CHAPTER 7

Chemistry and Metabolism of Nucleotides

Competency Achievement: **The student should be able to:**

BI6.2 Describe and discuss the metabolic processes in which nucleotides are involved
BI6.3 Describe the common disorders associated with nucleotide metabolism
BI6.4 Discuss the laboratory results of analytes associated with gout and Lesch-Nyhan syndrome

A **nucleotide** has 3 components, i.e. a pentose sugar, a nitrogenous base and phosphoric acid.

PENTOSE SUGARS

The presence of a pentose sugar defines the type of the nucleotide (such as nucleotide or deoxynucleotide) and nucleic acid (i.e. deoxyribonucleic acid or DNA, and ribonucleic acid or RNA). A nucleotide contains ribose sugar while a deoxynucleotide contains deoxyribose sugar.

- **Ribose** has three –OH groups at carbon numbers 1, 2 and 3 of the pentose molecule.
- **Deoxyribose**, on the other hand, does not contain an oxygen atom at the second carbon and thus has only two –OH groups, i.e. at carbon atoms, 1 and 3. Deoxyribose, thus, is also called 2-deoxyribose (Fig. 7.1).

  The sugar molecule is linked to a nitrogenous base on one side (through carbon atom one, i.e. C1) and to phosphoric acid on the other side (through carbon atom five, i.e. C5).

To differentiate between the carbon atoms of a sugar molecule from the carbon atoms of the nitrogenous base, in the nomenclature of a nucleotide, the position of the carbon atom of the sugar moiety is represented by a sign called **prime (′)**. These are, thus, written as C1′ or C5′, etc. On the other hand, the carbon and the nitrogen atoms of the nitrogenous bases are written without the prime sign, such as N1 or C2, etc.

NITROGENOUS BASES

Nitrogenous bases found in various nucleotides are either the derivatives of a purine ring or of a pyrimidine ring.

- A **Purine ring** is a nine-membered ring containing 5 carbon and 4 nitrogen atoms, whereas a **pyrimidine ring** is a six-membered ring which has 4 carbon and 2 nitrogen atoms (Fig. 7.2).

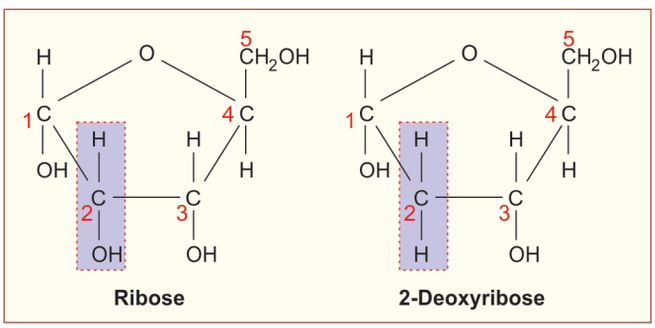

Fig. 7.1: Sugars present in nucleic acids

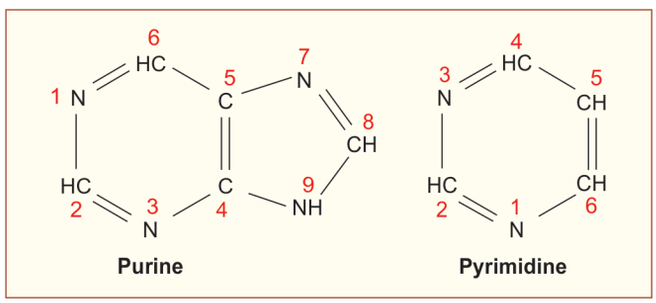

Fig. 7.2: Purine and pyrimidine rings

Various nitrogenous bases are formed with the appropriate substitutions on the parent heterocyclic ring, and are accordingly called the derivatives of purines and pyrimidines.

- **Purines:** There are two types of purine bases present in nucleic acids, viz. **adenine** (6-aminopurine) and **guanine** (2-amino-6-oxypurine).
- **Pyrimidines:** The pyrimidine bases, found in nucleic acids, are designated as **cytosine** (2-oxy-4-amino-pyrimidine), **uracil** (2,4-dioxypyrimidine) and **thymine** (2, 4-dioxy-5-methylpyrimidine, also called 5-methyluracil; Fig. 7.3).

Out of these three pyrimidines, only two are repeatedly found in a nucleic acid. Usually, cytosine and uracil are found in RNA while cytosine and thymine are found in DNA.

Unusual Nitrogenous Bases

- Besides the four nitrogenous bases (2 purines and 2 pyrimidines), which are commonly found in nucleic acids, there also occur some unusual nitrogenous bases in nucleic acids of certain bacteria and viruses. These are called minor bases, e.g. **5-methylcytosine**, **5-hydroxymethylcytosine**, **pseudouracil** (where structural positions of N1 and C5 in the uracil molecule are interchanged), **N6-methyladenine, N7-methylguanine**, etc. (Fig. 7.4).

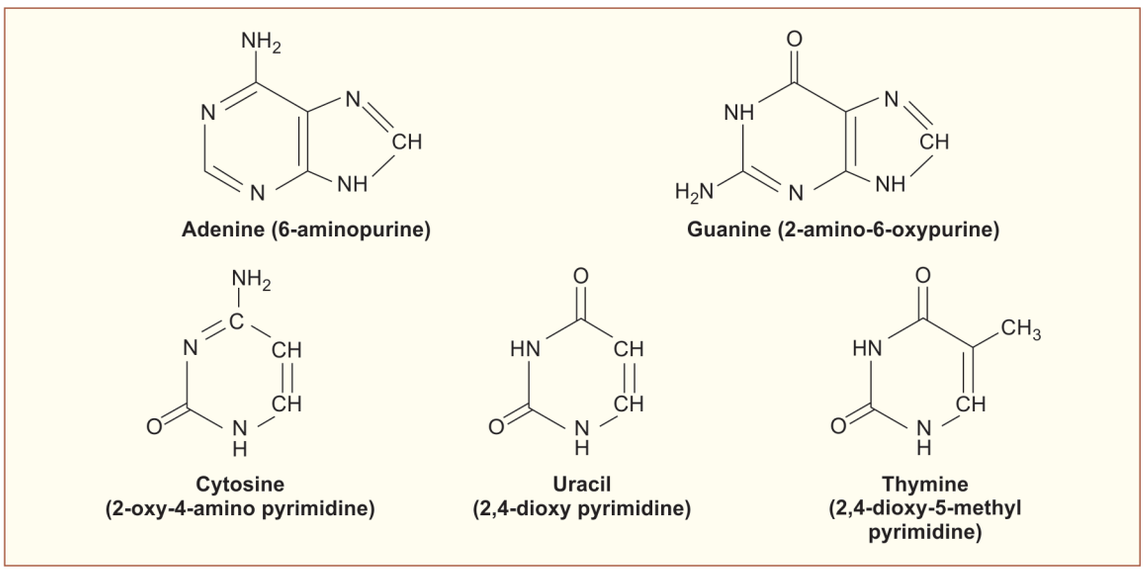

Fig. 7.3: Nitrogenous bases found in nucleic acids

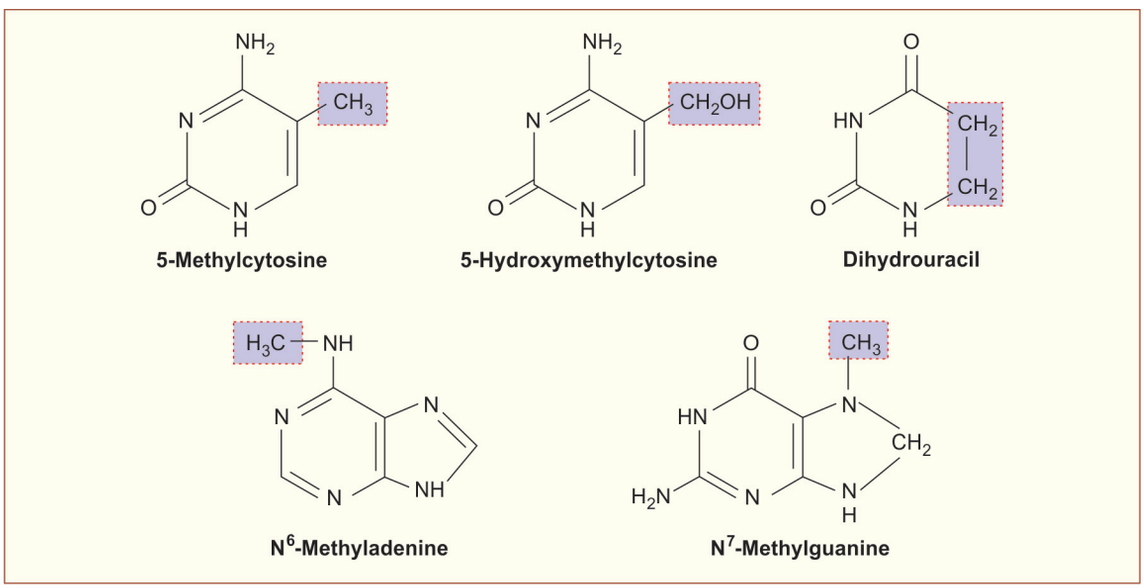

Fig. 7.4: Some unusual nitrogenous bases found in nucleic acids

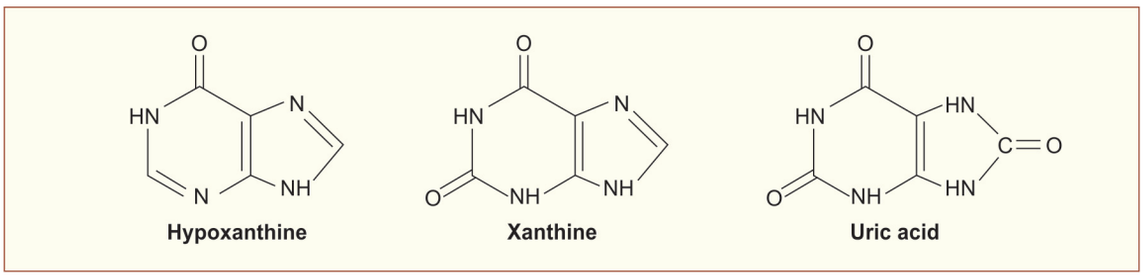

Fig. 7.5: Some purine bases of metabolic significance

- There are also some other purine derivatives which are of biological significance. These are either found as intermediates in purine biosynthesis, e.g. **hypoxanthine** and **xanthine**, or as the end product of their catabolism, such as **uric acid** (Fig. 7.5).

NUCLEOSIDES

A nucleoside has two components, i.e. it consists of a **nitrogenous base** and a molecule of **sugar**. In a nucleoside, a pentose sugar (ribose or deoxyribose) is linked to a nitrogenous base by an N-glycosidic linkage. The first carbon atom of the sugar moiety (C1) is linked either with the N1 of a pyrimidine or with N9 of a purine.

Base – N – (1/9)–C′ – **Sugar**

- **Ribonucleosides**
  - The nucleosides of ribose are called ribonucleosides. Ribonucleosides of adenine, guanine, cytosine and uracil are designated as **adenosine**, **guanosine**, **cytidine** and **uridine**, respectively (Fig. 7.6).

