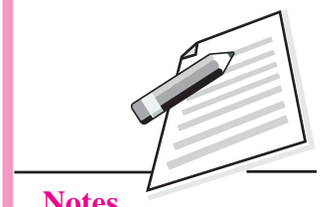


6

NUCLEOTIDES

6.1 INTRODUCTION

Nucleotides play major important roles in cellular metabolism. They are very essential for chemical links in the response of cells to hormones and other extracellular stimuli. They also act as the structural components of an array of enzyme cofactors and metabolic intermediates. Most importantly they are the constituents of nucleicacids: deoxyribonucleic acid (DNA) and ribonucleicacid (RNA). Nucleic acids are the molecular repositories of genetic information. The structure of every protein, and ultimately of every biomolecule and cellular component are programmed in form of nucleotide sequence of a cell's nucleic acids. The ability of nucleic acids to store and transmit genetic information from one generation to the next is a fundamental condition for life.

This chapter provides an overview of the structure of nucleic acids, chemistry of nucleotides, nucleotide metabolism, function of nucleic acid and diseases of error in nucleotide metabolism with special reference to gout.

OBJECTIVES

After reading this lesson, you will be able to:

- describe the chemistry of nucleotides
- describe the structure of nucleic acids
- explain the characteristics of nucleic acids
- describe the nucleotide metabolism
- enlist the functions of nucleic acid

6.2 STRUCTURE AND CHEMISTRY OF NUCLEIC ACIDS

In all living organisms the amino acid sequence of every protein and the nucleotide sequence of every RNA, is specified by a nucleotide sequence in the cell's DNA. Segment of DNA molecule that encodes a protein or RNA, is referred to as a gene.

6.2.1 Chemistry of nucleic acid

Nucleotides are organic compounds that are monomeric units of nucleic acids,they are of different types and structures and have variety of physiological functions. Nucleic acids are responsible for storage and transmission of genetic information. Nucleic acids are chemically composed of polymers of nucleotides joined together by phosphodiester linkages (bonds). Nucleic acids are broadly divided into two major types; Ribonucleic acid (RNA) which is single stranded containing Adenine (A), Uracil (U), Cytosine (C) and Guanine (G) ribonucleotides and deoxyribonucleic acid which is double stranded containing Adenine, Thymine (T), Cytosine and Guanine deoxyribonucleotides.

6.2.1.1 Nucleosides

The nitrogenous bases and pentose sugars associated structure gives a compound called nucleoside. Based on the type of nitrogenous base and the types of sugar it is liked to, different types of nucleotides are formed each having its own characteristic and structure.

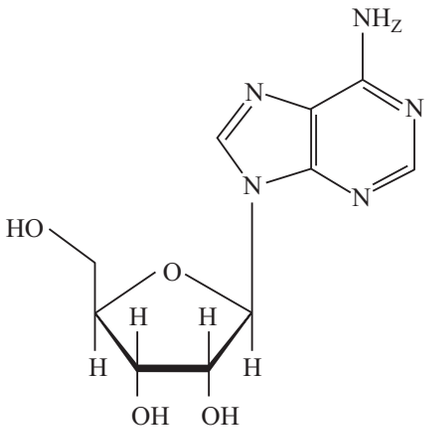


Fig. 6.1

Purines and pyrimidines are the components of nitrogenous bases. The purine bases contains the purine ring (double ringsystem) while the pyrimidine base contain pyrimidine ring (single ring structure). The purine bases include Adenine

and Guanine. The unusual forms of purines are hypoxathine, 1 methylguanine, 1 methylhypoxanthine etc. While the pyrimidine includes cytosine, thymine anduracil and its unusual forms are 5-methylcytosine, Thiouracil etc.

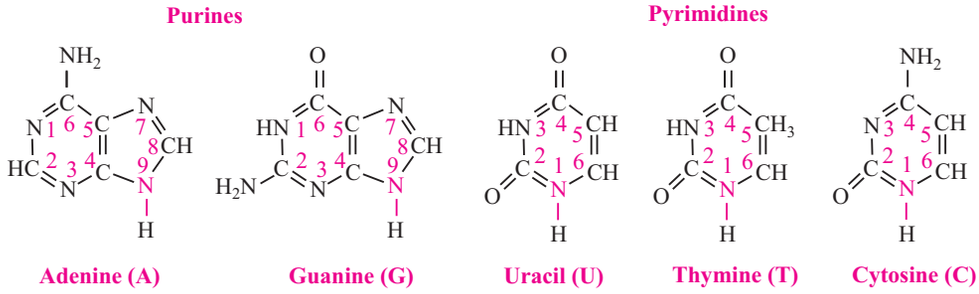


Fig. 6.2

In nucleosides, nitrogenous bases are joined to pentose sugar through the hemiacetal hydroxyl group on the C-1 (first carbon atom of the sugar). The purines are attached to the sugar through the N-9 nitrogen atom while pyrimidine are attached through the N-1 nitrogen atom.

