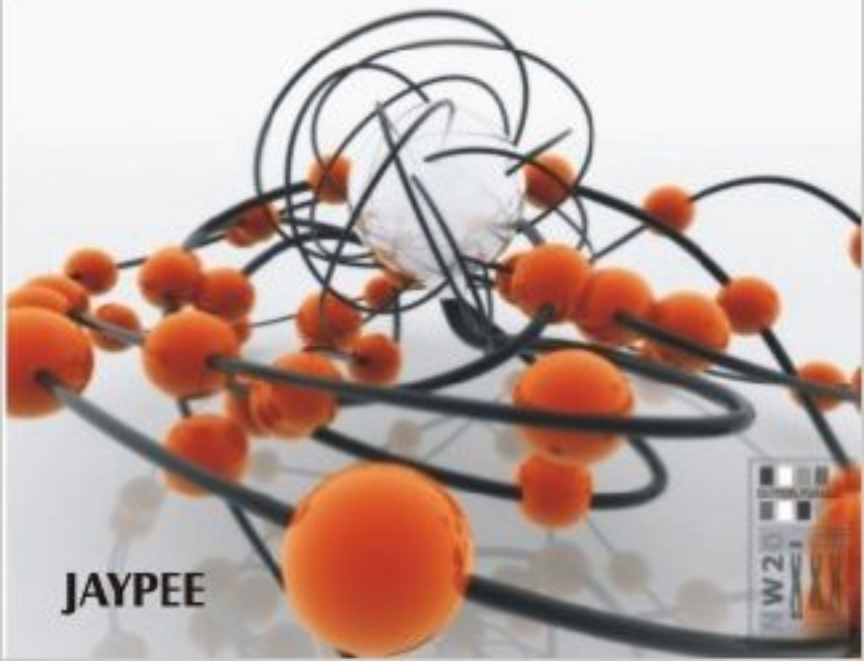
2nd Edition

Viva in Biochemistry

MN Chatterjea



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VIVA IN BIOCHEMISTRY

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SECOND EDITION

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Preface to the Second Edition

I feel it a great pleasure and satisfaction to present the second edition of *Viva in Biochemistry* (after a revised reprint in 2004), to my beloved students and esteemed teachers. The book got a good response from all quarters, some good words from different professors also came. As pointed out in preface of first edition, the book can be considered as a "companion" to my *Textbook of Medical Biochemistry*. I hope the book will be of great help before examination for quick and rapid revision.

The book has been revised, new questions and answers have been put forward. A new chapter on "Acid Base Balance and Imbalance" has been added.

I am confident that the second edition will fulfil the requirements of the undergraduates and postgraduates to some extent.

I sincerely thank to Shri Jitendar P Vij (Chairman and Managing Director), Mr PG Bandhu (Director-Sales), and Mr Tarun Duneja (Director-Publishing) of M/s Jaypee Brothers Medical Publishers (P) Ltd., New Delhi for their untiring work and keen efforts to bring out the new edition.

Dr (Brig) MN Chatterjea

Preface to the First Edition

The discipline of Biochemistry has expanded by leaps and bounds. It is now an independent and separate subject of examination for Ist year MBBS students, in all medical colleges. The Medical Council of India has revised the curriculum in Biochemistry, which is rather increased but at the same time reduced the time period. Now the students have to complete the syllabi in one year and face the university examination.

Viva-voce examination constitutes a sizeable portion of total marks in Biochemistry in university examination. This covers the questions asked not only related to theory but also the table-viva related to practical problems as well.

As a teacher and examiner in Biochemistry, I observed that the students are usually not confident enough in answering the questions accurately and to the point in viva-voce examination. The students are nervous and shaky while in appearing the viva examination. The reason is obvious, they have to study so much in too short a time!

Keeping these problems of the students in mind, I have attempted to prepare the book entitled *Viva in Biochemistry.* The book has been made as companion to my *Textbook of Medical Biochemistry*. I have attempted to prepare simple, short and accurate answers to the questions generally asked in viva-voce examination in Biochemistry. I am sure that by making appropriate use of this book, the students will be able to face the viva and answer any question asked, accurately and more confidently, to the complete satisfaction of the examiners.

I hope the efforts put in by me in preparation of the book will not go waste and the book will be appreciated and get a warm welcome from the students.

In spite of careful and scrupulous preparation, it is likely that a few mistakes might have crept in inadvertently. I shall welcome

fruitful suggestions and constructive criticisms from the teachers in Biochemistry and the students as well for betterment in future.

I am extremely grateful to Shri Jitendar P Vij (Chairman and Managing Director) and Mr RK Yadav, Editorial Consultant and staff members of M/s Jaypee Brothers Medical Publishers (P) Ltd. for their sincere, and untiring efforts to bring out the book.

Dr (Brig) MN Chatterjea

Contents

CHAPTER

1 Chemistry of Carbohydrates

Q.1. What are carbohydrates?

Carbohydrates are organic substances containing C, H and O. Hydrogen atoms are present usually in the ratio of 2:1 as it occurs in a water molecule. ***Example:*** glucose, fructose, lactose, starch etc. There may be exception to the above, e.g. $C_2H_4O_2$ is acetic acid and not a carbohydrate though H and O are in the ratio of 2:1.

Q.2. Define carbohydrates in chemical term.

Carbohydrates are defined chemically as aldehyde or ketone derivatives of the higher polyhydric alcohols or compounds which yield these derivatives on hydrolysis.

Q.3. How will you classify carbohydrates?

Carbohydrates are classified into *four major groups:*

- ***Monosaccharide ("simple sugars"):*** They cannot be hydrolyzed into simpler forms.
- ***Disaccharides:*** They yield two molecules of same or different monosaccharide units on hydrolysis.
- ***Oligosaccharides:*** They yield three to six molecules of monosaccharides on hydrolysis.
- ***Polysaccharides (glycans):*** They yield more than 6 molecules of monosaccharides on hydrolysis.

Q.4. How are monosaccharides further classified?

Monosaccharides are further classified into two groups depending on:

- The number of carbon atoms they possess, e.g. trioses, tetroses, pentoses, hexoses, etc.
- Whether aldehyde (-CHO) or ketone (-CO) group is present, e.g. aldoses, ketoses.

Q.5. Give an example of an aldohexose and a ketohexose which is of biological importance.

- Aldhexose: D-glucose
- Ketohexose: D-fructose

Q.6. How will you classify polysaccharides?

Polysaccharides are classified into two main groups:

- ***Homopolydisaccharides (hemoglycans):*** Polymer of same monosaccharide units, e.g. starch, glycogen, insulin, dextrins, cellulose, etc.
- ***Heteropolysaccharides (heteroglycans):*** Polymer of different monosaccharide units or their derivatives, e.g. mucopolysaccharides (MPS).

Q.7. What is an asymmetric carbon?

A carbon atom to which four different atoms or groups of atoms are attached is said to be an asymmetric carbon.

Q.8. What are the effects of presence of asymmetric carbon in a compound?

The presence of asymmetric carbon atoms in a compound produces the following effects:

- Gives rise to the formation of stereoisomers of that compound.
- Also confers optical activity to the compound.

Q.9. What are stereoisomers?

The compounds which are identical in composition and differs only in spatial configuration are called stereoisomers. Two such stereoisomers of glucose, D-glucose and L-glucose are mirror-images of each other.

Q.10. What are optical isomers?

When a beam of plane polarized light is passed through sugar solution exhibiting optical activity, it will be rotated to the right or left according to the type of the compound. Such compounds are called ***"optical isomers" (or "enantiomorphs").***

Q.11. Explain 'D' and 'L' forms of sugar and d(+) and l(–) sugars.

- D and L (capital letters) are used to represent configuration of the sugar molecules. The orientation of H and OH groups around the carbon atom just adjacent to the terminal primary alcohol group -CH_2OH, i.e. C

atom 5 in glucose determines the series. ***When the -OH group is on the right it belongs to D-Series*** and ***-OH Group is on the left it is a member of L-series.***

- d(+) and l(–) denote the nature of optical activity. When the plane polarized light is rotated to the right, the compound is called ***"dextrorotatory"*** or d(+) and when rotated to left, the compound is called ***"laevo-rotatory"*** or l(–).

Q.12. What is Vant Hoff's rule of n?

According to Vant Hoff's rule of 'n'; $\mathbf{2^n}$ gives the possible stereoisomers of that compound; where n represents the number of asymmetric carbon atoms in a compound.

Q.13. How many stereoisomers are formed by glucose?

Glucose has 4 asymmetric carbon atoms, hence as per Vant Hoff's rule of 'n'; glucose will have $2^n = 16$ stereoisomers; of which 8 belongs to D-series and 8 belongs to L-series.

Q.14. What are α and β forms of glucose?

When aldehyde group of C-1, on cyclization, condenses with alcoholic-OH group on C-5 on the same molecule, two different cyclic forms are produced. When -OH group on C-1 of cyclic form extends to right, it is called α-D-glucose and when -OH group extends to left, it is called β-D-glucose.

Q.15. What is an anomer and what is an anomeric carbon?

- After cyclization, the two cyclic compounds α and β have different optical rotation but they are not "mirror-image" of each other. Such compounds are called ***"anomers".***
- Carbon 1 after cyclization becomes asymmetric as it has new 4 different groups attached to it and is called ***"anomeric carbon".***

Q.16. What do you mean by mutarotation?

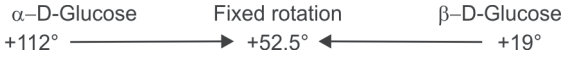
When an aldohexose is first dissolved in water and the solution is put in the optical path so that the plane-polarized light is passed, the initial optical rotation shown by the sugar gradually changes until a ***"constant fixed"*** rotation characteristic of the sugar is reached. ***This phenomenon of change of rotation is called mutarotation.***

Q.17. What is the mechanism of mutarotation? Explain.

Ordinary crystalline glucose is an α form. The mutarotation represents a conversion of α glucose when in solution to an equilibrium mixture of α and β forms. This involves the opening of the hemiacetal ring to form traces of the "free" aldehydeform which is transitory and recondensation to the cyclic forms again.

Q.18. What are the optical rotations shown by glucose in solution?

The glucose solution shows rotation according to its form: α form shows + 112º and β-form shows + 19º. When the solution has an equilibrium mixture of α and β forms, it shows fixed rotation of + 52.5°.



Q.19. What is meant by pyranose form?

The pyranose forms of the sugars are internal hemiacetals formed by combination of the aldehyde or ketone group of the sugar with the -OH group on the 5th carbon atom from the aldehyde or ketone group.

Q.20. What is meant by the furanose form of the sugar?

The furanose forms of the sugars are formed by reaction between the aldehyde or ketone group with the -OH group on the 4th carbon from the aldehyde or ketone group.

Q.21. What is an epimer? Give two examples.

- When two sugars differ from one another only in configuration of H and OH around a single carbon atom, they are called ***"epimers". Examples:*** Glucose and galactose are epimeric pairs which differ in orientation of H and OH groups on C-4.
- Similarly glucose and mannose are epimers in respect of C-2.

Q.22. What is epimerization? Give one example.

- The process by which one epimer is converted to other is called ***"epimerization"*** and it requires the enzyme" ***"epimerase".***
- ***Example:*** Conversion of UDP-glucose to UDP-galactose in liver (reversible reaction) by the enzyme "epimerase".

Q.23. What are osazones? What is its importance?

Osazones are crystalline derivatives of sugars. They have characteristic features:

- Melting points
- Crystal structures and
- Precipitation times.

Thus, they are valuable in identification of sugars.

Q.24. Describe the osazones of different sugars.

Different sugars form characteristic osazone crystals which can be seen under the microscope. Thus,

Osazones of	*Time taken*	*Appearance*
• **Glucose and fructose (same osazone)**	2-5 minutes	***"Haylike"*** structure (corn leaf life)
• **Maltose**	10-15 minutes	***Sunflower like***
• **Lactose**	10-12 minutes	***"Powder puff"*** like
• **Galactose**	7 minutes	Sunflower like but narrow blades

Q.25. How many molecules of phenyl hydrazine are required in the reaction and what are the by-products?

- Three molecules of phenylhydrazine react with the sugar molecule.
- **NH_3** and **aniline** are by-products in the reaction.

Q.26. Why galactose forms different osazone than glucosazone?

Structure of galactose differs on C-4 and this part of the molecule is unaffected in osazone formation. Hence, galactose forms different osazone from glucose and fructose.

Q.27. What are the oxidation products of glucose?

Oxidation products of glucose depends on type of oxidation:

- With mild oxidation with Br_2—water, it forms D-gluconic acid (aldonic acid)
- With strong oxidizing agent like conc-HNO_3, it forms dicarboxylic acid called D-glucaric acid (saccharic/ aldaric acid).

- If the -CHO group is protected and the primary -CH_2OH group is oxidized to COOH group, it forms D-glucuronic acid (uronic acid).

Q.28. What happens when D-galactose is oxidized with hot conc HNO_3?

Galactose on oxidation with hot conc HNO_3 acid produces dicarboxylic acid called ***Mucic acid***. The crystals of mucic acid have characteristic shape, hence, it can be used for indentification of galactose.

Q.29. What are the reduction products of glucose, mannose, galactose and fructose?

On reduction, the monoaccharides produce ***sugar alcohols.*** Thus,

- D-glucose → D-sorbitol
- D-galactose → D-dulcitol
- D-mannose → D-mannitol
- D-fructose → D-mannitol + D-sorbitol

Q.30. If glucose is dissolved in weak alkali such as $Ba(OH)_2$/ or $Ca(OH)_2$ and kept for some time, one can detect mannose and fructose in the solution. Explain.

Glucose, fructose and mannose are interconvertible in solutions of weak alkalinity such as $Ba(OH)_2$ or $Ca(OH)_2$. These interconversions is due to the fact that all the three give same ***Enediol*** form, which tautomerizes to all three sugars, the reaction is called ***Lobry de Bryan Van Ekenstein reaction***.

Q.31. Name one biological fluid which is rich in fructose. What is the source of fructose in this fluid and its importance?

Seminal fluid is rich in fructose.

- ***Source:*** It is formed from glucose in the seminiferous tubular epithelial cells.
- ***Importance:*** Spermatozoa utilizes fructose for energy.

Q.32. Describe the chemistry of reduction of Benedict's qualitative reagent by glucose and fructose.

Glucose/fructose having free -CHO/= CO group respectively undergo enolization in weak alkaline solution. ***The "enediol" forms of the sugars are highly reactive.*** The Cu^{++}/(ic) ions take electrons from the enediols and

oxidize them to sugar acids and are in turn reduced to Cu^{+} (ous) ions. Cu^{+}(ous) ions combine with -OH to form ***yellow cuprous hydroxide,*** which upon heating is converted to ***red cuprous oxide.***

Q.33. Name the ingredients present in Benedict's qualitative reagent and mention their functions.

- Benedict's Qualitative reagent contains $CuSO_4$, sodium citrate, sodium carbonate.
  Functions: Sodium citrate in the reagent prevents precipitation of cupric carbonate by forming soluble, slightly dissociable complexes which dissociate sufficiently to provide supply of readily available Cu^{++} (ic) ions for oxidation.
- Sodium carbonate, a weak alkali enolizes the sugars and thereby causes them to be strong reducing agents. ***Enolization is better in weak alkali than strong alkali.***

Q.34. What are aminosugars?

Sugars containing an NH_2 group in their structure are called aminosugars. The alcoholic OH group on carbon 2 is usually replaced by -NH_2 group.
Examples: D-glucosamine, D-galactosamine.

Q.35. What is the biomedical importance of aminosugars?

- N-acetyl derivatives of D-glucosamine and D-galactosamine occur as constituents of certain mucopolysaccharides (MPS).
- Certain antibiotics such as erythromycin, carbomycin, etc. contain aminosugars which are probably responsible for the antibiotic activity.

Q.36. What are glycosides?

Glycosides are compounds containing a carbohydrate and a non-carbohydrate residue in the same molecule; Carbon 1 of carbohydrate is attached to the non-carbohydrate residue ***by an acetal linkage.***

Q.37. What is aglycone? And mention its nature.

The non-carbohydrate residue present in the glycoside is called an ***aglycone***. The aglycone can be a simple substance like methyl alcohol, glycerol, phenol or complex substances like sterol, hydrosquinone or anthraquinones.

Q.38. Name some glycosides and mention their biomedical importance.

Some of the important glycosides and their functions are as follows:

- ***Cardiac glycosides:*** used in cardiac insufficiency. ***Examples*** are digitonin, strophanthin, etc.
- ***Ouabain:*** a sodium pump inhibitor.
- ***Phlorhizin:*** displaces Na^+ from the binding site of "carrier protein" and thus prevents the transport of glucose across the mucosal cells of small intestine and renal tubular epithelial cells (producing glycosuria).

Q.39. Name three disaccharides of biological importance.

They are: maltose, lactose and sucrose.

Q.40. Mention the hydrolytic products of these three disaccharides.

Hydrolytic products

- Maltose (Malt sugar) → D-glucose + D-glucose.
- Lactose (Milk sugar) → One mol of D-glucose + One mol of D-galactose.
- Sucrose (Table sugar) → One molecule of D-glucose + one mol of D-fructose.

Q.41. What will be the oxidative product of lactose after prolonged boiling with conc HNO_3 acid?

After boiling with conc HNO_3 acid lactose is hydrolyzed to glucose and galactose. Galactose on oxidation produces ***mucic acid***.

Q.42. Name the disaccharide which is non-reducing and why it is so?

Sucrose is a disaccharide which is non-reducing.

- ***Reason:*** In sucrose, both aldehyde group of glucose and ketone group of fructose are linked together by $\alpha 1 \rightarrow 2$ linkage. Hence, no free aldehyde or ketone group is available for enolization and reducing action.

Q.43. What are invert sugars? What is meant by inversion?

- Sucrose is dextrorotatory (+ 62.5°) but its hydrolytic products are laevorotatory (– 19.5°), as fructose has a greater specific laevorotation than the dextrorotation of glucose.

- As the hydrolytic products invert the rotation from + to –, the resulting mixtures of glucose and fructose, i.e. the hydrolytic products is called ***"invert sugars"*** and the process is called as ***"inversion".***

Q.44. Mention the biomedical importance of maltose.

- Various baby food preparations contain maltose which is easily digested and thus of nutritional significance.
- Dietary maltose is hydrolyzed in small intestine by the enzyme ***maltase*** to two molecules of glucose which are absorbed and utilized for energy by the body.

Q.45. State the biomedical importance of lactose.

- Dietary lactose is hydrolyzed in small intestine by the enzyme ***lactase*** to glucose and galactose and absorbed for utilization in the body.
- In lactating mammary gland, lactose is synthesized from glucose by duct epithelium. Lactose, present in breast milk is a good source of energy for the newborn.
- Lactose is fermented by *E.coli* which is usually non-pathogenic and not fermented by the typhoid bacilli which is pathogenic. Thus, ***lactose fermentation is utilized to differentiate non-pathogenic and pathogenic bacteria.***
- Many organisms that are found in milk, e.g. *E.coli*, Str. lactis, A. aerogenes convert lactose of milk to lactic acid (LA) causing "souring of milk".

Q.46. What is the effect of sucrose when given parentally?

Sucrose cannot be hydrolyzed when introduced parentally due to lack of enzyme ***sucrase*** in blood. Presence of sucrose in the blood changes the osmotic condition and causes a flow of water from the tissues to the blood. Thus, ***clinicians use it in edema like cerebral edema.***

Q.47. What is the biomedical importance of oligosaccharides?

- Antibodies, blood group substances and coagulation factors contain oligosaccharides.
- The oligosaccharides units of glycoproteins are rich in information and are functionally important.

Q.48. What is sialic acid and its importance?

- Sialic acid is N-acetyl neuraminic acid (NANA). Neuraminic acid is an amino sugar acid and structurally an aldol condensation product of pyruvic acid (PA) and D-mannosamine.
- Neuraminic acid and siliac acids (NANA) occur in a number of mucopolysaccharides (MPS) and in glycolipids like gangliosides.

Q.49. Name the enzyme which hydrolyses sialic acids.

"Neuraminidase" is the enzyme which hydrolizes to split NANA from the compound.

Q.50. Name some homopolysaccharides (homoglycans).

Some of the important homopolysaccharides are: starch, glycogen, dextrins, dextran, insulin, cellulose, agar, etc.

Q.51. What are the polymeric units present in the starch granule?

Two polymeric units of glucose present in a starch granule are:

- Amylose and
- Amylopectin

Q.52. Enumerate the essential differences between amylose and amylopectin.

Essential differences between amylose and amylopectin are:

	Amylose	*Amylopectin*
1. Amount	15-20%	80-85%
2. Mol Wt	Low (approx 60,000)	High (approx 500,000)
3. Reaction with dilute I_2 solution	Blue color	Reddish violet color
4. Structure	• Unbranched straight chain • 250-300 D-glucose units joined by $\alpha\ 1 \rightarrow 4$ linkages. • Twists into a helix, 6 glucose units per turn.	• Highly branched • Main stem and branches-$\alpha\ 1 \rightarrow 4$ linkages; at branch point $\alpha\ 1 \rightarrow 6$ linkages. • Approx 80 branches, one branch after every 24 to 30 glucose units.

Q.53. What are hydrolytic products of starch and state their reaction with I_2 solution?

- Hydrolysis of starch yields succession of polysaccharides of diminishing molecular size as follows:

Course of hydrolysis	Reaction with I_2 solution
Starch	Blue
↓	
Soluble starch	Blue
↓	
Amylodextrin	Purple
↓	
Erythrodextrin	Red
↓	
Achroodextrin	Colorless
↓	
Maltose	

Q.54. Mention the end products of hydrolysis of starch by enzyme (amylase) and by acids.

- Enzyme *(amylase)* hydrolysis of starch ends in maltose. For formation of glucose, it requires another enzyme *maltase*.
- Acid hydrolysis of starch always ends in glucose.

Q.55. Name one homopolysaccharide which is a polymer of fructose. State its biomedical importance.

- Polymer of D-fructose is ***inulin,*** a homopolysaccharide.
- ***Biomedical importance:***
  - Used in determination of the rate of glomerular filtration rate (GFR)
  - Also used for estimation of body water (ECF) volume.

Q.56. What is animal starch?

Animal starch is glycogen, a storage form of D-glucose.

Q.57. Describe the structure of glycogen.

Glycogen has a highly branched structure resembling Amylopectin of starch granule. D-glucose units in main stem and the branches are joined together by α-1-4 glucoacidic linkage. ***The linkage at branch point is α 1-6.*** Glycogen is more bushy and branches occur for every 12 to 18 glucose units.

Q.58. State the biomedical importance of glycogen?

- Glycogen is a storage form of glucose and occurs principally in liver and muscle. Formation of glycogen from glucose is called ***glycogenesis***.
- Liver supplies glucose-1-P by breakdown of glycogen, called ***glycogenolysis***, which is converted to glucose. Hence, it serves as ready source of glucose in body in time of need.

Q.59. How much glycogen can be stored in liver and muscle?

- Storage capacity is limited.
- Liver can store up to 4 to 6% of its weight, i.e. 72 to 108 gm.
- Muscle can store 0.7% of its weight, i.e. 245 gm.

Q.60. What is agar and what is its biomedical importance?

- Agar is a ***polymer of sulfated galactose*** units and obtained from seaweeds.
- ***Biomedical importance:***
  - Used in constipation. It is not utilized by humans when taken orally. It adds bulk to the feces and helps in its propulsion.
  - Agar is used as a culture medium for bacterial growth and isolation.

Q.61. What is cellulose?

- Cellulose is a polymer of D-glucose, a homopolysaccharide. Heating with high concentrations of cellulose with acids yield ***cellobiose*** (a disaccharide) and
- D-glucose.

Q.62. What is cellobiose?

It is a disaccharide made up of two molecules of D-glucose linked together by β-glucosidic linkage between C-1 and C-4 of the glucose units. A hydrolytic product of the cellulose.

Q.63. State the biomedical importance of cellulose.

Cellulose is the main constituent of the supporting tissues of plants and it forms considerable part of our vegetable food. In humans, there is no cellulose-splitting enzyme in

the gut, hence, ***it is not of any nutritional value.*** But it gives bulk to the intestinal contents ***(roughage)*** and helps in removal of constipation.

Q.64. Differentiate dextrins and dextrans.

Dextrins	*Dextrans*
• Are hydrolytic products of starch	• Synthetic polymer of D-glucose
• Used in infant feeding	• ***Used as a plasma expander*** when given IV in cases of hemorrhage (blood loss), it increases the blood volume.

Q.65. What precaution should be taken before administering dextran in a case of hemorrhage?

Only drawback is that ***dextran can inferfere with grouping and crossmatching,*** as it forms ***false agglutination (Roleux formation).*** Hence, blood sample for grouping and crossmatching should be collected before administration of dextran in a case of hemorrhage and blood loss, where blood transfusion may be needed, otherwise it can give wrong grouping.

Q.66. What are mucopolysaccharides (MPS)?

Mucopolysaccharides (MPS) are ***heteropolysaccharides,*** usually composed of repeating units of aminosugars and uronic acids, though some are chiefly made up of aminosugars and monosaccharide units without the presence of uronic acid. The hexosamine present is generally acetylated.

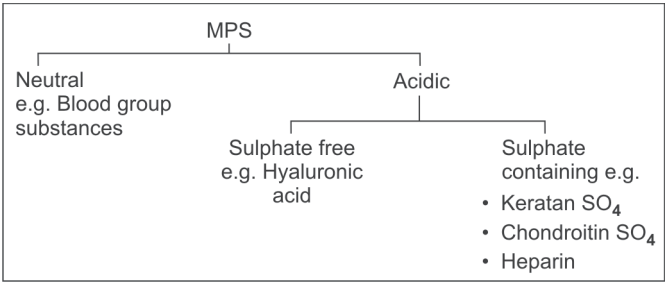
Q.67. What is the other name of mucopolysaccharide?

The other name is ***glycosaminoglycan (GAG).***

Q.68. How will you classify MPS?

Though there is no unanimity regarding classification, MPS may be classified based on two properties:

- On its reaction, and
- Whether contains sulphate or not



Q.69. Name one acidic sulphate free MPS which is of biological importance.

Hyaluronic acid which is a polymer of N-acetyl glucosamine and D-glucuronic acid.

Q.70. Sate the biomedical importance of hyaluronic acid.

- ***Acts as a barrier in tissues:*** Hyaluronic acid in tissues acts as a cementing substance and contributes to tissue barrier. It permits metabolites to pass through but resist penetration of bacteria.
- ***Acts as lubricants and shock absorber*** in joints and has capacity to hold water.
- ***Present in high concentration in embryonic*** tissues and has a role in cell migration during morphogenesis and wound repair.
- ***Present in storage and secretory granules*** where they play part in release of the contents of granules.
- ***Present in Basement membrane (BM)*** of renal glomeruli, where it plays an important role in charge selectiveness and wound repair.

Q.71. What is hyaluronidase? Why is it called spreading factor?

- ***Hyaluronidase*** is an enzyme present in certain tissues, particularly in testicular tissue and spleen. Also produced by certain pathogenic micro-organisms like pneumococci and hemolytic streptococci.
- The enzyme is called ***"spreading factor"*** because it catalyzes depolymerization of hyaluronic acid, thus reduces its viscosity, and facilitates diffusion of materials in the spaces.

