College of Veterinary Medicine
Department of Physiology, Biochemistry & Pharmacology

Nucleotides Metabolism

Dr. Nashaat Ghalib Mustafa

Biochemical Importance

1. Deoxyribonucleotides are required for DNA synthesis.
2. Ribonucleotides are required for RNA synthesis.
3. Biosynthesis of purine and pyrimidine nucleotides is essential for DNA replication, (cell division), and growth of all types of mammalian cells, bacteria and virus. If the supply of nucleotides is blocked, cell division (viral replication) and growth is arrest. So, compounds, which can block nucleotide biosynthesis effectively stop growth of cells, bacteria and virus. Indeed, many anti-tumor, anti-bacterial and anti-viral agents currently used are inhibitors of nucleotide (nucleic acid) biosynthesis.

4. Nucleotide metabolism is defective in diseases like gout, and immunodeficiency syndrome.
5. Nucleotides are required for few co-enzymes formation.
6. Nucleotide metabolism in malarial parasite differs from its human host. These differences in metabolic pathways between parasites and host are used for development of new anti-malarial agents.

7. *Giardia lamblia,* which causes giardiasis in humans and *trichomonas fetus* that causes embryonic death in cows, are unable to synthesize purines via *de novo* pathways. They depend mainly on salvage pathways. Hence, enzymes in salvage pathways are potential targets of therapeutic agents for the treatment of diseases caused by these parasites.

Biosynthesis of Nucleotides

There are two types of pathways for nucleotide biosynthesis.

1. *De novo* pathways.
2. Salvage pathways.

De novo pathways for nucleotide biosynthesis

1. Purine and pyrimidine nucleotides are synthesized by two separate pathways present in cytosol of most of the cells. They are:

(*a*) *De novo* pathway for purine nucleotide biosynthesis, and

(*b*) *De novo* pathway for pyrimidine nucleotide biosynthesis.

2. Both purine and pyrimidine nucleotide biosynthetic pathways are energy intensive processes.

3. Both pathways are linked to HMP (hexose monophosphate) shunt as well as glycolysis.

De novo purine nucleotide biosynthesis

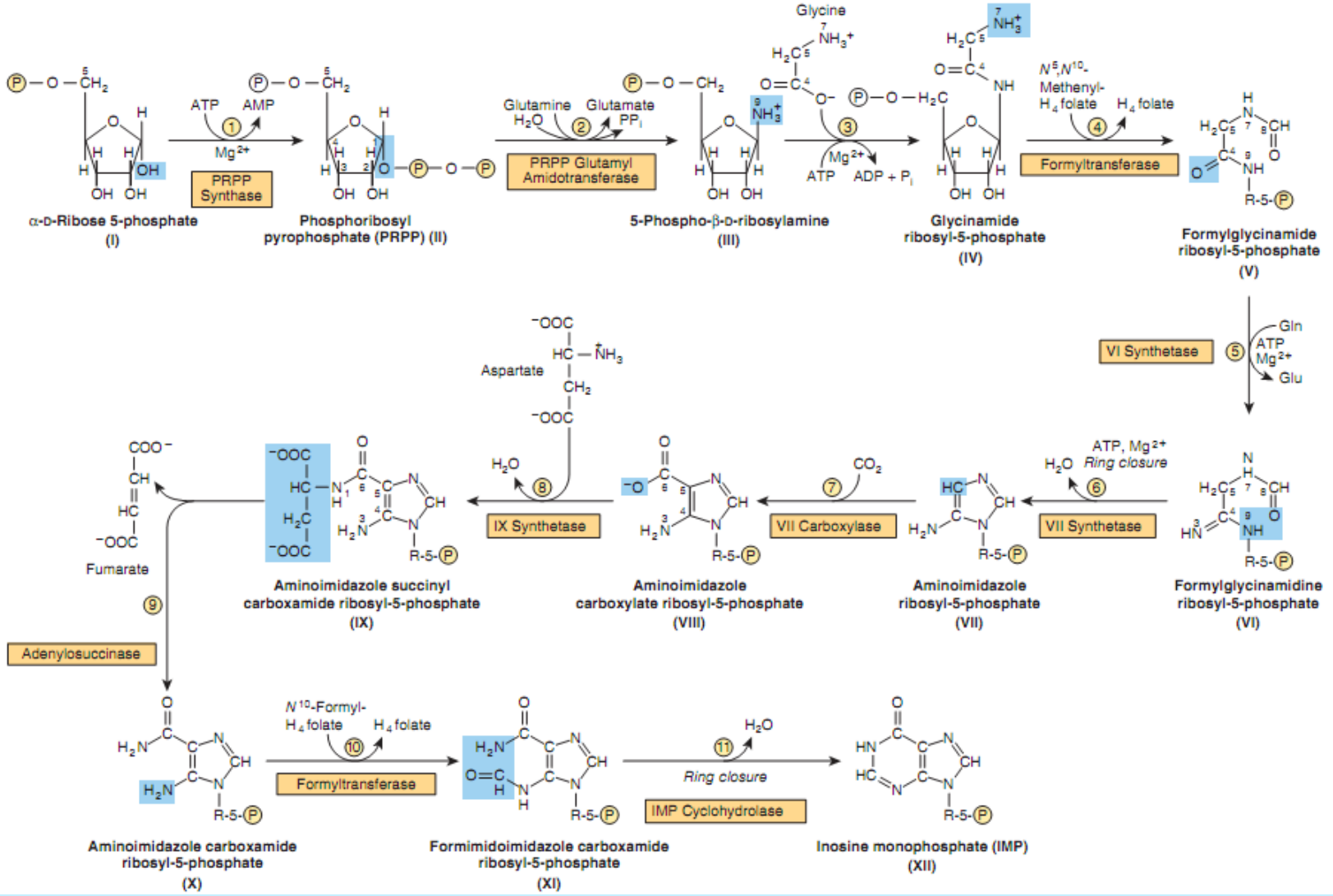
1. Liver is the major site of purine nucleotide biosynthesis.
2. Purine nucleotide biosynthesis involves construction of purine ring on ribose-5-phosphate. So, intermediates of purine nucleotide biosynthesis are bound to ribose-5-phosphate.

