Folic acid (THF) is essential for the synthesis of purine nucleotides (reactions 4 and 10). ***Sulfonamides*** are the structural analogs of para-aminobenzoic acid (PABA). These sulfa drugs can be used to ***inhibit the synthesis of folic acid by microorganisms***. This indirectly reduces the synthesis of purines and, therefore, the nucleic acids (DNA and RNA). Sulfonamides have no influence on humans, since folic acid is not synthesized and is supplied through diet.

The structural ***analogs of folic acid*** (e.g. ***methotrexate***) are widely ***used to control cancer***. They inhibit the synthesis of purine nucleotides (reaction 4 and 10) and, thus, nucleic acids. Both these reactions are concerned with the transfer of one-carbon moiety (formyl group). These inhibitors also affect the proliferation of normally growing cells. This causes many side-effects including anemia, baldness, scaly skin etc.



Salvage pathway for purines

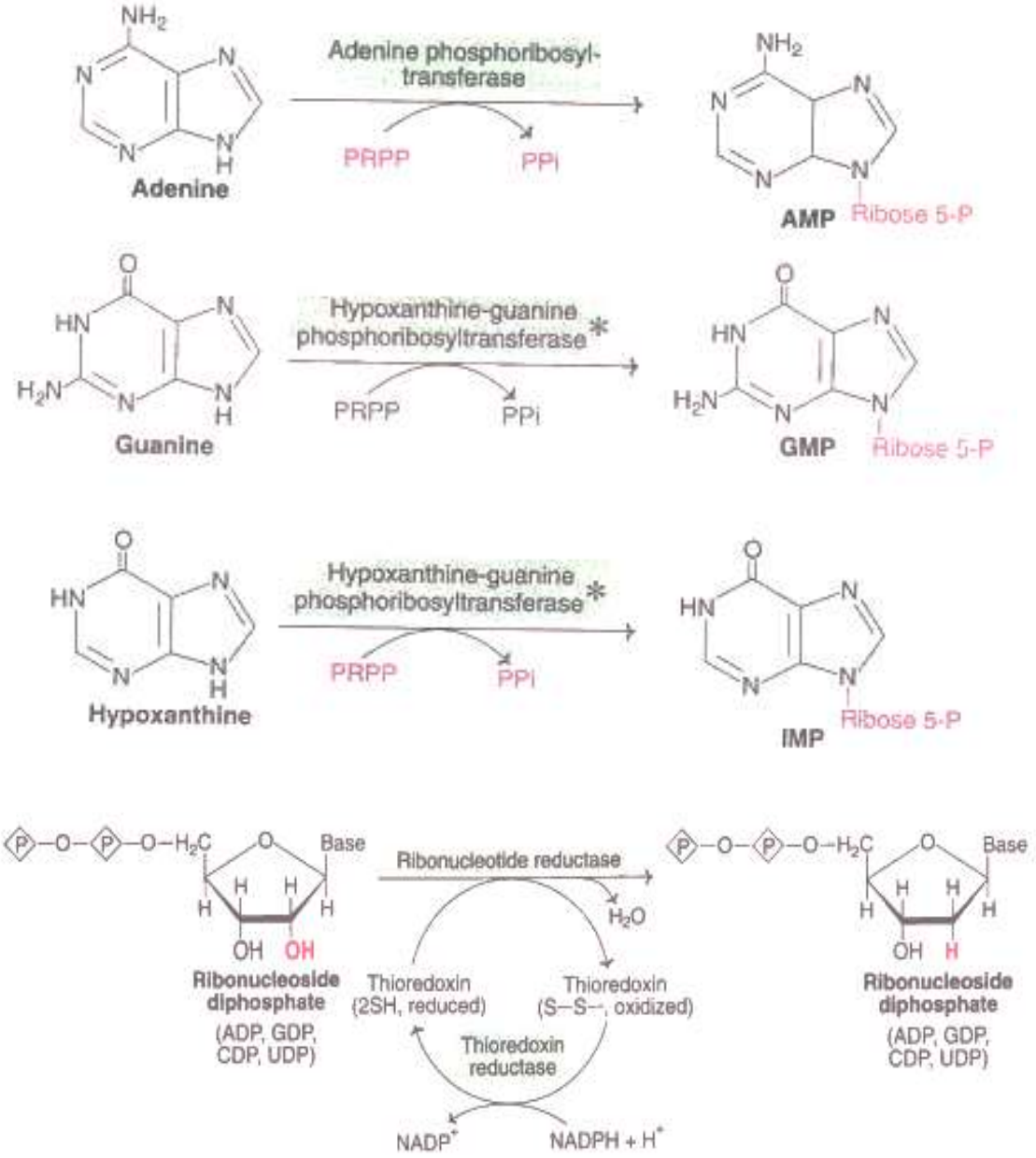
The free purines (adenine, guanine and hypoxanthine) are formed in the normal turnover of nucleic acids (particularly RNA), and also obtained from the dietary sources. The ***purines*** can be ***directly converted to the corresponding nucleotides***, and this process is known as 'salvage pathway' (***Fig.17.5***).