Q.72. State the biomedical importance of hyaluronidase.

- The invasive power of some bacteriae is increased as they secrete ***hyaluronidase.***
- In testicular secretions, it may dissolve the viscid substances surrounding the ovum to permit penetration of spermatozoa and fertilization.
- Clinically the enzyme is used to increase the efficiency of absorption of solutions when administered by ***clysis***.

Q.73. Name the sulphate containing acid MPS which does not contain uronic acid.

- ***Keratan sulphate I and II*** does not contain uronic acid.
- Keratan SO_4 is composed of repeating units of N-acetyl glucosamine and galactose.

Q.74. State the biomedical importance of keratan sulphate.

Keratan SO_4 I is present in cornea of the eye and lie between the collagen fibrils and plays an important role in maintaining corneal transparency.

Q.75. Name the different types of chondroitin sulphates and its constituents.

Principally **4 types:**

- ***Chondroitin SO_4 A:*** repeating units of sulphated N-acetyl galactosamine and D-glucuronic acid.
- ***Chondroitin SO_4 B:*** Repeating units of sulphated N-acetyl galactosamine and ***L-iduronic acid.***
- ***Chondroitin SO_4 C:*** Similar to chondroitin SO_4 A but SO_4 group is present at C_6 of galactosamine molecule.
- ***Chondroitin SO_4 D:*** Not found in humans, isolated from cartilage of shark.

Q.76. State the Biomedical importance of chondroitin sulphates?

- ***Role in compressibility of cartilages:*** chondroitin sulphates and hyaluronic acid are present in high concentrations in cartilages and have a role in compressibility of cartilage and weight bearing.
- ***Role in sclera of eye:*** chondroitin SO_4B (also called Dermatam SO_4) is present in sclera of the eye where it has an important function in maintaining over-all shape of the eye.

- ***Regulate flow*** and ***concentration of cations*** round the cells.

Q.77. What is β-heparin?

Chondroitin SO_4 B is called as β-heparin as it has weak anticoagulant property.

Q.78. What is α-heparin? Give its structure.

- α-Heparin is ***sulphate containing acid MPS,*** an anticoagulant present in liver (produced mainly by mast cells of liver). Also found in lungs, thymus, spleen, walls of large arteries, in skin and in small quantities in circulating blood.
- Structurally it is a polymer of sulphated D-glucosamine and either of the two uronic acids D-glucuronic acid or L-iduronic acid.

Q.79. State the biomedical importance of heparin.

- It is an ***important anticoagulant in vitro*** and also ***in vivo*****.** It inhibits thrombin.
- Acts as a coenzyme:

  Heparin acts in the body to increase the activity of the enzyme ***lipoprotein lipase*** and hence it is called as ***clearing factor.***

Q.80. What is haparitin SO_4 (or heparan SO_4)?

Structurally it is similar to α-heparin but it ***differs*** in following respects:

- negligible anticoagulant activity,
- has a lower molecular weight,
- Some of the-NH_2 groups are acetylated,
- Percentage of SO_4 groups are less, hence less acidic,
- Predominant uronic acid is D-glucuronic acid.

Q.81. State the biomedical importance of heparan SO_4.

Heparan SO_4 in combination with proteins are components of plasma membrane of cells, where they may act as ***"receptors"*** and participate in cell-adhesion and cell-cell interactions.

Q.82. Mention typical example of neutral MPS.

Typical examples of neutral MPS are blood group substances (ABO system).

Q.83. Describe salient features of composition of blood group substances.

Blood group substances contain peptide or amino acids as well as carbohydrates. ***Four monosaccharides*** are found in all types of blood group substances viz. galactose, L-fucose, N-acetyl galactosamine, and N-acetyl glucosamine. Non-reducing end groups of acetyl glucosamine, galactose and L-fucose are associated with blood group specificities of A, B, and H respectively.

Q.84. What are glyco and mucoproteins? What is the essential difference?

Glyco and mucoproteins are proteins which contain carbohydrates like mannose, galactose, fucose, sialic acid, etc. ***Glycoproteins*** are proteins ***with less than 4% of carbohydrates,*** whereas ***mucoproteins*** have ***higher 4% of carbohydrates.*** Glyco and mucoproteins give purple color with Molisch's reagent.

Example:

- Glycoproteins:
  - α-globulins
  - Gonadotropins etc.
- Mucoproteins:
  - Orosomucoid etc.

Q.85. What are proteoglycans?

Proteoglycans are conjugated proteins (called "core" proteins) covalently linked to any of the Glycosaminoglycans (GAGs). The amount of carbohydrates in proteoglycans is much greater (up to 95%) as compared to glycoproteins.

Q.86. State the type of linkages found in proteoglycans?

Three types of linkages with core protein and GAG is observed. They are:

- ***O-glycosidic linkage:*** between N-acetyl galactosamine (Gal NAc) and serine/threonine of core protein.
- ***N-glycosyl amine linkage:*** Formed between N-acetyl glucosamine (Glc NAc) and amide N of asparagine (ASn) of core protein.
- ***O-Glycosidic linkage with xylose:*** formed between xylose and serine of core protein.

Q.87. What are mucopolysaccharidosis?

The mucopolysaccharidosis are a group of related disorders, due to inherited enzyme deficiency, in which mental retardation, skeletal changes, visceral involvement and corneal clouding are manifested to varying degrees. Defect/defects in these disorders result in:

- Widespread deposits in tissues of a particular MPS.
- In excessive excretion of a particular MPS in urine.

Depending on the type of enzyme deficiency, at least **six types** of mucopolysaccharidosis have been described.

Q.88. Describe a screening test for detecting mucopolysaccharidosis.

Cetyl trimethyl ammonium bromide test can be used for detection of MPS in urine.

- To 5 ml of fresh urine from the suspected patient in a test tube. Add 1.0 ml of 5% cetyl trimethyl ammonium bromide (cetar – 10n) in 1 M citrate buffer (pH 6.0).
- Mix and allow to stand at room temperature for 1/2 hour. A **heavy precipitate** is obtained in gargoylism.

CHAPTER

2 Chemistry of Lipids and Eicosanoids

Q.1. What are lipids? Define.

The lipids are a heterogeneous group of organic compounds present in plant and animal tissues and related either actually or potentially to the fatty acids and chemically they are various types of esters of different alcohols.

Q.2. What are Bloor's criteria for a substance to be called a lipids?

Bloor gave the following criteria for a substance to be called as lipid:

- Insoluble in water
- Soluble in one or more organic solvents such as ether, chloroform, Benzene, acetone etc, so called ***"fat solvents".***
- Related to fatty acids as ester either actual or potential
- Possibility of utilization by living organisms.

Q.3. What is an oil? How it is related to lipids?

An oil is a lipid which is liquid at ordinary temperature. Distinction between fats and oil is purely physical one; chemically they are all esters of fatty acids and alcohol.

Q.4. State the biomedical importance of Lipids.

- Important dietary constituent and acts as ***"fuel" in body yielding energy.***
- Lipids supply essential fatty acids (EFA) in the diet.
- Some vitamins like A, D, E and K are fat soluble.
  - Nervous system is rich in lipids and are essential for proper functioning.

- Breakdown products of fats like ***acetyl CoA*** can be used by the body for synthesis of cholesterol, steroid hormones, etc.
- Lipoproteins and phospholipids (PL) are important constituents of many natural membranes such as cell walls and cell organelles like mitochondria, etc.
- Some lipid deposits exert an insulating effect, while lipids deposited around internal organs have protective function.
- ***Lipoproteins are "carriers" of trigylcerides (TG),*** phospholipids (PL) and cholesterol in the blood.
- Phosphatides of platelets, called ***platelet factor 3*** are involved in the production of thromboplastin activity in the early stages of blood coagulation.
- ***Dipalmitoyl lecithin (DPL),*** a derivative of lecithin (PL) acts as a surfactant and lowers the surface tension in the lung alveoli.

Q.5. How are lipids classified? Give examples of each group.

Bloor has classified lipids as:

Simple lipids: Esters of FA with various alcohols.

Examples:

- ***Neutral fats (triacyl glycerol)*** are esters of fatty acids with glycerol.
- ***Waxes:*** Esters of fatty acids with monohydroxy aliphatic alcohols other than glycerol.

Compound lipids: Esters of fatty acids containing groups other than and in addition to an alcohol and fatty acids. They are of various types as follows:

- ***Phospholipids (PL):*** Consists of fatty acids + glycerol + phosphoric acid + a nitrogenous base or other constituents.

  Examples:
  - Phosphatidyl choline (lecithin),
  - Phosphatidyl ethanolamine (cephalin),
  - Sphingomyelin etc.
- ***Glycolipids:*** Consists of sphingosine (alcohol) + fatty acids + carbohydrate + carbohydrate derivative. They *do not contain glycerol or phosphoric acid.*

Examples:

- cerebrosides
- gangliosides

- ***Sulfolipids:*** Lipids containing sulphate groups
- ***Aminolipids (proteolipids)***
- ***Lipoproteins:*** Lipids as a prosthetic group to proteins called apo-proteins + PL + cholesterol/and cholesteryl esters.

Derived lipids: Substances derived from above groups by hydrolysis e.g. fatty acids, glycerol and other alcohols, cholesterol.

Miscellaneous: Aliphatic hydrocarbons, carotenoids, squalene, vit E, vit K, etc.

Q.6. What is a fatty acid? Define.

A fatty acid (FA) may be defined as an organic acid that occurs in a natural triglyceride and is a monocarboxylic acid ranging in chain length from $\mathbf{C_4}$ to about $\mathbf{C_{24}}$.

Q.7. What are saturated fatty acids? Give examples.

- Fatty acids which do not have any double bond in their structure are called saturated fatty acids.

  Examples: Acetic acid ($CH_3.COOH$), propionic acid (C_2H_5COOH), butyric acid (C_3H_7COOH), etc. Higher homologues like palmitic acid ($C_{15}H_{31}COOH$), stearic acid ($C_{17}H_{35}COOH$), etc.

Q.8. What are unsaturated fatty acids? What are the types?

- Fatty acids which contain double bonds in their structure are called unsaturated fatty acids **(UFA).**
- ***Types:*** Depending on the degree of unsaturation they are divided into ***two groups:***
  - ***Monounsaturated (mono-ethenoid):*** containing only one double bond.
  - ***Polyunsaturated (polyethenoids):*** containing more than one double bond in their structure.

Q.9. Give an example of monosaturated FA (monoethenoid) found in our body fat.

Oleic acid $C_{17}H_{33}COOH$ **(Formula 18: 1; 9)** is found in abundance in our body fat.

Q.10. Name the three polyunsaturated fatty acids (polyethenoids).

Three polyunsaturated fatty acids of biological importance are:

- ***Linoleic acid: Two double bonds*** between C_9 and C_{10} and another between C_{12} and C_{13} (formula: 18: 2; 9, 12).
- ***Linolenic acid:*** contains ***three double bonds*** between carbons 9 and 10, 12 and 13, and 15 and 16. (Formula: **18: 3; 9, 12, 15**).
- ***Arachidonic acid:*** It is a 20 C fatty acid and contains ***four double bonds*** between 5 and 6, 8 and 9, 11 and 12, and 14 and 15. (Formula: **20: 4; 5, 8, 11, 14**).

Q.11. Name two cyclic fatty acids of clinical importance.

- Chaulmoogric acid and
- Hydnocarpie acid

Both of them were used earlier for a long time for treatment of leprosy.

Q.12. Name essential fatty acids (EFA). Why are they called essential?

Three polyunsaturated fatty acids:

- ***Linoleic acid***
- ***Linolenic acid*** and
- ***Arachidonic acid*** are called essential fatty acids (EFA). They are called essential as they are necessary for normal growth and body functions. They ***cannot be synthesized in the body*** and ***must be provided in the diet.***

Q.13. Which EFA is important?

- Linoleic acid is most important as arachidonic acid can be formed in the body from linoleic acid.
- Biologically arachidonic acid is very important as prostaglandins (PGs) and leukotrienes (LTs) are formed in the body from it.

Q.14. Monounsaturated fatty acid oleic acid is found in plenty in the body, then why EFA cannot be synthesized in the body from oleic acid?

Introduction of additional double bonds (called desaturation) in an unsaturated FA can take place between COOH group and existing double bond but it cannot do

so between existing double bond and CH_3 group (omega carbon). This explains why body cannot synthesize EFA from oleic acid.

Q.15. State the deficiency manifestations of EFA.

EFA deficiency causes:

- Cessation of growth
- Skin lesions (Dermatitis)
- Kidney damage
- Abnormalities in pregnancy and lactation in adult females
- Fatty liver and
- Decreased resistance to stress.

Q.16. Enumerate the important functions of EFA.

- Are structural elements of various tissues.
- Lipids of gonads are rich in EFA and are involved in reproductive function.
- Prostaglandins (PGs) and leukotrienes (LTs) are synthesized from arachidonic acid.
- Lowers cholesterol level in blood.
- Prolongation of clotting time.
- Increase in fibrinolytic activity.
- EFA deficiency produces fatty liver.
- Linoleic acid is necessary for synsthesis of Docosahexenoic acid present in retinal photoreceptor membrane.

Q.17. What is glycerol?

Glycerol is commonly called as glycerine. Chemically it is a trihydric alcohol containing three -OH groups in the molecule.

Q.18. What are the sources of glycerol in the body?

Sources of glycerol in the body:

- ***Exogenous:*** From dietary fats, approximately 22% of glycerol formed in the gut by lipolysis of dietary TG is absorbed directly to portal blood.
- ***Endogenous:*** From lipolysis of fats (TG) in adipose tissue.

Q.19. What is acrolein test?

The *presence of glycerol in a compound is detected by acrolein* test. Glycerol, when heated with $KHSO_4$, two molecules of water are removed and it produces ***acryl aldehyde*** which has characteristic pungent or acrid odour.

Q.20. Is glycerol produced in the body by lipolysis a waste product or useful?
Glycerol produced in the body is ***not a wasteproduct.*** It is useful in that:
- Re-esterified to form TG again
- It has ***nutritive value.*** It can be converted to glucose/and glycogen by the process called gluconeogenesis.

Q.21. Can glycerol be used clinically in medicine?
Glycerol has been used orally or IV in cases of cerebrovascular diseases. It is nontoxic and it reduces cerebral edema with improvement in CS fluid. There is no rebound increase in intracranial pressure on discontinuation of therapy.

Q.22. What is nitroglycerine? State its clinical use.
A derivative of glycerol. It is ***used as a vasodilator.***

Q.23. What are steroids? Give a few examples.
The steroids are often found in association with fats. These are compounds having a special ring called ***Cyclopentano perhydrophenanthrene nucleus.***
Examples: Steroid hormones, like cortisol and aldosterone, sex hormones, bile acids, vit D, etc.

Q.24. What is a sterol? How does it differ from steroid? Give one example of sterol which is biologically most important.
If the compound contains cyclopentanoperhydrophenanthrene nucleus like a steroid, but has one or more -OH groups and ***no carbonyl or carboxyl groups,*** it is called a "Sterol".
Example: Most important sterol of biological importance in the body is cholesterol.

Q.25. What is the normal blood level of cholesterol?
Normal blood level of cholesterol is 150 to 250 mg/dl of blood.

Q.26. What is the appearance of cholesterol crystals under the microscope?
Crystals of cholesterol when seen under the microscope appears as *rhombic plates* with characteristic *broken notches* at *the corners.*

Q.27. State the characteristic features of cholesterol structure.

Characteristic features of cholesterol structure are:

- Possesses *"cyclopentanoperhydrophenanthrene"* nucleus.
- α -OH group at C_3
- an unsaturated double bond between C_5 and C_6.
- has two-CH_3 groups at C_{10} and C_{13}
- and has an eight carbon side chain attached to C_{17}.

Q.28. What are the sources of cholesterol in the body?

Two sources:

- ***Exogenous:*** Dietary cholesterol approximately 0.3 gm/day. Diet rich in cholesterol are butter, egg yolk, milk, cream, meat, etc.
- ***Endogenous:*** Synthesized in the body from two carbon units, acetyl CoA (CH_3. C ~ S .CoA). Approximately 1.0 gm/day.

Q.29. What are the forms in which cholesterol exist in blood and tissues?

Cholesterol occurs both in ***free*** form in which -OH group on C_3 is free and in ***ester*** form in which -OH group is esterified with fatty acids.

Q.30. How does cholesterol esterified?

Two ways:

- Some cholesterol esters are formed in tissues ***by the transfer of "acyl" groups*** from acyl-CoA to cholesterol by the enzyme ***acyl transferase*.**
- ***By interaction of lecithin and cholesterol:*** Plasma cholesterol esters are produced in plasma by transfer of an acyl group, usually unsaturated, from β-position of lecithin to cholesterol by the enzyme ***lecithincholesterol acyl transferase (LCAT).***

Q.31. What is Norum's disease?

An inherited disorder due to deficiency of the enzyme LCAT. Hence esterification of cholesterol in plasma does not occur.

The disease is characterized by:

- Rise in free cholesterol↑
- Rise in lecithin in plasma↑
- Fall in the cholesterol ester, lysolecithin and α-lipoproteins ↓ in plasma.

Q.32. Metabolically which form of cholesterol is in demand free or ester cholesterol?

Free cholesterol is in greater demand metabolically as it is converted to steroid hormones and bile acids. If ester cholesterol has to be utilized it has to be first hydrolyzed by the enzyme ***cholesterol ester hydrolase.***

Q.33. What are bad effects of cholesterol?

Excessive cholesterol is harmful to body in that it gets deposited in arterial walls producting atherorsclerosis. This can narrow the lumen of the blood vessel impeding blood flow which can cause thrombosis.

Q.34. Is cholesterol good for the body? What are the good effects?

In normal quantities cholesterol has number of good effects. They are:

- Bile acids are produced from cholesterol by its oxidation in liver.
- Cholesterol is converted to steroid hormones in adrenal cortex and to sex hormones in gonads.
- Cholesterol forms 7-dehydrocholesterol and in skin it is converted to vit D_3 by UV rays.
- Cholesterol is poor conductor of heat and hence acts as an insulator.
- It is also a poor conductor of electricity and has a high dielectric constant.
- Cholesterol is in abundance in brain and nervous tissues where it functions as an insulating covering of structures which generate and transmit electrical impulses.

Q.35. What is Liebermann-Burchard reaction?

A chloroform solution of cholesterol when treated with acetic anhydride and conc. H_2SO_4 gives a grass green color (cholesta-polyene sylphonic acid is formed). This reaction is utilized in colorimetric estimation of cholesterol in blood **by Sackett's method.**

Q.36. What is Zak's reaction?
When solution of cholesterol is treated with glacial acetic acid, ferric chloride and conc H_2SO_4, it gives purple red color (cholestapolyene carbonium ion). This reaction forms the basis for the colorimetric estimation of cholesterol **by Zak's method.**

Q.37. What precaution is necessary in Zak's method?
Glacial acetic acid used must be made ***aldehyde free***, otherwise it yields higher result. Aldehyde, if present gives additional color with tryptophan in blood.

Q.38. What reagent is used to estimate ester cholesterol?
Digitonin is used for the above. Digitonin reacts with alcoholic -OH group of free cholesterol and forms cholesterol digitonide. Total cholesterol is first estimated by a standard method. Total cholesterol – free cholesterol = ester cholesterol.

Q.39. What is 7-dehydrocholesterol? Why it is called pro-vitamin D_3?
7-dehydrocholesterol is produced in the body from cholesterol and it is present in skin epidermis. Ultra violet ray of sunlight changes 7-dehydrocholesterol to vitamin D_3 (Cholecalciferol). Hence it is called as ***pro-vitamin D_3***.

Q.40. What is pro-vitamin D_2?
Ergosterol is a plant sterol. When it is irradiated with UV rays [long wave 265 mμ (millimicron)] it changes to vitamin D_2. Over irradiation may produce toxic substances viz. Toxisterols and suprasterols.

Q.41. What is coprosterol (Coprostanol)?
Cholesterol is reduced in the gut (by hydrogenation of double bond) by the intestinal bacteria and is converted to coprosterol (coprostanol) which is excreted in faeces.

Q.42. What is saponification and saponification number?
- ***Saponification: Hydrolysis of fat by an alkali*** is called saponification. The resultant products are glycerol and the alkali salts of the fatty acids which are called "soaps".
- ***Saponification number:*** The number of mgms of KOH required to saponify the free and combined FA in one gram of a given fat is called its saponification number. ***It measures the number of COOH groups***.

Q.43. What is Reichert-Meissl number?
It is the number of milli-liters of 0.1 (N) alkali required to neutralize the soluble volatile fatty acids distilled from 5 gm of fat. ***It measures the amount of volatile soluble fatty acids present in fat.***

Q.44. What is Iodine number?
Iodine number is defined as the number of grams of iodine absorbed by 100 grams of fat. ***It measures the degree of unsaturation of a fat.***

Q.45. What is acetyl number? What is its significance?
- The number of mgms of KOH required to neutralize the acetic acid obtained by saponification of 1 gm of fat after it has been acetylated.
- ***Significance:*** It is the measure of the number of – OH groups present. It is ***used to detect adulteration.***

Q.46. Name the enzyme which hydrolyzes TG?
Lipases are enzymes which hydrolyzes a triglyceride (TG) yielding fatty acids and glycerol.

Q.47. Name the different lipases found in the body mentioning their sites?
Lipases are found in human body in the following sites:
- ***Lingual lipase:*** in saliva in the mouth
- ***Gastric lipase:*** in gastric juice in the stomach
- ***Pancreatic lipase:*** in pancreatic juice in the duodenum.
- ***Intestinal lipase:*** in intestinal epithelial cells
- ***Adipolytic lipase:*** in adipose tissue and
- Serum lipase.

Q.48. What is rancidity?
The unpleasant odor and taste developed by most natural fats ***on aging*** is referred to as rancidity.

Q.49. What are the causes of rancidity?
Rancidity are caused by the following:
- ***By hydrolysis of fats:*** producing hydrolytic products viz., fatty acids, glycerol, and/or mono and diglycerides.
- ***Oxidation of unsaturated fatty acids:*** may form ***"peroxides",*** which then decompose to form aldehydes of objectionable taste and odor. The process is aggravated when exposed to air, light, warmth and moisture.

Q.50. Explain why vegetable fats are preserved for longer periods than animal fats?

Vegetable fats contain certain substances like vit E, phenols, hydroquinones, tannins, and others which are ***anti-oxidants*** and t*hey prevent development of rancidity.*

Q.51. What are phospholipids?

Phospholipids are compound lipids that contain in addition to fatty acids, and glycerol/or other alcohol, a phosphoric acid residue, nitrogen containing base and other constituents.

Q.52. State how phospholipids are classified?

As per **Celmer** and **Cartar's** classification phospholipids are classified into **3 groups:**

- ***Glycerophosphatides:*** containing glycerol as alcohol group e.g., phosphatidyl choline (lecithin), phosphatidyl ethanolamine (cephalin), phosphatidyl serine, plasmalogens, etc.
- ***Phosphoinositides:*** Containing inositols as alcohol e.g. phosphatidyl inositol (lipositol).
- ***Phospho sphingosides:*** Containing an unsaturated 18 carbon aminoalcohol called sphingosine (sphingol) e.g. sphingomyelin.

Q.53. What are phospholipases? What are the types? Mention their site of action.

Phospholipases are enzymes which hydrolyze phospholipid in a characteristic way. **Five types** described. Their specific site of action on lecithin are as follows:

- ***Phospholipase A_2:*** attacks **β** position and forms lysolecithin + one mole of FA.
- ***Phospholipase A_1:*** attacks ester bond in position 1 of lecithin.
- ***Phospholipase B:*** Substrate is lysolecithin. *(lysophospholipase).* It hydrolyzes ester bond in **α** position and forms glyceryl phosphoryl choline + one mole of FA.
- ***Phospholipase C:*** Hydrolyzes phosphate ester bond and forms **α, β**-diacyl glycerol + phosphoryl choline.
- ***Phospholipase D:*** Splits off choline and forms phosphatidic acid.

Q.54. What is sphingomyelin?

It is a phospholipid. It ***does not contain glycerol,*** instead contains an 18 carbon unsaturated aminoalcohol called as ***sphingosine (sphingol).***

- ***On hydrolysis,*** sphingomyelin yields one molecule FA + phosphoric acid + nitrogenous base choline + sphingosine (alcohol).

Q.55. State the biomedical importance of sphingomyelin.

- Normally found in considerable quantities in brain and nervous system. ***Sphingomyelinase*** is the enzyme, which normally degrades sphingomyelin.
- In inherited deficiency of the enzyme, sphingomyelin cannot be catabolized and large quantities of sphingomyelin accumulate in brain, liver and spleen leading to inherited disorder called **"Niemann-Pick disease".** The disease affects children and is manifested by progressive mental retardation, hepatomegaly and splenomegaly.

Q.56. What are plasmalogens? How does it differ from lecithin/cephalin?

- Plasmalogens are phospholipids. They are predominantly found in brain and nervous tissue.
- ***On hydrolysis,*** they yield one molecule of FA + glycerol + phosphoric acid + a nitrogenous base usually ethanolamine (some times choline) + one molecule of long chain aliphatic aldehyde. Thus it differs from lecithin cephalin in having a long chain aliphatic aldehyde.

Q.57. What is cardiolipin?

It is a phospholipid found in mitochondrial inner membrane. Chemically, it is diphosphatidyl glycerol and is formed form phsophatidyl choline.

Q.58. Enumerate the important functions of phospholipids?

- Constitutes structural components of cell wall and membrane, the myelinsheath, cell organelles like mitochondria and microsomes.
- Acts as prosthetic group of certain enzymes and required for the enzyme activity.
- Plays an essential part in blood coagulation.

- Phospholipids are implicated in ion transport and secretions.
- Participates in electron transport and oxidative phosphorylation in mitochondria.
- Lecithin lowers surface tension and aids in emulsification of fats and thus helps in absorption of lipids from GI tract.
- Helps in transport of lipids from intestine: Exogenous TG is carried as lipoprotein complex ***chylomicrons***, in which PL takes active part.
- Choline part of lecithin acts as a lipotropic agent and prevents formation of fatty liver.
- Helps in transport of lipids from liver: Endogenous TG formed in liver is carried in blood as a lipoprotein complex called ***very low density lipoprotein*** (VLDL) which requires PL.
- Phospholipids required as a cofactor for the activity of the enzyme ***lipoproteinlipase*** and ***TG lipase.***
- Membrane PL serves as a source of arachidonic acid, which is used for synthesis of prostaglandins (PGs) and leukotrienes (LTs).
- Phospholipids of myelin sheaths provide insulation around the nerve fibers.
- Metabolites of phosphatidyl inositides have been implicated in hormone action.

Q.59. What is dipalmityl lecithin (DPL)? What is its clinical importance?

- Dipalmityl lecithin (DPL), is secreted in Lung alveoli by Type II granular pneumocytes lining the alveolar wall. It ***acts as a surfactant and lowers the surface tension in Lung alveoli*** and prevents collapse of lung alveoli.
- Absence of DPL, in premature infants, may produce collapse of Lung alveoli producing difficulties in respiration and death. It is called as ***respiratory distress syndrome (RDS) or hyaline membrane disease.***

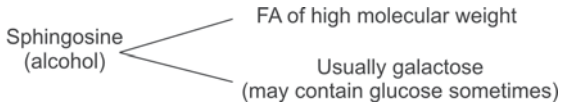
Q.60. What are glycolipids? Give examples.