Adenosine has attained considerable clinical importance (Chemistry to Clinics 7.1).

Chemistry to Clinics 7.1: Significance of Adenosine

- Adenosine is a local hormone, known as an **autacoid**. During exercise, contracting muscles release adenosine which causes local vasodilation, thereby enhancing the supply of nutrients including oxygen to the muscles.
- Adenosine participates in sleep regulation. Prolonged wakefulness increases the extracellular adenosine levels in the brain which induce sleepiness by binding to specific adenosine receptors. Interestingly, this interaction is blocked by caffeine, explaining the tendency of coffee to promote wakefulness.
- Intravenous administration of adenosine is employed in the emergency treatment of supraventricular tachycardia characterized by uncontrolled rapid heartbeats (Fig. 7.7).

Deoxyribonucleosides

- Nucleosides of deoxyribose are designated as deoxyribonucleosides. Various deoxyribonucleosides are written with a prefix d-(deoxy). Thus,

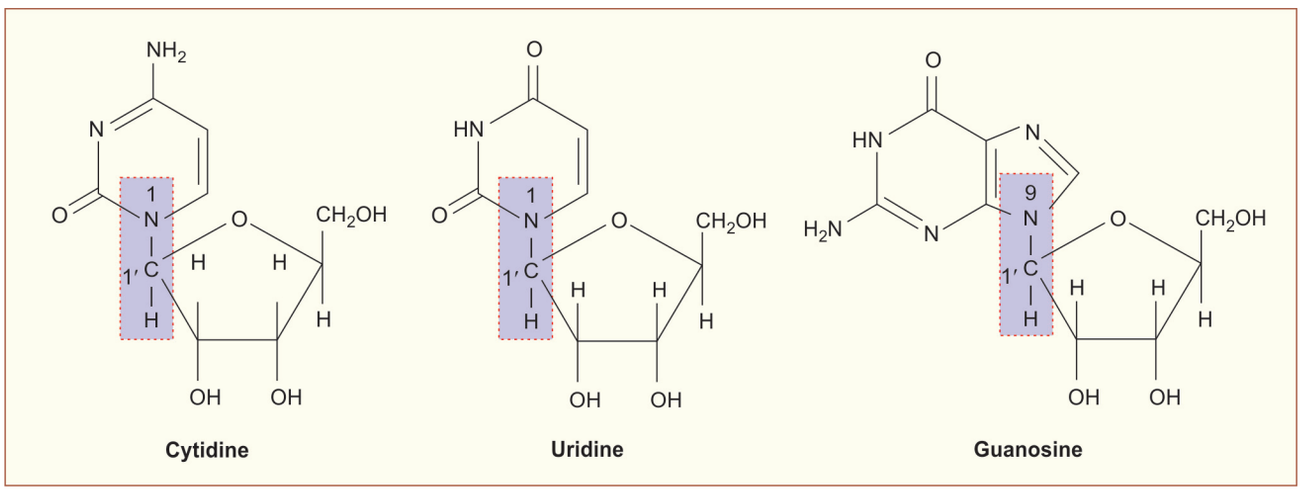

Fig. 7.6: Ribonucleosides

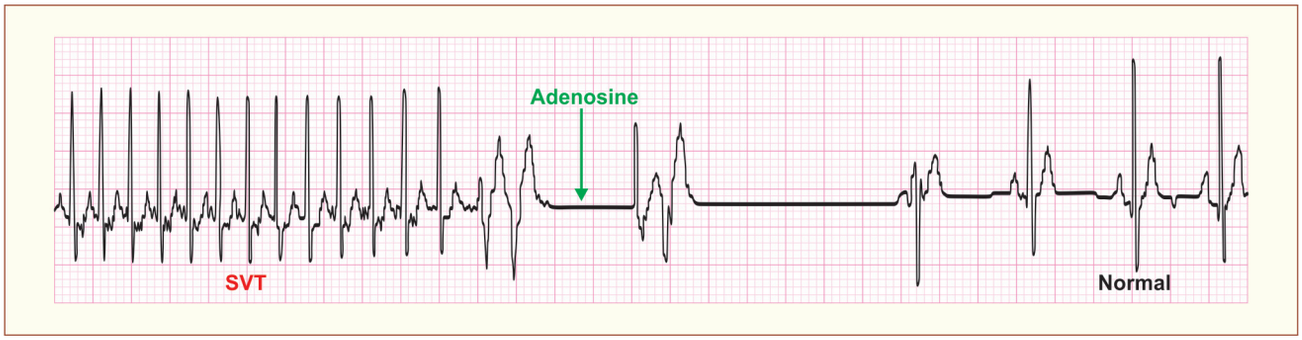

Fig. 7.7: ECG showing supraventricular tachycardia (SVT: Abnormally rapid heart rate which may be life-threatening) being normalized by intravenous **adenosine**

deoxyadenosine, deoxyguanosine, deoxycytidine and **deoxythymidine** are the nucleosides of deoxyribose with adenine, guanine, cytosine, and thymine respectively. Since thymine is usually found in DNA where it is linked to a deoxyribose only, its nucleoside is commonly designated as thymidine and not deoxythymidine (Fig. 7.8).

NUCLEOTIDES

In a nucleotide, the **sugar** molecule (ribose or deoxyribose) is linked both to a **nitrogenous base** on one side and to **phosphoric acid** on the other side. As in a nucleoside, C1 of the sugar molecule is linked to a nitrogenous base either through N1 of a pyrimidine or N9 of a purine. The sugar is also linked to phosphoric acid. Although any hydroxyl group (–OH) of the sugar can get esterified with phosphoric acid, more commonly C5 of the sugar is linked to a phosphate moiety.

Base – N – (1/9) –C1′ – **Sugar** – 5′ – **Phosphate**

During hydrolysis of a nucleic acid, the phosphate moiety may get attached even onto the –OH group at C3′, forming deoxyribonucleoside-3′-monophosphate (dN-3′-MP). The N refers to a nucleoside of any nitrogenous base.

In a ribose, since the –OH group is also present at C2′, in addition to N-3′-MP (nucleoside-3′-monophosphate) and N-5′-MP, N-2′-MP is also obtained during hydrolysis of an RNA molecule.

Ribonucleotides

- A nucleotide containing a ribose sugar is called ribonucleotide. Four ribonucleotides obtained on hydrolysis of RNA are designated as adenosine monophosphate or adenylate (**AMP**), guanylate (**GMP**), cytidylate (**CMP**) and uridylate (**UMP**; Fig. 7.9).

Deoxyribonucleotides

- The corresponding deoxyribonucleotides obtained on hydrolysis of a DNA molecule are designated as **dAMP, dGMP, dCMP** and **TMP**, respectively. Some of the deoxyribonucleotides are shown in Fig. 7.10.
- Various nitrogenous bases, nucleosides and nucleotides found in nucleic acids are shown in Table 7.1.