Reaction sequence

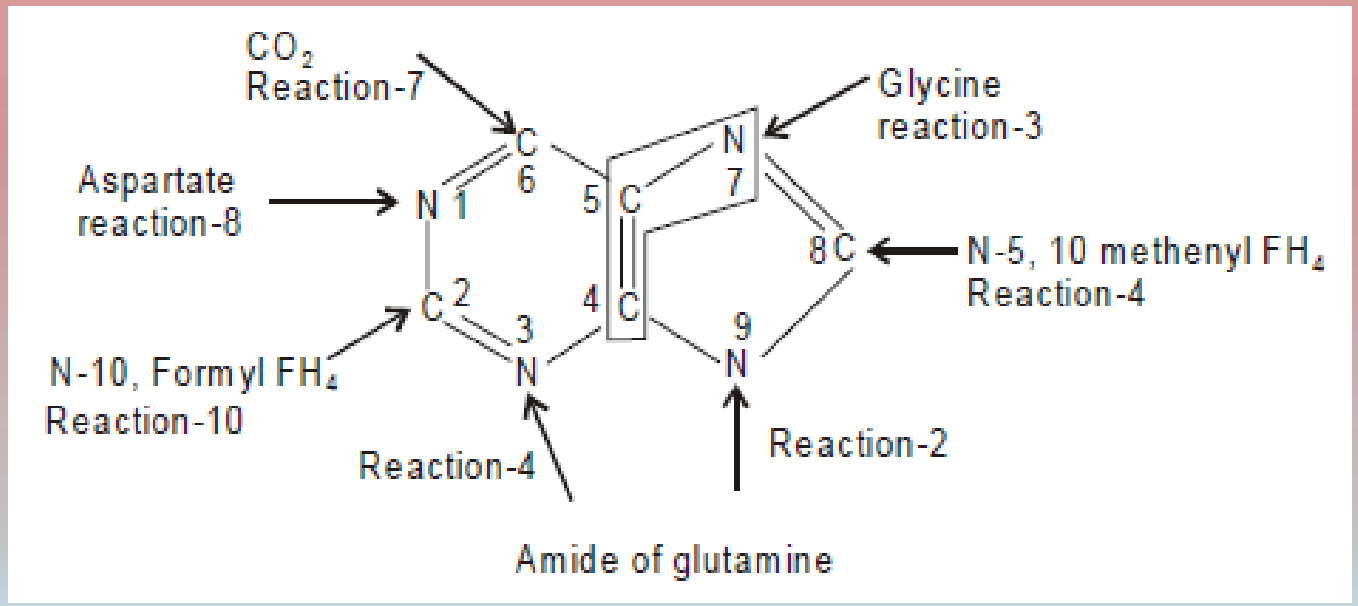
1. Formation of phosphoribosyl pyrophosphate (PRPP) from ribose-5-phosphate is the first reaction of *de novo* pathway for purine nucleotide biosynthesis. The reaction is catalyzed by PRPP synthetase in presence of ATP and Mg^{2+}.
2. In this reaction, NH_2 of glutamine (amide) displaces PPi from PRPP to yield β-5-phospho ribosyl-1-amine.
3. β-5-phosphoribosyl-1-amine reacts with carboxyl group of glycine to form 5-phosphoribosyl.
4. The 5-phosphoribosyl glycinamide is formylated in this reaction by transformylase to 5-phosphoribosyl-N-formyl glycinamide.