- Glycolipids are lipids that contain carbohydrate moiety in their molecule.
- They are **mainly of 2 types:**

- ***Cerebrosides,*** and
- ***Gangliosides,***

Q.61. What are cerebrosides?

Cerebrosides are glycolipids. A cerebroside is built as follows:



Q.62. What are the different types of cerebrosides? How do they differ from each other?

Cerebrosides are mainly **4 types.** They differ from each other by the fatty acid content. They are:

- ***Kerasin:*** Contains FA lignoceric acid
- ***Cerebron:*** Contains hydroxy lignoceric acid called **"cerebronic acid (phrenosin)".**
- ***Nervon:*** Contains an unsaturated homologue of lignoceric acid called ***"nervonic acid".***
- ***Oxynervon:*** Contains hydroxy derivative of nervonic acid.

Q.63. What is a ceramide?

- Ceramide is formed by esterification of sphingosine with FA of high molecular weight.
- Principally found in white matter of brain, in myelin sheaths and medullated nerves.

Q.64. What is psychosin?

By prolonged hydrolysis of any cerebroside with $Ba(OH)_2$, FA is removed and it yields ***psychosin***. Thus psychosin is sphingosine (sphingol) + sugar (galactose/or glucose).

Q.65. What is Gaucher's disease?

- It is in inherited disorder of cerebrosides metabolism. Normally in health, cerebrosides are degraded by the enzyme ***β-gluco-cerebrosidase*** to form ceramide and galactose/or glucose. In this condition, there is inherited deficiency of the enzyme, so that cerebrosides are not degraded and large amounts of ***kerasin*** accumulate in RE cells viz. liver, spleen, bone marrow and brain. The disease is characterized by

hepatomegaly, progressive enlargement of spleen (splenomegaly) which may reach to umbilicus or below and progressive mental retardation.

- Biochemically there occurs elevation of ***acid phosphatase*** in serum.

Q.66. What are gangliosides?

- Gangliosides are most complex carbohydrate rich glycolipids which occurs in ganglion cells, neuronal bodies and dendrites, spleen and RB cells stroma.
- Highest concentrations are found in gray matter of brain.

Q.67. How does ganglioside differ structurally from a cerebroside?

- Similar to cerebroside, a ganglioside, contains the alcohol sphingosine, a long chain FA and galactose/or glucose. But ***it differs from cerebroside in carbohydrate content*** in that it contains carbohydrate derivatives:
  - One molecule of N-acetyl galactosamine (Gal NAC).
  - One or more molecules of N-acetyl neuraminic acid, NANA (also called as sialic acid).

Q.68. What is Tay-Sachs disease (GM_2 gangliosidosis)? What is the enzyme defect?

- Tay-Sachs disease is inherited disorder of ganglioside, GM_2 metabolism.
- Enzyme deficiency is ***hexosaminidase A.*** In absence of the enzyme GM_2 cannot be degraded and it accumulates in brain. There occurs widespread injury to ganglion cells in brain and retina.

Q.69. Mention important characteristic clinical findings in GM_2 gangliosidosis.

- Progressive development of idiocy and blindness in infants soon after birth.
- A ***cherry-red*** spot about the macula, seen ophthalmoscopically, is ***pathognomonic of this disease.*** This is caused by destruction of retinal ganglion cells exposing the underlying vasculature.

Q.70. What are eicosanoids? How will you classify?

Eicosanoids are compounds derived from "eicosa (20C) polyenoic acid". They consist mainly of **2 groups:**

- Prostanoids (PGs)
- Leukotrienes (LTs) and lipoxins (LYs)

Prostanoids are further subdivided into **3 main types:**

- Prostaglandins (PGs)
- Prostacyclins (PGI)
- Thromboxanes (Tx)

Q.71. What are prostaglandins (PGs)? State briefly their characteristics.

- Prostaglandins are eicosanoids, a 20C FA with a ***cyclopentane ring in the middle.***
- Have been detected in almost every mammalian tissue and body fluids.
- Produced in minute amounts.
- Their production increases/or decreases with diverse stimuli or drugs.
- Biologically very active compound and duration of action is very short.
- Broad spectrum of activity and diverse biological effects.
- ***Not stored in the body.***
- Have been found to modulate the activity of cyclic AMP and cyclic GMP in cells.

Q.72. How many prostaglandins have been isolated so far? What are the different types?

Fourteen PGs isolated in the begining and they are divided into ***4 main groups:***

- ***PGE group:*** PG-E_1, PG-E_2, and PG-E_3
- ***PGF group:*** PG-$F_1\alpha$, PG-$F_2\alpha$, and PG-$F_3\alpha$
- ***PGA group:*** PG-A_1, PG-A_2, 19-OHPG-A_1 and 19-OHPG-A_2
- ***PGB group:*** PG-B_1, PG-B_2, 19-OHPG-B_1 and 19-OHPG-B_2
- Besides the above 14 PGs recently:
  - PG-C and PG-D groups found
  - A new type of PG has been isolated from human seminal fluid, which has been designated as PGx.

Q.73. Which PG is absent in seminal fluid?

PG-$F_3\alpha$ has been found to be conspicuously absent in human seminal fluid.

Q.74. Which are called as primary PGs?
Six PGs of E and F series are referred to as "primary" prostaglandins as none is precursor of the other.

Q.75. Name the enzyme and its nature alongwith the cofactors required for PG synthesis.
- PGs are synthesized ***aerobically*** from polyunsaturated 20C FA, arachidonic acid with the help of a multienzyme complex, now called *prostaglandin synthase (PGHS),* present as two isoenzyrnes PGHS-1 and PGHS-2 which consists of 2 components:
  - Cyclo-oxygenase system and
  - Peroxidase system
- The following cofactors are required for the enzyme action:
  - presence of molecular O_2
  - reduced glutathione (G-SH)
  - Tetrahydrofolate (FH_4) and
  - Heme

Q.76. How much prostaglandin is synthesized in human per day?
Approximately 1 mg (one mg) of PG is synthesized normally in man every day.

Q.77. Why cyclo-oxygenase is called suicide enzyme?
Cyclo-oxygenase is called ***suicide enzyme*** because of self catalyzed destruction and switching off PG synthesis.

Q.78. What are cyclic endoperoxides?
PG-G_2 and PG-H_2 are cyclic endo-peroxides. PG-G_2 is first formed which is immediately converted to PG-H_2 by the enzyme ***peroxidase*.** Both the cyclic endoperoxides have a very short half-life (1/2-5 minutes) but they are biologically very active.

Q.79. Which are the first PGs produced from cyclic endoperoxides?
PG-E_2 and PG-$F_2\alpha$ are produced first from the cyclic endoperoxide PG-H_2 and then other PGs are derived from them.

Q.80. Enumerate briefly the functions of PGs.
- ***Antihypertensive***-Lowers BP↓
- ***Inhibits platelets aggregation***

- ***Inhibits gastric secretion:*** thus useful in alleviating gastric ulcers.
- ***Stimulates GI musculature:*** Thus have "Purgative action".
- ***Produces bronchodilatation:*** Thus useful in the treatment of bronchial asthma
- ***Increases uterine contraction:*** Thus can be used as "abortifacient" and "induction of labour".
- ***Renal action:*** increases RPF↑, increases GFR↑, diuresis, natriuresis and kaliuresis.
- ***Stimulates renin secretion*** from JG cells.
- ***Metabolic effects:***
  - Decreases lipolysis
  - Insulin-like effects
  - PTH-like action produces hypercalcaemia
  - TSH-like activity
  - Steroidogenesis
  - Produces luteolysis

Q.81. What are the blood levels of PGs? How much is excreted in urine?

- PG levels in blood are as follows:
  - PGE = 385 + 30 Pico g/ml
  - PGF = 141 + 15 Pico g/ml
  - PGA = 1062 + 107 Pico g/ml
- ***Urinary excretion:***

PG-E = 0.2 to 1.2 microgram/ 24 hrs.

Q.82. How are prostaglandins catabolized in the body?

- PGs are catabolized very rapidly. Some 80 to 90% or more is destroyed during a single passage through the liver lungs.
- It is achieved by:
  - Oxidation of secondary alcohol group at C_{15} by the enzyme **15-OH PG dehydrogenase** (PGDH). This is the ***rate-limiting step.***
  - Above is followed by reduction of Δ^{13} double bond.

Q.83. Name the inhibitors of PG synthesis and their mode of action.

- Non-steroidal anti-inflammatory drugs (NSAIDs) viz. aspirin, indomethacin, phenylbutazone, ibuprofen (Brufen), diclofenac, naproxin, piroxycam, etc.
- ***Mechanism:*** They prevent conversion of Arachidonic acid to PG-G_2 (cyclic endoperoxide) by inhibiting cyclo-oxygenase system and thus stop PG synthesis.
- Gluco-corticoids (GC): inhibits ***phospholipase A_2.***

Q.84. Why prostaglandins are not suitable as a drug?

Use of PGs as drugs is limited due to:

- Short duration of action
- Lack of tissue specificity e.g. when PG-E_2 is used for induction of labor it produces cramping and diarrhea.

Q.85. What is prostacyclin (PG-I_2)? How is it synthesized?

Prostacyclin (PG-I_2) is one of the prostanoids formed in vascular endothelium. It is formed from PG-H_2, cyclic endoperoxide by the action of the microsomal enzyme ***prostacyclin synthase.***

Q.86. Mention two most important functions of PG-I_2.

- PG-I_2 inhibits platelet aggregation, increases cyclic AMP.
- Produces vasodilatation and decreases B.P.↓

Q.87. What is thromboxane (Tx)? How is it formed in the body?

- Thromboxanes are prostanoids. It differs structurally from PGs and PG-I_2 in that it contains an **oxane ring** instead of cyclopentane ring. It is formed principally in platelets, but also in lungs, brain, polymorphs etc. There are of **2 types:** TxA_1 and TxA_2. In humans TxA_2 is the main type.
- It is synthesized from cyclic endoperoxide PG-H_2 by the action of the enzyme ***thromboxane synthase.***

Q.88. Mention two most important functions of TxA_2.

- TxA_2 enhances platelets aggregation and thrombus formation. Decreases cyclic AMP↓ in platelets.
- Produces vacoconstriction and increases BP ↑

  Note: TxA_2 actions are opposite to PG-I_2.

Q.89. How aspirin prevents thrombus formation?

- Aspirin has been found to be a most affective drug which prevents platelet aggregation and thrombus formation. A small amount 30 mg of aspirin can act as anti-platelet aggregator.

  Mechanism:

  – Aspirin irreversibly acetylates the platelets cyclo-oxygenase system and inhibits TxA_2 formation.
  – At the same time, it also inhibits PG-I_2 synthesis in vascular endothelial cells.
  – But unlike platelets, the vascular endothelial cells regenerate cyclo-oxygenase very soon.
  – Thus over all balance shifts towards the formation of PG-I_2 which prevents platelets aggregation and thrombus formation.

Q.90. What are leukotrienes (LTs)?

Leukotrienes are conjugated trienes formed from eicosanoic acids in leucocytes, mast cells and macrophages by the ***lipo-oxygenase*** enzyme system in response to immunologic and non-inflammatory stimuli. LTs do not have any ring in its structure but have three characteristic conjugated double bonds.

Q.91. How LTs are synthesized in the body?

LTs are synthesized from arachidonic acid by the addition of hydroperoxy groups to arachidonic acid and produces ***"hydroperoxy eicosa tetranoates"*** **(HPETE)** by lipo-oxygenase system. Leukotrienes are formed in our body mainly from 5 HPETE.

Q.92. What are the different types of LTs? Which LT is first formed in synthetic pathway?

- First LT formed from 5 HPETE is LT-A_4.
- Other LTs are LT-B_4, LT-D_4 and LT-E_4 which are all derived from LT-A_4.

Q.93. State the clinical significance of leukotrienes (LTs).

- LTs in general appear to act as mediators in inflammation and anaphylaxis.

- Produces capillary dilatation and increases vascular permeability.
- Inhalation of LTs (C_4, D_4 or E_4) causes bronchospasm. They are about 30 times (E_4) to 1000 times (C_4 and D_4) more potent bronchoconstrictors in normal subjects as compared to histamine.
- Leukotrienes C_4 and D_4 are found to be potent stimulators of mucus secretion from human air-way tissues.
- SRS-A (Slow reacting substance of anaphylaxis) produced by mast cells during anaphylactic reaction has now been shown to be mixtures of LTs like LT-C_4, D_4 and E_4.

Q.94. What are Lipoxins? Name the types.

Lipoxins are a family of conjugated tetraenes recently discovered arising in ***leucocytes*** by lipo-oxygenase pathway.

Several lipoxins have been found viz. LxA_4 to LxE_4.

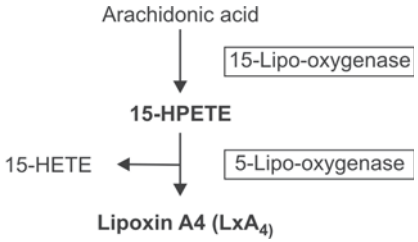
Q.95. Name the starting material required for formation of Lipoxins.

Arachidonic acid is the starting material required formation of Lipoxins.

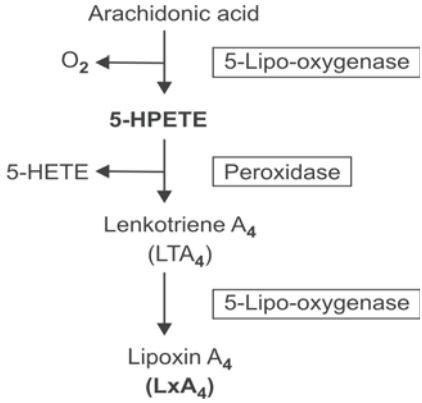
Q.96. How Lipoxin A4 (LxA_4) is formed?

Lipoxin A_4 (LxA_4) can be formed in **two ways** either from 15-HPETE or 5-HPETE as shown below:

- **Formation from 15-HPETE:**



- **Formation from 5-HPETE:**



Q.97. State the functions of Lipoxins.

Evidences support a role of lipoxins in vasoactive and immunoregulatory function e.g. as counter-regulatory compounds (chalones) of the immune response.

CHAPTER

3 Chemistry of Proteins and Amino Acids

Q.1. Define α-amino acids.

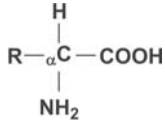
Amino acids are organic compounds which contain the two functional groups:

- The basic -NH_2 group (amino group)
- The -COOH group (carboxylic group)

When the -NH_2 group is attached to the carbon atom carrying the -COOH group, it is called the α-amino acid.

Q.2. Give the general formula of α-amino acid.

- Most of the amino acids in the body are α-amino acids and the general formula is as shown below:



R represents a side chain and can be a hydrogen, an aliphatic chain, aromatic or heterocyclic group.

Q.3. What are proteins?

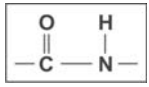
Proteins are organic substances of high molecular weight formed by a number of **α**-amino acids united by a ***peptide linkage*****.** Thus proteins are polymers of monomeric units **α**-amino acids.

Q.4. Which amino acids are involved in protein synthesis?

Only naturally occurring 20 L-**α**-amino acids are involved in protein formation.

Q.5. What is the significance of D-amino acids?
More than 20 D-amino acids occur naturally. Some of them occur in certain bacterial cell-walls and a variety of D-amino acids are found in antibiotics.

Q.6. What is peptide linkage or peptide bond?
The -COOH group of one amino acid can be joined to -NH_2 group of another amino acid by a covalent bond called as ***peptide bond*** or ***peptide linkage***. In the process of formation of a peptide bond, a molecule of water is eliminated. A peptide bond is



Q.7. State the difference between a polypeptide and protein.
Polypeptides do not contain amino acids more than 100, whereas proteins contain amino acids more than 100. In both, amino acids are joined together by peptide bonds.

Q.8. How are amino acids classified?
Amino acids are classified mainly into **three groups** depending on their reaction in solution as follows:
- Neutral amino acids
- Acidic amino acids
- Basic amino acids

Q.9. What are neutral amino acids? Give examples.
Neutral amino acids form the largest group and they can be further classified as follows:
- ***Aliphatic amino acids:*** having an aliphatic side chain and are called simple monoamino monocarboxylic acids. e.g. glycine (gly), alanine (ala), valline (val), leucine (leu) and isoleucine (Ile).
- ***Hydroxyamino acids:*** having an—OH group e.g. serine (ser), threonine (thr).
- ***Aromatic amino acids:*** Contain a benzene ring e.g. phenylalanine (phe), tyrosine (tyr).
- ***Heterocyclic amino acids:*** Containing heterocyclic group like indole ring or imidazole ring e.g. tryptophan (trp), histidine (his).

- ***Sulphur containing amino acids:*** Containing Sulphur e.g. methionine (met), cysteine (cys) and cystine.
- ***Imino acids:*** They do not have a free -NH_2 group but have a basic Pyrrolidone ring e.g proline (Pro), hydroxyproline (Hyp).

Q.10. What is the relationship between cysteine and cystine?
Two molecules of cysteine are joined together by S–S– bond to form one molecule of cystine. One molecule of cystine on oxidation gives 2 molecules of cysteine.

Q.11. What are acidic amino acids? Give examples.
Acidic amino acids have two -COOH groups and one NH_2 group and they are monoamino dicarboxylic acids e.g. aspartic acid (Asp), glutamic acid (Glu).

Q.12. Name the amides of acidic amino acids.
Amide of Aspartic acid is called Asparagine (Asn) and amide of Glutamic acid is called Glutamine (Gln).

Q.13. What are basic amino acids? Give examples.
Basic amino acids have one -COOH group and two –NH_2 groups and they are diaminomonocarboxylic acids e.g. arginine (Arg), lysine (Lys) and hydroxy-lysine (Hyl).
Note: ***Histidine (His) can also be classified as basic amino acid due to presence of imidazole ring.***

Q.14. Which is the simplest of the amino acids?
Glycine is the simplest of the amino acids.

Q.15. What is the chemical name of glycine?
Chemically glycine is called as ***aminoacetic acid***.

Q.16. Which amino acids do not have an asymmetric carbon and do not show optical activity?
Glycine is the only amino acid which does not have an asymmetric carbon and does not exhibit optical activity.

Q.17. Name the amino acids that donot occur in proteins. Give 5 examples.
There are compounds similar to basic skeletal structure of amino acid but do not occur in proteins. **Examples:**
- **β-**alanine found in coenzyme A
- **α-**amino butyrate-a neurotransmitter
- Taurine—present in bile salt
- Ornithine and citrulline—metabolities of urea cycle
- DOPA—precursor of pigment melanin.

Q.18. What are essential amino acids? Name them.

- The amino acids which are essential for tissue synthesis and growth and required for positive nitrogen balance are called as ***essential amino acids***. They are "essential" because they cannot be synthesized in the body and must be provided in the diet.
- They are 8 in number:
  Methionine, Tryptophan, Threonine, Valine, Isoleucine Leucine, Phenylalanine, and Lysine.

Note: For remembering use the formula
"MTTVILPLY"

Q.19. What are semi-essential amino acids? Name them and state why they are called semi-essential?

Arginine and ***histidine*** are called ***semi-essential*** amino acids. They are growth promoting factors since they are not synthesized in sufficient quantity during growth. They are sufficient for normal persons but they ***become*** inadequate and ***essential*** in ***"growing children, pregnancy*** and ***lactating women".*** Hence, they are called semi-essential.

Q.20. Give examples of three amino acids which produce specific biologic compounds.

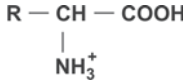
- ***Tyrosine:*** produces:
  - Thyroid hormones,
  - Catecholamines, and
  - Melanin pigments
- ***Glycine:*** required for
  - Heme synthesis
  - Formation of creatine
  - Formation of Glutathione
- ***Tryptophan:*** necessary for
  - Formation of serotonin
  - Forms vitamin nicotinic acid

Q.21. How does an amino acid behave in an acidic and basic media?

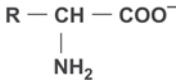
Every amino acid has at least two ionizable groups:

- The $-NH_2$ group and
- The -COOH group.

- ***In acidic medium:*** The -NH_2 group behaves as a base and accepts a proton and becomes ***positively charged (cationic form).***



- ***In basic medium:*** The -COOH group acts as proton donor and the amino acid becomes ***negatively charged (anionic form).***



Q.22. What is iso-electric pH and what are zwitterion (or hybrid ion)?

At a specific pH the amino acids carry both the charges in equal number and thus exists as ***dipolar ion or zwitterion***. At this point the net charge on the amino acids is zero i.e. the positive charges and negative charges on the amino acids equalizes. ***The pH at which the amino acid or protein is in zwitterion form is called isoelectric pH (pI).***

Q.23. What is the iso-electric pH (pI) for albumin, haemoglobin and casein?

Isoelectric pH (pI) of:

- Albumin is 4.7
- Haemoglobin is 6.7
- Casein is 4.6

Q.24. What is Sorensen's formol titration? Explain the mechanism.

- Titration of amino acids in presence of excess of formalin to determine total acidity is called "Sorensen's formol titration".
- ***Mechanism: Explanation***

  It becomes difficult to estimate the total acidity of amino acid solution by direct titration against standard NaOH solution in view of presence of -NH_2

groups which will take up the H^+ released by the ionization of -COOH. But, if neutral formalin (solution of formaldehyde in water) in excess is added, ***the interference from -NH_2 group is eliminated.*** This is due to the fact that the NH_2 groups react with form-aldehyde and forms ***dymethylol or methylene deri-vative.*** In such a condition, an amino acid solution can be titrated to complete neutralization point and the total acidity can be determined.

Q.25. What is Sanger's reagent? What is it used for?

- Sanger's reagent contains ***l-fluoro-2, 4-dinitro benzene*** **(FDNB)**
- ***Use:*** It is used to study the sequence of amino acids in protein molecule. It reacts with free NH_2 group of an amino acid or N-terminal amino group (-NH_2 group) of the protein molecule, in an alkaline medium to form the yellow substances called DNP amino acid. The compound so formed can be isolated after protein hydrolysis and identified.

Q.26. What other reagent can be used to identify N-terminal residue of amino acid?

The N-terminal-NH_2 group can also combine with ***Dansyl chloride (1-dimethyl-amino naphthalene-5-sul-phonyl chloride),*** to form a fluorescent dansyl derivative which can be isolated and identified.

Q.27. What is Edman reaction?

Edman reaction also enables the identification of the N-terminal residue of amino acid. Edman reagent contains ***phenyl isothiocyanate.*** Under mildly acidic condition, it reacts with -NH_2 group and forms phenyl thiocarbamyl derivative, which is cleaved off the peptide. It cyclizes to form a phenyl thiohydantoin derivative that can be identified by chromatography.

Q.28. Name two dipeptides found in our body.

The two dipeptides present in muscle tissues are:

- ***Carnosine:*** **β-**alanine + histidine
- ***Anserine:*** **β-**alanine + methyl-histidine

Q.29. Name tripeptide of immense biological importance.

- Glutathione is a tripeptide consisting of three amino acids—Glutamic acid + Cysteine + and Glycine.
- It functions in the body in oxidation-reduction system.

Q.30. Name some antibiotics which are peptides.

Gramicidin A, polymyxin, chloramphenicol, penicillin, bacitracin, actinomycin.

Q.31. Name a peptide which act as an antitumor agent.

Bleomycin

Q.32. How will you classify proteins? Give suitable examples of each group.

Proteins can be classified on the basis of their solubility and physical properties into following ***three groups:***

- ***Simple proteins:*** e.g protamines, histones, albumins, globulins, gliadins, glutelins and scleroproteins (albuminoids)
- ***Conjugated proteins:*** They are simple proteins combined with a non-protein group called prosthetic group: e.g nucleoproteins, chromoproteins, phospho-proteins, lipoproteins metalloproteins.
- ***Derived proteins:*** are mainly **two types:**
  - ***Primary derived proteins*** e.g proteans, metaproteins, and coagulated proteins
  - ***Secondary derived proteins*** e.g proteoses, peptones, peptides.

Q.33. What are fibrous proteins? Give two examples.

When the axial ratio of length: Width of a protein molecule is more than 10, it is called a fibrous protein.

Examples:

- **α-**keratin from hair
- Collagen of connective tissues

Q.34. What are globular proteins? Give two examples.

When the axial ratio of length: width of a protein molecule is less than 10, it is called a globular protein.

Examples:

- Haemoglobin
- Myoglobin

Q.35. What are chromoproteins? Give suitable examples.

Chromoproteins are conjugated proteins that contain a simple protein with colored substance as the prosthetic group.

Examples:

- All haemoproteins are chromoproteins which contain "heme" as prosthetic group e.g haemoglobin, cytochromes, catalase, peroxidase.
- Flavoproteins contain riboflavin, a yellow colored substance.
- Visual purple (rhodopsin) contains protein 'opsin' + prosthetic group 11-cisretinal.

Q.36. What are phosphoproteins? Give two examples which have dietary importance.

Phosphoproteins are conjugated proteins containing phosphoric acid as the prosthetic group. The phosphoric acid is esterified through the -OH groups of serine and threonine.

Examples:

- Caseinogen of milk
- Vitellin of egg yolk

Q.37. What are metalloproteins? Give three examples.

Metalloproteins are conjugated proteins which contain a metal ion as their prosthetic groups.

Examples:

- ***Carbonic anhydrase*** contains zinc
- Caeruloplasmin contains copper
- Ferritin contains Fe

Q.38. What is the essential difference between glycoproteins and mucoproteins?

- Both are conjugated proteins and contain carbohydrate moiety as prosthetic group.
- They differ by carbohydrate content in that glycoproteins contain less than 4% of carbohydrates whereas mucoproteins contain more than 4% of carbohydrates.

Q.39. How does primary derived proteins differ from secondary derived proteins? Give examples of each group.

- ***Primary derived proteins are synonymous with denatured proteins.*** In this, primary structure is not disturbed and peptide bonds remain intact.
  Examples: Proteans, metaproteins acid/alkaline, coagulated proteins.
- ***Secondary derived proteins are*** formed by progressive hydrolysis of the peptide bonds. Protein is gradually hydrolyzed to produce smaller molecules.
  Examples: Proteoses (albumoses), peptones, peptides.

Q.40. State the different structural organization of protein molecules.

Linderström Lang suggested mainly **three levels** of structural organization of a protein molecule. They are:

- Primary structure
- Secondary structure
- Tertiary structure

- A **fourth level,** quarternary organization can be added further.

Q.41. What is primary structure?

Primary structure indicates the sequence of amino acids linked through peptide bonds. It has an N-terminus and for the other end C-terminus.

Q.42. What is secondary structure?

- Secondary structure refers to the twisting of the peptide chain into a helical form. Hydrogen bonds are responsible for the secondary structure.
- Hydrogen bonds in secondary structure may form either:
  - **α-helix** or
  - **β-pleated sheet**

Q.43. Which amino acid is conspicuously absent in α-Helix?

Proline is never found in **α-**helix and is conspicuously absent.