6.2.1.2 Nucleotides

Nucleotides are phosphoric acid esters of nucleosides. Nucleotides contain nitrogenous bases, sugars and phosphoric acids in ester linkage. The nitrogenous base, presentin nucleotides are purines: Adenine and Guanine; pyrimidines: Cytosine,Thymine and Uracil. The uracil can only be found in ribonucleotides while thymine base can only be found in deoxyribonucleotides.

The sugar in nucleotides is the pentose sugar which could be ribose and deoxyribose. Sugar are esterified to a phosphoric acid residue at positions (2, 3 or 5) in ribose and (3 or 5) in the deoxyribose where the ester bonds could be formed. In addition, the nucleotides could be in form of mono,di and triphosphates.

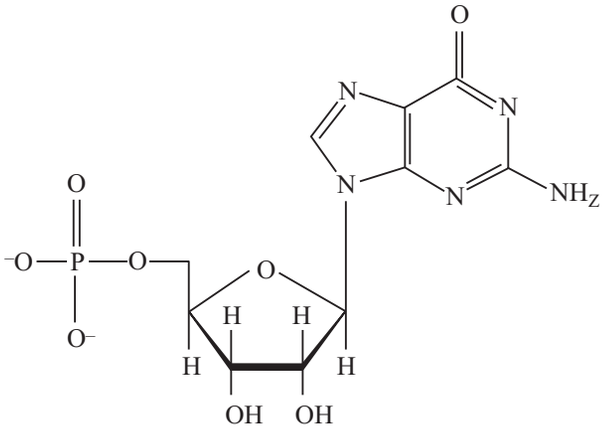
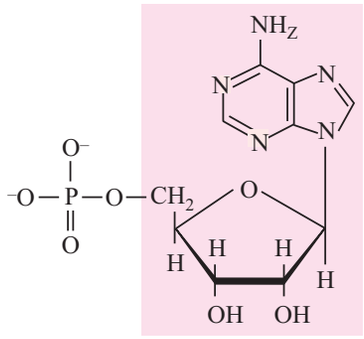


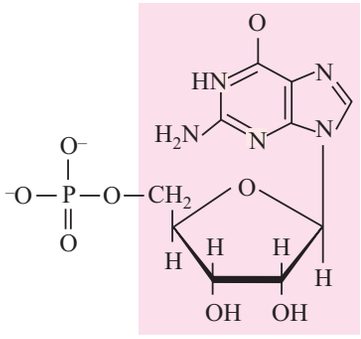
Fig. 6.3



Nucleotide: Deoxyadenylate (deoxyadenosine 5′-monophosphate)

Symbols: A. dA. dAMP

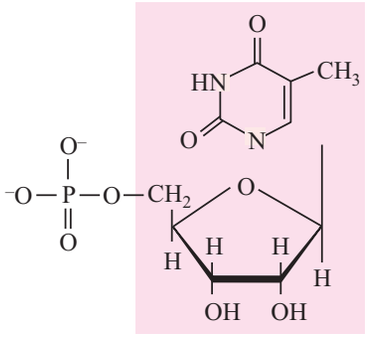
Nucleoside: Deoxyadenosine



Deoxyguanylate (deoxyguanosine 5′-monophosphate)

G. dG. dGMP

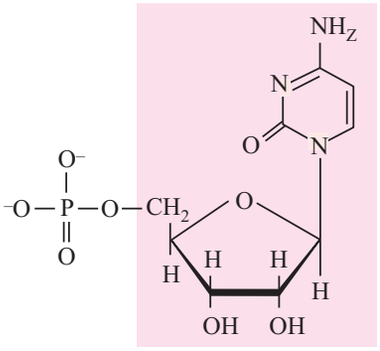
Deoxyadenosine



Deoxythymidylate (deoxythymidine 5′-monophosphate)

T. dT. dTMP

Deoxythymidine

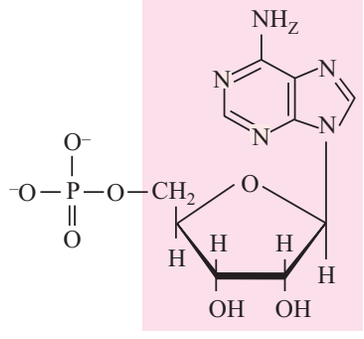


Deoxycytidylate (deoxycytidine 5′-monophosphate)