5. In this reaction, carbonyl oxygen of amide is replaced with $–NH_2$ of (amide) of glutamine to form amidine.
6. In this step, 5-phosphoribosyl aminoimidazole synthetase catalyzes imidazole ring formation in an intramolecular ATP-dependent reaction.
7. A carboxylase introduces CO_2 into C-4 of imidazole ring in this reaction.
8. In this step, an amide bond is formed between carboxylate introduced in the preceding reaction and amino group of aspartate in presence of ATP.

9. Elimination of aspartate by a lyase in this reaction.
10. A second transformylase introduces formyl group into 5-amino group.
11. Finally six membered ring is formed by dehydration between formyl group and carboxamide group.


α-D-Ribose 5-phosphate (I)
ATP
AMP
Mg2+
PRPP Synthase
Phosphoribosyl pyrophosphate (PRPP) (II)
Glutamine
Glutamate
PPi
PRPP Glutamyl Amidotransferase
5-Phospho-β-D-ribosylamine (III)
Glycine
ATP
ADP + Pi
Glycinamide ribosyl-5-phosphate (IV)
N5,N10-Methenyl-H4folate
H4folate
Formyltransferase
Formylglycinamide ribosyl-5-phosphate (V)
VI Synthetase
Gln
Glu
Formylglycinamidine ribosyl-5-phosphate (VI)
ATP, Mg2+ Ring closure
VII Synthetase
Aminoimidazole ribosyl-5-phosphate (VII)
CO2
VII Carboxylase
Aminoimidazole carboxylate ribosyl-5-phosphate (VIII)
Aspartate
IX Synthetase
Aminoimidazole succinyl carboxamide ribosyl-5-phosphate (IX)
Fumarate
Adenylosuccinase
Aminoimidazole carboxamide ribosyl-5-phosphate (X)
N10-Formyl-H4folate
Formyltransferase
Formimidoimidazole carboxamide ribosyl-5-phosphate (XI)
Ring closure
IMP Cyclohydrolase
Inosine monophosphate (IMP) (XII)