Q.44. Which amino acids tend to destabilize α-helix?

More polar residues such as arginine, glutamic acid and serine may repel and destablize **α-**helix.

Q.45. Which protein tends to form triple helix?

Collagen is rich in proline and hydroxy-proline, hence it cannot form **α**-helix or **β**-pleated sheet; it forms a triple helix. The triple helix is stabilized by both non-covalent as well as covalent bonds.

Q.46. What is tertiary structure? Which bonds are found in tertiary structure?

- Tertiary structure arises due to the folding of the helical structure into globular, ellipsoidal or any other conformations.
- The folding is brought about by:
  - Disulfide bonds (S-S)
  - Polar or/salt linkages between atoms with positive and negative charges.
  - hydrophobic bonds, and
  - van der Waals forces.

Q.47. What is quarternary structure?

When proteins consists of 2 or more peptide chains held together by non-covalent interactions or by covalent cross-links, it is referred to as the quarternary structure.

Examples:

- ***Haemoglobin:*** a tetramer having 4 polypeptide chains
- ***Lactate dehydrogenase (LDH):*** four polypeptide chains

Q.48. What are prions?

Prions are proteins that contain no nucleic acid and earlier thought to be an infectious agent or a virus. The protein prions was discovered in 1982 by Stanley Prusiner.

Q.49. What are the types of prions?

Two isoforms have been found:

- Normal or physiologic prp—called Pr Pc or PrP – Sen
- Abnormal or pathologic PrP—called Pr Psc or Pr P-res.

Q.50. State the basic defect in the two forms.

The basic defect involves alteration of α-helical structure into β-pleated sheet.

- Both forms have identical primary structural and post translational modification but different tertiary and quarternary structures.

- PrPc is rich in α-helix but Prp-Sc consists predominantly β-sheet. This structural change occurs when PrPc interacts with the pathological isoform Pr-Psc.

Q.51. What are Prion diseases?
Abnormal or pathological Prions PrP-sc cause several fatal neurodegenerative disorders both in humans and in animals known as Prion's diseases—a transmissible spongi form encephalopathies (TSEs).

Q.52. Name one Prion disease that occurs in human.
In humans, the disease is called "Creutzfeldt-Jacob disease" (CJD).

Q.53. Name one Prion disease that occurs in animal.
Bovine spongiform encephalopathy (BSE) in cattle (also known as "Mad cow disease").

Q.54. State the pathological changes that occur in brain.
Prion disease is characterized by

- Spongiform changes,
- Astrocytic gliosis, and
- Neuronal loss, resulting from deposition of insoluble proteins in stable amyloid fibrils.
- The protofilaments of amyloid fibrils contain pairs of β-sheets in a helical form that are continuously hydrogen bonded in all along the fibrils.

Q.55. How proteins are precipitated from aqueous solution?
Proteins being colloidal in nature are easily precipitated by a variety of methods. They include:

- Full saturation with solid ammonium sulphate
- Half saturation with a saturated solution of ammonium sulphate.
- By addition of metal ions (+ve ions)
- By addition of sulphosalicylic acid, picric acid, tungstic acid, etc. (-ve ions)
- By heat coagulation at isoelectric pH (pI)
- By addition of alcohol.

Q.56. What is meant by +ve ion precipitation?
The +ve ions most commonly used are those of heavy metals like Pb^{2+}, Zn^{++}, Ca^{++}, Hg^{++} etc. The metals precipitate proteins at the ***pH alkaline*** to its isoelectric

pH (pI). At this pH, proteins behave as anions and +ve metal ions combine with -COO group to give insoluble precipitate of metal proteinate.

Q.57. What is meant by –ve ions precipitation?

Negative ions like tungstic acid, trichloroacetic acid, picric acid, tannic acid, etc. combine with proteins when the pH of the medium is on ***acidic side*** of its isoelectric pH (pI). In acidic pH, proteins exist as Pr^+ and forms precipitate with -ve ions.

Q.58. What is the clinical use of protein precipitation?

Protein free filtrate (PFF) obtained after precipitation of proteins is used for estimation of certain body constituents in blood or serum like estimation of blood sugar or blood urea, etc.

Q.59. What do you mean by the term denaturation of proteins?

Denaturation of protiens may be defined as a disruption of the secondary, tertiary and wherever applicable quarternary organization of a protein molecule due to cleavage of non-covalent bonds.

Note: ***In denaturation, the primary structure of protein molecule i.e. Peptide bonds are not affected and remain intact.***

Q.60. Name the various agents that bring about denaturation of proteins?

Various agents that can bring about denaturation of proteins are:

- ***Physical agents:*** heat, UV light, ultrasound, and high pressure. Even violent shaking can bring about denaturation of proteins.
- ***Chemical agents:*** organic solvents, acids/alkalies, urea and various detergents.

Q.61. State the changes that occur in proteins after denaturation and its effects.

Alterations brought about are:

- ***Physical changes:***
  - Confers increased viscosity of the solution
  - Rate of diffusion decreases

- ***Chemical changes:***
  - Greatly decreased solubility at pI.
  - Maximum precipitation as floccules occur at pI. Many chemical groups which were inactive becomes exposed, e.g -SH group.
- ***Biological changes:***
  - Biologically becomes inactive-denaturation destroys enzyme and protein hormone activity.
  - Increased digestibility by proteolytic enzymes found in case of certain denatured proteins.

Q.62. Differentiate flocculation and coagulum formation of proteins.

- Denatured protein ***is soluble in extremes of pH.*** Maximum precipitation occurs as ***floccules*** at pI of the protein (flocculation). The flocculation is a ***reversible*** process and soluble in extremes of pH.
- When floccules at pI is heated further, it becomes dense white ***coagulum*** (coagulation) which is an ***irreversible*** process and is not soluble in extremes of pH.

Q.63. Give one common reaction for all amino acids.

All amino acids on heating with a solution of Ninhydrin in acetone to about 100°C give a purple-blue colored compound ***(ninhydrin reaction)***.

Q.64. Which amino acids do not give purple-blue color in ninhydrin reaction?

Proline and hydroxyproline are exceptions and they give yellow color.

Q.65. What is biuret reaction?

Protein solution in water, two or the drops of dilute copper sulphate and excess of NaOH (about one ml) produces a pink or purple-violet color.

Q.66. What is the mechanism of biuret reaction?

The colour ***depends upon the presence of 2 or more peptide bonds.*** It is due to co-ordination of cupric ions with the unshared electron pairs of peptide nitrogen and the oxygen of water to form the colored co-ordinate complex.
Note: ***Thus di-peptides and free amino acids do not give the biuret test.***

Q.67. Name one amino acid which can give +ve biuret reaction.
Only Histidine can give a +ve biuret reaction.

Q.68. What is xanthoproteic reaction?
The aromatic amino acids like phenylalanine, tyrosine and tryptophan present in protein molecule give yellow precipitate when heated with conc HNO_3. The reaction is due to nitration of aromatic ring.
Note: ***Collagen and Gelatin do not give a positive reaction.***

Q.69. Give a specific colour test for tyrosine in protein.
- ***Millon's test:*** It is a specific colour test for tyrosine of protein. Proteins having tyrosine give a ***white precipitate*** with Millon's reagent (10% mercurous chloride in H_2SO_4) on heating. ***On addition of $NaNO_2$ the precipitate turns pink-red.***

Q.70. Mention a specific color test for arginine in protein.
- ***Sakaguchi test:*** It is a specific color test for Arginine of protein. Sakaguchi reagent consists of alcoholic α-naphthol and a drop of sodium hypobromite. Guanidine group of arginine reacts to give the ***red color.***

Q.71. Mention a specific color test for sulphur containing amino acids.
- ***Lead acetate test:*** It is specific for sulphur containing amino acids. The proteins having S-containing amino acids when boiled with strong alkali split out sulphur as sodium sulphide which reacts with lead acetate to give ***black precipitate*** of lead sulphide (PbS).

Q.72. List the different methods for separation of a mixture of proteins.
The following methods can be used:
- Different concentrations of alcohol (***Cohn's fractionation*** of plasma proteins).
- Different concentrations of salt solutions like sodium sulphate ***("salting out" method).***
- Electrophoresis: Commonly employed is paper elect-rophoresis and cellulose acetate membrane (CAM) electrophoresis. Also can be used agar gel electrophoresis, polyacryl amide disc electrophoresis.

- Ion exchange chromatography using modified cellulose resins e.g DEAE cellulose.
- High performance liquid chromatography (HPLC).

Q.73. Define electrophoresis.
Electrophoresis is defined as the movement of electrically charged particles towards the anode or the cathode under the influence of an applied electrical field.

Q.74. Name the buffer commonly used in paper electrophoresis? What is the pH of the buffer?
For routine work, ***veronal buffer or barbitone buffer*** of pH 8.6 is used. In alkaline pH, protein molecules are -vely charged and they move to +ve pole when subjected to electrical field.

Q.75. What are the fractions obtained in paper electrophoresis of serum?
Albumin being lighter moves fast. It is followed by α_1 globulin, α_2 globulin, β-globulin and last γ-globulin near the origin.

Q.76. What is Rf value?
The ratio of the distance moved by a compound/ amino acid in paper chromatography to the distance moved by the solvent front is known as Rf value.

Q.77. Name six important plasma proteins.
Six plasma proteins are:
- Albumin, α_1-globulin, α_2-globulin, β-globulin, fibrinogen, and γ-globulin.

Q.78. Which fraction will be absent in serum proteins and why?
Fibrinogen is absent in serum protein because fibrinogen is used up in clot formation in separation of serum.
Serum = Plasma – Fibrinogen.

Q.79. Which fraction has the highest and lowest molecular weight among the plasma proteins?
Albumin has the lowest molecular weight of approximately 69,000 and γ-globulins have the highest molecular weight.

Q.80. Where is albumin synthesized in the body?
Albumin is mainly synthesized in the liver. Rate of synthesis is approximately 14.0 gm/day.

Q.81. State the important functions of albumin.

- Nutritive function.
- Exerts low viscosity
- Contributes 70 to 80% of osmotic pressure.
- Plays important role in exchange of fluids between blood and tissue.
- Helps in transport of several substances viz. FFA, unconjugated bilirubin, Ca^{++} and steroid hormones.
- Binding of certain drugs: Sulphonamides, aspirin, penicillin are bound to albumin and carried in the blood.

Q.82. At what level of plasma proteins and albumin edema can occur?

Edema occurs when total proteins of plasma fall below about 5.0 gm% and albumin level below approximately 2.5 gm%.

Q.83. Where are the globulins synthesized in the body?

α and **β**-globulins are synthesized in the liver, but **γ**-globulins are synthesized by plasma cells and B-cells of lymphoid tissues.

Q.84. What is oroso-mucoid? What is its functions?

Orosomucoid is α_1-acid glycoprotein. Orosomucoids are considered to be a reliable indicator of acute inflammation. Aslo functions as a transport protein for progesterone.

Q.85. What is α_1-fetoprotein? What is its clinical importance?

- It is an α_1-globulin present in high concentrations in foetal blood during pregnancy. Normal adult blood has very little of this protein, it is less than 1 μg/100 ml.
- ***Clinical importance:***
  Presence of increased amount of α_1-fetoprotein in human blood indicate hepato-cellular carcinoma or teratoblastomas.

Q.86. What is haptoglobin? What is its clinical importance?

Haptoglobin (Hp) is an α_2-globulin synthesized in Liver. Haptoglobin binds "free" Hb if present in blood. Average binding capacity of Hp irrespective of phenotype is approximately 100 mg/dl. Hp-Hb complex circulates in the blood and ultimately destroyed by RE cells.

Q.87. What is methaemalbumin? What does it signify?
Normally blood does not contain methaemalbumin. When the degree of intravascular (IV) haemolysis is rapid and severe, as may happen in incompatible blood transfusion, free Hb released exceeds the binding capacity of Hp. The free Hb combines with albumin to form ***methaemalbumin***, which can be detected by a sensitive test called ***Schumm's Test***. ***Detection of methaemalbumin points to IV haemolysis.***

Q.88. What is Bence Jones protein? What is the clinical significance?
Bence Jones protein is an abnormal protein which occurs in blood and urine of people suffering from a disease called ***multiple myeloma*** (a plasma cell tumor). It is monoclonal light chain either "K" or **"λ"** and excreted in urine of multiple myeloma patient.

Q.89. How Bence Jones protein can be detected in urine in clinical laboratory?
Presence of Bence Jones protein in urine can be identified easily by a simple **"heat test".** Take urine in a clean test-tube and heat up to 50° to 60°C, when Bence Jones protein, if present, are precipitated. But when heated further the precipitate dissolves again. Reverse occurs on cooling.

Q.90. What is caeruloplasmin?
Caeruloplasmin is copper-containing α_2-globulin, a glycoprotein with enzyme activities. **It has eight sites for binding copper** and contains about eight atoms of copper per molecule, ½ as cuprous (Cu^{+}) and ½ as cupric (Cu^{++}). It carried 0.35% copper by weight and 7 to 10% carbohydrates. It is synthesized in Liver as ***"apocaeruleplasmin"*** protein to which eight copper atoms are attached.

Q.91. What is the normal blood level of caeruloplasmin?
Normal plasma level is approximately 30 mg/dl.

Q.92. What are the functions of caeruloplasmin?
- Caeruloplasmin is ***not involved in copper transport,*** though 90% or more of total serum copper is contained in caeruloplasmin.

- ***Mainly functions as ferroxidase*** and helps in oxidation of Fe^{++} (ous) to Fe^{+++} (ic) which can be incorporated into transferrin.
- Acts as an ***antioxidant.*** By its ferroxidase activity it can convert Fe^{++} to Fe^{+++} and halts the ***Haber-weiss-Fenten*** reaction, thus preventing formation of "free" hydroxy radical $OH^{\cdot}$ and signet oxygen (O_2) from superoxide anion O_2^- and H_2O_2.

Q.93. What are acute phase proteins or reactants?

Levels of certain proteins in plasma increase during acute inflammatory states or secondary to certain types of tissue damage. These proteins are called "acute phase proteins or reactants".

Q.94. Name some of the "acute phase proteins or reactants".

Acute phase proteins or reactants include the following:

- C-reactive protein (CRP)
- Haptoglobin (HP)
- α_1-antitrypsin
- α_1-acid glycoprotein ("oroso-mucoid") and
- Fibrinogen.

Q.95. What is C-reactive Protein?

- It is a **β**-globulin, present in concentration of less than 1 mg per 100 ml in the adult males.
- It precipitates with group C polysaccharide of pneumococci, in presence of Ca^{++}, hence called as C-reactive protein.

Q.96. What are the electrophoretic forms of C-reactive protein?

Two electrophoretic forms have been found in immuno electrophoresis:

- CRP alone in the **α**-band.
- A CRP complex with acidic mucopolysaccharide called m-CRP in **β**-region.

Q.97. State the clinical significance and functions of C-reactive protein.

- A sensitive indicator of the early phase of an inflammatory process.
- Remains increased in presence of solid tumors.

- A role in the formation of heme proteins.
- Can bind to T-lymphocytes and can activate complement.

Q.98. What is α_1-antitrypsin (α_1 – AT)?

α_1-antitrypsin is an α_1-antiprotease having molecular weight approx. 45,000 to 54,000.

- It inhibits trypsin, elastase and certain other proteases by forming complexes with them.
- It is synthesized by liver.

Q.99. What is the normal value in adults?

Normal value in adults varies from 2 to 4 gm/liter.

Q.100. How can you suspect α_1-AT deficiency?

A very low or absent α_1 globulin band in electrophoresis suggests α_1-antitrypsin deficiency (α_1-AT).

Q.101. Name some clinical conditions which are associated with α_1-AT deficiency.

α_1-AT deficiency has been found to be associated with following clinical conditions:

- Emphysema lung
- Juvenile cirrhosis of liver.

Q.102. What are the normal values of total proteins and differential proteins?

- Total proteins: 7.0 to 7.5 gm%
- Differential protein:

gm% *(by precipitation)*	*% of total proteins (by electrophoresis)*
• Albumin-3.7 to 5.3	50 to 70%
• Globulins-1.8 to 3.6	29.5 to 54%
• α_1-globulins-0.1 to 0.4	2.0 to 6.0%
• α_2-globulins-0.4 to 0.8	5.0 to 11.0%
• β-globulins-0.5 to 1.3	7.0 to 16%
• γ-globulins-0.6 to 1.5	11.0 to 22.0%

Q.103. What is the normal level of fibrinogen in plasma?

0.2 to 0.4 gm% (200 to 400 mg%).

Q.104. What is the normal A:G ratio? In which condition the A:G ratio is reversed?

- Normal A:G ratio is 2.5 to 1.0 (usually 2:1)
- It is reversed characteristically in cirrhosis liver.

Q.105. Enumerate the principal functions of plasma proteins in the body.

- Nutritive functions
- Fluid exchange in capillary bed
- Buffering action
- Binding and transport function
- Responsible for viscosity of blood. Fibrinogen molecules are large and asymmetric and contributes maximum.
- Role in blood coagulation and fibrinolysis
- Defensive action (immunological function) by **γ**-globulins.
- Enzyme action: enzymes are proteins
- Hormones: certain harmones are proteins like TSH ACTH, oxytocin, vasopressin, insulin, parathormone, etc.

Q.106. What is hyperproteinemia?

Increase of total proteins above normal is called hyperproteinemia.

Q.107. State the causes of hyperproteinemia.

Hyperproteinemia can occur as follows:

- ***Hemoconcentration:*** Due to dehydration. In such a situation both albumin and globulins are increased, A:G ratio remians unaltered.
- ***Hypergammaglobulinemia:*** Diseases resulting in high levels of plasma globulins, mainly **γ**-globulins. In such a situation, albumin remains either normal or reduced. A:G ratio is usually reversed.

Q.108. What are the types of hypergammaglobulinemias? Give a few examples.

- ***Polyclonal gammopathies:***
  - Chronic infections e.g TB, kala-azar
  - Chronic liver disease—Cirrhosis liver
  - Sarcoidosis
  - Autoimmune diseases e.g rheumatoid arthritis, systemic lupus erythematosus (SLE)
- ***Monolclonal gammopathies:***
  - Multiple myeloma
  - Macroglobulinemia etc.

Q.109. What is hypoproteinemia?

Decrease of total proteins below normal is called hypoproteinemia.

Q.110. State some causes of hypoproteinemia.

Hypoproteinemia can occur as follows,

- ***Hemodilution:*** Water intoxication (overload), IV infusion of fluids. In this both albumin and globulins are decreased ↓ and A:G ratio remains unaltered.
- ***Hypoalbuminemia:***
  Conditions resulting in low albumin level, accompanied either by no increase in globulin or by an increase which is less than the fall in albumin. A:G ratio is decreased.
  Examples:
  – Loss through kidney: nephrotic syndrome
  – Loss through GI Tract: protein losing enteropathy etc.
- **Hypogammaglobulinemia:**
  – Conditions due to decrease in **γ-**globulin.

Q.111. Name four transport binding proteins.

- ***Transferrin (siderophillin):*** Binding and transport of Fe.
- ***Thyroxine binding globulin (TBG):*** Binds and transport thyroxine (T_4)
- ***Transcortin:*** binding and transport of cortisol
- ***Retinol binding protein (RBP):*** Binding and transport of retinol (vit A).

CHAPTER

4 Immunoglobulins—Chemistry and Functions

Q.1. What is an antigen?

Antigen is a substance which when introduced into an animal can induce a detectable immune response, which may be humoral, cellular or both.

Q.2. What is the nature of an antigen?

Most antigens are macromolecules of proteins though synthetic polypeptides and certain synthetic polymers like polyvinyl pyrolidone are known to behave like antigens.

Q.3. What is meant by the term antigenic determinant and valence?

The portion of the antigen molecule which binds to its specific antibody is called the "antigenic determinant" (or "epitope").

The total number of epitopes for antigen molecule is the valence of that antigen.

Q.4. What are haptens?

Haptens are small molecules which by themselves are not capable of evoking an antibody response upon introduction into an animal. But when they are conjugated with a ***carrier*** molecule such as a protein, they behave like typical antigens.

Q.5. How the word hapten derived?

Hapten is derived from the Greek word ***haptein*** which means ***to fasten***.

Q.6. What is understood by the term immune response?

"Immune response" may be defined as a defensive reaction by the body to the entry of a foreign substance

viz. bacteria, viruses or even a foreign protein into human body.

- The immune response is a complex interplay of cells and their products. By this mechanism the organism tries to eliminate or destroy the invading foreign agent.

Q.7. What are the types of immune response?

Immune responses are of **2 types:**

- Humoral immune response and
- Cellular immune response.

Q.8. What is humoral immune response?

The humoral immune response is mediated by specific protein molecules, usually γ-globulins, called antibodies or immunoglobulins (Igs); these are formed by B-lymphocytes.

Q.9. What is cellular immune response?

In cellular immune response, another type of lymphocytes called **T-lymphocytes** with the co-ordination with other cell systems, recognize the invading agent i.e. the antigen by virtue of certain unique molecules on their cell surface and destroy the antigen **(cell-mediated immunity).**

Q.10. Define immunoglobulins?

The immunoglobulins are defined to constitute a heterogenous family of serum proteins, which either function as antibodies or are chemically related to antibodies, and on electrophoresis they mainly occupy the γ-globulin position but also may occur in β-or α_2-regions.

Q.11. What are the criteria laid down by WHO to call a substance immunoglobulin?

WHO's criteria for immunoglobulins are as follows:

- Should be proteins
- Should be of animal origin
- Should have a common structure and
- Should function as antibodies.

Q.12. What is the WHO classification of immunoglobulins? Give the corresponding old nomenclature.

Immunoglobulins (Igs) have been divided into **five main classes** by WHO according to the molecular weight,

electrophoretic mobility, ultracentrifugal sedimentation and structural characteristics.

Classes	*Corresponding old nomenclatures*
• IgG (γG)—	7Sγ, γ_2, γss, 6.6 sγ
• IgA (γA)—	γ_1A, β_2A, 7 sγ^1
• IgM (γm)—	19 sγ, γ_1m, β_2M
• IgD (γD)—	—
• IgE (γE)—	—

Q.13. Why are the immunoglobulin classes and types so named?

Classes G, M, A, D and E are so named as per the nature of "heavy chains". Thus:

- G means **γ**-chains (gamma)
- M means **μ** chains (mew)
- A means **α** chains (alpha)
- D means **δ** chains (delta)
- E means **ε** chains (epsilon)

Two types 'L' and 'K' stand for **λ** (Lambda) and **κ** (Kappa) light chains.

Q.14. How many immunoglobulins are there?

There are total **10** different Igs as follows:

- Five classes of Igs depending on types of heavy chains IgG, IgM, IgA, IgD, and IgE.
- There are two types 'L' and 'K' for each class possessing λ (Lambda) and '**κ**' light chains. A given Ig molecule contains identical λ (Lambda) and 'κ' chains but never both.
- Thus, there are 5 × 2 = 10 different immunoglobulins.

Q.15. State the salient features of structure of an immunoglobulin molecule?

- An immunoglobulin has Y-shape.
- The molecule is about 250-300 A° long and 40 A° wide and possesses a ***"hinge portion"***.
- Each molecule is composed of two heavy chains ('H'-chains) and two light chains ('L' chains).
- Two light chains **'κ'** (Kappa) or **λ** (Lambda), each of molecular weight 23,000 and consist of 214 amino acids.

- Two heavy chains **γ, μ, α, δ,** and **ε** each molecular weight 50,000 to 70,000 and consist of 446 amino acids.
- The 'H' chains are held together by non-covalent forces and usually covalent inter-chain disulfide bridges (Total of 3 to 4).
- The proportion of 'κ' to 'λ' chains varies from species to species being about 2:1 in humans.

Q.16. Differentiate the five classes of immunoglobulins on the following properties: Serum concentration, molecular weight, electrophoretic mobility, sedimentation co-efficient (S_f) and placental transfer.

- Different properties of 5 classes of Igs are as follows:

Properties	*Classes of Igs*				
	IgG	*IgA*	*IgM*	*IgD*	*IgE*
• **Serum concentration (mg/dl average)**	1200	200	120	3	0.05 (50 μg%)
• **Molecular weight**	145,000	150,000 to 500,000	900,000 to 1,000,000	180,000	190,000
• **Electrophoretic mobility**	γ	Fast γ→β	Fast γ→β	Fast γ	Fast γ
• **Sf**	7S	7S-13S	19S	7S to 8S	8S
• **Placental transfer**	+++	—	—	—	—

Q.17. Name five antibodies contained by IgG.

The following five antibodies have been identified in this class:

- Immune anti-A and anti, B
- Anti-Rh antibodies—incomplete type
- Antiviral antibodies
- Antistreptolysin
- Auto-antibodies to thyroid

Q.18. Which immunoglobulins exist in polymeric forms?

- IgM normally exists as **pentamer**
- IgA molecule also exists in polymeric forms specially as a **dimer.**

Q.19. What is J-chain?

J-chain is a small glycopeptide with an unusually high content of aspartic acid (Asp) and glutamic acid. It is found

in each IgM pentamer or in polymeric IgA. It is claimed that J-chain facilitates the polymerization.

Q.20. Name five antibodies contained in IgM.

The following five antibodies have been identified in IgM class:

- Naturally occurring anti-A and anti-B.
- Anti-Rh antibodies-saline type
- Cold antibodies (anti-i type)
- LE factor
- Rheumatoid factor (RF).

Q.21. In the evolutionary scale, which antibody appears first in human body?

In terms of evolution, IgG claimed to have evolved later than IgM. The sequence in the animal's response to antigenic stimulation usually consists of IgM produced initially, followed later and ultimately replaced by IgG.

Q.22. Name one condition in which there occurs excessive production of IgM.

Excessive production of IgM occurs in ***Walden Ströms macroglobulinemia*** due to malignant proliferation of lympho-cytoid cells.

Q.23. What is SIA test?

It is a simple screening test used for Walden Ströms macroglobulinemia for presence of excessive IgM.

Q.24. Which immunoglobulin is found predominantly in external secretions like tears, saliva, GI fluids, etc.?

IgA is found predominantly in external secretions and it is called as ***secretory IgA***.

Q.25. How does secretory IgA differ from vascular IgA?

Secretory IgA differs from vascular IgA as follows:

- Tend to be a higher polymer consists of 4 chains of basic units and one molecule of J-chain.
- Molecular weight of secretory IgA is higher, approximately 400,000.
- Structurally it **possesses** another **small protein** called **"T-protein"** (Transport protein or Transport Piece).
- Plays an important role in host defence mechanisms against viral/bacterial infections.

Q.26. What is T-piece?

- T-piece is a single polypeptide chain of approximate mol wt of 70,000. It is produced by the ductal epithelial cells of salivary glands, lacrymal glands and mucosal glands of GI Tract.
- Secretory IgA molecule is a dimer, and T-piece is attached to the L-chains (Fc regions) of the two IgA molecules.

Q.27. What are the functions of T-Piece of secretory IgA molecule?

T-protein (T-Piece) is responsible for two important biological properties of secretory (non-vascular) IgA:

- Its selective transport to secretions.
- Its protective function to IgA, probably prevents digestion by proteolytic enzymes found in GI secretions.