G. dG. dGMP

Deoxycytidine

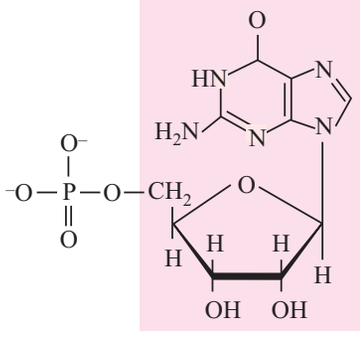
(a) Deoxyribonucleotides



Nucleotide: Adenylate (adenosine) 5′-monophosphate

Symbols: A. AMP

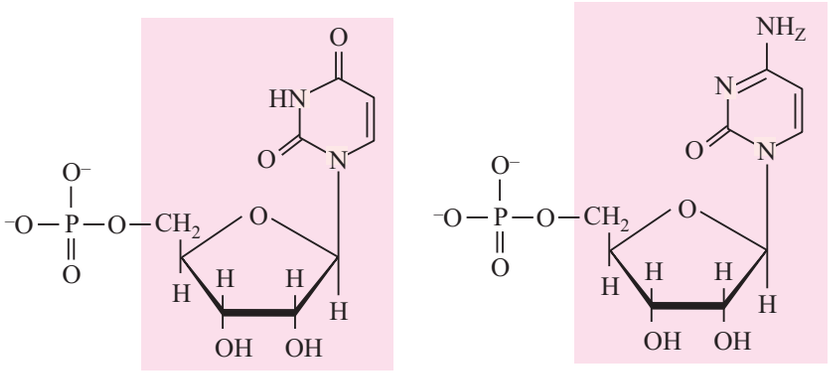
Nucleoside: Adenosine



Guanylate (guanosine 5′-monophosphate)

G. dGMP

Deoxyadenosine



Uridylate (uridine 5′-monophosphate)
U. UMP
Uridine

Cytidylate (cytidine 5′-monophosphate)
C. CMP
Cytidine

(b) Ribonucleotides

Fig. 6.4

Compound that have their structures derived from nucleotide structures are called as nucleotide derivatives. They share close structural features of nucleotides. Nicotinamide Adenine dinucleotide (NAD), Nicotinamide Adenine dinucleotide phosphate (NADP), flavine adeninedinucleotide (FAD) are some of the examples for nucleotide derivatives.

INTEXT QUESTIONS 6.1

1. Segment of DNA molecule that encodes a protein is called as .....
   (a) RNA (b) gene (c) interon (d) operon
2. Nucleotides are organic compounds that are monomeric units of .....
3. The nitrogenous bases and ..... associated structure gives a compound called nucleoside
4. ..... are phosphoric acid esters of nucleosides

6.2.1.3 Structure of different types of DNA

The primary structure of a nucleicacid is based on nucleotide sequence. Any regular, stable structure taken up by some or all of the nucleotides in a nucleic acid can be referred to as secondary structure. The complex folding of large chromosomes within eukaryotic chromatin and bacterial nucleoids is generally considered tertiary structure. The structure of DNA was worked out by bringing together a number of observations from various sources such as

(a) That DNA from different sources have remarkable similarity in their Xray diffraction patterns; suggesting that DNA molecules have uniform molecular pattern and consists polynucleotide chains arranged in helical structure.

(b) The ratio of the bases (A: T and C: G) is very close to one. In base pairing event A and T can be paired with a maximum of two hydrogen bonds between them while C and G will have a maximum of three bonds.

(c) The long polynucleotide chains were held together through bonds between these residues.

Using these observations, Watson and Crick constructed a model of DNA structures in 1953.

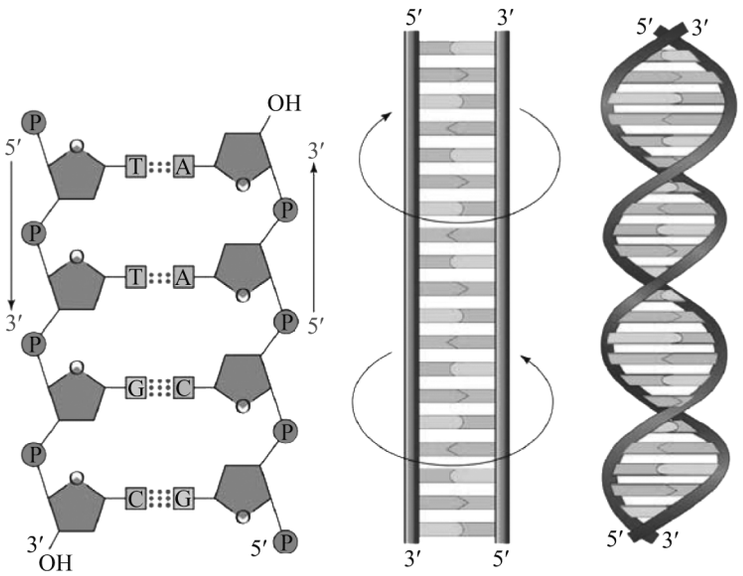
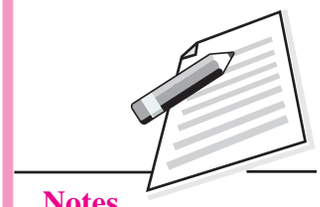