Adenine phosphoribosyl transferase catalyses the formation of AMP from adenine. Hypoxanthine-guanine phosphoribosyl transferase (HGPRT) converts guanine and hypoxanthine, respectively, to GMP and IMP. Phosphoribosyl pyrophosphate (PRPP) is the donor of ribose 5-phosphate in the salvage

The salvage pathway is particularly important in certain tissues such as erythrocytes and brain where *de novo* (a new) synthesis of purine nucleotides is not operative.

A ***defect*** in the enzyme ***HGPRT*** causes ***Lesch-Nyhan syndrome*** (details given later).

Lesch-Nyhan syndrome is a ***sex-linked metabolic disorder*** since the structural gene for HGPRT is located on the X-chromosome. It ***affects*** only the ***males*** and is characterized by excessive uric acid production (often gouty arthritis), and ***neurological abnormalities*** such as mental retardation, aggressive behavior, learning disability etc. The patients of this disorder have an irresistible urge to bite their fingers and lips, often causing self-mutilation.



DEGRADATION OF PURINE NUCLEOTIDES

The ***end product*** of purine metabolism in humans is ***uric acid***. The sequence of reactions in purine nucleotide degradation is given in

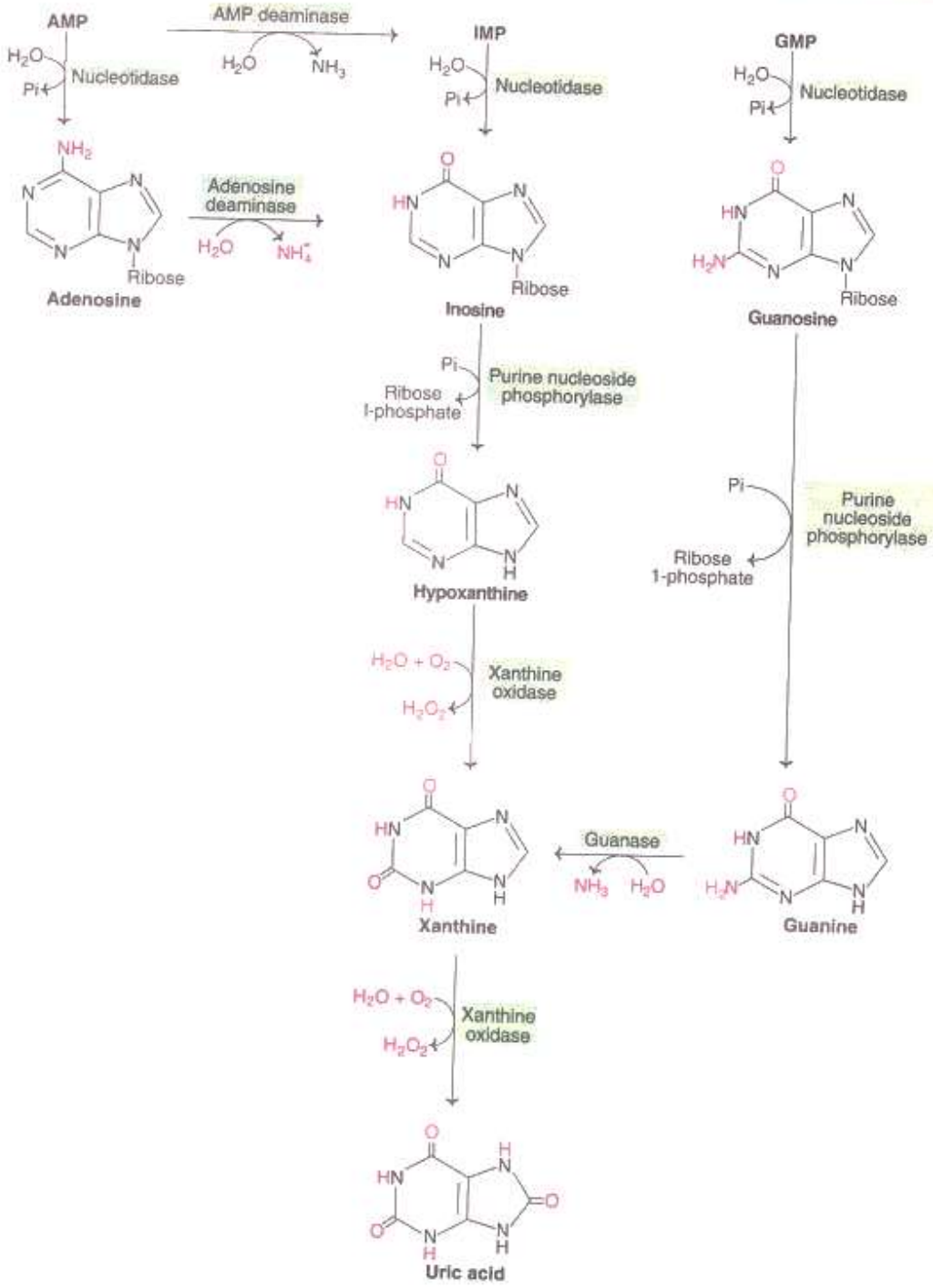
1. The nucleotide monophosphates (AMP, IMP and GMP) are converted to their respective nucleoside forms (adenosine, inosine and guanosine) by the action of ***nucleotidase***.