Q.28. What is the percentage of the different classes of immunoglobulins in blood?

- IgG = 70 to 80%
- IgA = 10 to 20%
- IgM = 3 to 10%
- IgD = 0.2%
- IgE = 0.004%

Q.29. What are reagins (or Reaginic antibodies)?

- IgE antibodies are called "reagins" (or Reaginic antibodies).

Q.30. How IgE is responsible for allergic response?

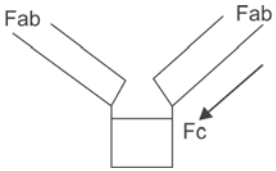
When IgE antibodies combine with certain specific allergens (antigens), triggers the release from mast cells pharmacologic mediators which are responsible for the characteristic "wheal" and "flare" skin reactions evoked by the exposure of the skin of allergic individuals to allergens.

Q.31. Name the enzymes used to cleave the Ig molecule.

Two enzymes are used:

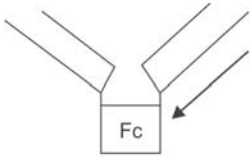
- Pepsin
- Papain

Q.32. How does papain cleave the Ig molecule?
Papain **cleaves** the Ig molecule at the **"hinge"** region and forms two fragments:
- 'Fab' fragments-contains entire 'L' chain and the V_H and CH-1 domain of H-chain and
- One "Fc" fragment composed C-terminal halves of the H-chains



Site of action of papain in the ***"hinge". region***, ∴ Products = 2 Fab + 1 Fc.

Q.33. How does papsin cleave the Ig molecule?
Pepsin cuts the Ig molecule at long arm below the S-S linkage of 'H' chains so that it forms:
- One large fragment of 2 Fab $(Fab)_2$
- 'Fc' fragment is degraded extensively.



Site of action of pepsin ***below s-s linkage*** of H-chains.
∴ Products = $(Fab)_2$ + degraded Fc

Q.34. Which Ig passes through the placental barrier?
Only immunoglobulin molecule which is easily transported across the placenta is the IgG molecule. This accounts for the immunity to the newborn babies.

Q.35. Though more than 80% of serum IgA are similar to IgG in molecular size and molecular weight, IgA molecule cannot pass through the placental barrier. Why?
Reason is not very clear. Probably it is ***due to the difference in the "Fc" fragments*** of both molecules. There is 8 to 10.5% carbohydrates in IgA against only 2.5% in IgG. Over 90% of carbohydrates are present in 'Fc' fragment. This probably explains the absence of IgA in newborn babies.

Q.36. What are "domains" of Ig molecule?
The polypeptide chains do not exist as linear sequences of amino acids but are folded by disulfide bonds into globular regions called ***domains***. The domains in 'H-chains' are called V_H and CH-1, CH-2, CH-3 and CH-4. The "domains" on L-chains are called VL and CL.

Q.37. What is the antigen binding site of Ig molecule?
The part of the antibody molecule which binds antigen is formed by only small number of aminoacids in the "V" regions of 'H' and 'L' chains. These amino acids are brought into close relationship by the folding of the 'V' regions.

Q.38. How antigen-antibody complexes are formed?
- Antigens bind to antibody molecules with varying degrees of affinity. The interaction involves physical forces. The "Fab" portion of the antibody recognizes the corresponding "epitopes" on the antigen and the complex that is formed is due to physical forces viz electrostatic, hydrogen bonding, hydrophobic and van der Waals forces.
  Ab + Ag ⟶ Ab-Ag complex.
- The antigen-antibody reaction is reversible and the affinity of the antibody to the antigen determines the equilibrium of the reaction.

Q.39. What are polyclonal antibodies? Give examples.
In response to an antigenic challenge body produces different types of antibodies against various antigenic determinants (epitopes) of the antigen. The antibodies thus ***produced by the different "clones" of antibody forming cells*** are called polyclonal antibodies.
Examples: Body produces polyclonal antibodies in response to all types of microbial infections (Polyclonal gammopathy).

Q.40. What is monoclonal antibody? Give example.
When one particular "clone" of antibody producing cells secrete a particular type of antibody against a particular antigenic determinant (epitope), it is called monoclonal antibody.
Example: Monoclonal antibodies are typically produced in "multiple myeloma" (a plasma cell tumour). In this

condition paraprotein is produced which gives a ***sharp paraprotein band called "M-band"*** in electrophoresis. (monoclonal gammopathy).

Q.41. Can monoclonal antibodies be produced in the laboratory? Who first produced?

Kölher and Milstein in 1975, first produced monoclonal antibodies in the laboratory from ***hybridoma*** cells.

Q.42. What is hybridoma? How it is produced?

Hybridoma is a "hybrid" cell capable of producing monoclonal antibodies. Cultured splenic cells from mouse immunized with specific antigen (against whom the monoclonal antibodies to be raised), can be **fused** with that of cultured myeloma cells. The hybrid cell (a hybridoma) thus produced remain immortal in culture like myeloma cells and produce monoclonal antibodies like the immunized splenic cells.

Q.43. What are the properties of hybridoma cells?

Can propagate in culture for indefinite period (Immortal)

- Can secrete Igs
- Has *HGPRTase* enzyme, hence "salvage" pathway of purine synthesis can operate.

Q.44. State the uses of monoclonal antibodies.

(a) ***Diagnostic uses:***

- Monoclonal antibodies have been raised for the diagnosis of many bacterial, viral and parasitic diseases.
- Used for blood grouping
- Used for standardization and leucocyte identification through the cluster differentiate antigen (CD antigen).
- Recently used against HLA antigens for phenotype screening purposes.

(b) ***Therapeutic uses:***

- Used for antitumor therapy
- Immunosuppression in organ transplants
- Used in autoimmune diseases e.g. recently in rheumatoid arthritis monoclonal CD4 antibody tried.

CHAPTER

5 Chemistry of Nucleotides and Nucleic Acid

Q.1. What are nucleoproteins?

Nucleoproteins are conjugated proteins, characterized by presence of non-protein prosthetic group nucleic acid attached to one or more molecules of a simple protein, a basic protein histone or protamine. Nucleoproteins → Nucleic acid + proteins (Histone or Protamine).

Q.2. What are purines and pyrimidines?

Purines and pyrimidines are heterocyclic compounds having "N" in their molecule. They are nitrogen bases present in nucleic acids. In purines, there are four 'N' atoms while in pyrimidines, there are only two.

Q.3. Name the purine bases. Give their chemical names.

Purine bases are ***mainly two:***

- ***Adenine:*** Chemically it is 6-amino purine.
- ***Guanine:*** Chemically it is 2-amino-6-oxy purine.

Note: ***Guanine can exist as "lactam" and "lactim" forms, adenine cannot.***

Q.4. Name the pyrimidine bases. Give their chemical names.

Pyrimidines are ***mainly three:***

- ***Cytosine:*** Chemically 2-deoxy-6-amino pyrimidine.
- ***Thymine:*** It is 5-methyl uracil. Chemically it is 2, 6-deoxy-5-methyl pyrimidine.
- ***Uracil:*** Chemically it is 2, 6-di-oxy pyrimidine.

Note: ***All the pyrimidine bases can occur both in "lactam" and "lactim" forms.***

Q.5. Name the nucleic acids which are of biomedical importance.

Nucleic acids of biomedical importance are mainly two:

- ***DNA:*** Deoxyribonucleic acid—it constitutes the ***genetic material.***
- ***RNA:*** Ribonucleic acid—involved in ***protein synthesis.***

Q.6. Name the common nitrogen bases in DNA.

Adenine, guanine, cytosine and thymine

Note: ***Uracil is absent.***

Q.7. Name the common bases in RNA.

Adenine, guanine, cytosine and uracil.

Note: ***Thymine is absent.***

Q.8. What sugars are present in nucleic acids?

D-ribose and D-2-deoxy ribose are the only two pentose sugars found in nucleic acids. Both sugars are present as the β-furanoside ring structures. ***D-ribose is present in RNA, while D-2-deoxy ribose is present in DNAs.***

Q.9. Name same minor bases found in nucleic acids.

- Some minor bases found in nucleic acids are:
- 5-OH methyl cytosine in bacteriophages.
- Both bacterial and human DNA have been found to contain significant quantities of 5-methyl cytosine.
- Certain unusual bases found in m-RNA molecules of mammalian cells viz., N^6-methyl adenine, N^7-methyl guanine, N^6-N^6 dimethyl adenine.

Q.10. Name the methylated purines of plant origin which have pharmacologic properties.

- ***Theophylline:*** found in tea. Chemically it is 1, 3-dimethyl xanthine.
- ***Theobromine:*** found in cocoa. Chemically it is 3, 7-dimethyl xanthine.
- ***Caffeine:*** present in Coffee. It is Chemically 1, 3, 7-trimethyl xanthine.

Q.11. What is nucleoside? Give two examples.

- The nucleosides are composed of purine or pyrimidine base + either D-ribose (as in RNA) or D-2-deoxy ribose (as in DNA).

Examples:

1. Purine nucleoside; **adenosine**
   (purine base Adenine + D-ribose)
2. Pyrimidine nucleoside: **uridine**
   (Pyrimidine base uracil + D-ribose)

Q.12. What is the linkage in purine nucleoside?

The linkage in purine nucleoside is at position 9 of purine base and carbon 1' of D-ribose or D-2-deoxyribose (**β**-N-glycosidic linkage).

Q.13. What is the linkage in pyrimidine nucleoside?

The linkage in pyrimidine nucleoside is at position 3 of pyrimidine base and carbon-1' of D-ribose or D-2-deoxy ribose (**β**-N-glycosidic linkage).

Q.14. Name the nucleosides of adenine, guanine, cytosine and uracil with D-ribose.

Adenosine, guanosine, cytidine, uridine.

Q.15. Name the nucleosides of adenine, guanine, cytosine, and thymine with D-2-deoxy ribose.

Deoxy adenosine, Deoxy guanosine, Deoxy cytidine, and Thymidine.

Q.16. What is a nucleotide?

A nucleotide is a nucleoside to which a phosphoric acid group is attached to the sugar molecule by esterification of a definite -OH group.

Thus general composition will be:

purine or pyrimidine base-sugar-PO_4

Q.17. Name the nucleotides present in RNA.

Name	*Base*	*Sugar*	*Acid*
• **Adenylic acid (Adenylate or AMP)**	– Adenine	+ Ribose	+ Phosphoric acid
• **Guanylic acid (Guanylate or GMP)**	– Guanine	+ Ribose	+ Phosphoric acid
• **Cytidylic acid (Cytidylate or CMP)**	– Cytosine	+ Ribose	+ Phosphoric acid
• **Uridylic acid (Uridylate or UMP)**	– Uracil	+ Ribose	+ Phosphoric acid

Note: ***There is no Thymidylic acid in RNA.***

Q.18. Name the nucleotides present in DNA.

Name	*Base*	*Sugar*	*Acid*
• **deoxy adenylic acid (deoxy adenylate or d Amp)**	– Adenine	+ deoxy ribose	+ Phosphoric acid
• **deoxyguanylic acid (deoxyguanylate or d Gmp)**	– Guanine	+ deoxy ribose	+ Phosphoric acid
• **deoxycytidylic acid (deoxy cytidylate or d cmp)**	– Cytosine	+ deoxy ribose	+ Phosphoric acid
• **Thymidylic acid (Thymidylate or Tmp)**	– Thymine	+ deoxy ribose	+ Phosphoric acid

Note: ***There is no deoxy uridylic acid in DNA.***

Q.19. Name the synthetic nucleosides in which ribose is replaced by arabinose. What is the clinical importance?

They are:

- Cytabrine (arabinosyl cytosine or Ara c)
- Vidarabine (arabinosyl adenine or Ara a)

Both are synthetic nucleosides containing arabinose in place of ribose. They are used in chemotherapy of cancers and certain viral infections.

Q.20. What is the clinical importance of 5-iodo-deoxy uridine?

5-iodo-deoxy uridine is a recent synthetic nucleoside analogue. It possesses anti-viral activities and found to be ***effective in treatment of herpetic keratitis.***

Q.21. What is allopurinol? What is its clinical importance?

- Allopurinol is a synthetic purine analogue. It is chemically 4-OH-pyrazolo pyrimidine.
- It is used as a drug in treatment of gout and hyperuricaemias. ***It inhibits the enzyme xanthine oxidase*** by competitive inhibition and thus lowers uric acid level in blood preventing uric acid formation.

Q.22. Classify and name the nucleotides found in tissues and cells which are biologically important.

Various nucleotides which can occur in tissues and cells and have diverse biochemical functions are:

- **Adenosine nucleotides:** ATP, ADP, AMP and cyclic AMP.
- **Guanosine nucleotides:** GTP, GDP, GMP and cyclic GMP.
- **Uridine nucleotides:** UTP, UDP, UMP and UDP-G
- **Cytidine nucleotides:** CTP, CDP, CMP

Q.23. What is ATP?

ATP is a ***nucleotide,*** chemically it is ***adenosine triphosphate.*** It is store house of energy and is called the **"storage battery"** of the tissues. Two of the three phosphate residues are "high energy phosphate" (~p) and on hydrolysis each releases energy (7.6 K. Cal); the energy is utilized for endergonic/synthetic reactions.

Q.24. Enumerate some important functions of ATP in the body.

- ATP is an important source of energy and used for:
  - muscle contraction
  - transmission of nerve impulses
  - transport of nutrients across cell membranes.
  - motility of spermatozoa.
- Many synthetic reactions in body require energy ("endergonic" reactions)
- Required for formation of "active Methionine" (S adenosyl methionine), required for methylation reactions.
- Required for formation of "active sulphate" which is necessary for incorporation of sulphates.
- ATP donates phosphate for a variety of "phosphotransferase" reactions.
- In body, ATP is converted to ADP, AMP and cyclic nucleotides like 3 '-5'-cyclic AMP which have important role in regulation of metabolic reactions.

Q.25. Enumerate three important functions of GTP.

GTP is chemically guanosine triphosphate. Three important functions are:

- GTP is required for protein synthesis.
- GTP is required for formation of 3'-5'-cyclic AMP.
- Oxidation of succinyl-CoA in the TCA cycle involves phosphorylation of GDP to form ATP.

Q.26. Name two cyclic nucleosides of biological importance.

- **S-adenosyl methionine, SAM ("active" methionine)** required for methylation reactions.
- **Phospho adenosine phosphosulphate (PAPS)**-also called **"active sulphate".** It is formed from ATP and SO_4 and required for incorporation of SO_4 in biomolecules.

Q.27. What is DNA?

DNA is deoxyribonucleic acid. It is a ***polymer of deoxy-ribonucleotides*** and is found in chromosomes of nucleus and also in mitochondria.

Q.28. State the structural organizations of DNA.

Like proteins, DNA has **three** structural organizations:

- Primary structure
- Secondary structure
- Tertiary structure

Q.29. What is the primary structure of DNA?

The primary structure constitutes the number and sequence of different deoxy-ribonucleotides in its strand joined together by phosphodiester linkages.

Q.30. How many deoxy ribonucleotides are there in DNA? Name them.

Four deoxyribonucleotides are found. They are:

- Adenosine deoxyribonucleotide (dA)
- Thymine deoxyribonucleotide (dT)
- Guanine deoxyribonucleotide (dG)
- Cytosine deoxyribonucleotide (dC)

Q.31. What is the secondary structure of DNA?

According to **Watson** and **Crick,** the DNA molecule consists of two right handed helical chains of deoxy-ribonucleotides coiled around the same axis and held together as a **"double helix"**. The two strands run in opposite directions i.e. **'antiparallel';** the terminal phosphate groups being at opposite ends of the two helical strands. The strands are held together by interchain hydrogen bonds and hydrophobic forces.

Q.32. What is Chargaff's rule?

Chargaff observed that molar content of adenine is equal to thymine (A = T) and the molar content of guanine is equal to cytosine (G = C). Hence in secondary structure there should be equal number of A and T and equal number of G and C.

Q.33. State the hydrogen Bonds present in between the bases.

- **Two hydrogen bonds** between adenine (A) and thymine (T)
- **Three hydrogen bonds** between guanine (G) and cytosine (C).

The length of hydrogen bond is approximately 3.0 A^o.

Q.34. What is the tertiary structure of DNA?

- DNA double strands are held by dense cylindrical flatfaced particles called **"nucleosome"**.
- Each nucleosome is an 8 member structure of different histones.
- Nucleosomes with coiled DNA form the fibril axis of 10 nm. These fibrils are arranged one above the other to form 25 to 30 nm fibre axis.

Q.35. What are the different conformations of DNA?

The conformations of DNA have been studied by X-ray crystallography. They are:

- B DNA
- A DNA and
- Z DNA (zig-zag DNA)

Q.36. Which is the commonest conformation found?

- B-DNA

Q.37. What is Z-DNA (zig-zag DNA)?

- A-DNA form with left-handed double helix in which the phospho-diester backbone zig-zags along the molecule. Hence, it is called Z-DNA (zig-zag DNA).
- Z-DNA is longer and thinner than B-DNA and is least twisted.

Q.38. Which regions of DNA are more prone to form Z-conformation?

The regions rich in guanine and cytosine base pairs are more prone to assume Z-conformation.

Q.39. What is meant by Tm of DNA?

The temperature at which DNA is half-denatured is called the melting temperature or "Tm" of DNA.

Q.40. Which DNA has higher Tm?

DNA rich in "guanine-cytosine" base pairs has a higher Tm than DNA with high proportion of A-T pairs.

Q.41. What is annealing?

If a melted sample of DNA is slowly cooled, the absorbance of the solution decreases. This indicates the complementary strands being paired again. This process is called ***annealing*** and it can occur only at a temperature below Tm of DNA.

Q.42. What is mitochondrial DNA? What is the function?
The proteins synthesized in cytoplasm by protein synthesizing apparatus cannot pass through the mitochondrial membrane. Hence for synthesizing the proteins of mitochondrial matrix and membrane, there is a second genetic system with DNA and RNAs in the mitochondrion itself. The mitochondrial DNA is **also double stranded** like nuclear DNA but it is **circular**.

Q.43. What are the different sequences of bases in eukaryote DNA?
Three types of base sequences seen in eukaryote DNA:
- ***Unique sequences:*** This region codes for proteins.
- ***Repetitive sequences:*** 20 to 30% of DNA has sequences of N-bases which are repetitive i.e. the same sequences report here and there. They can be **of** 2 types:
  i. Moderately repetitive
  ii. Highly repetitive

Q.44. What are exons and introns?
- Unique sequences constitute ***exons***. Exon transcripts are spliced and form the m-RNA proper which codes for proteins.
- Moderately repetitive sequence which occurs in between unique sequences constitute the ***introns***. The ***introns transcripts are non-coding.***

Q.45. What is a gene (or cistron)?
Gene is the biological unit of heredity. It is the segment of a DNA molecule specifying one complete polydeoxy nucleotide chain. Biochemically, it is called as cistron.

Q.46. Why eukaryote gene is monocistronic?
Eukaryote cistron (gene) is discontinuous strand in which "exons" are separated by "introns". After transcription, the introns-transcripts are excised out, and the exons-transcripts spliced to give m-RNA proper. ***Only a single m-RNA is formed*** and ***hence the eukaryote DNA is monocistronic.*** (cf. prokaryote DNA which is polycistronic).

Q.47. What is an RNA?
RNA is chemically Ribonucleic acid. It is a polymer of ribonucleotides of adenine, guanine, cytosine and uracil joined together by 3'-5' phosphodiesterase bonds.
Note: ***Thymine is absent in RNA.***

Q.48. What are the different types of RNAs?

There are **three different types** of RNAs. They are:

- **Ribosomal RNA** (r-RNA) or high molecular weight RNA.
- **Transfer RNA** (t-RNA). Also called as soluble RNA (S-RNA).
- **Messenger RNA** (m-RNA). Also known as informational RNA.

Q.49. Mention the biochemical and functional characteristics of ribosomal RNA (r-RNA).

- 50 to 80% of total RNA is associated with small particles called ribosomes.
- Contains 50% RNA + 50% proteins.
- Have relatively higher molecular weight than the other RNAs.
- Most of the ribosomes are attached to the endoplasmic reticulum and form the platform for protein synthesis. The individual ribosomes are held together by a strand of m-RNA as a cluster.

Q.50. What is t-RNA? What is its role?

- t-RNA constitutes 10 to 20% total RNAs.
- Molecular weight is low, hence it is soluble and called also as S-RNA.
- There are 20 or more t-RNA molecules, ***one for each amino acid.***
- The individual amino acids are carried by t-RNA to the protein synthesis. The anti-codon of t-RNA is recognized by the codon of m-RNA for incorporation of a specified amino acid in a protein molecule.

Q.51. What is hn-RNA? State the characteristics of hn-RNA and its functions.

- The m-RNA that comes out to cytoplasm is the product of processing of a precursor called ***"heterogeneous nuclear RNA or hn-RNA".***
- ***Characteristics:***
  - synthesized in the nucleus.
  - has a half life of 23 minutes
  - has 400 to 4000 nucleotides and it is 10 to 100 times bigger than m-RNA.

- It is bound to macromolecular proteins called ***informofers*** and exists as heterogenous ribonuclear proteins **(hn RNP)**
- ***Function:***
  75% of hn RNA is degraded in the nucleus. Only 25% of the hn-RNA forms a precursor of m-RNA called **pre-m-RNA**.

Q.52. What is pre-m-RNA?

- It is precursor of m-RNA and formed from hn-RNA.
- Approximately 80% of length of pre-m-RNA is removed as "introns inscripts" and only 20% of pre-m-RNA the "exons inscripts" are spliced to form m-RNA.

Q.53. What is m-RNA? State the biochemical and functional characteristics.

- m-RNA is formed from pre-m-RNA and accounts for less than 10% of total RNA.
- It is short lived and its molecular weight is high.
- It is synthesized from DNA by transcription and carries a specific sequence of nucleotides in triplets called codons, responsible for synthesis of a specific protein molecule.

Q.54. Describe a typical t-RNA molecule.

- RNA is a single stranded and coiled, appearing like a ***"clover-leaf".***
- It contains the N-bases adenine, guanine, cytosine and uracil (thymine in a few), and sugar ribose and phosphoric acid.
- Folds of secondary structure are stabilized by H-bonds between complementary bases in different portions of the same strand.
- The ***terminus of all RNAs is the same-C-C-A.***
- ***Three loops*** in the RNA structure:
  - ***Anticodon loop:*** it is picked up by "codon" of m-RNA by base pairing.
  - ***TyC loop:*** it binds t-RNA amino acid to ribosomal surface.
  - ***DHU loop:*** it is for recognition by the concerned enzyme ***"t-RNA aminoacyl synthetase".***

Q.55. In which portion of the molecule the amino acid is attached?

Amino acid is attached to t-RNA molecule to the free C-C-A end to the 3'-carbon of adenosine.

Q.56. How are the nucleic acids DNA and RNAs linked to protein histone in nucleoproteins?

Due to presence of phosphoric acid, DNA and RNAs are charged negatively. On the other hand, protein histone is + vely charged. Thus – vely charged nucleic acids and + vely charged proteins are held together by ***"electrostatic attraction"*** and DNA is wound round histones in the nucleosomes.

Q.57. What are satellite DNAs?

The highly repetitive sequence of chromosomal DNA, when separated after isopycnic centrifugation in C_sCl after shearing the DNA into segments, the DNA distributes into a main band and a set of smaller bands termed "satellite bands" of DNA. In humans, four satellite DNAs constitute 6% of the chromosomal DNA.

Q.58. Name two cyclic nucleotides of biomedical importance.

- They are:
  - Cyclic AMP (3', 5'-adenosine monophosphate)
  - Cyclic GMP (3', 5'-guanosine monophosphate)

Q.59. What is cyclic AMP? How it is formed in the body?

- Cyclic AMP is a cyclic nucleotide and chemically it is 3', 5'-adenosine monophosphate.
- It is formed in the cells from ATP under the influence of an enzyme ***adenyl cyclase*** in presence of Mg^{++} ions and GTP.

Q.60. What is the role of GTP in adenyl cyclase activity?

- The ***adenyl cyclase*** enzyme can be activated or inhibited by two independent GTP-dependent regulatory proteins Gs and Gi, each of which has α, β and δ subunits.
- Gs activates the enzymes through formation of GTPαs and Gi inhibits through, GTPα.

Q.61. How cyclic AMP is inactivated or degraded?

Cyclic AMP is rapidly inactivated by an enzyme, a cyclic nucleotide ***phosphodiesterase*** which opens the 3'-5' phosphate bond at the 3'-position leaving ordinary 5' AMP as the product which is inactive.

Q.62. State some known activators and inhibitors of the enzyme phosphodiesterase.

Activators	Inhibitors
Promotes degradation of cyclic AMP thus reducing its level in the cells.	Prevents degradation of cyclic AMP and thus increases cyclic AMP level in the cells.
Examples:	**Examples:**
• Imidazoles	• Methyl xanthines: Theophylline > caffeine > Theobromine
• Mg^{++}	• Papaverine
• Mg^{++} ions	• Reserpine
• NH_4^+ ions	• Bromolysergic acid diethyl amide.

Q.63. State some important functions of Cyclic AMP.

Some important functions of cyclic AMP are as follows:

- Mediator of hormone action—acts as "second messenger" in the cells.
- Regulates Glycogen metabolism.
- Regulates TG metabolism.
- Stimulates protein kinases.
- Modulates both transcription and translation in protein biosynthesis.
- Activates different steps of steroidogenesis.
- Inhibits cholesterol biosynthesis.
- Regulates permeability of cell membranes to water, sodium, potassium and calcium.
- Plays an important role in cell differentiation.
- Histamine increases gastric secretion by increasing c-AMP in parietal cells of gastric mucosa.

Q.64. What is cyclic GMP? How it is formed?

Cyclic GMP is formed from GTP by the enzyme ***guanylate cyclase*** and requires Mn^{++} as a cofactor.

Q.65. How cyclic GMP is degraded?

Cyclic GMP is degraded by the enzyme ***phosphodiesterase***, a cyclic nucleotide, which opens the 3'-5' ring and forms 5'-AMP as an inactive product.

Q.66. State some important functions of cyclic GMP.

Some important functions of cyclic GMP are:

- Role in phosphorylation of proteins.
- Role in vasodilatation.
- Role in action of neurotransmitters.
- Role in PG action: PG-$F_2\alpha$ has been shown to use cyclic GMP as "second messenger" for its action.
- Role in insulin action.
- Role in retinal light-dark adaptation:
  c-GMP as "second messenger" regulates the opening and closing of Na^+ channels. In the dark, increase in c-GMP level occurs which binds to Na^+ channels causing them to open. Reverse occurs in light.

CHAPTER

6 Chemistry of Enzymes

Q.1. What are enzymes?

Enzymes are biocatalysts, which are heat-labile, soluble proteins produced by living cells and are highly specific in their action. They are ***colloidal*** in nature and may contain a non-protein moiety (co-enzyme or prosthetic group).

Q.2. What are the similarities of enzyme (biocatalysts) with chemical inorganic catalysts?

Enzymes (biocatalysts) share some of the properties of chemical inorganic catalysts. They are as follows:

- They are neither consumed nor produced during the course of a reaction.
- They do not cause reactions to take place; they ***speed up reactions*** that would ordinarily proceed in much a slower rate in their absence.