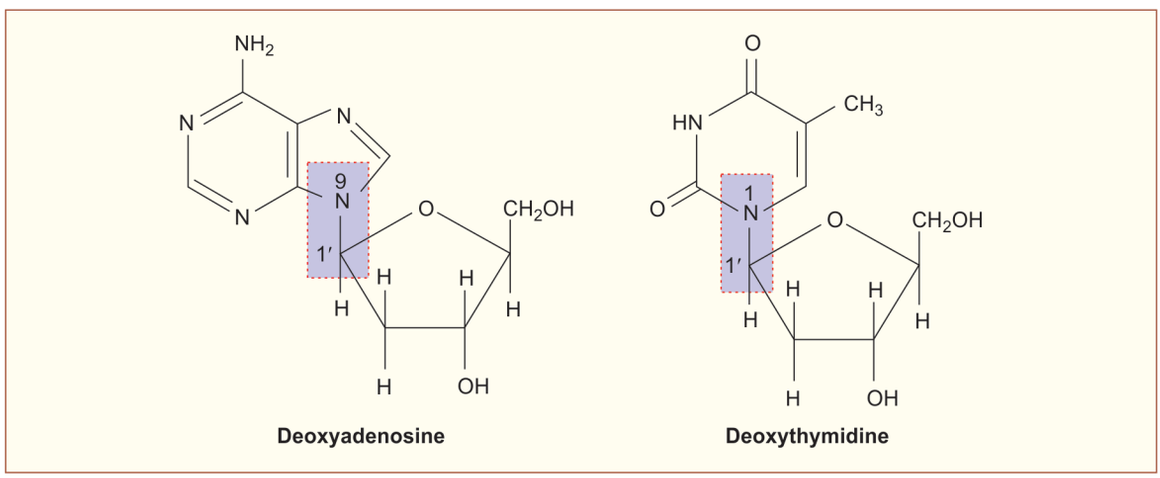

Fig. 7.8: Deoxyribonucleosides

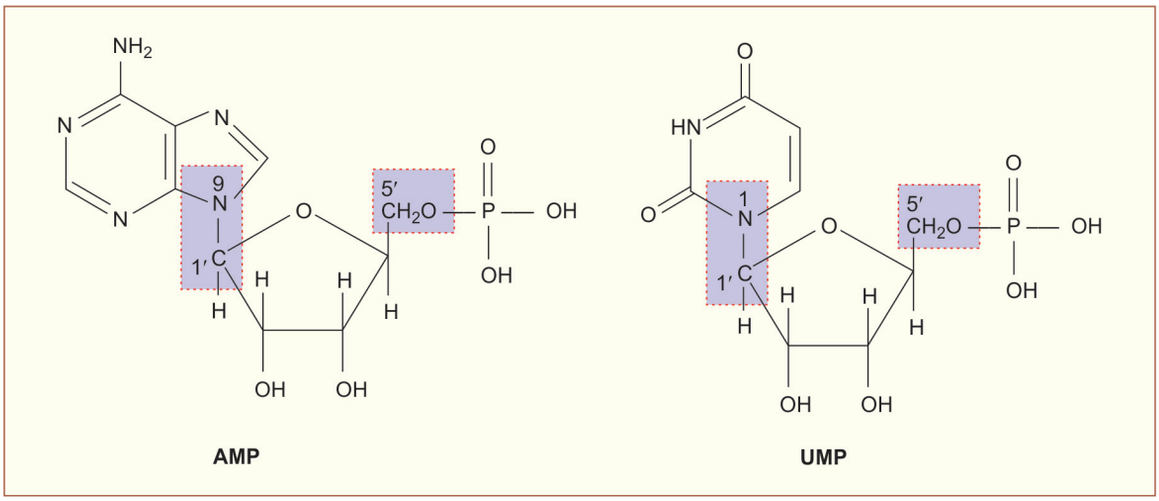

Fig. 7.9: Ribonucleotides

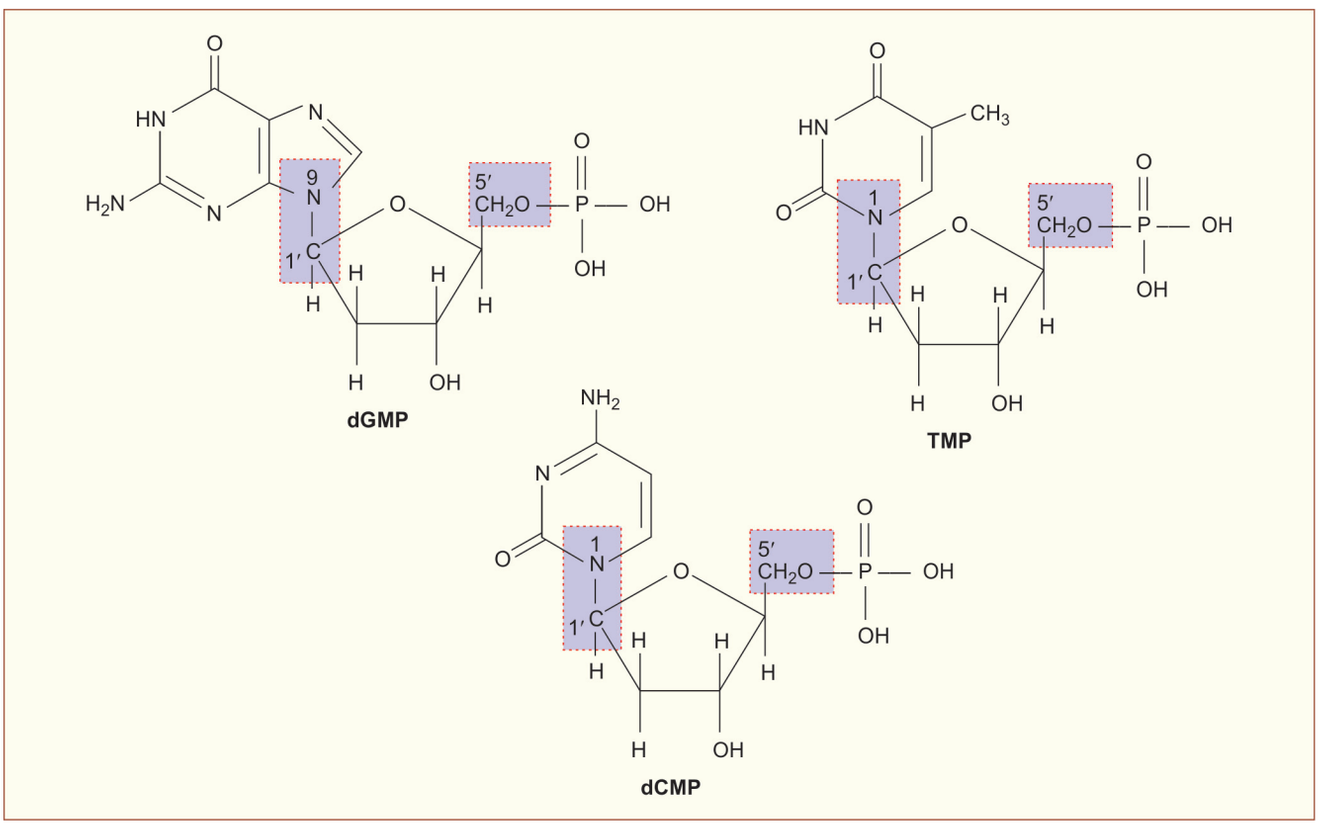

Fig. 7.10: Deoxyribonucleotides

Nucleotide Derivatives of Biological Significance

Besides the nucleotides of adenine, guanine, cytosine, uracil or thymine which are the hydrolyzed products of nucleic acids, there also occur di- and triphosphate derivatives of various nucleosides, e.g. ADP, ATP, GTP, CTP, UTP, etc. (Fig. 7.11).

Pharmacologically Active Purine/Pyrimidine Analogs

Any alteration either in the purine or pyrimidine ring, or in the sugar moiety, results in the production of various pharmacologically active compounds. When they get incorporated into the nucleic acid, they show toxic effects.

Some of them are inhibitory to several enzymes in DNA replication, inhibit DNA synthesis, and thus, are of therapeutic significance (Chemistry to Clinics 7.2).

Some analogs of sugar moiety, such as arabinosyl-cytosine (cytosine with arabinose instead of cytidine which contains cytosine and ribose) are also used in cancer chemotherapy (Fig. 7.12).

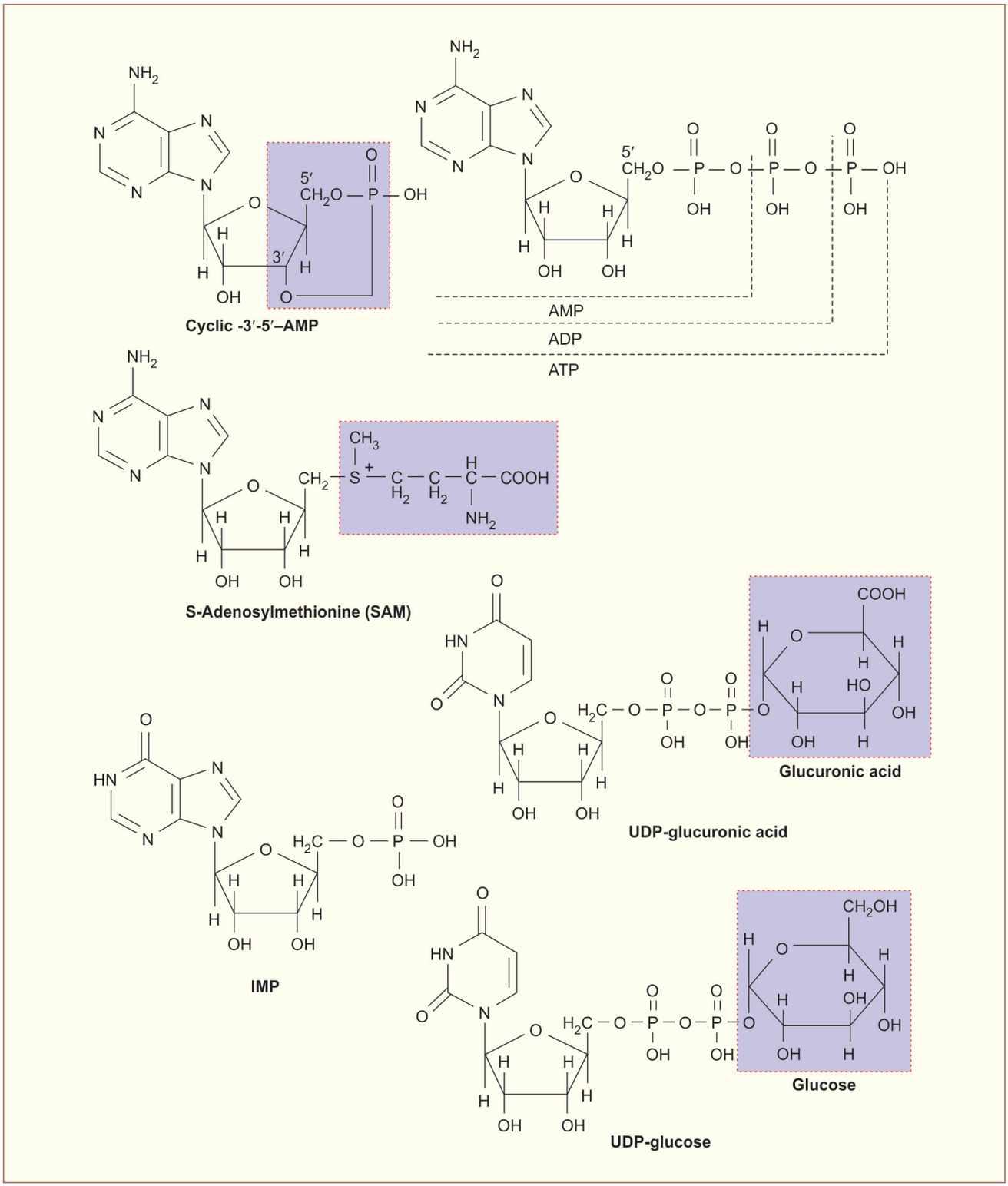

Fig. 7.11: Some nucleotide derivatives of biological significance

Table 7.1 Different nitrogenous bases, nucleosides and nucleotides found in nucleic acids

Nitrogenous base	Nucleoside (base + sugar)	Nucleotide (base + sugar + P)
RNA		
Adenine	Adenosine	AMP
Guanine	Guanosine	GMP
Cytosine	Cytidine	CMP
Uracil	Uridine	UMP
DNA		
Adenine	Deoxyadenosine	dAMP
Guanine	Deoxyguanosine	dGMP
Cytosine	Deoxycytidine	dCMP
Thymine	Thymidine	TMP

Chemistry to Clinics 7.2: Purine/Pyrimidine Analogs as Drugs

Some of the synthetic analogs, such as 5-fluorouracil (a derivative of pyrimidine with the analogous heterocyclic ring), 6-thioguanine and 6-mercaptopurine (sulfur substituted analogs of purine) are used as anticancer drugs. Allopurinol (4-hydroxy-pyrazolopyrimidine, an analog of purine) is an inhibitor of xanthine oxidase and is used in the treatment of hyperuricemia.

METABOLIC PROCESSES IN WHICH NUCLEOTIDES ARE INVOLVED

Nucleotides and their derivatives play important role in several metabolic processes:

- **Synthesis of nucleic acids: Ribonucleotides** (AMP, GMP, CMP and UMP) and **deoxyribonucleotides** (dAMP, dGMP, dCMP and TMP) are the **monomeric units of nucleic acids**. These are obtained on hydrolysis of RNA and DNA, respectively. At the same time **ribonucleotide triphosphates** (ATP, GTP, CTP and UTP) and **deoxyribonucleotide triphosphates** (dATP, dGTP, dCTP and TTP) are used in the **synthesis of RNA** and **DNA**, respectively.
- **As energy currency of cell:** Nucleotide triphosphate, e.g. **ATP** is **the energy currency of cell**.
- **As metabolic precursors:** Some of the nucleotide triphosphates are used as metabolic precursors, e.g. **GTP for tetrahydrobiopterin synthesis**.
- **Act as allosteric effectors:** Some of the nucleotides **act as allosteric effectors**. For example, hexokinase is allosterically activated by ADP but inhibited by ATP.
- **Act as second messenger: Cyclic nucleotides act as physiological mediators**, e.g. cAMP acts as second messenger for epinephrine.
- **Components of coenzymes:** Some of the **nucleotide derivatives act as components of coenzymes**, e.g. NAD, FAD, etc.
- **Activated metabolic intermediates:** Some **nucleotide derivatives act as activated metabolic intermediates**, e.g. S-adenosylmethionine (SAM).
- **Used in the interconversion of monosaccharides:** Some of the nucleotide derivatives, such as UDP-glucose and UDP-galactose are used in the **interconversion of monosaccharides**.
- **Used as a carrier of monosaccharide unit:** Some of the nucleotide derivatives, such as UDP-glucose and