2. The amino group, either from AMP or adenosine, can be removed to produce IMP or inosine, respectively.

3. Inosine and guanosine are, respectively, converted to hypoxanthine and guanine (purine bases) by *purine nucleoside phosphorylase*. *Adenosine is not degraded by this enzyme,* hence it has to be converted to inosine.

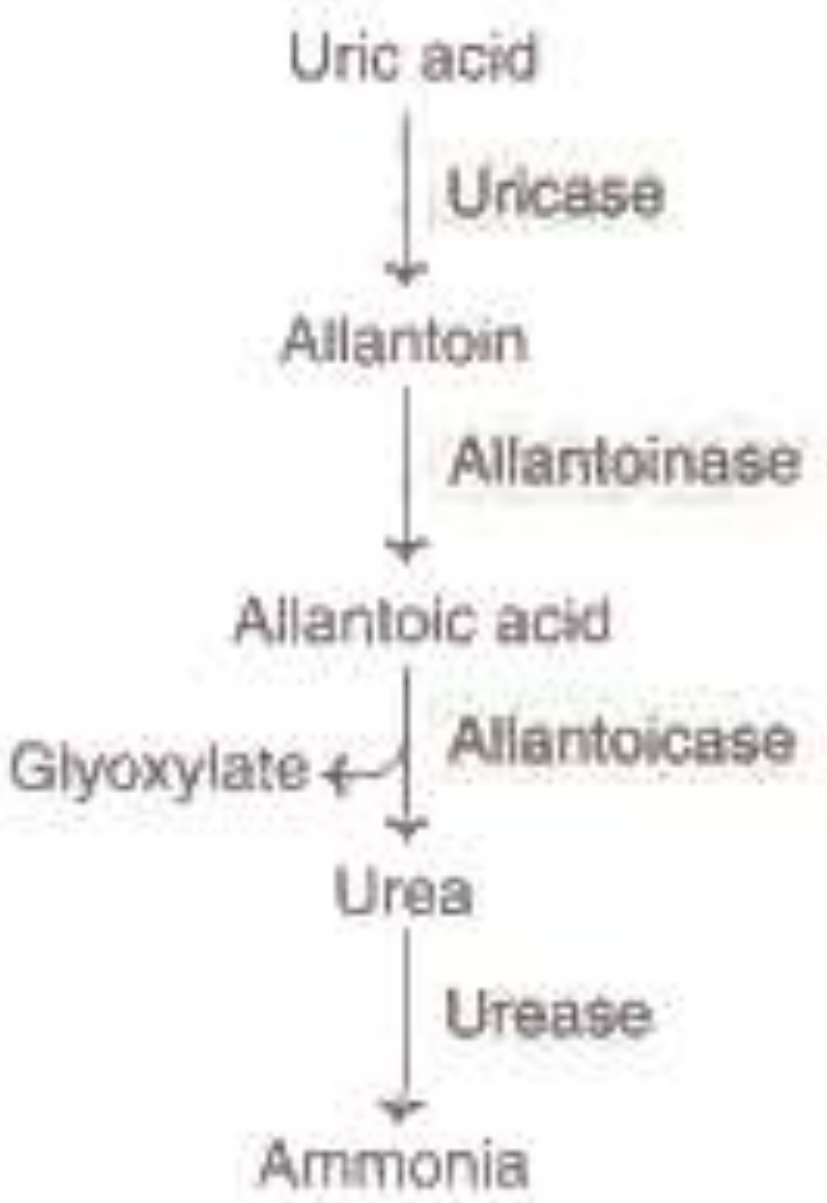
4. Guanine undergoes deamination by guanase to form ***xanthine***.