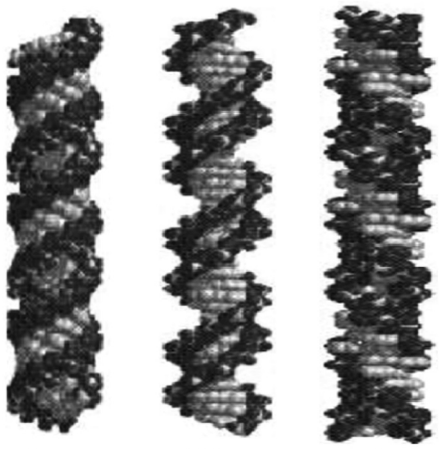
Fig. 6.5

The Watson-Crick Model consists of sugar molecule joined together by phosphate diesters. Bases were observed to be projecting perpendicularly from the chains into the central axis. For each adenine projecting inwards, there is a corresponding thymine from the other chain and for each cytosine, there is a guanine. A-T and C-G are held together by two and 3 hydrogen bonds respectively. The two chains are however not identical because of base pairings. The chains donot run in the same direction with respect to linkage between the nucleotides, rather they are anti-parallel.

Structurally DNA exists as three different forms namely: A, B and Z form. The A form of DNA posses' right handed helix with a diameter of 26Å. The A form of DNA contains 11 base pair per helix turn and base turns rise of helix is 2.6Å. The B form of the DNA is considered as Watson-Crick DNA structure. In B form, the DNA helix is arranged as left handed. The B form of DNA contains 0.34 nm



between bp, 3.4 nm per turn, about 10 bp per turn1.9 nm (about 2.0 nm or 20 Angstroms) in diameter. The Z form of DNA is a more radical departure from B-DNA with left handed helical rotation. Ion Z form of DNA each helical turn consist of 12 base pairs. Structures appear to be more slender and elongated.



A Form B Form Z Form

Fig. 6.6

Comparison between different forms of DNA

Character	A form	B form	Z form
helix sense	Right handed	Right handed	Left handed
bp per turn	10	11	12
vertical rise per bp	3.4 Å	2.56 Å	3.7Å
rotation per bp	+36°	+33°	+30°
helical diameter	19 Å	23 Å	18 Å

INTEXT QUESTIONS 6.2

1. The primary structure of a nucleic acid is based on ..... nucleotide sequence
2. In base pairing event A and T can be paired with a maximum of two hydrogen bonds

   (a) 1 (b) 2 (c) 3 (d) 4
3. Watson and Crick constructed a model of DNA structures in the year 1953
4. Structurally DNA exists as three different forms namely .....

Notes

6.2.2 Structure of different types of RNA

RNA in solution is dynamic molecules in solution. They undergo changes in conformation during synthesis, processing and functioning. RNA (Ribo nucleic acid) molecule exist as three different forms namely,

(a) Primary structure

(b) Secondary structure

(c) Tertiary structure

Unlike DNA, RNA molecules in primary structure are single stranded linear polymer containing nucleotides joined by phosphodiester linkage. The linkage of the ribonucleotides in RNA is 3'5' phosphodiester link involve 3'-OH group of ribose and 5'-phosphate group of another ribonucleotide. In eukaryotes the length of RNA molecule ranges from 65 nucleotides to 6000 nucleotides.

In secondary structure the single stranded RNA molecule may have double helical regions formed by hydrogen bonding between complimentary base sequences within the RNA molecule. In tertiary structure the association of RNA with proteins enables the RNA molecule to be stable and also fold into specific conformations. The "L shaped" conformation of the tRNA conformations held in position not only by the base pairing interactions but also other interactions.

RNA exist mainly as 3 major types namely:

(a) mRNA

(b) tRNA

(c) rRNA

6.2.2.1 Messenger RNA (mRNA)

The structure of messenger RNA (mRNA) especially the eukaryotic mRNA has some unique. The 5'terminus of mRNA is "capped" with a methylated base of Guanosine 5' triphosphate. The methylation is on the 2'-hydroxyl group of the ribose sugar. The methylated capping is followed by a non translated or "leader" sequence. The leader sequence is followed by an initiation codon, most often AUG. Coding region of mRNA are terminated by stop codon, usually UAA, UAG, UGA. The stop codon are followed by a second non translated sequence at the 3' end. A series of adenylic acids called poly A tail which makes up 3' terminus of the mRNA molecule.

6.2.2.2 Transfer RNA (tRNA)

The length of tRNAs ranges from 65-110 nucleotides with a corresponding molecular weight of 22, 000-37500 Daltons. The tRNA is a single stranded. As a result of intramolecular hydrogen bonding the 5' to 3' nucleotide stretch folded

into a conformation and forms different loops. The various loops are: the D-loop, anticodon loop, variable loop or arm, TøC loop and the acceptor system, each having its own function.

6.2.2.3 Ribosomal RNA (rRNA)

Ribosomal RNA are found associated with large number of proteins in an ordered complex. Ribosomal RNA has a helical structure resulting from the folding back of single stranded polymer and constitute about 74-80% of total RNA in a cell.