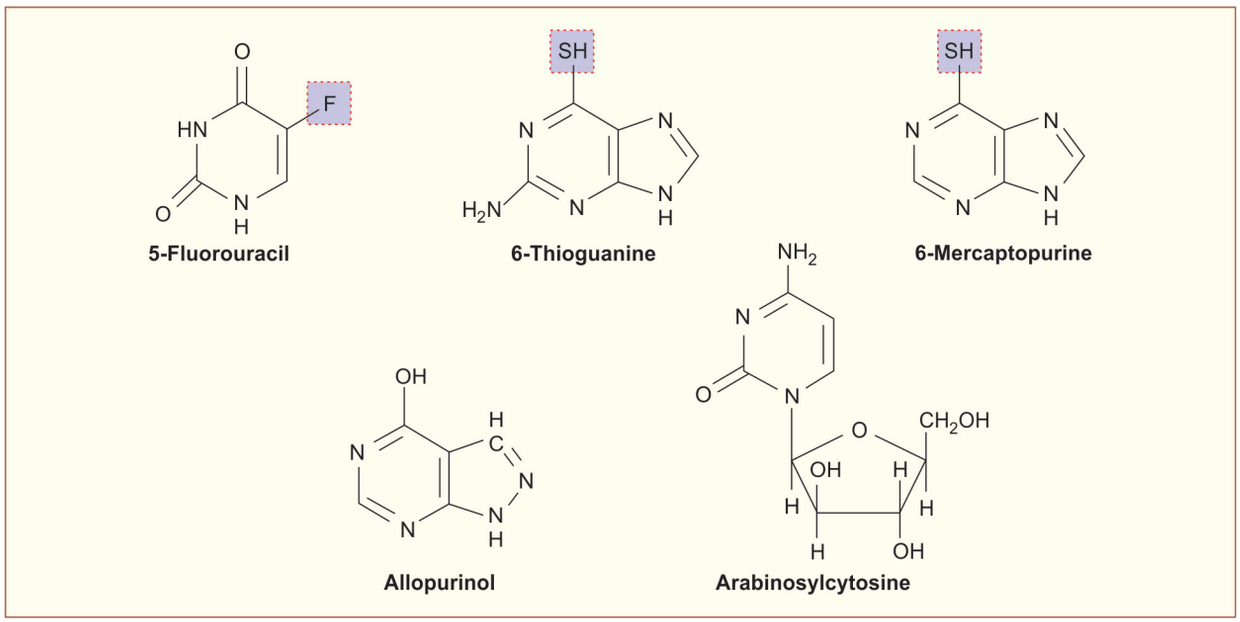

Fig. 7.12: Some pharmacologically active nucleotide analogs

UDP-galactose are also **used in glycogenesis** and **lactose synthesis**.

- **Used in conjugation reactions:** Some of the nucleotide derivatives are also used in **conjugation reactions**, e.g. UDP-glucuronic acid, for the conjugation of bilirubin.
- **Used as anticancer drugs:** Some of the **synthetic analogs**, such as 5-fluorouracil, are used **as anticancer drugs**.
- **Used in the treatment of hyperuricemia: Allopurinol** (an analog of purine) acts as an inhibitor of xanthine oxidase and is used in the **treatment of hyperuricemia**.

COMMON DISORDERS ASSOCIATED WITH NUCLEOTIDE METABOLISM

All cells can synthesize nucleotides, either by the biosynthetic process, called ***de novo*** synthesis, or from the degradation products of nucleic acids, i.e. free bases and nucleosides, by the process called **salvage pathway**.

METABOLISM OF PURINE NUCLEOTIDES

Synthesis of Purine Nucleotides

De novo Synthesis of Purine Nucleotides

The process of synthesis of complex end product(s) in a metabolic pathway from simple precursor molecules is called *de novo* synthesis (*de novo* = 'anew', i.e. starting 'from scratch'). A purine ring is synthesized by utilizing three amino acids (aspartate, glutamine and glycine) as carbon and nitrogen donors, besides respiratory CO_2 (HCO_3–) and two 1C moieties (transferred via tetrahydrofolate, FH_4) as the carbon sources.

The N1 of a purine ring is derived from the amino group (–NH_2) of aspartate, C2 and C8 from N^{10}-formyltetrahydrofolate (N^{10}-formyl-FH_4), N3 and N9 from amide nitrogen (–$CONH_2$) of glutamine, C6 from CO_2 (HCO_3^-), and C4, C5 and N7 from glycine (Fig. 7.13).

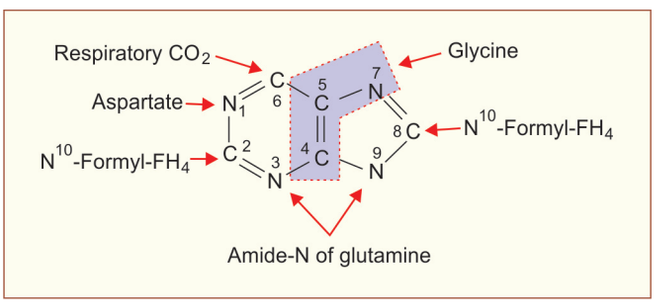

Fig. 7.13: Sources of carbon and nitrogen atoms in a purine ring

The purine ring is synthesized **not as a free base but as a part of the nucleotide** by a series of reactions by adding various carbon and nitrogen atoms onto ribose-5-phosphate.

Synthesis of IMP

De novo pathway for the synthesis of purine nucleotides leads to the synthesis of a nucleotide of hypoxanthine, i.e. inosine-5'-monophosphate (IMP) which in turn serves as a **precursor for AMP and GMP**:

1. The starting material for the synthesis of a purine ring is phosphoribosyl pyrophosphate (PRPP). It is synthesized by the addition of pyrophosphate from ATP, onto ribose-5-phosphate (produced in the HMP shunt). This reaction is catalyzed by phosphoribosyl pyrophosphate synthetase (PRPP synthetase), also called ribose phosphate pyrophosphokinase, which is inhibited by, both, ADP as well as GDP (Fig. 7.14). PRPP is used as a starting material, not only for the synthesis of purines but also for pyrimidines. PRPP is also used in the utilization of purine and pyrimidine bases by the salvage pathway.
2. The next step is the formation of 5'-phosphoribosylamine. The reaction is catalyzed by an amidotransferase (glutamine PRPP amidotransferase), also called phosphoribosylamine synthetase. Pyrophosphate group is replaced by the amide nitrogen of glutamine. This, in turn, results in the acquisition of N9 of the purine ring and is a committed step. The rate of the reaction is controlled by intracellular concentrations of both the substrates, i.e. glutamine as well as PRPP.
3. In the next step, glycine gets conjugated with the amino group of phosphoribosylamine and forms 5-phosphoribosylglycinamide. The reaction is catalyzed by phosphoribosylglycinamide synthetase.
4. In the next reaction, N^{10}-formyl-FH_4 donates a formyl group and results in the formation of 5-phosphoribosylformylglycinamide. The reaction is catalyzed by formyltransferase or transformylase (phosphoribosylglycinamide transformylase).
5. Thereafter, through a series of reactions, **IMP** is synthesized as the **first purine nucleotide**.

Various enzymes involved in the synthesis of a purine nucleotide are found in the **cytosol** of a cell whereas energy, to drive some of these reactions, is obtained from the hydrolysis of ATP. During this process 6 moles of ATP are utilized per mole of IMP synthesized. This implies that the ***de novo*** synthesis of purines is **costly** in terms of the energy investment. Hence, a purine 'salvage' pathway also exists as discussed later in this chapter.

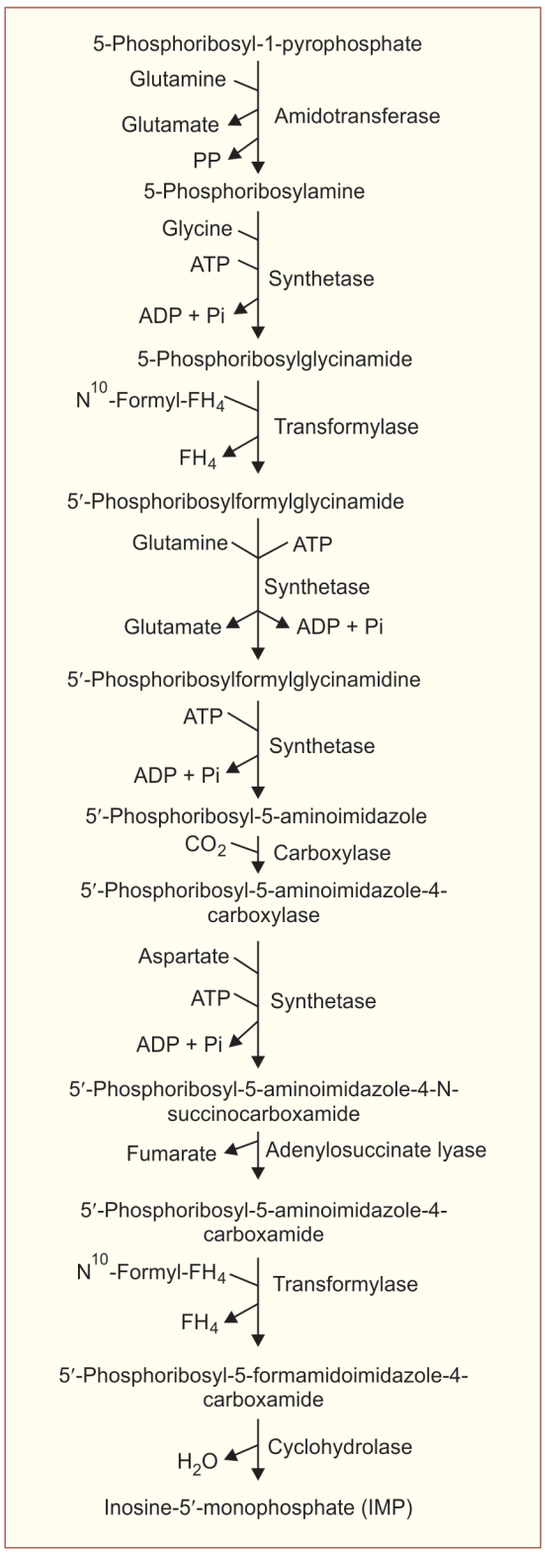

Fig. 7.14: Synthesis of IMP

Synthesis of AMP and GMP

The first ribonucleotide formed in the ***de novo*** pathway, i.e. IMP serves as a common precursor for the synthesis of AMP as well as GMP (Fig. 7.15).

- **Synthesis of AMP:** IMP reacts with aspartate and is changed to adenylosuccinate (AMPS). The reaction is catalyzed by adenylosuccinate synthetase. Thereafter, adenylosuccinase (adenylosuccinate lyase) removes fumarate and forms AMP. Adenylosuccinate synthetase is a rate-limiting enzyme with AMP acting as a competitive inhibitor. GTP is used as a source of energy.
- **Synthesis of GMP:** IMP is converted to xanthosine monophosphate (XMP) by IMP dehydrogenase. Thereafter, GMP synthetase converts XMP to GMP. IMP dehydrogenase is a rate-limiting enzyme. It is regulated by GMP which acts as a competitive inhibitor for the enzyme.

  Formation of GMP from IMP requires ATP as a source of energy.