5. ***Xanthine oxidase*** is an important enzyme that converts hypoxanthine to xanthine, and xanthine to uric acid. This enzyme contains FAD, molybdenum and iron, and is exclusively found in liver and small intestine. Xanthine oxidase liberates H_2O_2 which is harmful to the tissues. Catalase cleaves H_2O_2 to H_2O and O_2.



Uric acid (2,6,8-trioxypurine) is the final excretory product of purine metabolism in humans. Uric acid can serve as an important ***antioxidant*** by getting itself converted (non-enzymatically) to allantoin. It is believed that the antioxidant role of ascorbic acid in primates is replaced by uric acid, since these animals have lost the ability to synthesize ascorbic acid.

Most animals (other than primates) however, oxidize uric acid by the enzyme uricase to allantoin, where the purine ring is cleaved. Allantoin is then converted to allantoic acid and excreted in some fishes (***Fig.17.8***). Further degradation of allantoic acid may occur to produce urea (in amphibians, most fishes and some molluscs) and, later, to ammonia (in marine invertebrates).

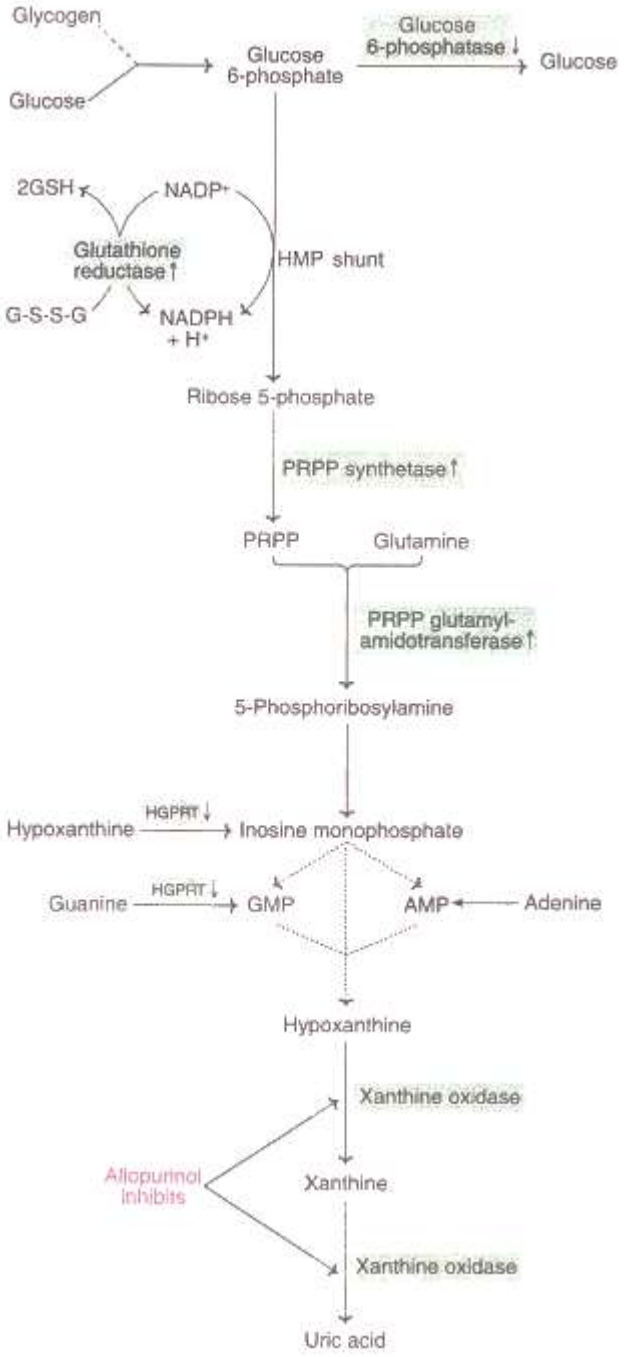


DISORDERS OF PURINE METABOLISM

Hyperuricemia and gout