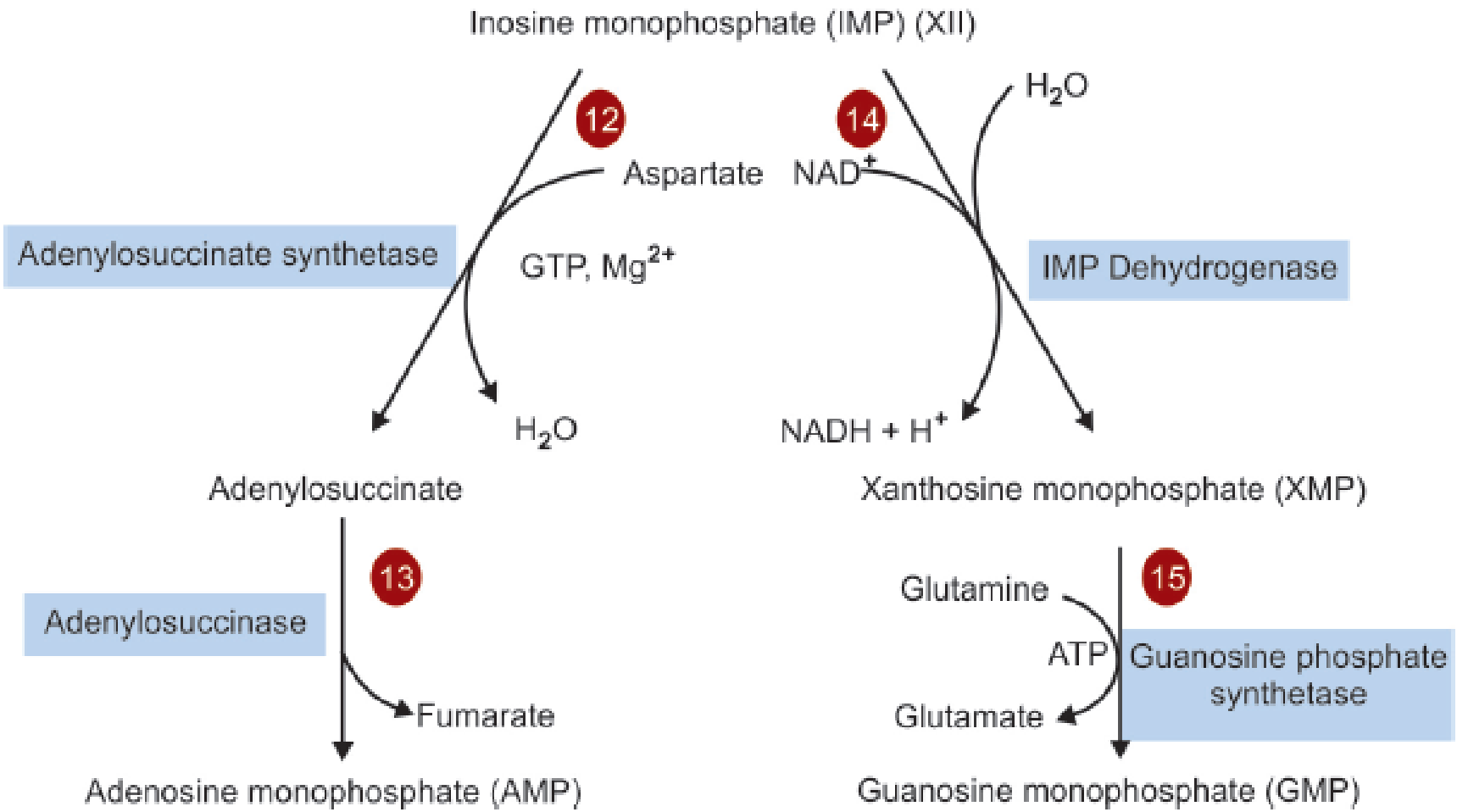
The first purine nucleotide inosine monophosphate (IMP) is thus produced. Total six high energy bonds are used for the formation of inosinic acid (IMP) from ribose-5-phosphate.



Origins of different atoms of purine ring

Formation of AMP and GMP from IMP

The initial product of purine biosynthetic pathway IMP is not found in nucleic acids. Hence, it is converted to AMP and GMP in two pathways. In AMP synthesis aspartate is the nitrogen source where as in GMP synthesis amide group of glutamine is the nitrogen source.


Inosine monophosphate (IMP) (XII)
12
Aspartate
GTP, Mg^{2+}
Adenylosuccinate synthetase
H_2O
Adenylosuccinate
13
Adenylosuccinase
Fumarate
Adenosine monophosphate (AMP)
14
H_2O
NAD^+
IMP Dehydrogenase
$NADH + H^+$
Xanthosine monophosphate (XMP)
15
Glutamine
ATP
Guanosine phosphate synthetase
Glutamate
Guanosine monophosphate (GMP)