INTEXT QUESTIONS 6.3

1. RNA molecules in primary structure are single stranded linear polymer containing nucleotides joined by ..... phosphodiester linkage
2. The three types of RNA are .....
3. The 5'terminus of mRNA is "capped" with a methylated base of .....
   (a) Guanosine 5' triphosphate (b) Adenosine 5' triphosphate
   (c) Cytocine 5' triphosphate (d) Thyamine 5' triphosphate
4. The length of tRNAs ranges from ..... to ..... nucleotides

6.2.3 Characteristics of nucleic acids

(a) The components of nucleic acids are nucleotides joined by phosphodiester linkage to one another.

(b) Based on the type of sugar in nucleic acids, nucleic acids can either be deoxyribonucleic acids in which case they contain deoxyribose sugar or ribonucleic acids when the sugar is a ribose one.

(c) The double stranded forms of both DNA or RNA can be denatured.

(d) DNA is highly viscous at pH 7.0 and room temperature (25°C).

(e) At extreme pH or temperature above 80°C the viscosity of DNA decreases sharply, which is an indicator of physical change.

(f) The denaturation of double helical DNA associated with the disruption of hydrogen bonds between the paired bases is called melting of the.

(g) The temperature midpoint in the transition is called melting temperature (tm).

(h) For nucleic acids the marked absorption in the ultraviolet (UV) region of the lights pectrum with Absorption maxima is near 260nm.

INTEXT QUESTIONS 6.4

1. The components of nucleic acids are nucleotides joined by .....
2. ..... is highly viscous at pH 7.0
3. At temperature above ..... the viscosity of DNA decreases
4. The Absorption maxima of nucleic acid is near .....

6.2.4 Metabolism of Nucleotides

Nucleotide metabolism involves several interconnected pathways. Nucleotides can be synthesized de novo, or from components "salvaged" from the degradation products of nucleic acids. When in excess, nucleotides are degraded to products that can either be consumed by other pathways or excreted. Defects in the pathways for de novo synthesis, salvage, and degradation of nucleotides result in clinical disorders, and many drugs target these pathways.

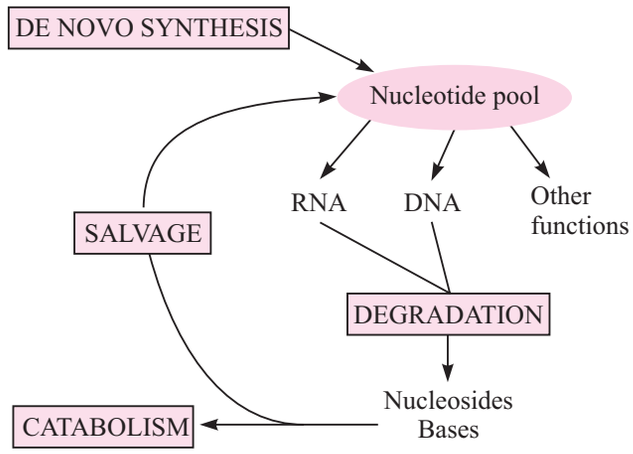
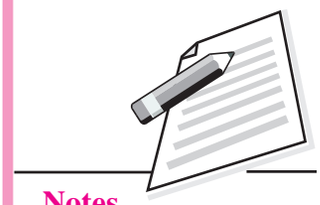


Fig. 6.7

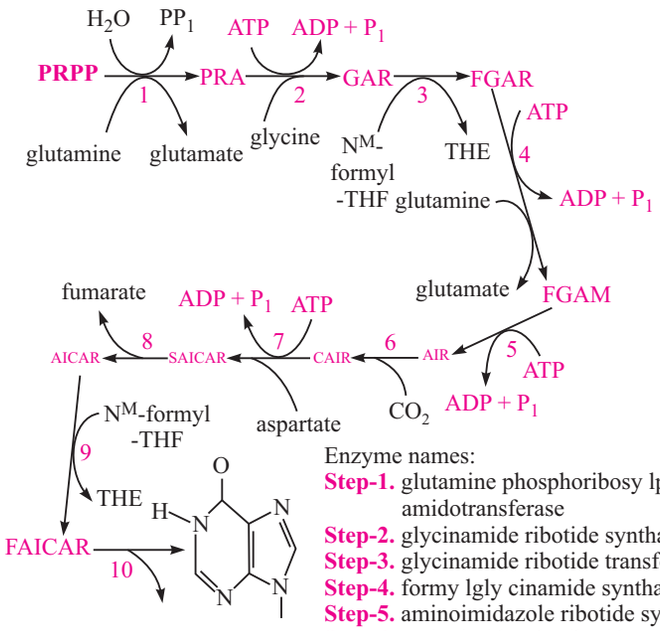
Nucleotide anabolism can be broadly characterized as purine and pyrimidine biosynthesis.