Gout *is a metabolic disease associated with* ***overproduction of uric acid.*** *At the physiological pH, uric acid is found in a more soluble form as sodium urate. In severe hyperuricemia, crystals of sodium urate get deposited in the soft tissues, particularly in the joints. Such deposits are commonly known as* ***tophi.*** *This causes inflammation in the joints resulting in a painful gouty* ***arthritis.*** *Sodium urate and/or uric acid may also precipitate in kidneys and ureters that results in renal damage and stone formation.*

Historically, gout was found to be often associated with high living, over-eating and ***alcohol consumption.*** In the previous centuries, alcohol was contaminated with lead during its manufacture and storage. ***Lead poisoning*** leads to ***kidney damage*** and decreased uric acid excretion causing ***gout.***



BIOSYNTHESIS OF PYRIMIDINE RIBONUCLEOTIDES

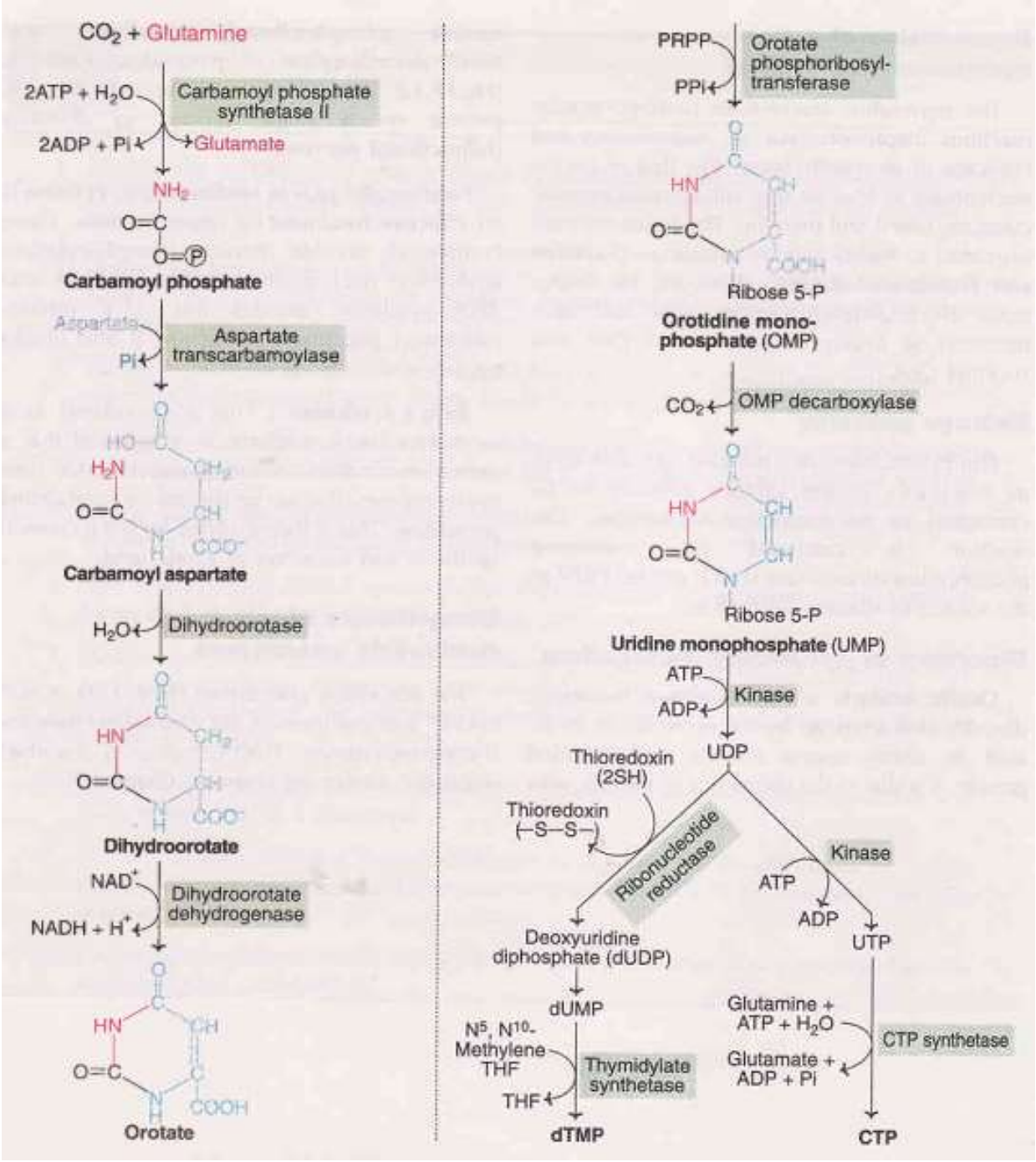
The synthesis of pyrimidines is a much simpler process compared to that of purines. *Aspartate, glutamine (amide group) and CO_2 contribute to atoms in the formation of* pyrimidine ring (***Fig.17.11***). Pyrimidine ring is first synthesized and then attached to ribose 5-phosphate. This is in contrast to purine nucleotide synthesis wherein purine ring is built upon a pre-existing ribose 5-phosphate. The pathway of pyrimidine synthesis is depicted in ***Fig.17.12***, and the salient features are described below.