Q.3. How does enzymes (biocatalysts) differ from inorganic chemical catalysts?

Enzymes differ from chemical catalysts in the following ways:

- Enzymes are invariably proteins in nature.
- They are highly specific for the reactions they catalyze.
- Produce only the expected products from the given substances or reactants ***(there are no side reactions).***
- They function within a moderate pH and temperature range.
- They often show a high specificity toward one substrate.

Although some enzymes have a broader specificity, using more than one substrate.

Q.4. What is holoenzyme?

Holoenzyme = Apo-enzyme + Coenzyme
(Protein part) (Non-protein part).

Q.5. State the clinical importance of enzymes.

- Act as biocatalysts and regulates metabolic activity in the body
- Measurement of increased or decreased concentration of certain enzymes in serum provides valuable diagnostic and prognostic significance to the clinicians.
- Digestive enzymes secreted in the GI tract brings about digestion of the food stuffs.
- Inherited deficiency of a particular enzyme can produce inherited diseases.

Q.6. What are coenzymes and cosubstrates? Give examples.

- Coenzymes are thermostable, dialyzable, low mol. wt., non-protein organic substances required for the activity of the enzymes.
- Coenzymes are also called cosubstrates (synonymous)

Examples:

NAD^+, FAD, FMN, TPP, etc.

Q.7. What are cofactors?

Activity of many enzymes depends on the presence of certain metal ions which are called as cofactors, e.g. Mg^{++}, Mn^{++}, Fe^{++}, K^+, Zn^{++}, Ca^{++}, Cu^{++}, etc.

Q.8. What are oligomeric enzymes? Give suitable examples.

- When enzymes possess more than one polypeptide chain they are called as oligomeric enzymes. Each single polypeptide chain of oligomeric enzyme is called a **"subunit"** or **"monomeric unit".**

Example:

Lactate dehydrogenase (LDH) contains 4 monomeric units. Thus it is a tetramer.

Q.9. What is a multienzyme complex? Give examples.

- When many different enzyme catalyzing reaction sites are located at different sites of the same macromolecule, it is called a ***multienzyme complex***. The complex becomes inactive when it is fractionated into smaller units each bearing individual enzyme activity.

- Examples:
  - *Pyruvate dehydrogenase complex.*
  - *Prostaglandin synthase*
  - *Fatty acid synthase etc.*

Q.10. What are intracellular and extracellular enzymes?

- Enzymes which are used in the cells which produce them are called as ***intracellular*** enzymes, e.g. all metabolic enzymes.
- Enzymes which are produced by other cells and are used to the other part of the body are called as ***extracellular*** enzymes, e.g. enzymes of digestive juices.

Q.11. Which enzymes do not require coenzymes for their activity?

Extracellular enzymes e.g. enzymes of digestive juices.

Q.12. What is zymase reaction?

The extracellular enzyme when secreted and ready for action is called ***zymase*** reaction e.g. salivary α-amylase.

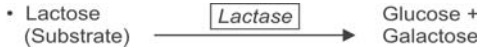
Q.13. What are zymogens?

- There are extracellular enzymes secreted in an "inactivated" form (zymogen form) and activated by other agents secreted by other cells are called as "zymogens" e.g.
- **Pepsinogen** (inactive form), it is activated to form active pepsin.
- **Trypsinogen** (inactive form) it is activated to form active trypsin, etc.

Q.14. What is a substrate?

- The substance on which an enzyme acts is called the substrate.

Example:



Q.15. How will you classify enzymes? Give examples of each group.

As **per IUB classification,** enzymes are divided into **six** main classes as follows:

- ***Oxidoreductases:*** Enzymes involved in oxidations and reductions of their substrates.

Examples:
Lactate dehydrogenase (LDH), xanthine oxidase, alcohol dehydrogenase, etc.

- ***Transferases:*** Enzymes that catalyze transfer of a particular group of one substrate to another. Examples:
  Aspartate aminotransferase (AST/SGOT)
  Alanin aminotransferase (ALT/SGPT)
- ***Hydrolases:*** Enzymes that bring about hydrolysis of the substrate.
  Examples:
  Amylases, pepsin, trypsin, glucose-6-phosphatase, etc.
- ***Lyases:*** Enzymes that facilitate removal of small molecule from a large substrate (break of -C-C Bonds)
  Examples:
  Fumarase, ATP-citrate lyase, HMG-CoA lyase, etc.
- ***Isomerases:*** Enzymes involved in isomerization of a substrate
  Examples:
  Phosphohexose isomerase, etc.
- ***Ligases:*** Enzymes involved in joining together two substrates (unite-C-C-bonds).
  Examples:
  DNA ligase, glutathione synthetase, etc.
  Note: To remember the six classes, the formula **OTHLIL** is used.

Q.16. Name some group transferring coenzymes.
Some group transferring coenzymes are:

- Thiamine pyrophosphate (TPP)
- Coenzyme A (CoA-SH)
- Pyridoxal phosphate (B_6-PO_4) etc.

Q.17. Name some hydrogen transferring coenzymes.

- Nicotinamide adenine dinucleotide (NAD^+)
- Nicotinamide adenine dinucleotide phosphate ($NADP^+$)
- Flavine adenine dinucleotide (FAD)
- Flavine mononucleotide (FMN) etc.

Q.18. State five important factors that affect enzyme action.
Activity of enzymes is markedly affected by certain factors. Five important factors are:

- Temperature
- pH

- Concentration of enzyme
- Concentration of substrate
- Presence of enzyme inhibitors

Q.19. What is meant by Q_{10}? Define.

- Q_{10} is also called as van't Hoff's temperature co-efficient.
- It is defined as increase in enzyme action, usually double, for an increase in temperature of 10°C.

Q.20. When does the maximum activity of the enzymes occur?

The enzyme shows maximum activity at the

- ***Optimum temperature:*** Which is 40°C to 50°C for animal enzymes and 60°C in case of plant enzymes, and
- ***Optimum pH:*** Which is usually in the region of 5 to 9.

Q.21. What is meant by Km value or Michaelis constant?

Km or Michaelis constant is equal to substrate concentration at which the velocity of reaction is half the maximum.

Q.22. What is Michaelis-Menten equation?

Kinetics of enzyme-substrate reaction is expressed as Michaelis-Menten equation as follows:

$$Vo = \frac{Vmax\ [S]}{[S] + Km}$$

Q.23. Explain the importance of Km in enzyme action.

Each enzyme has its Km value which indicates the efficiency of binding of the enzyme with the substrate to form E-S complex.

- If an enzyme has a low Km for a substrate, it indicates that the Vmax is reached even with such a low concentration i.e, maximum ES complex is formed. Thus,

Low Km enzyme → Maximum ES complex → high efficiency of the enzyme.

- If an enzyme has high Km for a substrate it requires higher concentration of substrate to bind, leading to less ES complex formation. Thus

High Km enzyme → ES complex Less → high concentration of substrate → low efficiency

Q.24. What is the mechanism of enzyme action?

- According to Michaelis and Menten hypothesis, the enzyme molecule (E) first combines with a substrate molecule (S) to form an enzyme-substrate complex (ES complex) which further dissociates to form product (P) and enzyme (E) back.
  E + S → ES Complex → P + E
- The interaction is between the ***active sites*** of the enzyme molecule and the substrate molecule. It should be understood that only if the ES complex is formed one can get the products.

Q.25. What is meant by active site?

The site to which a substrate can bind to the enzyme molecule is called ***active site*** (or ***catalytic site***) and it is extremely specific.

- The active sites are some specific amino acids, e.g.

Enzyme		Active site
• **Aldolase**	—	Lysine
• **Phosphoglucomutase**	—	Serine
• **Cholinesterase**	—	Serine
• **Chymotrypsin**	—	Serine and histidine, etc.

Q.26. Explain Fisher's Lock-Key or Template model of ES complex formation.

Emil Fisher proposed that active site provides a rigid, preshaped template which fits with the size and shape of the substrate molecule. Substrate fits into the active site of the enzyme as the key fits into a particular lock, hence called as ***Lock and Key model***.

Q.27. What is Koshland's "induced fit" model? Explain.

Koshland modified Fisher's concept. The important feature of this model is the ***flexibility of the region of active site.*** According to him, the active site is not a rigid, preformed template. The substrate during its binding ***induces conformational changes*** in the active site of enzyme, so that the substrate can bind to the enzyme.

Q.28. What is enzyme inhibition?

The chemical substances which inactivate the enzymes are called inhibitors and the process is called the enzyme inhibition. It may be ***reversible*** or ***irreversible.***

Q.29. What is competitive inhibition?

- If the inhibitor has molecular structure similar as the substrate, it competes with the substrate and binds to the active site of the enzyme thus inhibiting the enzyme action. This type of inhibition is called ***competitive inhibition.***
- By increasing the concentration of the genuine substrate, the inhibitor can be displaced from the active site thus it is ***"reversible" inhibition.***
- In competitive inhibition, both ES and EI complexes are formed, ***Km is high,*** and ***Vmax is the*** same.

Q.30. Mention three examples of drugs which act by competitive inhibition in biological systems and are used clinically.

- ***Allopurinol:*** Structurally resembles hypoxanthine and thus by competitive inhibition inhibits the enzyme ***"xanthine oxidase"*** thus reducing the uric acid formation. The drug allopurinol is used in treatment of gout.
- ***Ephedrine and Amphetamine:*** have similar structure to catecholamines, thus they can competitively inhibit the enzyme ***monoamine-oxidase (MAO)*** and prolong the action of pressor amines.
- ***Sulphonamides:*** Compete with para-amino benzoic acid (PABA) and competitively inhibit the enzyme action. Thus folic acid is not synthesized by bacteria and they die.

Q.31. What is non-competitive inhibition?

- Inhibitor does not resemble structurally to substrate. In this, sites of attachment of the substrate and inhibitor are different. The inhibitor binds reversibly with a site on enzyme other than the active site. So the inhibitor may combine with both free enzyme and ES complex. This probably brings about the changes in the three dimensional structure of the enzyme inactivating it catalytically.
- In non-competitive inhibition, **Km is kept constant,** but **Vmax is lowered.** This **type of inhibition can be "reversible" or "irreversible".**

Q.32. State the salient differentiating points of competitive and non-competitive inhibitions.

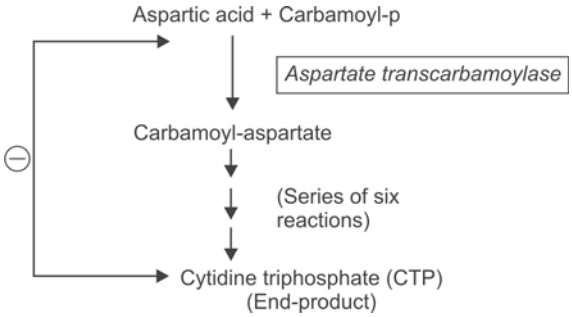
Competitive inhibitions	*Non-competitive inhibition*
• Inhibitors have similar structure like substrate	• Does not resemble in structure
• Reversible	• Reversible or irreversible
• Inhibitor binds to the active site	• Inhibitor binds to some other site other than active site.
• Vmax is same	• Vmax is lowered
• Km is increased ↑	• Km is unaltered
• Complex is EI	• Complex is EI or ESI

Q.33. What is allosteric inhibition and allosteric site?

- Inhibitor binds to the enzyme at a site other than the "active site" on a different region in the enzyme molecule called as ***allosteric site***.
- Binding of the inhibitor to "allosteric site" ***changes the conformation of the enzyme in the active site,*** so that the substrate cannot bind to the active site thus inhibiting the enzyme action. This type of inhibition is called ***allosteric inhibition***.

Q.34. Give a typical example of allosteric feed-back inhibition.

- CTP (cytidine triphosphate) is formed by a series of enzyme reactions from aspartic acid and carbamoyl-P.
- CTP the end product inhibits the first enzyme "Aspartate transcarbamoylase" and stop CTP synthesis.



Q.35. What are metal-activated enzymes?

In certain enzymes the metals form a loose and easily dissociable complex. Such enzymes are called metal-

activated enzymes. The metal ions can be removed by dialysis or any other such method from the enzyme without causing any denaturation of apo-enzyme.

Q.36. What are metallo-enzymes?

In this ***metal ion is bound tightly*** to the enzyme and forms an integral part of the enzyme molecule and is not dissociated even after several extensive steps of purification.

Q.37. What is meant by activation of enzyme? Give examples.

Enzymes may be activated by a variety of methods:

- ***Inorganic activators:*** **Examples:**
- Ca^{++} activates the enzyme lipase, Mg^{++} activates ATP requiring enzymes, H^{+} ions pepsinogen, etc. In these enzyme-substrate-metal complex is formed helping in better exposure of active centers.
- ***Organic activators:*** **Examples:**
- Glutathione and cysteine on -SH containing enzymes; Trypsin on chymotrypsin.
- ***Allosteric activator:*** **Examples:**
  - Acetyl CoA for pyruvate carboxylase (+ve-modifier), N-acetyl glutamate (NAG) for carbamoyl-P Synthetase I in urea synthesis.

Q.38. How does an enzyme reacts in a bi-substrate reactions?

Three different types of reactions can occur in an enzyme reaction with two substrates. Viz.

- ***Random:*** Between two substrates say 'X' and 'Y' either of the two can react first.
- ***Ordered:*** Always 'X' substrate reacts first, then it can act on 'Y'.
- ***Ping Pong:*** In this type, the enzyme alternates between form E and E and the products are formed stepwise.

Q.39. What is meant by covalent modifications of enzymes? Give examples.

Some enzymes are subjected to covalent modifications which are post translational. A group like phosphate may be added or removed or portions of the enzyme may be cleaved off. The ***covalent modifications make the enzyme "active".***

Examples:

- "Phosphorylase" inactive hepatic enzyme, the key enzyme involved in glycogenolysis is activated by phosphorylation.

- Inactive glycogen synthetase enzyme is dephosphorylated to form active enzyme.
- Muscle phosphorylase is "cleaved" to form inactive enzyme.

Q.40. What is lysozyme? What is its function?

- Lysozyme is an enzyme present in tears, sputum, nasal secretions, milk and in egg white, its mol wt is approximately 15,000. It is a single polypeptide chain consisting of 129 amino acids. It does not require any coenzyme or cofactor for its activity.
- ***Function:*** It destroys the cell walls of many air-borne gram +ve bacteria in tears and nasal mucous secretions.

Q.41. What is enzyme specificity? Give some suitable examples.

The biocatalysts (enzymes) differ from inorganic catalysts in possessing extraordinary specificity.

Types of enzyme specificity:

- ***Substrate specificity:*** An enzyme can react specifically on a particular substrate only, e.g ***Arginase,*** enzyme acts on arginine only. Similarly, ***urease"*** acts an urea only.
- ***Optical specificity:*** An enzyme can act only on a particular optical isomer of that compound e.g. L-amino acids oxidase can act only on L-amino acid and not on D-amino acid.
- ***Group specificity:*** A particular enzyme can act only on particular chemical groupings, e.g.
  - Alcohol dehydrogenase on alcohols,
  - Glycosidases: On glycosides
  - Pepsin and trypsin on peptide bonds

Q.42. What are ribozymes?

- Ribozymes catalyze the hydrolysis of phosphate diester bonds in RNA molecules.
- They play a key role in "intron" splicing which is essential for the conversion or pre-m-RNA to m-RNA formation.

Q.43. What is meant by constitutive enzymes?

Enzymes whose concentration in a cell is independent of an added inducer are termed constitutive enzymes.

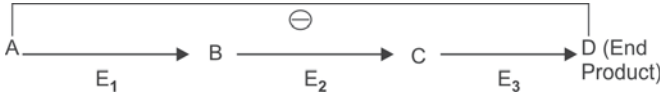
Q.44. What is enzyme induction?

The concentration of a given metabolite within the cell affects the activity of enzyme system which are involved in the synthesis or metabolism of metabolite itself. This is known as enzyme induction.

Q.45. Explain feed-back inhibition.

- In biosynthetic processes, the end-product may inhibit the first enzyme thus stopping the further formation of the product.

  Example:



- In the above reactions, D is formed from A and three enzymes are required E_1, E_2 and E_3. A high concentration of D, the end-product, binds to E_1 and inhibits the enzyme to further synthesis of D. This is known as ***feed-back-inhibition.***

Q.46. What are antienzymes?

- Enzymes are protein in nature. Enzyme which does not occur in blood and antibody forming apparatus is not exposed to that enzyme, can act as a foreign antigen.
- When such an enzyme is repeatedly injected into an animal, it produces antibodies to that enzyme which are called as ***antienzymes*.**

Q.47. Name two enzymes which are used systematically for therapeutic purposes.

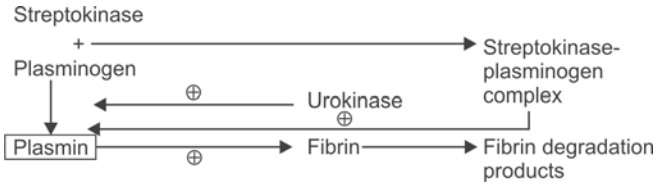
- ***Streptokinase*** and ***urokinase***:
- Used in treatment of acute myocardial infarction, deep vein thrombosis, acute thrombosis of arteries, pulmonary embolism, etc.
- ***Digestive enzymes: (amylase, lipase*** *and* ***protease)***
  Used as replacement therapy in pancreatic insufficiency e.g. in cystic fibrosis, chronic pancreatitis, following pancreatectomy, etc.

Q.48. How does streptokinase and urokinase act?

They increase amount of proteolytic enzyme "plasmin" by either–

- Increasing the circulatory level of its precursor "plasminogen", or
- By increasing the conversion of plasminogen to plasmin.
  - Plasmin acts directly on "fibrin", breaking it down to achieve thrombolysis.

Mechanism of action shown schematically below:



Q.49. Mention one enzyme which can be used locally for therapeutic purposes.

Hyaluronidase ("Hyalase") is given locally with subcutaneous fluids administration. It brings about deploymerization of ground substance and helps in rapid absorption of fluids given S.C.

Q.50. What are lysosomes?

Lysosomes are cell organelles found in cells which contain packet of enzymes.

Q.51. Who discovered lysosomes?

Lysosomes were discovered and described for the first time as a new cell organelle by the **Belgian Biochemist deDuve in 1955.**

Q.52. Which cell in the body does not contain lysosome?

Lysosomes are found in all animal cells in varying number and types ***except erythrocytes (RB cells).***

Q.53. What is the optimal pH for activity of lysosomal enzymes?

Optimal pH activity of lysosomal enzymes is around pH5.

Q.54. Name some of the essential enzymes found in lysosomes.
Essentially the enzymes present in lysosomes are **hydrolytic in nature.** Some of the important enzymes found are as follows:

- **Proteolytic enzymes:** cathepsins, collagenase, elastase
- **Nucleic acid hydrolyzing enzymes:** ribonucleases, Deoxyribonucleases
- **Lipid hydrolyzing enzymes:** lipases, phospholipases, fatty acyl esterases, etc.
- **Carbohydrate splitting enzymes:** α-glucosidase β-galactosidase hyaluronidase, etc.
- **Other enzymes:** acid phosphatase, catalase, etc.

Q.55. How does lysosomal enzymes bring about postmortem autolysis?
In the death of a cell, lysosomal bodies disintegrate releasing hydrolytic enzymes into the cytoplasm with the result that cells undergo autolysis.

Q.56. What is I-Cell disease?

- I-Cell disease is a rare inherited disorder in which lysosomes lack in almost all of the normal lysosomal enzymes.
- Plasma of these patients contain very high activities of lysosomal enzymes.
- Lysosomes, deficient in hydrolytic enzymes, do not function properly and accumulate partly digested cellular materials manifesting as ***inclusion bodies*.**

CHAPTER

7 Biologic Oxidation

Q.1. What is oxidation?
Oxidation is defined as the addition of O_2 or removal of hydrogens or removal of electrons.

Q.2. What is meant by reduction?
Reduction is defined as the gain of electrons or addition of hydrogen atoms.

Q.3. What is the biomedical importance of Biologic oxidation?
- To synthesize ATP molecules.
- As metabolic needs: If phenylalanine has to be metabolized in body, it has to be oxidized to Tyrosine which is needed for synthesis of thyroid hormones, catecholamines, melanin, etc.
- Molecular oxygen is incorporated into oxygenases. Many drugs, pollutants and chemical carcinogens are metabolized by enzymes of this class called as ***cytochrome P_{450} system.***

Q.4. What are dehydrogenases?
Enzymes that remove hydrogens from a substrate are called dehydrogenases.

Q.5. What are the types of dehydrogenases?
Two types:
- Aerobic dehydrogenases
- Anaerobic dehydrogenases

Q.6. How do the two types differ?
Essential differences between the two types of dehydrogenases are as follows:

Aerobic dehydrogenase	*Anaerobic dehydrogenase*
• Can react directly with O_2	Can not react directly. Transfers hydrogen and electrons from substrate to NAD or FP.
• H_2O_2 is produced	H_2O_2 is never formed. Forms NADH + H^+ or $FP.H_2$
• ATP is not produced	ATP is produced by oxidation of $NADH/FP.H_2$ in ETC

Q.7. What are oxidases?

Oxidases are enzymes that catalyze removal of hydrogen and electrons from a substrate by directly using molecular O_2 as acceptor.

Q.8. How does oxidases differ from aerobic dehydrogenases?

- ***Similarities:*** Both oxidases and aerobic dehydrogenases remove hydrogen and electrons from a substrate.
- ***Dissimilarities:***

Oxidases	*Aerobic dehydrogenases*
• Oxidases directly react with molecular O_2, usually H_2O is produced as product of oxidation.e.g. Cytochrome-oxidase	• Aerobic dehydrogenases can react with molecular O_2 or artificial hydrogen acceptors like methylene blue. The oxidation product is usually H_2O_2 e.g. L-amino acid oxidase, xanthine oxidase

Q.9. What are Hydroperoxidases? What is the clinical importance?

- Hydroperoxidases are enzymes which decompose H_2O_2 to H_2O and oxygen.
- ***Clinical importance:***
  Peroxides (H_2O_2) are harmful to body. Accumulation of peroxides lead to the genration of "free radicals" which can damage the biomembranes. Thus hydroperoxidases protect the body from harmful effects of H_2O_2.

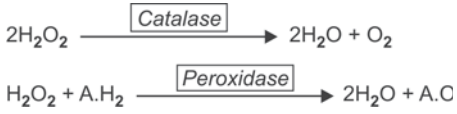
Q.10. What are the types of hydroperoxidases?

They are mainly two:

- Catalase
- Peroxidases e.g. glutathione peroxidase.

Q.11. State essential differences between catalase and glutathione peroxidase activity.

- Both the enzymes decompose $H_2O_2 \rightarrow H_2O + O_2$



- ***Essential differences are:***
  - Catalase can react directly with H_2O_2 but glutathione peroxidase requires reduced glutathione (G-SH)
  - Glutathione peroxidase is a selenium containing enzyme. Catalase does not contain selenium.
- Km of catalase for H_2O_2 is much greater than glutathione peroxidase. ***Hence glutathione peroxidase is the active enzyme to remove small amounts of H_2O_2 formed in cells like lens of eye and RB cells.***

Q.12. What are oxygenases?

Oxygenases are enzymes that catalyze the incorporation of O_2 into a substrate molecule.

Q.13. What are the types of oxygenases?

They are mainly **2 types:**

- Mono-oxygenases
- Di-oxygenases

Q.14. What are Di-oxygenases? Give examples.

- Di-oxygenases catalyze the incorporation of two atoms of O_2 into the substrate

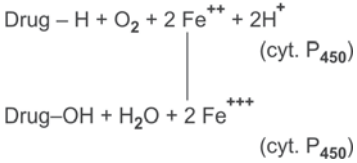
- **Examples** are:
- Homogentisate dioxygenase (or oxidase) contains Fe as prothetic group.
- L-tryptophan dioxygenase (or oxidase) contains 'heme' as prosthetic group.

Q.15. What are mono-oxygenases? Give examples.

Mono-oxygenases catalyze the incorporation of only one atom of O_2 into a substrate, the other oxygen atom is reduced to H_2O

Examples:
Many of the enzymes involved in steroid synthesis are mono-oxygenases and use NADPH as co-substrate.

Q.16. What is the role of mono-oxygenases in Drug hydroxylation?
Mono-oxygenases are also involved in metabolism of many drugs by hydroxylation. They are present in liver microsomes alongwith cytochrome P_{450} and cytochrome b, called as cytochrome P_{450} system.
Examples of Drugs metabolized are:
Aniline, Benzpyrine, Aminopyrine, Morphine, etc.



Q.17. Name a drug which stimulates microsomal enzyme including, Cyt . P_{450} system and used clinically.
- Phenobarbital.

Q.18. State the characteristic features of cytochrome P_{450} system.
Salient characteristic features are:
- Present in endoplasmic reticulum (ER), microsomol fraction of liver.
- An inducible enzyme, phenobarbital can stimulate production.
- At least six closely related species of cyt P_{450} in Liver found.
- Chemically they are **"heme-proteins"** and the enzyme is ***"NADPH-Cyt P_{450} system"***, and requires NADPH for its activity.
- The enzyme system contains lipids and most common lipid is phosphatidyl choline (lecithin).

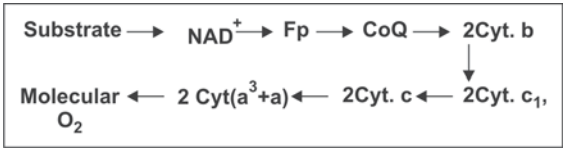
Q.19. What is meant by redox potential?
- In oxidation and reduction reactions, the "free energy" exchange is proportionate to the tendency of reactants to donate or accept electrons. This is expressed as ***"redox potential".***

- The pair consisting of the oxidant and reductant forms of an oxidizing and reducing agent is known as ***"redox couple"*** or ***"conjugate redox pair"*** e.g. $NAD^+/NADH + H^+$, FMN/FMN.H_2, Cyt C^{Fe++}/Cyt C^{Fe+++} etc.

Q.20. Define electron transport chain (ETC) or respiratory chain.
The sequence of enzymes and carriers responsible for the transport of reducing equivalents from substrate to molecular O_2 forming ATP and water is called as the ***electron transport chain*** (respiratory chain).

Q.21. Where the electron transport chian is located and how the enzymes are arranged?
- The enzymes of electron transport chain are embedded in the inner membrane of mitochondria in association with the enzymes of oxidative phosphorylation.
- The ***redox potentials in ETC are in increasing order*** ranging from – 0.3V to + 0.8 V in the terminal end except for coenzyme Q.

Q.22. Show schematically the accepted sequences of electron carriers in ETC.
The most accepted sequence is as follows:



Q.23. What are cytochromes?
Cytochromes are enzymes present in ETC involved in cellular-oxidation. They are classified as dehydrogenases except cytochrome oxidase.
- They are involved as "carriers" of electrons.
- They are iron-containing heme – Proteins in which the Fe atom oscillates between Fe^{+++} and Fe^{++} during oxidation and reduction.
- Varieties of cytochromes present in ETC are cytochromes b, c_1, c and a/a_3.

Q.24. What is the role of iron-sulphur protein (FeS; non-heme iron)?