6.2.4.1 Biosynthesis of purine nucleotides

Purine nucleotides are synthesized in cytoplasm of most of the tissues. The major site for purine synthesis is liver. Since purine ring are synthesized from different



small components, they are makorly denoted by de novo synthesis. Different sources such as respiratory CO_2, amino group from aspartate, formyl group, amide group from glutamine and glycine etc., are required for formation of purine ring. As a primary requirement of purine synthesis, purine ring are first built upon a ribose-5-phosphate molecule. De novo synthesis of purine is a multi enzyme reaction composed of 10 steps as follow as



Enzyme names:

Step-1. glutamine phosphoribosy lpyrophosphate amidotransferase
Step-2. glycinamide ribotide synthase
Step-3. glycinamide ribotide transformylase
Step-4. formy lgly cinamide synthase
Step-5. aminoimidazole ribotide synthase
Step-6. aminoimidazole ribotide carboxy lase
Step-7. succiny laminoimidazolecarboxamideribotide synthase
Step-8. adenylosuccinate lyase
Step-9. aminoimidazole carboxamide ribotide transformy lase
Step-10. IMP cyclohy drolase

Fig. 6.8

In salvage pathway purines were recycled from degraded nucleotide. Both nucleosides and deoxy-nucleosides can be salvaged. Phospho ribosyl phyrophosphate (PRPP) is the starting material for salvage pathway. In salvage pathway purines are salvaged by adenine phosphor ribosyl transferase (APRTase) and hypoxanthine guanine phosphoribosyl transferase (HGPRTase). Salvage pathway are encountered in tissues such as RBC and brain, where there is absence of de novo pathway.

6.2.4.2 Disorders of purine metabolism

The most common abnormality of purine metabolism is elevation of uric acid level in blood. Elevated level of uric acid in blood is called as hyper-uricemia. In hyperuricemia the serum uric acid level exceeds the level of 7 mg/dl for male

Notes

and 6 mg/dl in female. This increased uric acid may or may not be excreated via urine and the condition is called as uricosuria.

Gout

In gout the urate crystals accumulates in synovial fluid. The above condition leads to inflammation mediated acute arthritis. The solubility of uric acid is lowered to 4.5 mg/dl at 30°C. Thus the uric acid is deposited in cooler zone such as tophi. Deposition of uric acid crystrals are noticed with increase in excretion of uric acid in urine. The deposition of uric acid crystals leads to calculi or stone formation. The gout may be either primary or secondary in nature

Primary gout

Primary gout may show familial incidence and are about 1:500 in total population. About 10 % of primary gout are idiopathic in nature. The main cause of primary gout is because of error in synthesis of enzymes such as

(a) 5-phosphoribosyl amido transferase

The error in 5-phospho ribosyl amido transferase leads to over production of purine nucleotides. Even though the abnormal nucleotide is active, they are not sensitive to feedback inhibition by inhibitory nucleotides.

(b) Phospho ribosyl phyrophosphate (PRPP) synthetase

The abnormal leads to increase and accumulation of PRPP and are X-linked recessive in inheritance.

(c) Glucose-6-phosphatase

Deficiency in glucose-6-phosphatase leads to a glycogen storage disease called as Gierke's disease. In this case more glucose is channeled to the pentose-phosphate shunt pathway, leading increased availability of ribose5-phosphate. As a result of this there will be a increase in PRPP formation.

(d) Glutathione reductase

Abnormality in glutathione reductase leads to increased production of ribose-5-phosphate and thereby increases in PRPP.

Secondary gout

Secondary gout is characterized by increase in uric acid production and reduced excretion rate. The increase in uric acid is due to increase in turnover rate of nucleic acids. The increase in nucleic acid turnover rate are seen in

(a) Rapidly growing malignant tissues

(b) Increase in tissue breakdown after treatment of large malignant tumors

(c) Increase in tissue damage due to trauma

(d) Increase in rate of catabolism as in starvation

Reduction in excretion rate as encountered in secondary gout during

(a) Renal failure

(b) Treatment with thiazide diuretics

(c) Interfrence of tubular secretion due to lactic acidosis and keto-acidosis.

6.2.4.3 Pyrimidine synthesis

Purines are synthesized by building the ring system on the ribose. In contrast, the pyrimidine ring is constructed first, followed by attachment of the pyrimidine base to ribose using a phosphoribosyltransferase similar to those used for purine salvage reactions. In both purine and pyrimidine synthesis, PRPP is used as the ribose donor, but the stage of the pathway is different.

The first step of the pyrimidine synthesis pathway is the condensation of bicarbonate with nitrogen derived from glutamine to form carbamoyl phosphate. The enzyme involved is **carbamoyl phosphate synthetase II** and is different from the enzyme catalyzing the equivalent step in the urea cycle.