Regulation of biosynthesis of purine nucleotides: Biosynthesis of IMP is regulated at the first step of the pathway by the availability of ribose-5-phosphate and hence PRPP.

- The reaction catalyzed by **amidotransferase is a committed step**, i.e. the site of metabolic regulation. The enzyme glutamine: PRPP amidotransferase is regulated, allosterically, by the end products of the pathway, i.e. by IMP, GMP and AMP which serve as negative effectors. On the other hand, PRPP is a positive effector for the enzyme.
- Biosynthesis of AMP and GMP from IMP is also regulated. Both, AMP and GMP are competitive inhibitors of IMP in their own synthesis. From IMP to GMP, **IMP dehydrogenase is the rate limiting enzyme**. It is regulated by GMP which acts as a competitive inhibitor. Adenylosuccinate synthetase is a rate limiting enzyme in the conversion of IMP to AMP where AMP acts as a competitive inhibitor.
- The rate of GMP synthesis increases with the concentration of ATP while that of the AMP increases with the concentration of GTP (Fig. 7.16).

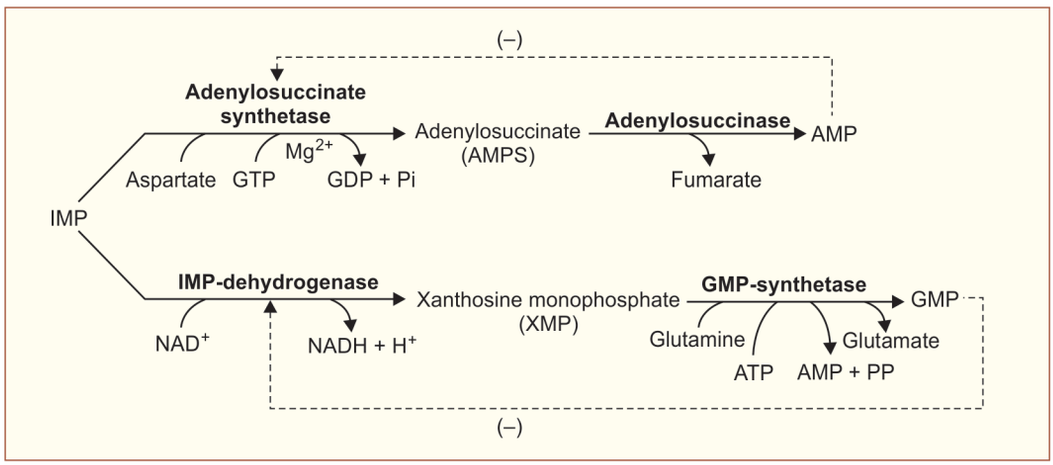

Fig. 7.15: Conversion of IMP to AMP and GMP

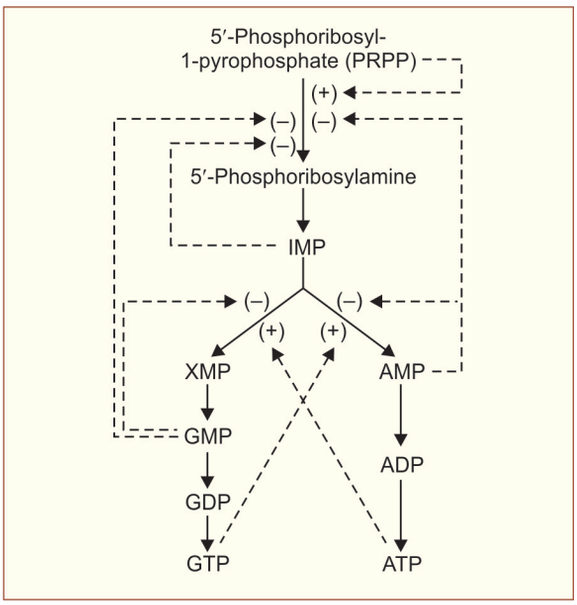

Fig. 7.16: Regulation of the synthesis of purine nucleotides

Salvage Pathway for Purine Nucleotides Synthesis

In mammals, **free purine bases**, i.e. hypoxanthine, guanine and adenine as well as their nucleosides can also be converted to the corresponding nucleotides in the salvage pathway.

The conversion of free base to the respective nucleotide requires phosphoribosyl transferase and PRPP as a source of ribose-5-phosphate. For the salvage, adenine mainly arises from the synthesis of polyamines, i.e. from 5'-methylthioadenosine. On the other hand, hypoxanthine and guanine arise from the degradation of purine nucleotides.

- **Adenine** is converted to AMP by adenine phosphoribosyltransferase (APRTase), whereas both **hypoxanthine** and **guanine** are converted to IMP and GMP respectively, by the same enzyme, i.e. hypoxanthine–guanine phosphoribosyltransferase (HGPRTase; Figs 7.17 and 7.18).

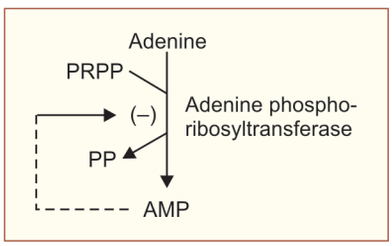

Fig. 7.17: Conversion of AMP by APRTase

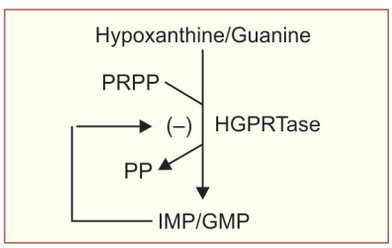

Fig. 7.18: Conversion of IMP/GMP by HGPRTase

Both the phosphoribosyltransferase reactions are regulated by their end products. Whereas AMP is a competitive inhibitor of APRTase, IMP and GMP competitively inhibit HGPRTase activity.

Lack of HGPRTase activity results in a condition designated as **Lesch-Nyhan syndrome** (Chemistry to Clinics 7.3).

- **Nucleosides** can be salvaged by the respective nucleoside kinases. For example, adenosine is salvaged by adenosine kinase, a 5′-phosphotransferase, that utilizes ATP as the phosphate donor (Fig. 7.19).

Chemistry to Clinics 7.3: Lesch-Nyhan Syndrome

Inability of the body to salvage hypoxanthine and guanine due to the complete deficiency of HGPRTase, leads to a condition called Lesch-Nyhan syndrome (partial deficiency causes Kelley-Seegmiller syndrome). Since the gene for HGPRTase is located on the X chromosome, this disease is limited to males only.

Features

1. In this condition, hypoxanthine and guanine are not salvaged. This, in turn, results in increased intracellular pool of PRPP and decreases IMP and GMP concentrations, thereby promoting *de novo* synthesis of purine nucleotides. This leads to excessive production of uric acid, hyperuricemia and gout.
2. Guanosine is an important modulator of glutamatergic neurotransmission, promoting glial reuptake of L-glutamate. A deficiency of guanosine could lead to dysregulated glutamatergic neurotransmission, including possible excitotoxic damage. This partly explains the mental retardation and some other neurological features.
3. Decreased GTP synthesis through salvage pathway reduces its availability for the enzyme GTP cyclohydrolase, an enzyme which initiates the synthesis of biopterins. The latter are essential for the synthesis of many neurotransmitters, e.g dopamine in the striatal dopaminergic pathways. This is thought to result in self-mutilation.

Diagnosis

i. Increase urinary urate/creatinine ratio.
ii. Absent/reduced enzyme activity in lymphocytes or fibroblasts.
iii. Mutation analysis of *HGPRTase* gene.

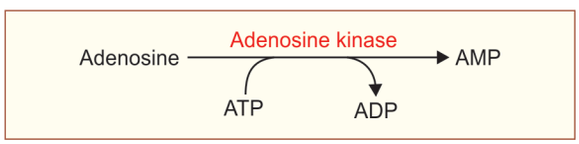

Fig. 7.19: A nucleoside kinase reaction

Significance of Salvage Pathway

1. The ***de novo*** purine synthesis is energetically expensive. Salvage reactions **conserve energy**.
2. Salvage reactions are important in cells like **erythrocytes** which lack amidotransferase and cannot synthesize 5-phosphoribosylamine.

Interconversion of Purine Nucleotides

To meet their need, cells can interconvert adenine and guanine nucleotides and thus maintain a balance between the two purine nucleotides. For their interconversion, GMP or AMP is first degraded to IMP which then, depending upon the need of the cell, can resynthesize any of the two nucleotides:

- By reductive deamination, GMP is converted to IMP. The reaction is catalyzed by GMP reductase. GTP activates this step, whereas XMP competitively inhibits the reaction.
- Conversion of AMP to IMP is catalyzed by AMP deaminase (5′-AMP aminohydrolase). This reaction is activated by K^+ and ATP but inhibited by Pi, GDP and GTP (Fig. 7.20).

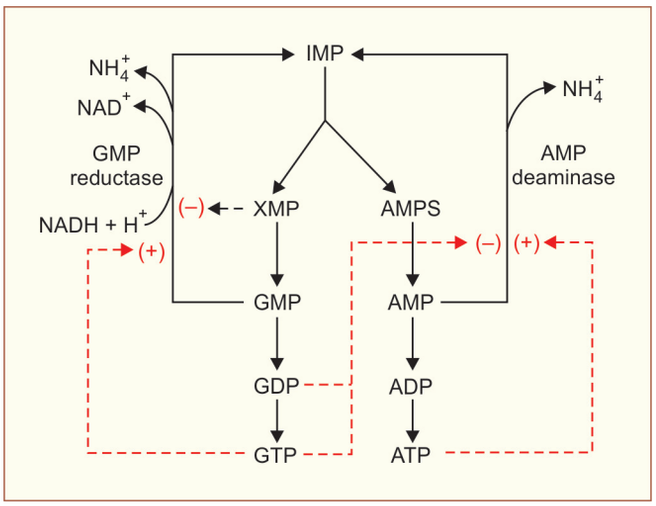

Fig. 7.20: Interconversion of purine nucleotides. (+) = Stimulation; (–) = Inhibition

Degradation of Purine Nucleotides

Degradation of purine nucleotides, i.e. AMP and GMP, leads to the formation of **uric acid**, in humans (Fig. 7.21).

Degradation of AMP

1. AMP is converted to adenosine, by 5'-AMP-nucleotidase which has high specificity for its substrate. Thereafter, adenosine deaminase (ADA) converts adenosine as well as deoxyadenosine to inosine. Alternatively, AMP deaminase, which is specific for AMP, removes ammonia and converts AMP to IMP. Subsequently, nucleotidase removes phosphoric acid and converts IMP to inosine.

   Activities of the above three enzymes, i.e. AMP-nucleotidase, ADA and AMP deaminase are several fold higher in muscle than in other tissues.
2. Purine nucleoside phosphorylase (PNP) then removes the ribose moiety from inosine and converts it to hypoxanthine.

Degradation of GMP

1. In the first step, phosphoric acid is removed by 5'-nucleotidase and GMP is converted to guanosine.
2. With the removal of the ribose moiety by PNP, guanosine is converted to guanine.
3. Guanase (an aminohydrolase) removes amino group from guanine and converts it to xanthine.