Pyrimidine nucleotide biosynthesis *De novo*

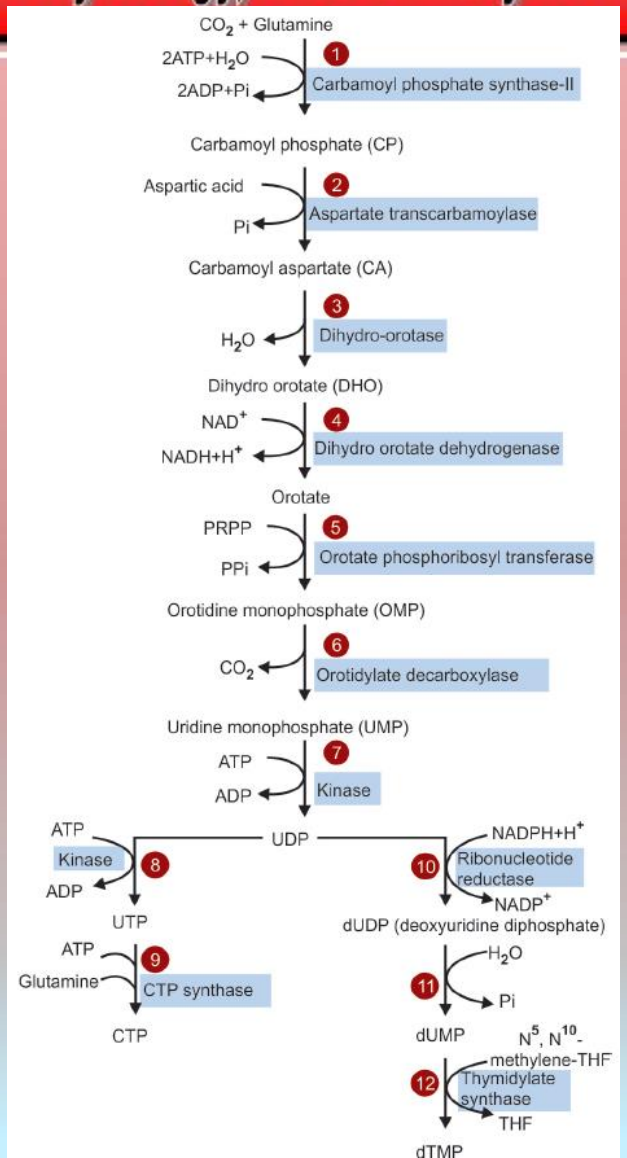
In pyrimidine nucleotide, biosynthesis the heterocyclic pyrimidine ring is constructed first from aspartate and carbamoyl phosphate and ribose-5-phosphate is added later.

Site

Cytosol of liver cells and most of the other cells have enzymes of pyrimidine nucleotide formation.

Reaction sequence

In mammals, first three reactions are catalyzed by single multicatalytic protein, 4th reaction is catalyzed unicatalytic protein and 5,6 reactions are catalyzed by another single multicatalytic polypeptide.


CO_2 + Glutamine
2ATP+H_2O
2ADP+Pi
1
Carbamoyl phosphate synthase-II
Carbamoyl phosphate (CP)
Aspartic acid
Pi
2
Aspartate transcarbamoylase
Carbamoyl aspartate (CA)
H_2O
3
Dihydro-orotase
Dihydro orotate (DHO)
NAD^+
$NADH+H^+$
4
Dihydro orotate dehydrogenase
Orotate
PRPP
PPi
5
Orotate phosphoribosyl transferase
Orotidine monophosphate (OMP)
CO_2
6
Orotidylate decarboxylase
Uridine monophosphate (UMP)
ATP
ADP
7
Kinase
UDP
ATP
Kinase
ADP
8
UTP
ATP
Glutamine
9
CTP synthase
CTP
$NADPH+H^+$
10
Ribonucleotide reductase
$NADP^+$
dUDP (deoxyuridine diphosphate)
H_2O
11
Pi
dUMP
N^5, N^{10}-methylene-THF
12
Thymidylate synthase
THF
dTMP

Biochemical importance

Bacterial growth, viral growth and tumor growth requires nucleic acid synthesis. This in turn depends on nucleotide biosynthesis. Hence, inhibitors of nucleotide biosynthesis are potential antibacterial, antiviral and anti-tumor agents. Since normal cell growth also requires nucleic acid biosynthesis these agents cause side or toxic affects.