Carbamoyl phosphate synthetase II has three **major differences**:

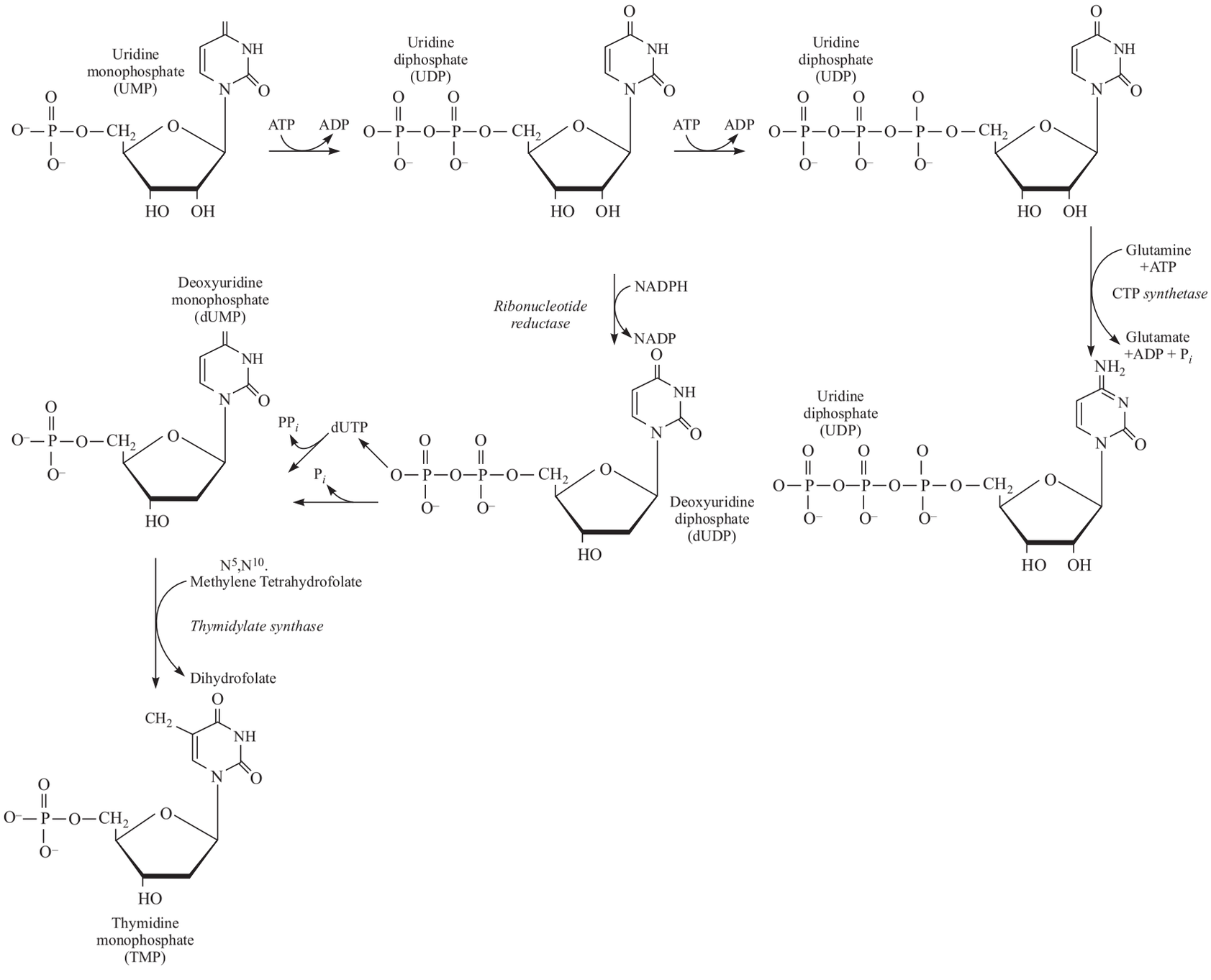
1. It uses nitrogen from glutamine rather than from ammonium
2. It is a cytosolic rather than a mitochondrial enzyme
3. Its regulation is completely different.

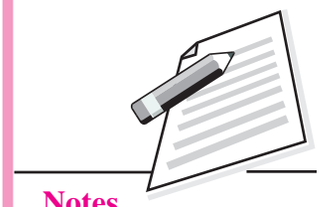
In animals two separate pools of carbamoyl phosphate were noticed, they are

1. Mitochondrial pool, used for the urea cycle
2. Cytosolic pool, used for pyrimidine synthesis.

While in bacteria a single pool of carbamoyl phosphate was used for both purposes, and therefore their pathways are regulated slightly differently. The pyrimidine ring skeleton comes from two molecules, the carbamoyl phosphate from the first step, and the aspartate added in the second step.

The ribose ring is not added until the synthesis of the pyrimidine orotic acid is complete. This orotic acid is then attached to PRPP with release of pyrophosphate. UMP is the first "completed" product. UMP can then be phosphorylated to produce UDP. UDP acts as a branch point; it can be converted to UTP and used as a nucleotide, or it can serve as a substrate for the synthesis of the two other major pyrimidine nucleotides. Both CTP and TTP were synthesized as described in the following reaction.