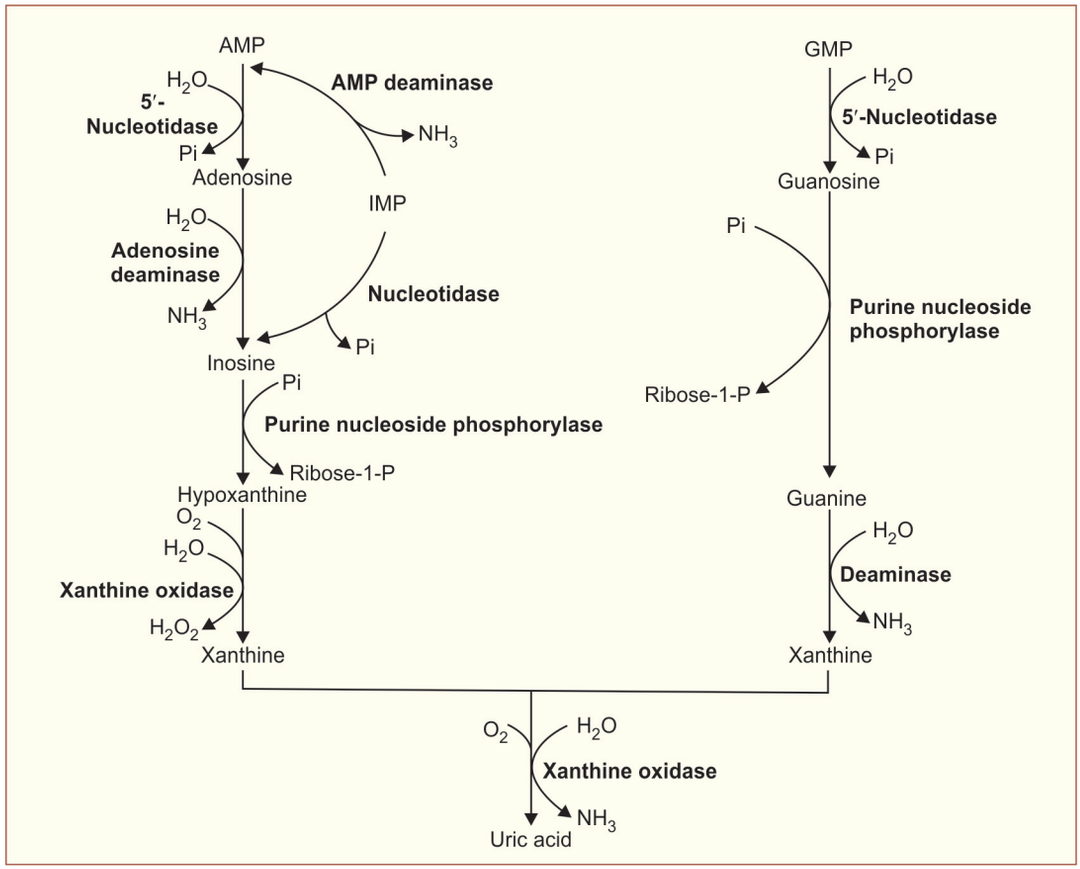

Fig. 7.21: Degradation of purine nucleotides

Immunodeficiency Diseases

Two distinct immunodeficiency diseases, i.e. **ADA deficiency** and **PNP deficiency** are associated with defects in the degradation of purine nucleosides (adenosine and guanosine; Chemistry to Clinics 7.4).

Synthesis of Uric Acid

Hypoxanthine and xanthine, produced as above, are catabolized by the enzyme **xanthine oxidase** and are converted to uric acid (Fig. 7.21).

Xanthine oxidase occurs in the liver and small intestinal mucosa. It is a dimeric protein, which contains FAD, a Mo complex and two different Fe-S clusters. It also requires molecular oxygen as a substrate.

METABOLISM OF PYRIMIDINE NUCLEOTIDES

De novo Synthesis of Pyrimidine Nucleotides

A pyrimidine ring is synthesized by utilizing two amino acids, i.e. aspartate and glutamine as carbon and nitrogen donors, and respiratory CO_2 (HCO_3^-) as a carbon donor. The N1, C4, C5 and C6 of the pyrimidine

Chemistry to Clinics 7.4: Immunodeficiency Diseases Associated with Degradation of Purine Nucleosides

- **Severe Combined Immunodeficiency:** A deficiency in ADA is associated with severe combined immunodeficiency (SCID) involving both T-cell and B-cell functions. In ADA deficient patients, intracellular concentration of 2′-deoxyadenosine is increased leading to accumulation of dAMP and dATP. This not only decreases ATP synthesis but also blocks DNA replication by reducing the synthesis of other dNTPs. Thus, proliferation and differentiation of immune cells are compromised. Treatment of such children includes blood transfusion, bone marrow transplantation, enzyme replacement therapy with ADA-polyethylene glycol (the first successful application of enzyme replacement therapy for an inherited disease) and gene therapy. Recently, ADA gene has been successfully transfected into stem cells of ADA-deficient children.
- **Purine Nucleoside Phosphorylase Deficiency:** It is associated with immunodeficiency involving T-cell functions, i.e. a defective T-cell immunity but with normal B-cell functions. In both the conditions, i.e. SCID and PNP deficiency, there is a decrease in uric acid formation which is accompanied with increased levels of purine nucleosides and nucleotides. Overwhelming infection in such children results in death within 2 years of age.

ring are derived from aspartate, C2 from HCO_3^- and N3 from glutamine (Fig. 7.22).

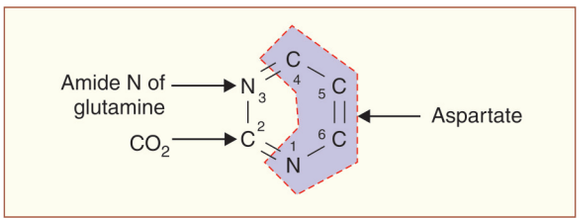

Fig. 7.22: Sources of carbons and nitrogens in pyrimidine ring

In the ***de novo*** synthesis of pyrimidine nucleotides, a **pyrimidine ring is formed first**. Thereafter, ribose-5-phosphate is added via PRPP. This leads to the synthesis of UMP, which serves as a precursor for other pyrimidine nucleotides.

Synthesis of UMP

1. The first step in the synthesis of a pyrimidine ring is the synthesis of carbamoyl phosphate from glutamine and HCO_3^-. This reaction consumes two molecules of ATP.

   This is a regulated step in the pathway and is catalyzed by carbamoyl phosphate synthetase II (**CPS II**), in the cytosol.
2. In the next step, carbamoyl phosphate condenses with aspartate and forms carbamoyl aspartate. The reaction is catalyzed by aspartate transcarbamoylase (aspartate carbamoyltransferase). This is the **committed step** in the synthesis of pyrimidine nucleotides.
3. Dihydro-orotase results in ring closure and forms dihydro-orotate. In animals, activities of the 3 enzymes, i.e. CPS II, aspartate transcarbamoylase and dihydro-orotase are present on a single trifunctional protein, termed **CAD**.
4. Dihydro-orotate dehydrogenase, a mitochondrial enzyme, oxidizes dihydro-orotate to orotate.
5. Orotate phosphoribosyltransferase transfers ribose-5-phosphate from PRPP and converts the pyrimidine base orotate to its nucleotide, orotidine monophosphate (OMP).
6. OMP is converted to UMP by OMP decarboxylase (orotidylate decarboxylase; Fig. 7.23).

   Orotate phosporibosyltransferase and OMP decarboxylase activities are present on a single **bifunctional protein**, termed UMP synthase (Chemistry to Clinics 7.5).

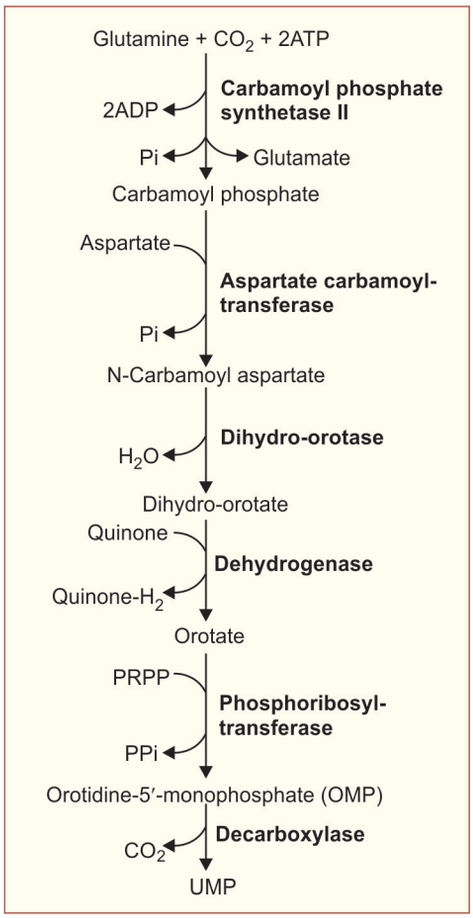

Fig. 7.23: Synthesis of UMP

Chemistry to Clinics 7.5: Orotic Aciduria

There are several types of the disease:

Type 1: It is due to a defect in *de novo* synthesis of pyrimidine nucleotides. There is a genetic deficiency of both the activities of the bifunctional protein UMP synthase, comprising of orotate phosphoribosyltransferase and OMP decarboxylase. High levels of orotic acid excretion in the urine and deficiency of enzymes in erythrocytes confirm the diagnosis.

Type 2: Reduced activity of OMP decarboxylase only.

Others

i. Ornithine transcarbamoylase (an enzyme of urea cycle) deficiency results in accumulation of carbamoyl phosphate which is channelized towards pyrimidine biosynthesis. Blood ammonia is increased, whereas urea is decreased, the two features not observed in type 1 and 2 orotic aciduria.
ii. Reye's syndrome.
iii. During allopurinol therapy.

Features

Megaloblastic anemia: Deficiency of UMP (precursor of UTP, CTP and TMP) leads to decreased nucleic acid synthesis in erythroid precursors in bone marrow, and megaloblastic anemia. It does not respond to vitamin B_{12} and/or folic acid.

Physical and mental retardation: This could be due to reduced supply of pyrimidine nucleosides in the neonatal period.

Treatment: It includes feeding uridine-rich diet which reduces formation of orotate and improves anemia. Uridine is taken up by the cells, salvaged to UMP and finally to UTP which inhibits CPS II, the regulatory step in pyrimidine biosynthesis. As a result, orotic acid synthesis is decreased. Exogenous uridine thus bypasses the defective step in pyrimidine biosynthesis and by its salvage utilization, uridine supplies UTP and CTP required by the cell.

Conversion of Nucleoside Monophosphates to Di- and Triphosphates

Various nucleoside monophosphates, i.e. AMP, GMP, CMP, UMP as well as dAMP, dGMP, dCMP and dTMP are sequentially converted to their nucleoside triphosphates.

- Firstly, a nucleoside monophosphate (NMP) is converted to the corresponding nucleoside diphosphate (NDP). The reaction is catalyzed by the base specific nucleoside monophosphate kinase.
- Thereafter, nucleoside diphosphate kinase converts a nucleoside diphosphate to the corresponding nucleoside triphosphate (Fig. 7.24).