Glutamine transfers its amido nitrogen to CO_2 to produce carbamoyl phosphate. This reaction is ATP-dependent and is catalysed by cytosomal enzyme carbamoyl phosphate synthetase II (CPS II).

CPS II is activated by ATP and PRPP and inhibited by UTP. Carbamoyl phosphate synthetase I (CPS I) is a mitochondrial enzyme which synthesizes carbamoyl phosphate from ammonia and CO_2 and, in turn urea (***Refer*** protein metabolism, ***Chapter 15***, for more details). Prokaryotes have only one carbamoyl phosphate synthetase which is responsible for the biosynthesis of arginine and pyrimidines.

Carbamoyl phosphate condenses with aspartate to form carbamoyl aspartate. This reaction is catalysed by aspartate transcarbamoylase. Dihydroorotase catalyses the pyrimidine ring closure with a loss of H_2O.

The three enzymes—CPS II, aspartate transcarbamoylase and dihydroorotase are the domains (functional units) of the same protein. This is a good example of a ***multifunctional enzyme***.



The next step in pyrimidine synthesis is an NAD^+ dependent dehydrogenation, leading to the formation of orotate.

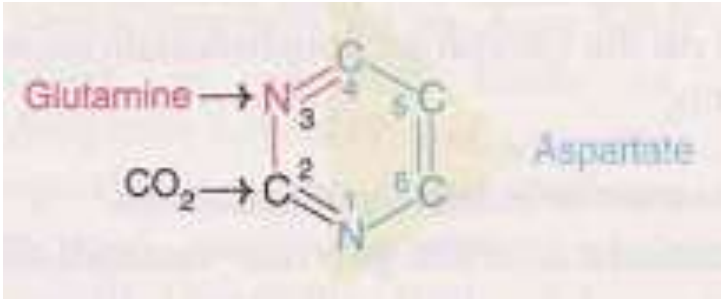
Ribose 5-phosphate is now added to orotate to produce orotidine monophosphate (OMP). This reaction is catalysed by orotate phosphoribosyltransferase, an enzyme comparable with HGPRT in its function. OMP undergoes decarboxylation to uridine mono-phosphate (UMP).

Orotate phosphoribosyltransferase and OMP decarboxylase are ***domains*** of a single protein. A *defect in this* ***bifunctional enzyme*** *causes orotic aciduria (details given later).*

By an ATP-dependent kinase reaction, UMP is converted to UDP which serves as a precursor for the synthesis of dUMP, dTMP, UTP and CTP.

Ribonucleotide reductase converts UDP to dUDP by a thioredoxin-dependent reaction. Thymidylate synthetase catalyses the transfer of a methyl group from N^5, N^{10}-methylene tetrahydrofolate to produce deoxythymidine monophosphate (dTMP).

UDP undergoes an ATP-dependent kinase reaction to produce UTP. Cytidine triphosphate (CTP) is synthesized from UTP by amination. CTP synthetase is the enzyme and glutamine provides the nitrogen.