Uridine monophosphate (UMP)
ATP
ADP
Uridine diphosphate (UDP)
ATP
ADP
Uridine diphosphate (UDP)
Glutamine +ATP
CTP synthetase
Glutamate +ADP + Pi
Deoxyuridine monophosphate (dUMP)
Ribonucleotide reductase
NADPH
NADP
PPi
dUTP
Pi
Deoxyuridine diphosphate (dUDP)
Uridine diphosphate (UDP)
N5,N10. Methylene Tetrahydrofolate
Thymidylate synthase
Dihydrofolate
Thymidine monophosphate (TMP)



INTEXT QUESTIONS 6.5

1. Nucleotide anabolism can be broadly characterized as ..... biosynthesis
2. Purine nucleotides are synthesized in ..... of most of the tissues and major site is .....
3. In salvage pathway purines both ..... and ..... can be salvaged
4. The most common abnorbality of purine metabolism is ..... in blood
5. In gout the ..... accumulates in synovial fluid

6.2.5 Functions of nucleic acids

Both structurally and chemically, the nucleic acids differ from other macromolecule found in living systems and poses their roles and functions. Nucleic acid itself exist as two different forms namely DNA and RNA, both differ chemically and structurally.

6.2.5.1 Functions of DNA

(a) DNA acts as a genetic material.

(b) It is worthwhile emphasizing that the main role of the DNA is storage and transmission of genetic information from parents to offsprings or from one generation to another.

(c) DNA is capable of undergoing replication (synthesis of another copy of DNA) and being transcribed into RNA (transcription).

(d) These two processes enables the genetic information encoded in the DNA found in the nucleus to be transformed into a functional biological material e.g protein in the cytoplasm.

6.2.5.2 Functions of RNA

(a) RNA, like DNA has shown to be a general constituent of prokaryotic and eukaryotic cells.

(b) RNA molecules are not as stable as DNA, they also serve as genetic information carrier in some organisms e.g some viruses where RNA is their genetic material.

(c) The main functions of tRNA include: Transportation of specific amino acids to the ribosome's decoding the genetic information in the messenger RNA in terms of the proper amino acid to be inserted in the sequence of protein/polypeptide synthesised.

Notes

(d) tRNA carries one amino acid and also possess an anticodon by which it recognizes the message on the mRNA template during protein synthesis.

(e) Transfer RNAs have two primary active sites, the 3'hydroxyl terminus to which specific aminoacid are attached covalently and the anticodon triplet.

(f) Ribosomal RNAs (rRNA) serve as a structural framework for the ribosomes. The hinging mechanism between the two ribosomal subunits enables translocation and mRNA movement.

(g) Messenger RNA (mRNA) are direct carriers of genetic information from the nucleus to the cytoplasmic ribosomes.

(h) Eukaryotic mRNA contains information for only one polypeptide and is therefore monocistronic whereas prokaryotic mRNA can contain information for more than one polypeptide chain and therefore designated polycistronic.

INTEXT QUESTIONS 6.6

1. Nucleic acid itself exist as two different forms namely ..... and .....
2. DNA acts as a .....
3. ..... are not as stable as DNA
4. ..... are direct carriers of genetic information from the nucleus to the cytoplasmic ribosomes

   (a) mRNA (b) rRNA (c) tRNA (d) DNA
5. Prokaryotic mRNA can contain information for more than one polypeptide chain and therefore designated .....

WHAT HAVE YOU LEARNT

- There are different types of nucleic acids, each having its own characteristic function.
- DNA has a structure different from RNA. Structurally there are 3 forms of DNA. Structures of mRNA, rRNA and rRNA have been described. RNA has primary, secondary and tertiary level structural organization.
- Nucleic acids are polymers of nucleotides. There are two types of nucleic acids, DNA and RNA.
- The DNA is further subdivided into three (3) forms namely: A form, B form and Z form.

- DNA can be denatured and renatured. DNA is highly condensed in the chromosome and its size can be determined under centrifugal field.
- RNA has a primary, secondary and tertiary structures. DNA and RNA have differences and similarities. DNA serves in storage and transmission of genetic material from one generation to another or from parents to offsprings.
- Messenger RNA carries genetic information from nucleus to the cytoplasm.
- Transfer RNA carries amino acids to the site of protein synthesis. Ribosomal rRNA serves as the structural framework of the ribosome.

TERMINAL QUESTIONS

1. Describe the structure of DNA
2. Write short notes on the mRNA and tRNA
3. Elaborate in detail about nucleotide metabolism
4. Give details on Gout.

ANSWERS FOR INTEXT QUESTIONS

6.1

1. Gene
2. Nucleic acids
3. Pentose sugars
4. Nucleotides

6.2

1. Nucleotide sequence
2. 2
3. 1953
4. A, B and Z form

6.3

1. Phosphodiester linkage

Notes

2. mRNA, rRNA and tRNA
3. Guanosine 5' triphosphate
4. 65 to 110

6.4

1. Phosphodiester linkage
2. DNA
3. 80°C
4. 260nm

6.5

1. Purine and pyrimidine
2. Cytoplasm and liver
3. Nucleosides and deoxy-nucleosides
4. Elevation of uric acid level
5. Urate crystals

6.6

1. DNA and RNA
2. Genetic material
3. RNA
4. Messenger RNA
5. Polycistronic