However, the severity of toxic effects varies from one drug to another drug. Rapidly dividing epithelial cells of gastrointestinal tract, bone marrow stem cells and hair follicles are affected most by these agents. Hence, symptoms like decreased blood count, gastrointestinal disturbances and hair loss may occur in individuals undergoing treatment with these agents.

Salvage pathways for nucleotide biosynthesis

1. These pathways produce nucleotides from preformed purine and pyrimidine bases and nucleosides.
2. Endogenous nucleic acid breakdown, foreign DNA and RNA, which enters body through infectious agents breakdown and digestion of dietary nucleic acids are the sources for preformed bases and nucleosides.
3. Synthesis of nucleotides from preformed bases and nucleosides saves considerable cellular energy.

4. Moreover certain cells like RBCs, WBCs, and brain tissue lack enzymes of *de novo* pathways and hence they entirely depend on salvage pathways for nucleotide biosynthesis.
5. Liver supplies free bases and nucleosides to salvage pathways of brain, erythrocytes and leukocytes.
6. These salvage pathways helps in recycling of 90% of preformed bases and nucleosides in the body.

Purine salvage pathways

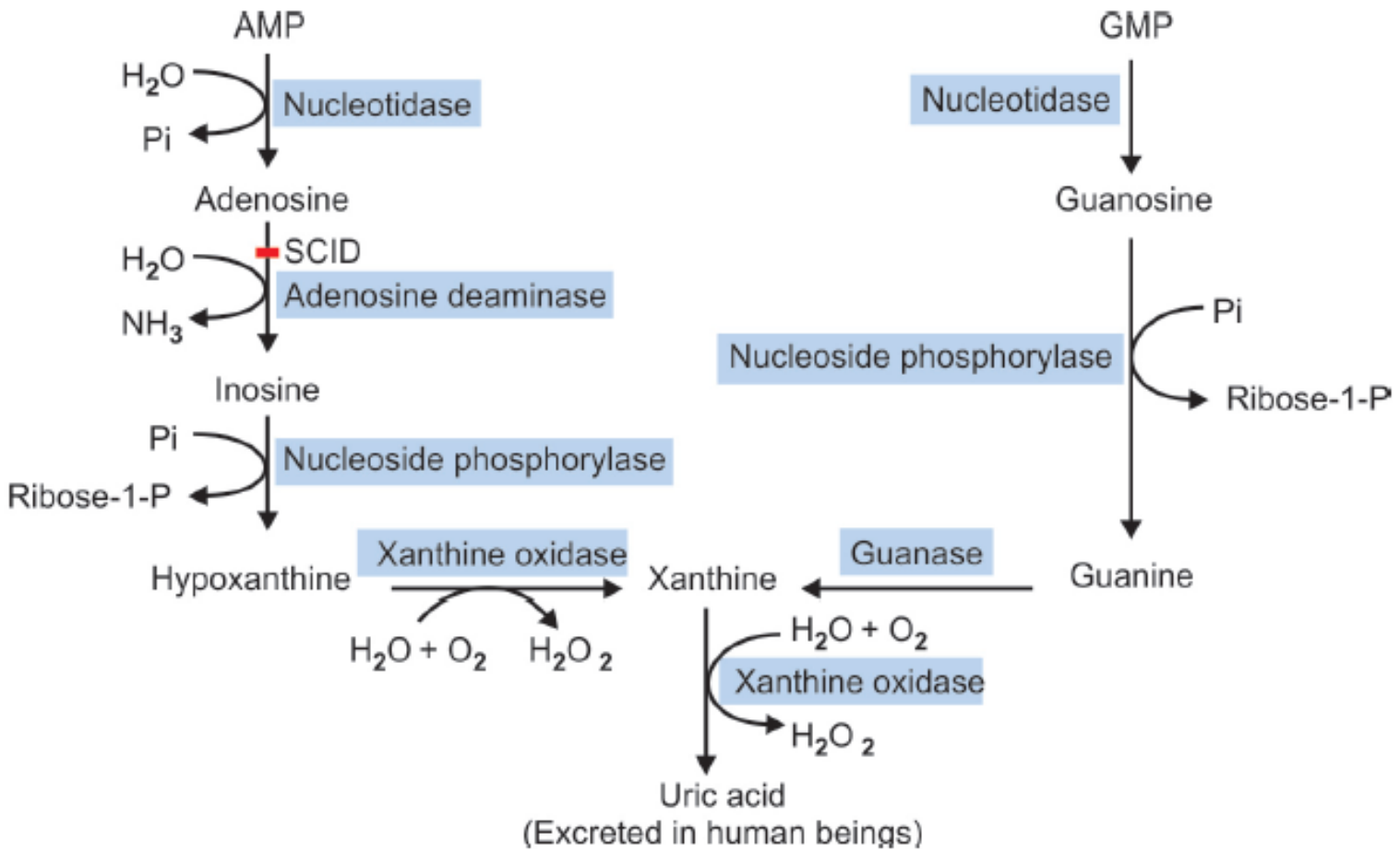
1. Free purine bases like guanine and hypoxanthine are salvaged by hypoxanthine-guanine phosphoribosyl transferase. This enzyme converts hypoxanthine and guanine to IMP and GMP respectively by using PRPP as donor of ribose-5-phosphate.