FeS is associated with the flavo-proteins and with cytochrome b. The sulphur and Fe are thought to take part in the oxidation-reduction mechanism between flavin and CoQ.

Q.25. What is CoQ?

- CoQ is also known as ***ubiquinone*** as it is ubiquitous in nature.
- It exists in mitochondria in the oxidized quinone form under aerobic conditions and in the reduced quinol form under anaerobic conditions.
- The structure of CoQ is very similar to vitamin K and vitamin E.
- It is a mobile lipid component of the ETC, it collects reducing equivalents from the more fixed flavoprotein complexes and passes the electrons only to the cytochromes.

Q.26. How the reducing equivalents are passed in ETC?

From NAD^+ system to CoQ both hydrogen and electrons are passed. Beyond CoQ, it is electrons which are transferred through various cytochromes to molecular O_2 and H^+ goes to the media.

Q.27. How do the cytochromes act in transferring the electrons removed from CoQ.H_2 ?

The cytochromes b, c_1, c, a/a_3 have Fe which can oscillate between Ferrous and Ferric forms by removal or acceptance of electrons

$$Fe^{+++} + e^- \xrightarrow{\text{reduction}} Fe^{++}$$
(Ferricytochrome) (Ferrocytochrome)

$$Fe^{++} - e^- \xrightarrow{\text{oxidation}} Fe^{+++}$$
(Ferricytochrome) (Ferrocytochrome)

Q.28. Name the various complexes of ETC.

The ETC has 4 complexes as follows:

- ***Complex I:*** NADH-CoQ reductase
- ***Complex II:*** Succinate-CoQ reductase
- ***Complex III:*** CoQ-cytochrome C reductase
- ***Complex IV:*** Cytochrome C oxidase

Q.29. What are the functions of complex I?

- ***Acts as a proton pump***
- Catalyzes transfer of two electrons from NADH + H^+ to CoQ via FMN and FeS clusters.
- ***Permits one ATP formation (site I)***

Q.30. What are the functions of complex II.

- Flow of electrons from succinate to CoQ via FAD.H_2.
- ***Cannot act as a proton pump.***
- ***Cannot form ATP.***

Q.31. What are the functions of complex III?

- ***Acts as a proton pump.***
- Catalyzes transfer of electrons only from CoQ .H_2 to cytochrome C via cyt.b and cyt.c_1.
- ***Can form one ATP (site II)***

Q.32. What are the functions of complex IV?

- ***Acts as a proton pump.***
- Catalyzes transfer of electrons only from cyt.c to molecular O_2 via cyt.a, Cu^{++} ions and a_3 to form H_2O. It is the terminal component of ETC. Flow of electrons is as follows:

  Cyt.c → Cyt.a → Cu^{++} → $Cyta_3$ → O_2

- ***Can form one ATP (site III).***

Q.33. Does cytochrome C constitute a part of the complexes?

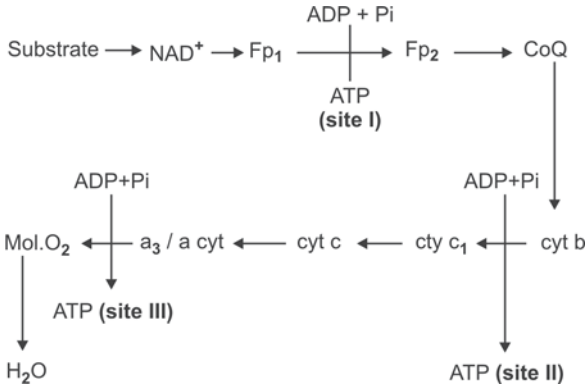
Cytochrome C does not form a part of any complexes. It is **free** and **mobile** and **acts as a shuttle** between complex III and IV to transfer electrons.

Q.34. What is the role of copper in cytochrome a/a_3 (complex IV)?

Copper atom adjacent to "cyt. heme a" is CuA (sub unit II) and "cyt.heme a_3" is close to CuB (subunit I). From cyt C, the electrons are transferred to heme-a-CuA cluster and then heme-a_3 -CuB cluster, and finally to molecular O_2 to form H_2O.

Q.35. Show schematically the exact sites of ATP production in the ETC.

There are 3 (three sites) of ATP formation. Exact sites are shown below schematically:



Q.36. What is minimum free energy requirement for formation of ATP in ETC?

- The formation of ATP from ADP + Pi require a "free energy" change which must **exceed 0.15V,** the minimum requirement to give the available energy of **greater than 7.4 K.cal** (Possible only in site I, II, and III)
- The formation of ATP is not possible at the sites where free energy released is less than 7.4 K.cal/mole (Not possible in complex II).

Q.37. Why does oxidation of succinate via FAD.H_2 in ETC produce 2 ATPs?

Because in oxidation of succinate via FAD.H_2, site I is bypassed by the flavoprotein-linked succinate dehydrogenase. Hence two ATPs are only formed in site II and site III.

Q.38. Name the inhibitors of site I of ETC

Inhibitors of site I are;

- Barbiturates: (Amobarbital and secobarbital) } Blocks electron transfer through NADH-Q reductase
- Rotenone (Fish poison)
- Antibiotic: (Piericidin A) } Blocks electron transfer by competing with CoQ.
- Drugs like chlorpromazine and hypotensive drug like guanethidine.

Q.39. Name the inhibitors of site II of ETC

Inhibitors of site II are:

- BAL (Dimercaprol): } Blocks electron
- Antibiotic antimycin A: } transfer form cyt b → c
- Hypoglycaemic drug like phenformin.

Q.40. State the inhibitors of site III.

Inhibitors of site III are:

- Cyanide: } Inhibits terminal transfer
- H_2S: } of electrons to molecular
- Azide: } O_2
- CO (carbon monoxide): inhibits cytochrome oxidase by combining with O_2 binding site.

Q.41. Name the inhibitors of complex II (succinate-CoQ reductase)

Inhibitors of complex II are:

- Carboxin: } Specifically inhibit
- TTFA: } transfer of reducing equivalent from succinate dehydrogenase to FAD.
- Malonate: } A competitive inhibitor of succinate dehydrogenase.

Q.42. What is ADP:O or P:O ratio?

P:O ratio is a measure of how many moles of ATP are formed from ADP by phosphorylation per gram atom of oxygen used. This is usually measured as the number of moles of ADP or Pi that disappear per gram atom of oxygen used.

P:O ratio of NADH system = 3
P:O ratio of $FAD.H_2$ = 2

Q.43. What is meant by oxidative phosphorylation?

The process by which biologic oxidation in respiratory chain (ETC) is coupled with phosphorylation, in which ADP is phosphorylated by Pi to form ATP is called oxidative phosphorylation.

Q.44. What is ATP synthase?

ATP synthase also called as ***H^+–ATPase*** or ***mitochondrial ATPase*** is an enzyme complex which helps in synthesis of ATP, present in inner mitochondrial membrane.

Q.45. State the structure of ATP synthase.

The enzyme ATP synthase consists of:

- **Headpiece** with several polypeptides
- **A stalk** which is attached to 8 peptides of headpiece.
- **A base piece** made up of proteolipid.

Q.46. Explain the functions of different parts.

- The stalk serves as the proton channel.
- The head piece protein assembly is called F_1 unit which protrudes from the inner surface of the plasma membrane. F_1 is considered to be $\alpha_3 \beta_3 \gamma\delta\varepsilon$.
- The base piece consists of transmembrane integrae protein called F_0 unit. It is thought to be $\alpha_1 \beta_2 l_{10-12}$.
- The F_1 unit functions as an ATPase. **F_1 unit when bound to F_0 serves as proton translocating ATP synthase and catalyses phosphorylation of ADP to ATP using the energy** from the down gradient proton flow through the proton channel of F_0.

Q.47. State the inhibitors of oxidative phosphorylation. How do they act?

Inhibitors of oxidative phosphorylation are:

- ***Oligomycin:*** Prevents stimulation of O_2 uptake by ADP and phosphorylation of ADP $\rightarrow$ ATP
- ***Atractyloside:*** A toxic glycoside, it blocks the ***translocase*** that is responsible for the movement of ADP and ATP across the inner mitochondrial membrane.
- ***Bongregate:*** A toxin produced by pseudomonads. Acts similar to atractyloside.

Q.48. What are uncouplers?

Uncouplers are substances that uncouple oxidative phosphorylation in the respiratory chain (ETC). They prevent the formation of ATP but permits biologic oxidation to proceed resulting in generation of heat.

Q.49. State the uncouplers of oxidative phosphorylation.

The ***uncouplers of oxidative phosphorylation*** are:

- 2:4 Dinitrophenol (DNP)
- Dicoumarol (Vitamin K analogue)
- Calcium: Transport of calcium into mitochondria can cause uncoupling
- CCCP (Chloro carbonyl cyanide phenyl hydrazone): most active uncoupler

- Valinomycin: Produced by a type of streptomyces. Transports K^+ from the cytosol into matrix and H^+ from matrix to cytosol thereby decreasing the proton gradient.

Q.50. Name some physiological uncouplers.

- Excessive thyroxine hormone
- EFA deficiency

} Both causes mitochondrial swelling

- Long cahin FA in Brown Adipose tissue
- Unconjugated hyperbilirubinemia.

Q.51. What do you know of chemiosmotic hypothesis proposed by Mitchell?

Mitchell's hypothesis proposes that biologic oxidation in the respiratory chain (ETC) generates protons (H^+) which are ejected to the outside of inner mitochondrial membrane of the mitochondrion. The electrochemical potential difference resulting from the asymmetric distribution of the H^+ is used to drive the mechanism for the formation of ATP.

Q.52. Name the antibiotic which completely blocks the biologic oxidation and phosphorylation in intact mitochondrion.

- Oligomycin.

Q.53. Name the antibiotics responsible for K^+ penetration through the mitochondrial membrane.

- Valinomycin
- Nigericin

Q.54. Mention the inherited disorders associated with biological oxidation. State the clinical features and enzyme deficiencies.

There are **2 types** of inherited disorders:

- ***Infantile mitochondrial myopathy:***
  - Condition is fatal
  - Is associated with renal dysfunction
  - ***Enzyme deficiency:*** There is severe diminution or absence of most of the oxidoreductases of ETC
- ***MELAS:*** An inherited disorder associated with
  - Mitochondrial myopathy
  - Encephalopathy
  - Stroke
  - Lactic acidosis
- ***Enzyme deficiency:***
  - NADH: ubiquinoue oxidoreductase (complex I)
  - Or deficiency of cytochrome oxidase.

CHAPTER

8 Chemistry of Hemoglobin and Hemoglobinopathies

Q.1. What is hemoglobin?
Hemoglobin is the red coloring matter of red blood cells. Chemically it is a conjugated protein, a chromoprotein containing **"heme"** as the prosthetic group and **"globin"** as the protein part-apoprotein.

Q.2. What is the normal concentration of Hb in blood?
The normal concentration of Hb in blood in an adult male varies from 14 to 16 gm%. In an adult female, the values are a little lower 12 to 14 gm%.

Q.3. How much hemoglobin (Hb) is present in total circulating blood of a normal adult?
Approximately 750 gm of Hb is present in total circulating blood of a 70-kg man.

Q.4. How much hemoglobin is produced and destroyed in the body daily?
Approximately 6.25 gm (90 mg/kg) of Hb are produced and destroyed in the body daily.

Q.5. State the biomedical importance of hemoglobin.
- Hb binds O_2 and carries O_2 to tissues.
- Part of CO_2, a waste-product of metabolism is carried from tissues by the globin part of Hb.
- Hb acts as a buffering agent.
- 2:3-biphosphoglycerate (BPG) is produced in the red blood cells by Rapaport-Leubering shunt.
- Conversion of Hb to methemoglobin by $NaNO_2$/or sodium thiosulfate forms the basis of treatment of cyanide poisoning.

- Study of Hb chemistry provides an insight into the molecular basis of genetic diseases such as abnormal Hbs and hemoglobinopathies.

Q.6. What is heme?

- Heme is the prosthetic group of Hb. It is a Fe-porphyrin compound.
- Porphyrins are **"tetrapyrrole"**, each pyrrole ring is joined by **"methylidene bridges"** or **"methyne" bridges.** Fe is in the center of "tetrapyrrole" and is in Fe^{++} (ous) state.

Q.7. What is globin?

Globin is the protein part of Hb. Globin is a **"tetramer"** and composed of 4 polypeptide chains. In normal adult Hb, there are two identical α-chains and two identical β-chains arranged in the configuration of "tetrahedron".

Q.8. State the essential differences between α-chains and β-chains.

- Essential differences between α-chains and β-chains of normal adult Hb A are as follows:

	α-Chain	***β-Chain***
• **Total amino acids**	141	146
• **C-terminal a.a.**	Arginine	Histidine
• **N-terminal a**.a.	Val-Leu	Val-His-Leu
• **Mol.wt.**	15126	15866
• **α-helices**	7	8
• **Heme-Pocket**	Adequate for entry of one mol. of O_2.	Entry of O_2 in heme-pocket is blocked by a valine residue.

Q.9. How many heme units are there in one Hb molecule?

Each polypeptic chain contains one "heme" unit in the so-called "heme-pocket". Thus ***one Hb molecule contains 4 "heme" units.***

Q.10. Name the different varieties of Normal human hemoglobins and state the nature of polypeptide chains in the globin.

Normal human hemoglobins are of several types, containing 4 polypeptide chains, 2 α-chains + 2 other chains

which may be β, γ, δ or ε depending on the type. Thus different types are:

- **Hb-A1:** Normal adult Hb (also called Hb A) consists of 2α-chains and 2β-chains **($\alpha_2\beta_2$)**
- **Hb-F:** Human foetal Hb is called Hb-F. It is **$\alpha_2\gamma_2$**
- **Hb-A_2:** A minor component, present only to the extent of 2.5%. It is **$\alpha_2\delta_2$**
- **Embryonic Hb:** Found in first three months of intra-uterine life of baby. It is **$\alpha_2\varepsilon_2$**
- **Hb A_3:** Appears to be an altered form of Hb-A, found chiefly in old red blood cells.
- **Hb-A_{1c}:** Glycosylated Hb, a minor glycosylated form of normal HbA. Present in concentration of 3 to 5% of total Hb.

Q.11. What is the clinical importance of glycosylated Hb?

- The level of glycosylated Hb appears to be an index of the blood sugar level for a period of several weeks, prior to the time of sampling. Once the Hb of RB cells get glycosylated, it ***remains for the life-span of the RB cells.***
- Its measurement would be a more reliable indicator of the adequacy of control of the diabetic state.

Q.12. How does Human foetal Hb differ from adult Hb-A_1?

Hb-F differs in many respects from adult Hb-A_1 as follows:

- Structurally it is $\alpha_2\gamma_2$
- It resists alkali denaturation
- Electrophoretically moves behind Hb-A_1
- BPG content is low↓ hence,
  - Affinity to O_2 is more↑
  - Delivery Power of O_2 is less↓.

  Present in fetal life and disappears after one year. ***Persistence of Hb-F after one year is pathological.***

Q.13. What is meant by heme-heme Interactions?

The increase in O_2 affinity by heme group on β-subunit, after combination of O_2 with α-subunit is called ***heme-heme interaction***.

Q.14. What is 'T' form of Hb?
'T' form of Hb is the **tense/taut state** and it is **deoxygenated Hb.**

Q.15. What is 'R' form of Hb?
"R" form of Hb is the ***relaxed state*** and it is the ***oxygenated Hb.***

Q.16. State the conformational changes that take place in Hb molecule in conversion from T → R form.
On oxygenation of "heme" of **α**-subunit which can easily admit one mole of O_2, the following conformational changes take place in T → R conversion:
- Rotation of one pair of rigid subunits $\alpha_2\beta_2$ through 15° along the long axis, to the other rigid pair of subunit $\alpha_1\beta_1$
- As a result of rotation, **salt bridges are broken.**
- BPG cannot be held in central pocket as it cannot form salt-bridges.
- Valine residue in "heme-pocket" of **β**-subunit is removed and this can now admit a molecule of O_2.

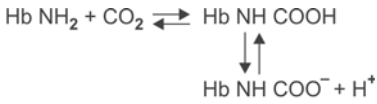
Q.17. What is oxy-hemoglobin (oxy-Hb)?
De-oxygenated Hb readily combines with O_2 in the lungs to form oxy-Hb. This combination is ***loose*** and ***reversible***. The attachment of O_2 occurs with Fe in the heme-portion. **Fe remains in the "ferrous" (Fe^{++}) state** both in de-oxygenated Hb and oxygenated Hb. It is in this form that O_2 is carried to tissues.

Q.18. How many molecules of O_2 can combine with one mol of Hb?
Each "heme" can bind only one mol of O_2. Since each mol of Hb contains 4 heme units, ***one mol of Hb can maximally combine with 4 mol of O_2.***

Q.19. State the absorption spectrum shown by oxy-Hb.
On spectroscopy, oxy-Hb shows two characteristic bands:
- ***α-Band:*** a narrow band in yellow portion of the spectrum at 597 mm nearer to D-line.
- ***β-Band:*** Wider broad band, nearer to E-line in green part of spectrum at 542 mμ.

Q.20. How does Hb combine with carbon dioxide (CO_2)?
CO_2 combines with the globin part and not with heme. It combines with -NH_2 group to form ***"Carbamino-***

compound". It is a reversible reaction, and accounts for 2 to 10% CO_2 transport in the blood.



Q.21. What is carboxy-Hb?

Carbon monoxide (CO) combines readily with "heme" portion of Hb to form carboxy-Hb (also called carbon monoxide Hb or carbonyl Hb).

Q.22. How carboxy-Hb differs from oxy-Hb?

- It is much firmer combination as compared to O_2 binding.
- **Not reversible**
- Affinity of Hb to CO is 210 times more than O_2.
- Absorption spectra: See below.

Q.23. What is the clinical importance of carboxy-Hb?

- Carbon monoxide poisoning can occur by inhalation of carbon monoxide gas (CO) which can be fatal.
- **Lethal action is due to inhibition of cytochrome oxidase a/a3** in electron transport chain (ETC) producing stoppage of cellular respiration.

Q.24. How will you test for presence of carboxy-Hb, in blood?

- ***Physical examination of blood:***
  shows a **cherry-red color.**
- ***Chemical test: Dilution test:***
  To dilute the suspected blood, after treating it with a little NaOH and compare the color with normal blood similarly treated. Normal blood shows a greenish hue after such treatment, whereas carboxy-Hb containing blood remains pink.
- ***Absorption spectra:*** It gives similar two bands like oxy-Hb and as such cannot be identified by absorption spectroscopy.

Q.25. How can you differentiate oxy-Hb and carboxy-Hb by absorption spectroscopy when both show similar two bands?

On treatment of blood with sodium hydrosulphite, there is no change of absorption bands, both the bands persist in case of carboxy-Hb. On the other hand, the two bands of

oxy-Hb are changed to one broad β-band (α-band disappears) as oxy-Hb is reduced to deoxy Hb.

Q.26. What is sulfhemoglobin? How can it be formed in the body? Is it harmful?

- Sulfhemoglobin a greenish pigment is formed by the **action of H_2S on oxy-Hb.**
- Sulfhemoglobin results from either:
  - Due to excess of H_2S: This can be formed in the gut by bacterial action on proteins in prolonged constipation and absorbed from the gut, or
  - After administration of compounds like aromatic amines which catalyze the formation of sulf-Hb from Hb.
- The condition is not harmful.

Q.27. What is cyanmethemoglobin?

Cyanides and HCN do not react directly with Hb but they react with methemoglobin to form the compound cyanmethemoglobin which is non-toxic.

Q.28. Why cyanides are lethal? What is the biochemical basis of treatment of cyanide poisoning?

- Cyanides are lethal as it inhibits cytochrome oxidase a_3 of ETC and stops cellular respiration.
- Biochemical basis for treatment would be to **enhance formation of methemoglobin** in the body so that cynadines combine with methemoglobin and spares cyt a_3. This can be achieved by:
  - Administering sodium nitrite to the patient.
  - Alternatively, by giving sodium thiosulfate IV.
    The drug reacts with cyanides, yielding thiocyanate, a non-toxic substance which is excreted in urine and thus spares cyt a_3.

Q.29. Name one derivative of Hb which is formed by true oxidation of Hb?

- **Methemoglobin—**it is a true oxidation product in which Fe is converted from Fe^{++}(ous) to Fe^{+++}(ic) state.

Q.30. What is methemoglobinemia?

Presence of increased amounts of Met-Hb in blood above normal is called methemoglobinemia.

Q.31. How methemoglobin can be formed in the laboratory?

By treating blood with potassium ferricyanide.

Q.32. How methemoglobin can be produced in the "Body in vivo?"

Methemoglobin can be produced in the body by certain oxidant group of drugs or by exposure to certain poisons e.g. nitrites, chlorates, nitrobenzene, phenacetin, sulphones, sulphonamide drugs, etc.

Q.33. What is the normal level of methemoglobin present in blood?

In normal healthy adult, small amount of methemoglobin is present approximately 0.3 gm/100 ml (about 1.7% of total Hb) which is harmless.

Q.34. Can body convert methemoglobin to normal Hb?

- Methemoglobin can be converted in the body to normal Hb again by the enzyme called ***methemoglobin reductase*** which requires $NADH+H^+$ as a coenzyme.
- Recently an additional enzyme called ***diaphorase I*** has been described which requires NADPH and can perform the same function.

Q.35. What is familial methemoglobinemia?

It is an inherited disorder due to lack or absence of the enzyme ***methemoglobin reductase*** which is responsible for conversion of met-Hb to normal Hb. In absence of the enzyme, methemoglobin goes on accumulating to produce methemoglobinemia.

Q.36. Name the abnormal Hb which can form methemoglobinaemia.

- Methemoglobinemia can be produced **by abnormal Hb, Hb-M.** In Hb-M, one amino acid sequence is altered either in **α** or **β**-chains. There are different types of Hb-M.
- In Hb-M (Iwate), histidine in position 87 of α-chain is replaced by tyrosine ($\alpha_2^{87Tyr}\beta_2A$). Heme Fe in the Hb of the variant chain is spontaneously oxidized to the Fe^{+++}(ic) state producing met-Hb.

Q.37. What is the action of acids/alkalies on Hb?

Acids and alkalies act upon Hb to form the acid or alkaline haematin respectively.

Q.38. How acid hematin can be formed? What is its chemical nature?

- Dilute HCl (or other acids) splits Hb→into Heme + Globin.
- Heme formed is **"ferro-heme"**, which in presence of O_2 is quickly oxidized to form **ferri-heme**. "Ferri-heme" has an additional +ve charge which is balanced by Cl^- ion (in case of HCl) and forms acid hematin which is chemically ***ferri-heme chloride***".

Q.39. How alkaline hematin is produced? What is its chemical nature?

- Alkalies split Hb into globin and ferro heme which is oxidized to **"ferriheme"** in presence of O_2. Ferriheme has an additional + ve charge which is balanced by -OH ion (in case of NaOH/or KOH) and forms alkaline hematin which is chemically ***ferri-heme hydroxide***.

Q.40. How will you differentiate the acid hematin and alkaline hematin by absorption spectroscopy?

By absorption spectroscopy:

- Acid hemation shows a **thinner band** of 650 mm between C and D lines, but nearer to D line.
- Alkaline hematin shows a **broader band** at 600 mm between C and D lines, but nearer to D line.

Q.41. What is hemochromogen?

Heme and the ferrous porphyrin complexes react readily with basic substances such as hydrazines, primary amines, pyridines or an imidazole (like histidine) to form ***hemochromogens*** (hemochromes).

Q.42. How hemochromogen identified by absorption spectroscopy?

On absorption spectroscopy, hemochromogen shows:

- **a "Soret" band:** a sharp absorption band near 400 mm (characteristic band of porphyrins).
- **Two additional bands:**
  - **α-band**-narrow band at 559 mm in green part, and
  - **β-band**-broader band at 527 mm in green part.

Q.43. What is heme-linked group?

The imidazole ring of histidine, No. 87 on **α**-subunit and No. 92 on **β**-subunit are linked to heme and undergoes ionization with pH changes and oxygenation/deoxygenation.

Q.44. Explain how oxy-Hb is a stronger acid than deoxygenated Hb.

On oxygenation of Hb, "N" of imidazole group of histidine acts as an acid and gives out protons H^+ in the media. The reverse occurs in deoxygenation. ***Thus oxy-Hb is a stronger acid than de-oxy Hb.***

Q.45. How Hb acts as a buffer?

- ***In acidic pH:*** The 'N' imidazole group of histidine acts ***as a base*** and ***takes up H^+*** (protons) from the medium (protonation).
- On the other hand, ***in alkaline pH:*** The 'N' of imidazole group of histidine, behaves ***like an acid*** and ***gives out a H^+*** (proton) to the medium (deprotonation).

Q.46. Describe Böhr effect.

- The CO_2 generated in peripheral tissues due to metabolism, combines with H_2O to form H_2CO_3 (Carbonic acid), which dissociates to form H^+ and HCO_3^-
- The deoxygenated Hb (T-state) acting as buffer take up two H^+ ions and deliver the H^+ ions to the lungs.
- In the lungs, the binding of O_2 to Hb (T $\rightarrow$ R state) forces the protons out of Hb. The H^+ combines with HCO_3 to form H_2CO_3 which breaks up to H_2O+CO_2. Carbon dioxide is exhaled out with expiration.

  The above is called as Böhr effect.

Q.47. What are the types of hemoglobinopathies?

Hemoglobinopathies are of **2 types:**

- If the mutation affects ***structural gene*****,** it results in replacement of a single amino acid residue of Hb-A_1 by some other amino acid resulting to an abnormal Hb.

  Examples are: Hb-S, Hb-C, Hb-D (Panjab), Hb-M, etc.
- If the mutation affects the ***regulator gene*****,** which affect the rate of synthesis of the peptide chains, the amino acid sequence remains unaffected. This produces **"thalassemias".**
- Depending on the chains affected it can be:
  - ***α-chain thalassemias.***
  - ***β-chain thalassemias.***
- Hemoglobinopathies produce hemolytic anemia.

Q.48. What is the abnormality in amino acid sequence in Hb-S?
In Hb-S, both the **α**-chains have the same amino acid sequence as those of normal **Hb-A_1**, but in both **β**-chains ***glutamic acid in 6th position is replaced by Valine.*** Thus molecular formula of Hb-S is $\alpha_2{}^{A}\beta_2{}^{6Val}$.

Q.49. What disease is produced by Hb-S?
- **Produces sickle-cell disease.**
- In homozygous patient manifests as full-blown disease, whereas in heterozygotes, the patient will have the "traits" (sickle-cell traits).

Q.50. Why the disease is called sickle cell disease?
When Hb-S is deoxygenated, the RB cells lose their normal morphology and they change to **"Crescent-shape" which look like "sickles".** Hence, it is called sickle-cell disease.

Q.51. What is the biochemied basis of sickling? Explain.
- Replacement of a "non-polar" residue valine in β-chain to 'Polar' residue glutamic acid generates ***sticky patch*** on the outside of the β-chains.
- The "sticky patch" is present in the 'oxygenated' (R-form) of Hb-S and 'deoxygenated' (T-form) Hb-S, but never present on oxy-Hb A (`R' form).
- On the surface of deoxygenated ('T' form) Hb-S, there exists a ***complementary site*** to the sticky patch, but in the oxygenated Hb-S ('R' form), the complementary site is masked and not present.
- When Hb-S is deoxygenated, the ***sticky patch of Hb-S can bind to the complementary site on another deoxy-genated Hb-S.*** This binding causes a polymerization of de-oxy Hb-S, forming long fibrous precipitates that mechanically distorts the red cells to produce ***crescent shape*** (or ***sickle cells***).