Conversion of UTP to CTP

CTP synthetase catalyzes the formation of CTP from UTP by using glutamine as a donor of the amino group. ATP is used as a source of energy. CTP is a negative effector of the reaction (Fig. 7.25).

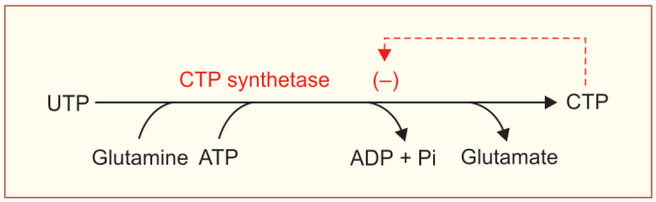

Fig. 7.25: Synthesis of CTP from UTP

Synthesis of TMP

Both UTP and CTP can serve as precursors of TMP. The two nucleoside triphosphates are first hydrolyzed to the corresponding nucleoside diphosphates, i.e. CDP and UDP which, in turn, act as precursors of dUMP.

Thereafter, thymidylate (deoxythymidylate, dTMP or TMP) is synthesized by methylation of the deoxyuridine monophosphate (dUMP). The reaction is catalyzed by thymidylate synthase. In this reaction, N^5, N^{10}-methylenetetrahydrofolate (N^5, N^{10}-methylene-FH_4) is used as a source of the methyl group (one carbon moiety) and also as a reducing agent. N^5, N^{10}-methylene-FH_4, in turn, is converted to FH_2 (*see* Figs 18.10 and 18.13).

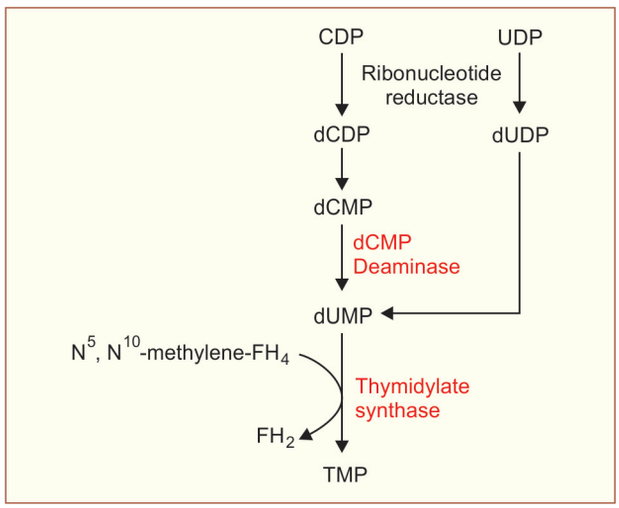

Fig. 7.26: Synthesis of TMP

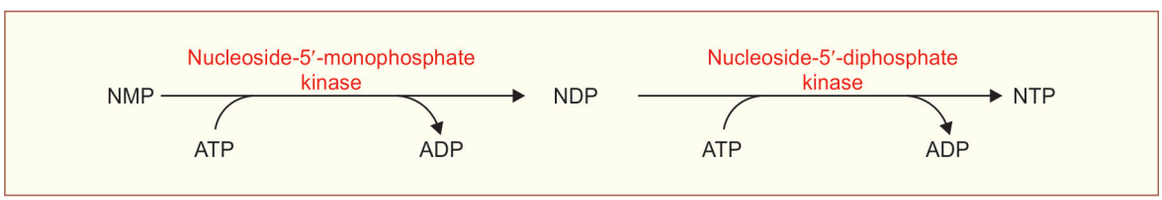

Fig. 7.24: Conversion of a nucleoside monophosphate to di- and triphosphate

Regulation of Biosynthesis of Pyrimidine Nucleotides

- Pyrimidine synthesis is regulated by CPS II. The enzyme is inhibited by CTP and is activated by ATP and PRPP (Fig. 7.27).

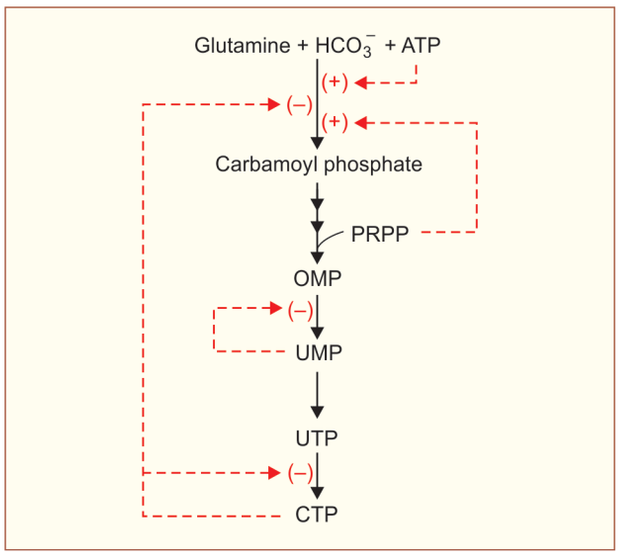

Fig. 7.27: Regulation of pyrimidine nucleotide biosynthesis (+) = Stimulation; (ñ) = Inhibition

- OMP decarboxylase is competitively inhibited by UMP.
- CTP synthetase is inhibited by CTP. This is an example of feedback inhibition. This, in turn, regulates the conversion of UTP to CTP. Cells thus maintain a balance between uridine and cytidine nucleotides.

Importance of DHFR and Thymidylate Synthase

TMP (dTMP) synthesis is essentially required for rapidly proliferating cells, such as cancer cells, for DNA synthesis. Inhibition of thymidylate synthase, or of DHFR, blocks the synthesis of dTMP and thus kills cancer cells. Various inhibitors of these enzymes are known and are used as effective anticancer/antibacterial agents (Chemistry to Clinics 7.6 and 7.7).

Chemistry to Clinics 7.6: Suicide Inhibitors

Analogs of dUMP, such as 5-fluorodeoxyuridylate (FdUMP) are irreversible inhibitors of thymidylate synthase. FdUMP binds to the enzyme thymidylate synthase like dUMP and forms an enzyme-FdUMP-FH_4 ternary covalent complex. This, in turn, inactivates the enzyme after undergoing some of the normal catalytic reactions. Such enzyme inhibitors are called mechanism-based inhibitors or suicide inhibitors or suicide substrates, as they force the enzyme to commit suicide.

Chemistry to Clinics 7.7: Antifolates

Antimetabolites, such as methotrexate (amethopterin), aminopterin and trimethoprim are analogs of FH_2 and are called antifolates. These drugs inhibit dihydrofolate reductase and block the regeneration of FH_4. This, in turn, affects dTMP biosynthesis. These drugs are used as effective anticancer agents, particularly against childhood leukemias.

Trimethoprim binds more tightly to bacterial enzymes than to mammalian cells. It is, therefore, used as an important antibacterial agent. It is an active ingredient of the popular antibiotic 'Septran'.

SALVAGE PATHWAY FOR PYRIMIDINE NUCLEOTIDES SYNTHESIS

Pyrimidine bases orotate, uracil and thymine, but not cytosine, can be salvaged and converted to the corresponding nucleotides by pyrimidine phosphoribosyltransferase. The enzyme utilizes PRPP as a source of ribose-5-phosphate (Fig. 7.28).

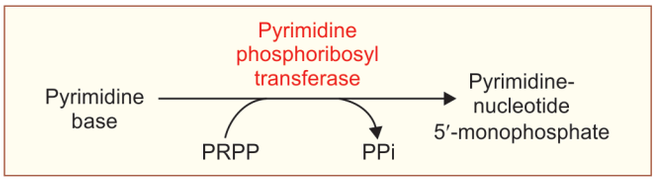

Fig. 7.28: Salvage pathway for pyrimidine nucleotides synthesis

DEGRADATION OF PYRIMIDINE NUCLEOTIDES SYNTHESIS

Pyrimidine nucleotides are first converted to free bases which are then degraded to form the end products.

Degradation of Pyrimidine Nucleotides to the Free Bases

- Pyrimidine nucleotides (CMP, UMP, dCMP and dTMP), released as a result of the turnover of nucleic acids, are converted to the corresponding nucleosides by the nonspecific phosphatases.
- Cytidine and deoxycytidine are thereafter deaminated by pyrimidine nucleoside deaminase to uridine and deoxyuridine, respectively.
- Uridine, deoxyuridine and deoxythymidine are degraded by uridine phosphorylase and result in the formation of uracil (from uridine and deoxyuridine) and thymine (from deoxythymidine; Fig. 7.29).

Degradation of Pyrimidine Bases

Uracil and thymine produced as above are further degraded to β-alanine and β-aminoisobutyric acid, respectively (Fig. 7.30; Chemistry to Clinics 7.8).

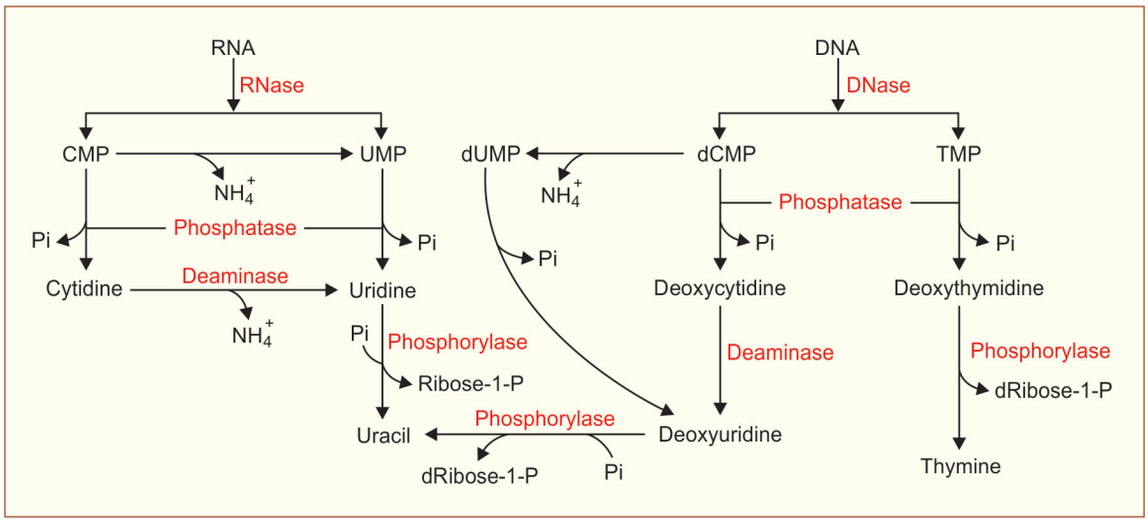

Fig. 7.29: Degradation of pyrimidine nucleotides

Chemistry to Clinics 7.8: β-Aminoisobutyric Acid

β-Aminoisobutyric acid is excreted in the urine and is a measure of turnover of DNA or thymine nucleotides. Its urinary excretion is increased in cancer patients undergoing chemotherapy or radiation therapy, due to the increased degradation of DNA.