2. Adenine is salvaged by adenine phosphoribosyl transferase. It converts adenine to AMP using PRPP as donor of ribose-5-phosphate.
3. Free guanine is formed from guanosine by removing ribose as ribose-1-phosphate. The reaction is catalyzed by purine nucleoside phosphorylase.

Free hypoxanthine is formed from adenosine via inosine. Adenosine deaminase (ADA) converts adenosine to inosine first, which is followed by release of inosine ribose as ribose-1-phosphate.

Degradation of Purine nucleotides

1. Liver is the major organ involved in degradation of purine nucleotides. Lysosomal enzymes converts nucleic acids to nucleotides. Majority of purine nucleotides so produced are AMP and GMP. AMP is converted to IMP by adenylate deaminase .



Catabolism of purine nucleotides

Medical Importance

Catabolism of purine nucleotides is abnormal in some diseases. Hence normal fate of uric acid which is end product of purine catabolism .

Gout

It is common disease associated with excessive purine catabolism. It is characterized by hyperuricemia and excessive excretion of uric acid in urine. It is more common in men (95%). Incidence rate is 3 in 1000.

Hyperuricemia or gout is due to:

(a) Over production of uric acid.

(b) Impaired excretion of uric acid.

Pyrimidine salvage pathways

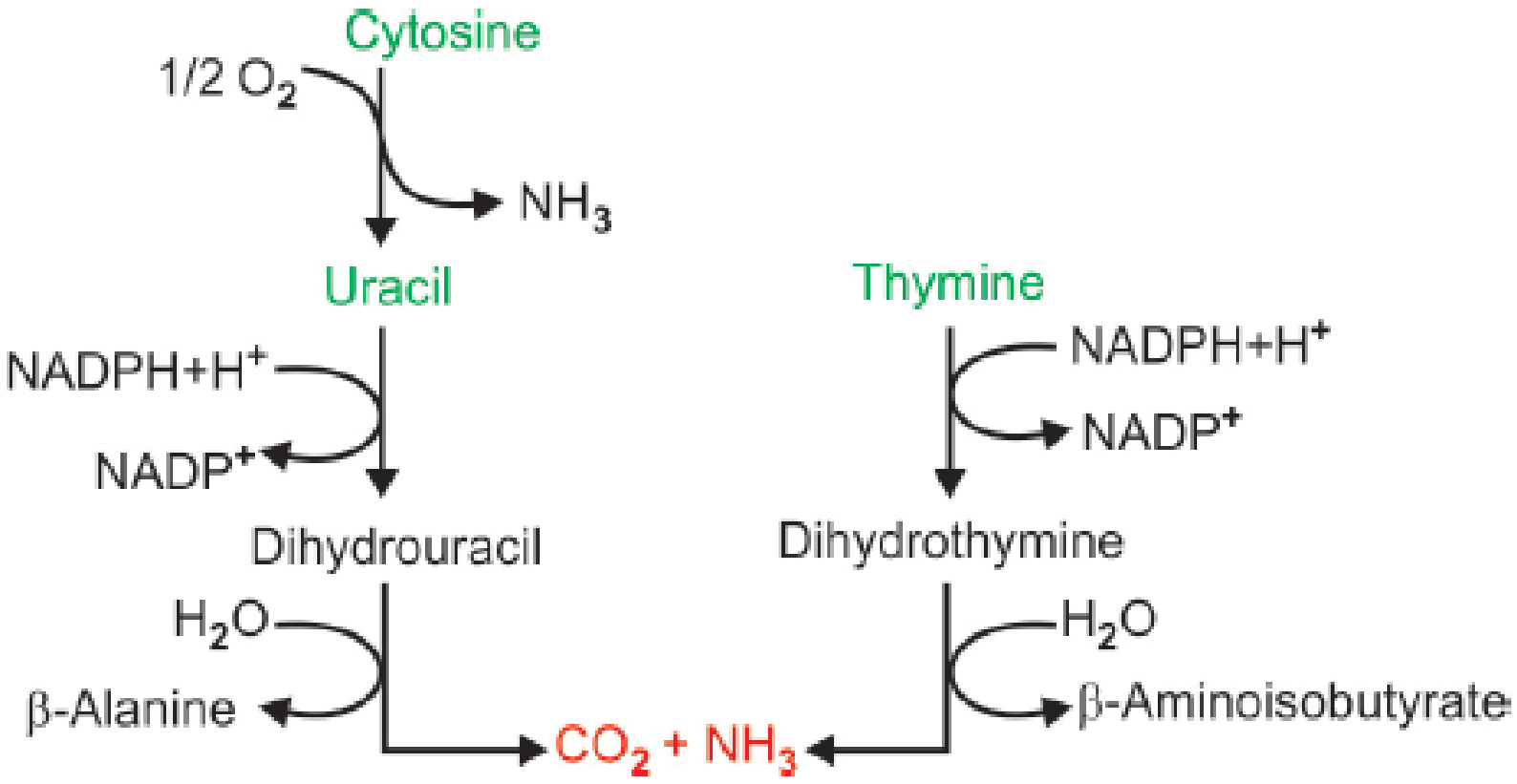
1. Free pyrimidine bases are salvaged by pyrimidine phosphoribosyl transferase. It catalyses conversion of uracil or thymine to UMP and TMP. It also acts on 5-fluorouracil and orotate.
2. Thymine is salvaged by thymidine phosphorylase. Pyrimidine nucleosides are salvaged by distinct pyrimidine nucleoside kinases. They phosphorylate nucleosides using ATP as phosphate donor.

Hypoxanthine analog

Allopurinol is the drug used in the treatment of gout. It is a hypoxanthine analog which is substrate for xanthine oxidase. Since allopurinol is structurally similar to hypoxanthine one might expect that it inhibits xanthine oxidase by binding at active site.

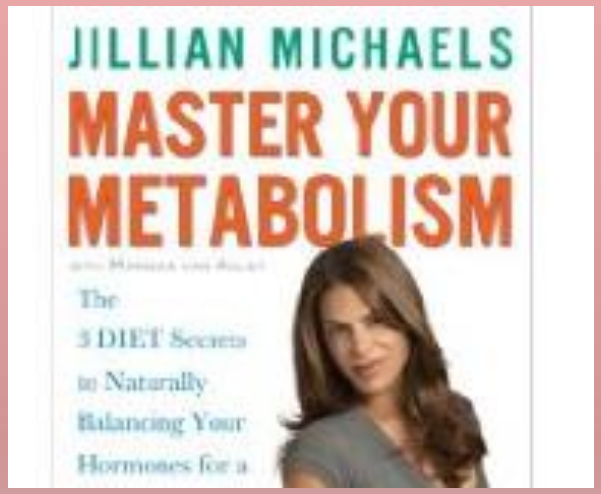
Degradation of pyrimidine nucleotides

Liver is the major organ involved in breakdown of pyrimidine nucleotides. Pyrimidine nucleotide are degraded to amino acids : alanine and Beta-amino isobutyric acid (BAIB) by cleaving pyrimidine ring.



Catabolism of pyrimidines

You can dramatically affect the expression of your metabolism and your biochemistry by the way you eat and the way you live.



Jillian Michaels

Thank you