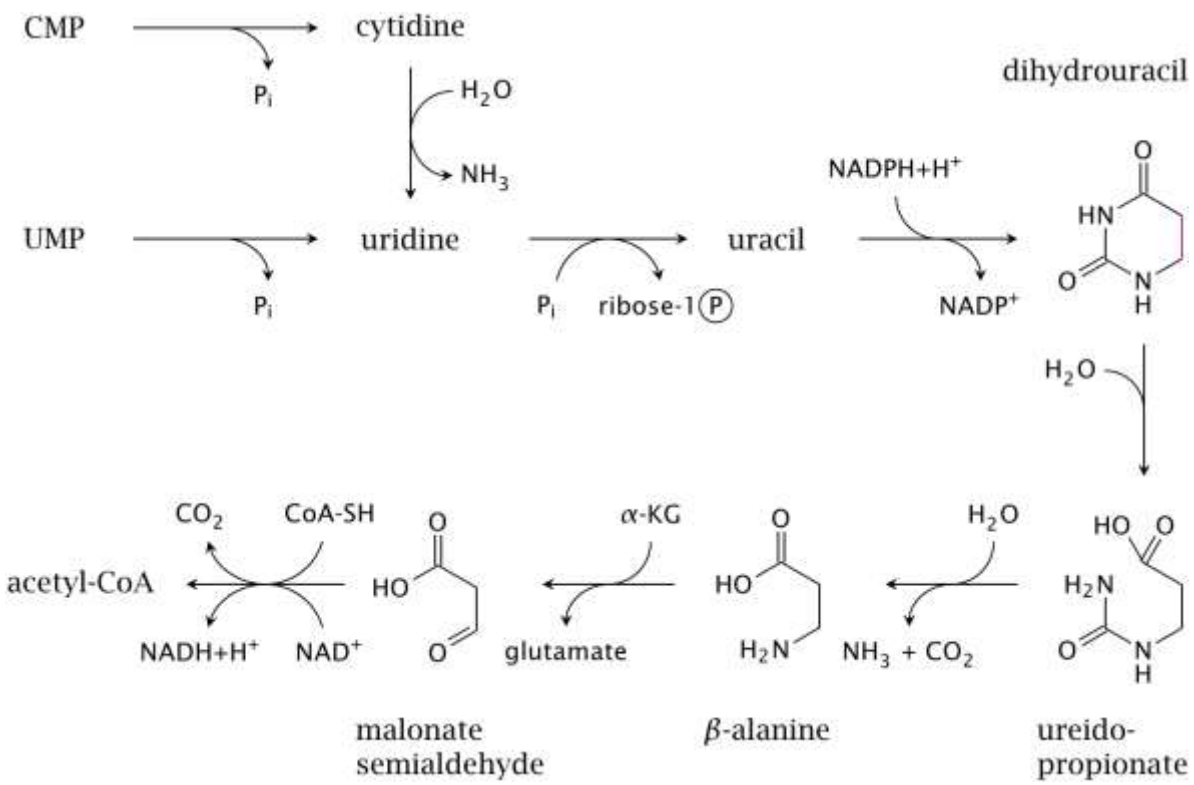
Regulation of pyrimidine synthesis

In bacteria, ***aspartate transcarbamoylase*** (ATCase) catalyses a ***committed step*** in pyrimidine biosynthesis. ATCase is a good example of an enzyme controlled by ***feedback mechanism*** by the end product CTP. In certain bacteria, UTP also inhibits ATCase. ATP, however, stimulates ATCase activity.

Carbamoyl phosphate synthetase II (CPS II) is the regulatory enzyme of pyrimidine synthesis in animals. It is activated by PRPP and ATP and inhibited by UDP and UTP. OMP decarboxylase, inhibited by UMP and CMP, also controls pyrimidine formation.