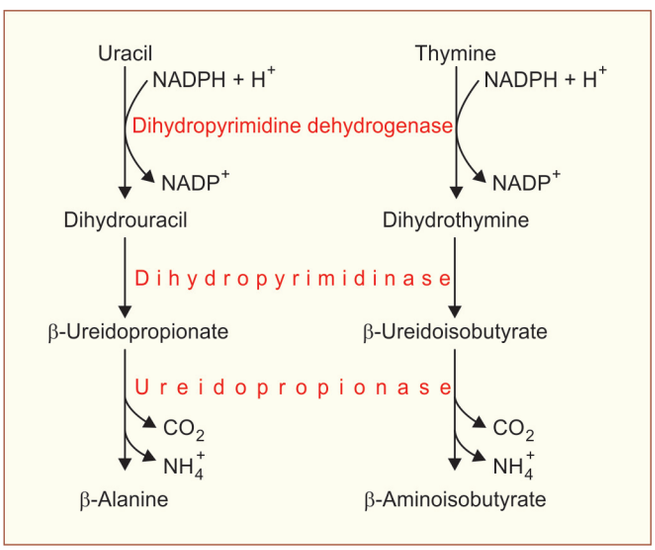

Fig. 7.30: Degradation of pyrimidine bases

CONVERSION OF RIBONUCLEOTIDES TO DEOXYRIBONUCLEOTIDES

Purine and pyrimidine ribonucleotides formed by the respective ***de novo*** pathways, are converted to their deoxyribonucleotides during **DNA replication**.

- Ribonucleoside diphosphates (NDPs), i.e. ADP, GDP, CDP and UDP are reduced at C2′ position to their deoxy forms, i.e. dADP, dGDP, dCDP and dUDP by ribonucleotide reductase (ribonucleoside diphosphate reductase). **Thioredoxin** (a peptide containing two cysteine residues) is used as a source of reducing equivalents. Reduced form of the coenzyme (reduced thioredoxin) gets oxidized and forms a disulfide bond.

  Mammalian ribonucleotide reductase is a heterodimer. The enzyme is under allosteric regulation where the products serve as potent negative effectors of the enzyme. dATP is a potent inhibitor of the reduction of all the four nucleoside diphosphates (CDP, UDP, GDP and ADP). dGTP inhibits the reduction of CDP, UDP and GDP while dTTP inhibits the reduction of CDP, UDP and ADP.
- The oxidized thioredoxin is then reduced by thioredoxin reductase which is a flavoprotein and contains FAD as a prosthetic group. NADPH + H^+ serves as the terminal reducing agent (Fig. 7.31).

  In place of thioredoxin, **glutaredoxin** can also be used which requires glutathione and glutathione reductase.
- Phosphorylation of dNDP produces dNTP which, in turn, is used in the biosynthesis of DNA. The reaction is catalyzed by nucleoside diphosphate kinase. Any of the NTP or dNTP, such as ATP, can function as the phosphoryl donor for this reaction (Fig. 7.32).

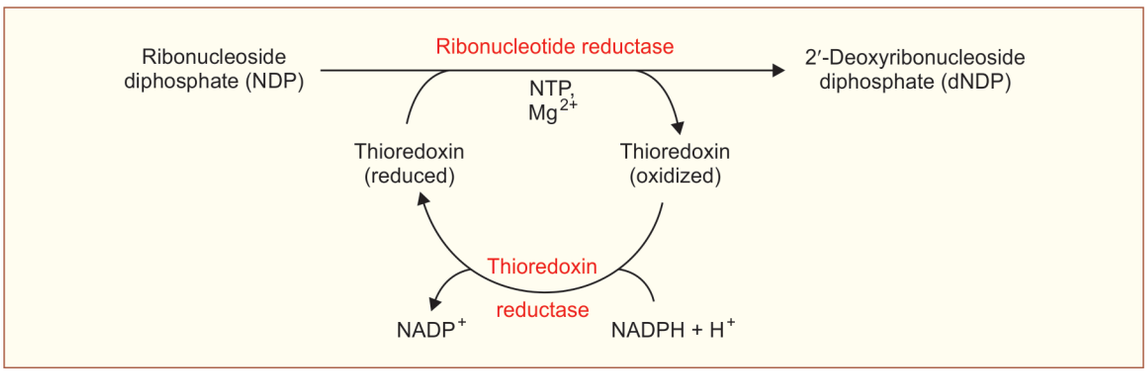

Fig. 7.31: Conversion of a ribonucleotide to deoxyribonucleotide

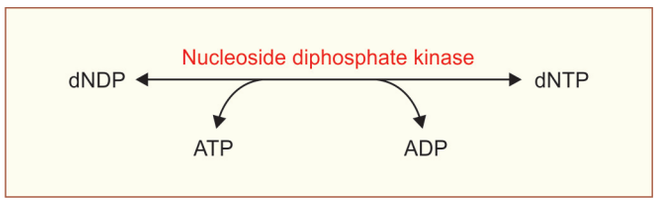

Fig. 7.32: Interconversion of dNDP and dNTP

ANALYTES ASSOCIATED WITH GOUT AND LESCH-NYHAN SYNDROME

As discussed above, Lesch-Nyhan syndrome is due to the deficiency of the enzyme HGPRTase, which, in turn, results in the accumulation of purines that are further degraded to **uric acid**. Thus, uric acid is, finally, the **analyte associated with gout** and **Lesch-Nyhan syndrome**.

Uric acid is sparingly soluble in an aqueous medium. **Normal serum uric acid concentration** is between **3 and 7 mg/dl**. Males have physiologically higher values because of higher rate of tubular urate reabsorption, and the uricosuric effect of estrogen in females.

Hyperuricemia

Elevated level of uric acid in the serum (>7 mg/dl, called hyperuricemia) is often asymptomatic. However, it may result in its deposition as sodium urate crystals primarily in the joints, in a clinical condition, called gout (Chemistry to Clinics 7.9).

There are two types of hyperuricemia:

- **Primary hyperuricemia:** This is due to some metabolic defect that results in increased synthesis of purine nucleotides via ***de novo*** pathway, such as:
  1. ***Increased PRPP synthetase activity:*** This results in increased intracellular concentration of PRPP which acts as a positive effector of the enzyme amidotransferase. This, in turn, increases *de novo* synthesis of purine nucleotides as well as their subsequent degradation to uric acid.
  2. ***HGPRTase deficiency:*** Deficiency of HGPRTase, such as in **Lesch-Nyhan syndrome**, causes a decrease in the salvage pathway of hypoxanthine and guanine to reform nucleotides. This, in turn, spares PRPP and results in overproduction of purine nucleotides and their degradation to uric acid.
  3. ***GIucose-6-phosphatase deficiency*:** GIucose-6-phosphatase deficiency, also known as **glycogen storage disease I** or **von Gierke's disease**, results in increased levels of glucose-6-phosphate which is diverted to HMP shunt. This, in turn, increases availability of ribose-5-phosphate and enhances the generation of PRPP. Raised levels of PRPP, increase ***de novo*** synthesis of purine nucleotides and the overproduction of uric acid. In addition, associated lactic acidosis elevates renal threshold for urate which further leads to hyperuricemia.

Chemistry to Clinics 7.9: Gout

Gout is characterized by elevated levels of uric acid in the blood (**hyperuricemia**) and its increased excretion in the urine (**uric aciduria**) due to a variety of metabolic abnormalities.

Most of the clinical conditions with hyperuricemia arise due to the poor aqueous solubility of uric acid. As a result of it sodium urate crystals are deposited in joints of the extremities (**gouty arthritis**) and/or in the renal interstitial tissue (**gouty nephropathy**). The crystals activate the complement pathway resulting in the recruitment of neutrophils, macrophages, release of inflammatory mediators and free radicals. In the affected joint, hyaluronic acid is reduced both due to decreased production and increased degradation. Hyperuricemia is, thus, associated with recurrent attacks of painful arthritic joints and inflammation, most often of big toe caused by the deposition of insoluble sodium urate crystals, called **tophi** (Fig. 7.33).

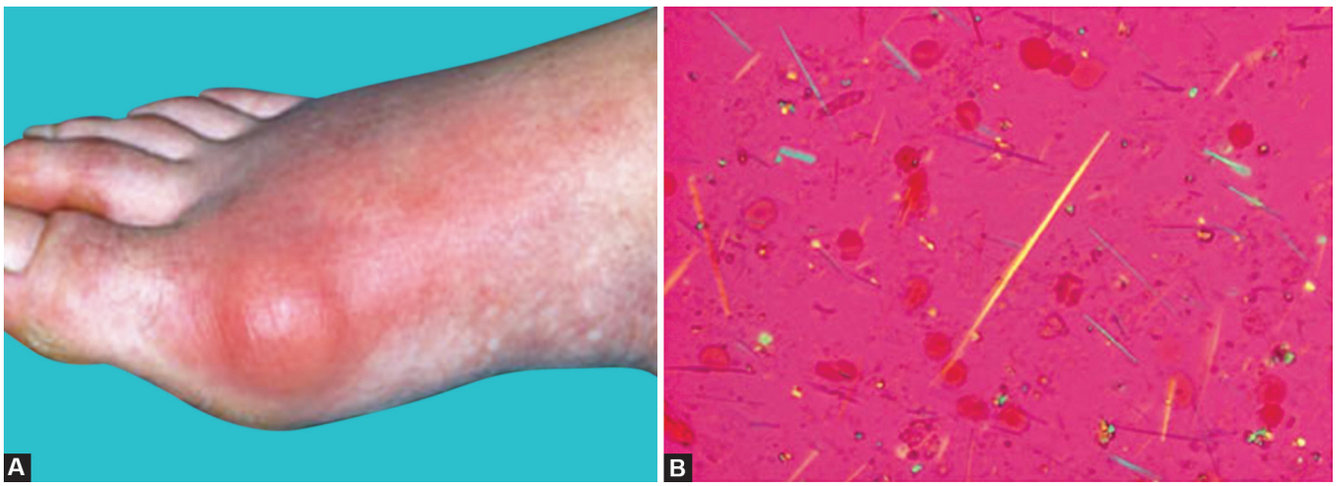

Fig. 7.33: Gout. (A) The condition most commonly affects the great toe (first metatarsophalangeal joint) when it is also called podagra; (B) Under polarized microscope, negatively birefringent needle-shaped monosodium urate crystals of various sizes and colors are seen in the joint aspirate

- **Secondary hyperuricemia:** It is secondary to some disease other than a metabolic disorder, such as a kidney disease or a disease associated with excessive cell turnover and increased breakdown of nucleic acids, e.g. radiation therapy/cancer chemotherapy, or psoriasis.

A better way of classifying hyperuricemia is based on its pathophysiology, i.e. overproduction of uric acid (e.g. in rapidly multiplying tumors, cancer chemotherapy), underexcretion (e.g. in lactic acidosis, diabetic ketoacidosis), or both (e.g. in alcoholism, von Gierke's disease).

Treatment: Most effective treatment of gout includes the use of **inhibitor of xanthine oxidase**, such as allopurinol or its metabolite alloxanthine. Allopurinol decreases both uric acid formation as well as ***de novo*** synthesis of purine nucleotides.