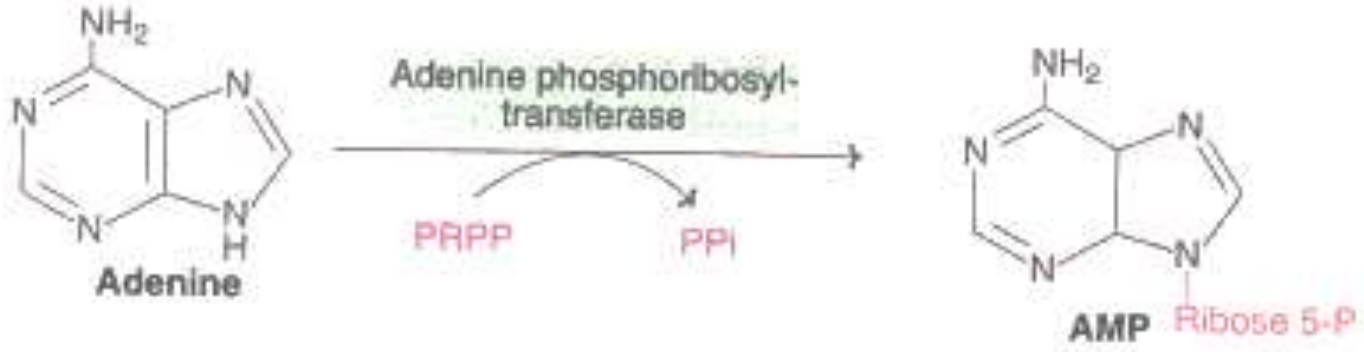
Degradation of pyrimidine nucleotides

The pyrimidine nucleotides undergo similar reactions (dephosphorylation, deamination and cleavage of glycosidic bond) like that of purine nucleotides to liberate the nitrogenous bases—cytosine, uracil and thymine. The bases are then degraded to highly soluble products—***β-alanine*** and ***β-aminoisobutyrate.*** These are the amino acids which undergo transamination and other reactions to finally produce acetyl CoA and succinyl CoA.



Salvage pathway

The pyrimidines (like purines) can also serve as precursors in the salvage pathway to be converted to the respective nucleotides. This reaction is catalysed by pyrimidine phosphoribosyltransferase which utilizes PRPP as the source of ribose 5-phosphate.



Disorders of pyrimidine metabolism

Orotic aciduria : This is a rare metabolic disorder characterized by the excretion of orotic acid in urine, severe anemia and retarded growth. It is due to the deficiency of the enzymes

orotate phosphoribosyl transferase and ***OMP decarboxylase*** of pyrimidine synthesis (***Fig.17.12***). Both these enzyme activities are present on a single protein as domains (***bifunctional enzyme***).

Feeding ***diet rich in uridine*** and/or ***cytidine*** is an ***effective treatment*** for orotic aciduria. These compounds provide (through phosphorylation) pyrimidine nucleotides required for DNA and RNA synthesis. Besides this, UTP inhibits carbamoyl phosphate synthetase II and blocks synthesis of orotic acid.

Reye's syndrome : This is considered as a secondary orotic aciduria. It is believed that a defect in ornithine transcarbamoylase (of urea cycle) causes the accumulation of carbamoyl phosphate. This is then diverted for the increased synthesis and excretion of orotic acid.

Reye syndrome is a rapidly progressive encephalopathy. Symptoms may include vomiting, personality changes, confusion, seizures, and loss of consciousness

Interconversion of the Nucleotides

During the catabolism of nucleic acids, nucleoside mono- and diphosphates are released. The nucleosides do not accumulate to any significant degree, owing to the action of nucleoside kinases. These include both nucleoside monophosphate (NMP) kinases and nucleoside diphosphate (NDP) kinases. The NMP kinases catalyze ATP-dependent reactions of the type:

(d)NMP + ATP <——> (d)NDP + ADP

There are four classes of NMP kinases that catalyze, respectively, the phosphorylation of:
1. AMP and dAMP; this kinase is known as adenylate kinase.
2. GMP and dGMP.
3. CMP, UMP and dCMP.
4. dTMP.
The enzyme adenylate kinase is important for ensuring adequate levels of energy in cells such as liver and muscle. The predominant reaction catalyzed by adenylate kinase is:
2ADP <——> AMP + ATP

The NDP kinases catalyze reaction of the type:
$N_1TP + N_2DP$ <——> $N_1DP + N_2TP$
N_1 can represent a purine ribo– or deoxyribonucleotide; N_2 a pyrimidine ribo– or deoxyribonucleotide. The activity of the NDP kinases can range from 10 to 100 times higher than that of the NMP kinases. This difference in activity maintains a relatively high intracellular level of (d)NTPs relative to that of (d)NDPs. Unlike the substrate specificity seen for the NMP kinases, the NDP kinases recognize a wide spectrum of (d)NDPs and (d)NTPs.

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All the original contributors of the concept and findings published are gratefully acknowledged while preparing the e-content for the students of Biotechnology and allied sciences