Q.52. What is α-chain thalassemias?
- Synthesis of **α**-chains are repressed and there occurs a compensatory increase in synthesis of other chains either **β** or **γ**.

Q.53. What are the types of α-chain thalassemias?
Mainly **2 types:**
- Hb-H **(β_4)**
- Hb-Barts **(γ_4)**

Q.54. What is the clinical significance of Hb-Barts?

Pregnant ladies suffering from this type of hemoglobinopathy delivers still-born babies with clinical picture of ***hydrops fetalis***. This disorder is found in SE Asia and 80% of still-born infants are due to Hb-barts.

Q.55. What is β-chain thalassemias?

- When the thalassemia mutant regulator gene represses the β-chain synthesis, an excess of α-chains occur which can combine with δ-chains producing **an increase in Hb-A_2↑** or with γ-chains producing **an increase in Hb-F↑.**
- It produces a servere type of hemolytic anemia, occurs around Mediterranean Sea. Also called as ***Cooley's anemia***.

CHAPTER

9 Vitamins

Q.1. Define vitamins.

Vitamins have been defined as organic compounds occurring in natural foods either as such or as utilizable "precursors", which are required in minute amounts for normal growth, maintenance and reproduction, for normal nutrition and health.

Q.2. How the name vitamin derived?

The first vitamin isolated from rice polishing was found to be an "amine" and as it was found to be vital for growth, it was called as "vitamine", Later on "e" was omitted.

Q.3. How will you classify vitamins?

Vitamins are classified according to its solubility into **two major groups:**

- Fat soluble vitamins, and
- Water soluble vitamins.

Q.4. Name the fat soluble vitamins.

Fat soluble vitamins are:

- Vitamin A, Vitamin D, Vitamin E, and Vitamin K.

Q.5. What are retinoids?

Retinoids are **three forms** of vitamin A:

- Vitamin A alcohol (retinol)
- Vitamin A aldehyde (retinal or retinene)
- Vitamin A acid (retinoic acid)

Q.6. What is the structural characteristics of retinoids?

- All the three compounds contain as common structural unit:
- A trimethyl cyclohexenyl ring (β-ionone ring), and

- An all "trans" configurated polyene chain (isoprenoid chain) with 4 double bonds.

Q.7. What are provitamins A?

Provitamins A are the carotenoid pigments which are hydrocarbon polyene pigments, yellow or red, widely distributed in nature. They are:

- α-carotene, β-carotene, γ-carotene, cryptoxanthine and lycopene.

Q.8. Why β-carotene gives 2 mols of vitamin A whereas other carotenoids give only one molecule of vitamin A?

- Carotenoids undergo symmetrical oxidative scission to yield vitamin A.
- β-carotene contains two β-ionone rings at both ends connected by 18 carbon hydrocarbon chain. Hence oxidative scission in the middle of the molecule yields 2 mols of vitamin A
- Other carotenoids viz., **α, γ** and cryptoxanthine contains only one **β**-ionone ring, so they yield only 1 mole of vitamin A.

Q.9. Where in the body the carotenoids are converted to vitamin A?

In man, liver is the only organ where carotenes undergo oxidative scission and converted to vitamin A.

Q.10. How retinal and retinoic acid are carried in the blood?

- Retinal is transported in the blood bound to a specific ***retinol binding protein*** (RBP), and
- Retinoic acid is carried in the blood bound to another specific ***retinoic binding protein*** (RBP).

Q.11. What is rhodopsin (visual purple)?

- Rhodopsin (visual purple) is a photosensitive pigment ***present in rod cells of retina*** responsible for visual acuity in dim light (night vision).
- Chemically it is a ***conjugated protein*** with mol wt 40,000. It contains ***opsin*** as its apo-protein and ***retinene*** (retinal or retinaldehyde) as 11-cis retinal as prosthetic group.

Q.12. What is Wald's visual cycle" (or rhodopsin cycle)?

- When light falls on rhodopsin it is split to form "opsin" + all "trans"-retinal after a series of changes viz. bathorhodopsin → lumirhodopsin → metarhodopsin I and II, and finally to all transretinal.

- All trans-retinal is inactive to resynthesize rhodopsin. It has to be converted to 11-cis-retinal the active form.
- Retina picks up "cis" retinal from blood which is converted to "11-cis" retinal (active form) by the enzyme ***retinene reductase*** and NAD^+.
- "11-cis" retinal now combines with "opsin" to regenerate rhodopsin.

Q.13. What are the functions of retinoic acid?

- Prevents keratinization of epithelium of respiratory tract, urogenital tract, lacrymal duct epithelium.
- Plays an important role in synthesis of glycoproteins as "carriers" of oligosaccharide chains.
- Role in synthesis of mucopolysaccharides (MPS)
- Inhibits the enzyme ***collagenase*.**

Q.14. State the deficiency manifestations of vitamin A.

- Night blindness ***(nyctalopia)***
- Keratinization of epithelial structures. Effects of keratinization are:
  - Lacrymal glands: produces **xerophthalmia**
  - Cornea: produces white opaque spots called ***Bitot's spots***
  - ***Keratomalacia:*** cornea becomes opaque, softened and ulcerated.
  - Respiratory tract: increased susceptibility to infections.
  - Urogenital tract: leads to ***calculi*** formation.
  - Skin: produces ***toad skin***.

Q.15. What are the effects of excessive vitamin A intake?

- Produces series of toxic effects known as ***hypervitaminosis A syndrome*.** In man, produces hepatic dysfunction, headache, drowsiness and peeling of skin around the mouth and elsewhere.
  Characteristically seen in Eskimos after eating the raw livers of polar bears and arctic foxes (rich in vitamin A).

Q.16. What are the types of vitamin D?

Two types:

- ***Vitamin D_3 (cholecalciferol):*** most common type. Occurs in fish livers and produced in human skin from ***"7-dehydro cholesterol"*** by UV irradiation from sun's rays.
- ***Vitamin D_2 (calciferol).***

Q.17. What is the biological active form of vitamin D?
The biological active form is ***"1, 25-dihydroxy-vit D_3"***, known as ***calcitriol***.

Q.18. What is 25-OH-D_3? Where it is formed?
- It is the precursor of "1,25, di-OH-D_3"
- It is formed in the Liver from vitamin D in the endoplasmic reticulum of the mitochondria, where vitamin D undergoes hydroxylation at 25 position. Hydroxylation requires Mg^{++}, NADPH and molecular O_2. Cytochrome P_{450} system is necessary for the hydroxylation.

Q.19. Where and how "Calcitriol" (1, 25-di-OH-D_3) is formed?
25-OH-D_3 after its formation in the liver is ***carried to kidney*** where it undergoes further hydroxylation at 1-Position by the enzyme, ***1a-hydroxylase*** in renal proximal convoluted tubule. It is mono-oxygenase reaction requiring NADPH, Mg^{++}, molecular O_2 and at least three enzymes ***ferredoxin reductase***, ***ferrodoxin*** and ***cytochrome*** P_{450}.

Q.20. State two important functions of vitamin D.
- "Active" vitamin D_3 increases the absorption of Ca and P from intestine by increasing the synthesis of a ***calcium-binding protein***.
- It increases the synthesis of vitamin-K dependent Calcium-binding Protein ***osteocalcin*** of the bone, thereby increasing the mineralization of bone.

Q.21. Why vitamin D_3 is called a prohormone?
Vit D_3 as such is inactive. It has to be converted to biologically active form "1, 25-di-OH-D_3" (Calcitriol) which acts like a steroid hormone. Hence Vit-D is called a prohormone.

Q.22. State reasons for calling "calcitriol a hormone".
- Structurally similar to steroid hormone, contains "cyclopentanoperhydrophenanthrene nucleus".
- It is synthesized by the human body.
- It has definite "target" organs, viz. bone, kidneys and small intestine (a property of hormone).
- The formation of calcitriol is subject to "feed-back" control (a property of hormone)
- Acts like a steroid hormone. It enters the intestinal epithelial cell, binds to a specific cytoplasmic "receptor",

the bound complex is translocated to nucleus, where it transcripts m-RNA, increases the synthesis of Ca-binding protein thereby increasing absorption of Ca.
- It maintains calcium homeostasis alongwith two other hormones, the parathormone (PTH) and calcitonin.

Q.23. What are the deficiency manifestations of vitamin D?

Vit D deficiency produces:
- Rickets in children, and
- Osteomalacia in adults.

Q.24. How does a child suffering from rickets look like?
- Child's growth is affected, becomes thin,
- Bending of long bones giving rise to deformities viz. ***bow legs*** and ***knock knees***.
- The ankles, knees, wrists and elbows are swollen due to swelling of epiphyseal cartilages.
- The fontanelles do not close properly giving rise to ***hot-cross bun*** appearance of head.
- Ribs give ***beaded*** appearance due to swelling of cartilages and chest gives a ***pigeon-breast*** appearance.

Q.25. What is Ca × P ratio in rickets?

The product of Ca × P of normal blood is:
- 50 to 60 in growing children
- 30 to 40 in adults.
- In rickets and osteomalacia, the ratio decreases and it falls below 30.

Q.26. What is hypervitaminosis D?

Vitamin D taken in large doses can produce deleterious effects, which are mainly due to induced hypercalcemia. Effects are:
- ***Immediate effects:*** Include anorexia, thirst, lassitude, constipation and polyuria. Later on, it is followed by nausea, vomiting and diarrhea.
- ***Delayed effects:*** Persistent hypercalcaemia and hyperphosphataemia may produce:
  - **Urinary lithiasis.**
  - **Metastatic calcification** which may affect kidneys, bronchi, pulmonary alveoli, arteries, muscles and gastric mucosa. Renal failure may develop and can lead to death.

Q.27. What is vitamin E? What is its chemical name?
Vitamin E is a fat-soluble vitamin obtained from vegetable oils. Its chemical name is ***tocopherol***.

Q.28. What are the different types of vitamin E? and how do they differ?
Different types of vitamin E are:
- **α**-tocopherol
- **β**-tocopherol
- **γ**-tocopherol, and
- **δ**-tocopherol

They differ from each other in the number and position of methyl groups. **α**-tocopherol is the most active in vitamin E activity.

Q.29. State the biochemical functions of vitamin E.
- ***Acts as an anti-oxidant:*** Mops up "free" radicals like O_2^-, OH^-, etc. and prevents their peroxidative effects on unsaturated lipids of membranes and thus help maintain the integrity of cell membranes.
- Cysteine, selenium containing organic compound and vitamin E act synergistically in ***preventing hepatic necrosis.***
- Vitamin E is required for the ***storage of creatine*** in the muscles. Vitamin E deficiency causes muscular weakness with creatinuria.
- Tocopherol derivative tocopheranolactone may be involved in ***synthesis of coenzyme Q (ubiquinone).***
- Vitamin E may have some role in ***nucleic acid synthesis.***

Q.30. What is the active group involved in anti-oxidant action of vitamin E?
Phenolic –OH group on the 6th carbon of the Chromane ring of vitamin E molecule is responsible for anti-oxidant property.

Q.31. How can the requirement of vitamin E be increased?
By greater intake of polyunsaturated fats.

Q.32. Name some diseases which are likely to produce vitamin E deficiency.
In chronic steatorrhea, malabsorption syndrome, cholestatic liver disease, cystic fibrosis of pancreas, Abetalipoproteinemia.

Q.33. How does selenium help in vitamin E activity?

- Selenium as a component of the enzyme ***glutathione peroxidase*** helps to destroy peroxides (H_2O_2) and thereby reduces the peroxidation of polyunsaturated FA present in lipids of membranes. This diminished peroxidation reduces the vitamin E requirement.
- Vitamin E itself reduces selenium requirements by preventing loss of selenium from the body.
- Selenium is required for normal pancreatic function which is necessary for digestion and absorption of lipids including vitamin E.

Q.34. What are the deficiency manifestations of vitamin E?

- No definite disease described. But vitamin E deficiency produces the following:
  - ***Impaired reproductive function:*** Permanent sterility in rats, atrophy of spermatogenesis, resorption of foetus "in utero" in females.
  - ***Muscular dystrophy:*** Degenerative changes in muscles, muscle creatine becomes low, produces creatinuria.
  - A haemolytic or macrocytic anemia.
  - ***Hepatic necrosis:*** vitamin E and selenium prevent hepatic necrosis.

Q.35. Mention some diseases where vitamin E has been found useful therapeutically.

- Nocturnal muscle cramps (NMC)
- Intermittent claudication (IC)
- Fibrocystic breast disease (FBD)
- Atherosclerosis.

Q.36. What is vitamin K?

Vitamin K is a fat soluble vitamin, chemically a naphthoquinone derivative.

Q.37. What are the types of naturally occurring vitamin K? Mention their sources.

Naturally occurring vitamin K are:

- ***Vitamin K_1:*** Chemically 2-methyl, 3 phytyl, 1,4 naphthoquinone. (Also called ***phylloquinone*** or ***mephyton***)

  Sources: Chiefly green leafy vegetables like alfalfa, spinach, cabbage, soyabean, tomatoes, etc.

- ***Vitamin K_2:*** Chemically it is 2-methyl-3-difarnesyl 1, 4-naphthoquinone (also called as farnoquinone). First isolated from putrid fish meal, it is produced by bacterial metabolism.

Q.38. What is vitamin K_3?

Vitamin K_3 is a synthetic analogue of vitamin K. It is known as ***menadione***, chemically it is 2-methyl, 1, 4-naphthoquinone, without any side chains or -OH group.

Q.39. What are the advantages of vitamin K_3?

Vitamin K_3 or menadione is superior to naturally occurring vitamin K_1 or K_2 in that:

- It is ***water soluble, can be given parenterally***, less painful injections. Also can be given orally, as it is watersoluble and absorbed easily.
- It is three times more potent than the natural varieties.

Q.40. What is the active group in menadione activity?

Its activity is related to presence of $-CH_3$ group at position 2 of the chromane ring.

Q.41. Which type of vitamin K is produced by the bacterial flora of intestine in humans?

Vitamin K_2

Q.42. What are the biochemical functions of vitamin K?

- ***Blood coagulation:*** The main function of vitamin k is the promotion of blood coagulation by helping in the post-transcriptional modifications of blood coagulation factors prothrombin, VII, IX and X so that they are converted from "inactive" to "active" forms.
- ***Calcium binding proteins:*** Like osteocalcin of bone,
- ***Oxidative phosphorylation:*** Cofactor with mitochondrial lipids.

Q.43. What is the role of vitamin K as a cofactor?

Carboxylation of glutamate residue of "precursors" of prothrombin, other coagulation factors and Ca-binding proteins which are inactive is carried out by the enzyme ***carboxylase*** which requires vitamin K as a cofactor and converts them into "active" forms.

Q.44. How does vitamin K deficiency be produced?

- ***In malabsorption of fats:*** In sprue, steatorrhoea, Celiac disease, atrophy of intestinal mucosa, biliary tract obstruction and pancreatic dysfunction, etc. in these vitamin K absorption suffers.
- ***Sterilization of the gut:*** By prolonged use of broad spectrum antibiotics orally and sulphadrugs: Synthesis by bacterial flora stops.

Q.45. What is hemorrhagic disease of the newborn?

In immediate postnatal life, a newborn may develop bleeding disorder due to hypoprothrombinemia and deficiency of other coagulation factors. This is called as ***hemorrhagic disease of the newborn***.

Q.46. Why a newborn baby is more prone to develop vitamin K deficiency and hypoprothrombinemia?

- Because the placenta does not pass the vitamin K from maternal blood to fetus readily and sufficiently.
- Because the intestinal bacterial flora not yet developed fully in the newborn baby.

Q.47. How will you prevent development of vitamin K deficiency in newborn baby?

Vitamin K deficiency and hypoprothrombinemia can be prevented by ***administering vitamin K to the mother before parturition or*** by giving the infant a small dose of vitamin K.

Q.48. Name the vitamin K antagonist.

- Dicoumarol.

Q.49. State the daily requirements of the fat soluble vitamins.

The daily requirements of fat soluble vitamins are:

Vit-A	Vit-D	Vit-E	Vit-K
• Adults=5000 I.U. • Children=200 to 3000 I.U. • During pregnancy and lactation= 6000 to 8000 I.U. 1 IU=0.3 mg of retinol	• Adults=200 I.U. • Children=400 I.U. During pregnancy & lactation=400 I.U. 1 I.U.=0.025 mg of cholecalciferol	15 to 30 mg	1 to 2 mg.

Q.50. State the sources of fatsoluble vitamins.

The sources are as follows:

Vit-A	Vit-D	Vit-E	Vit-K
• ***Vegetable sources:*** All green leafy vegetables, yellow/red vegetables like carrots, papaya, sweet potatoes, etc. (contain carotenoids) • ***Animal sources:*** Cod liver oil, halibut liver oil, milk, butter eggs	• 7-dehydro-cholesterol in skin. • Animal sources: oily fish, egg yolk, butter, liver	• Seed oils-Sunflower oil, ground nut oil, etc., wheat germ, corn and soyabean oil, fish liver oil	• ***Plant sources:*** Alfalfa, Spinach leafy vegetables • Intestinal synthesis by bacterial flora

Q.51. Classify the water soluble vitamins.

- Vitamin B-complex group.
- Vitamin C (Ascorbic acid)

Q.52. List the water soluble vitamins B-complex group.

- Water soluble vitamins B-complex group are:
- Thiamine or aneurin (vitamin B_1)
- Riboflavin or lactoflavin (vitamin B_2)
- Niacin or nicotinic acid (P-P factor/Vitamin B_3)
- Pyridoxine (vitamin B_6)
- Pantothenic acid (vitamin B_5)
- Lipoic acid (thioctic acid)
- Biotin (vitamin H)
- Folic acid group.
- Cobalamine (vitamin B_{12})
- Miscellaneous group: inositol, choline, para amino benzoic acid (PABA)

Q.53. Name the Sulphur containing vitamins.

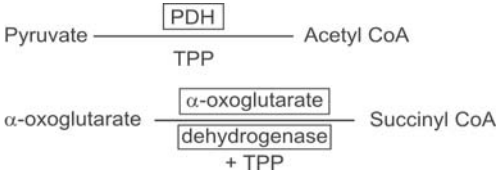
- Thiamine, biotin, and lipoic acid
- Coenzyme A also can be included as it contains –SH group.

Q.54. What is the "Biological active" form of thiamine?

- Thiamine pyrophosphate (TPP)

Q.55. Give two examples of metabolic reactions where TPP acts as a coenzyme.

- **Oxidative decarboxylation of pyruvic acid (PA)** to acetyl CoA and **α**-oxoglutarate to succinyl CoA.



- ***Transketolation reaction*** in HMP pathway of glucose oxidation.

Q.56. What is the deficiency manifestation of thiamine?

- In man, thiamine deficiency produces the disease called ***beriberi***. The disease is characterized by:
  polyneuritis with muscular atrophy, cardiovascular changes and edema, anorexia is an early symptom.

  Note: When associated with edema it is called as ***"wet-beriberi"***, if edema is absent, it is called as ***"dry beriberi"***.

Q.57. What is the normal daily requirement of thiamine?

Recommended daily allowance for thiamine is:

- For adult men: 1.5 to 2.0 mg
- For women: 1.0 to 1.2 mg.

Q.58. What is the relation of B_1 intake with diet?

The requirement of thiamine is dependent upon the carbohydrate intake. If carbohydrate intake is more, the greater is the thiamine requirement.

Q.59. What is the biochemical indicator that points to thiamine deficiency?

- ***Lactic acid/pyruvic acid (LA/PA) ratio:***
  In thiamine deficiency, pyruvic acid cannot be converted to acetyl CoA; instead it is converted to lactic acid (LA) which increases in blood. Hence abnormal LA/PA ratio is said to be more specific indicator of B_1 deficiency.

Q.60. What is chastek paralysis?

It is observed in foxes eating raw fish. Raw fish contains heat-labile thiamine-splitting enzyme ***thiaminase*** which destroys thiamine and produces deficiency. The disease is characterized by extreme board-like rigidity with retraction of head.

Q.61. What is Bracken disease?

It is found in grazing animals feeding on ferns and related species of plants, which contain ***thiaminase.*** The enzyme destroys thiamine and produces B_1 deficiency.

Q.62. What is Wernicke's encephalopathy?

This is associated with thiamine deficiency. The neurological manifestations like ascending symmetrical peripheral polyneuritis when accompanied occasionally by an acute hemorrhagic polioencephalitis is called ***Wernicke's encephalopathy***. It is frequently found in chronic alcoholics.

Q.63. State the sources of thiamine.

Thiamine is widely distributed in many plants and animal foods. Whole grains, rice bran, nuts and yeasts are some of the best plant sources while eggs, fish, liver are good animal sources.

***Note*:** Polishing of rice removes 80% of vitamin B_1 content.

Q.64. What is the difference between absorption of thiamine and riboflavin?

- ***"Free"*** thiamine is absorbed readily from the intestine whereas the phosphorylated form (TPP) is not.
- In case of riboflavin, ***"free" form is not absorbed*** readily. Free riboflavin undergoes phosphorylation, a prerequisite for absorption.

Q.65. What are the biological active forms of riboflavin?

- **FMN** (Flavin mononucleotide): In this the phosphoric acid is attached to ribityl alcoholic group in position 5.

Flavin-Ribityl-PO_4

- **FAD** (Flavin adenine dinucleotide): It is linked to an adenine nucleotide through pyrophosphate bridge.

Flavin-Ribityl-P-P-ribose-adenine

Q.66. What is the metabolic role of the active forms?
FMN and FAD, the biological "active" forms of riboflavin act as coenzymes with ***dehydrogenases*** in H-transfer reactions. The hydrogen is transported by reversible reduction of the coenzyme by two hydrogen atoms added to "N" at positions 1 and 10 to form FMN H_2 and FAD H_2 respectively.

67. Name three enzymes where FMN is a coenzyme.
- ***L-amino acid oxidase:*** brings about oxidative deamination of L-amino acid. FP is **auto-oxidizable** at Substrate level by molecular O_2 and forms H_2O_2.
- Cytochrome C reductase in ETC.
- Warburg's yellow enzyme.

Q.68. Name five enzymes where FAD acts as a coenzyme.
- ***Succinate dehydrogenase*** in TCA cycle (succinate → fumarate)
- ***Acyl CoA dehydrogenase*** in β-oxidation of FA
- ***Dihydrolipoyl dehydrogenase*** in oxidative decarboxylation of pyruvic acid (PA) and α-oxo-glutarate.
- ***Xanthine oxidase*** in purine degradation.
- ***Aldehyde dehydrogenase in degradation*** of aldehydes.

Q.69. What is the daily requirement of vitamin B_2?
- Adults = 1.5 to 1.8 mg.
- Children = 0.8 to 1.2 mg.
- Pregnancy and lactation = 1.5 to 2.0 mg.

Q.70. State the sources of riboflavin.
- ***Vegetable sources:*** Whole grains, dry beans and peas, nuts, green vegetables, yeasts.
- ***Animal sources:*** Liver, kidney, eggs, milk, crab meat has high content.

Q.71. What is the disease produced by riboflavin deficiency?
No specific disease described. But deficiency is manifested by certain symptoms and signs. They are:
- Redness of Lips.
- **Cheilosis:** Painful fissures at mucocutaneous junction of angle of mouth.
- Painful glossitis (red-purple/magenta color)

- Seborrheic dermatitis.
- Corneal vascularization and inflammation.

Q.72. What is the relation of vitamin B_2 intake with diet?

- Riboflavin (B_2) intake is related with **dietary protein intake**
- Requirement of the vitamin increases with increased protein intake.

Q.73. What is niacin?

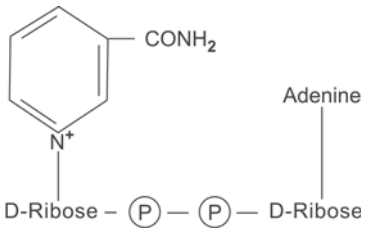
- Niacin is a water-soluble vitamin of B-complex group. Also called as nicotinic acid or vitamin B_3. Chemically it is pyridine-3 carboxylic acid.
- In tissues, it principally occurs as the amide form nicotinamide/or niacinamide.

Q.74. What are the biological active forms of niacin?

- NAD (nicotinamide adenine dinucleotide)
- NADP (nicotinamide adenine dinucleotide phosphate)

Q.75. Show the structure of NAD schematically. How does NADP differ from it?

- NAD can be represented schematically as follows:



- NADP differs from NAD^+ in that it contains an additional phosphate attached to 2-position of D-ribose attached to N_9 of adenine.

Q.76. What are the biochemical functions of NAD^+ and NADP?

Both of them act as coenzymes as **an acceptor** of **hydrogen** from the substrates in dehydrogenation reactions catalyzed by the enzymes ***dehydrogenases*** and gets reduced to $NADH+H^+$ and NADPH.

Q.77. Name Five important enzymes in which NAD^+ acts as coenzyme.

- ***Pyruvate dehydrogenase complex (PDH):*** PA → Acetyl CoA
- ***Lactate dehydrogenase (LDH):*** PA → LA.
- ***Alcohol dehydrogenase:*** Ethanol → Acetaldehyde
- ***Malate dehydrogenase:*** Malate → OAA.
- ***Glyceraldehyde 3-P dehydrogenase:*** Gly-3-P → 1. 3 bi-phosphoglycerate.

Q.78. Name one important enzyme in which NADP acts as a coenzyme?

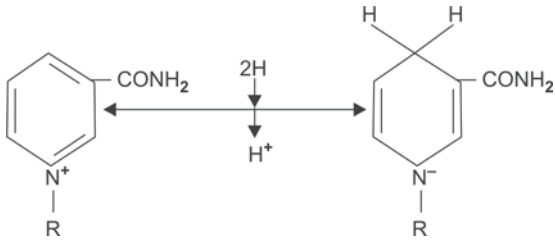
Glucose-6-P-dehydrogenase (G-6-PD)—The first reaction in HMP shunt of glucose oxidation.

Q.79. Name one important reaction in which either NAD^+ or NADP can act as a coenzyme.

Glutamate dehydrogenase: Glutamate → α-ketoglutarate + NH_3

Q.80. State the mechanism of reduction of NAD^+ to form $NADH + H^+$

- The mechanism of the transfer of hydrogen from a metabolite $A.H_2$ to oxidized NAD^+, thus completing the oxidation of the metabolite and the formation of reduced NAD ($NADH+H^+$) is shown below:



- Reduction of NAD^+ occurs at para position; one H loses an electron and enters the medium as H^+ (a Proton)

$$\underset{\text{(Substrate)}}{A.H_2} + NAD^+ \longrightarrow \underset{\text{(Oxidized substrate)}}{A.} + NADH + H^+$$

Q.81. In Physiological conditions, which is present in higher amounts, (a) NAD^+ or NADH (b) $NADP^+$ or NADPH? What is the main biochemical function so present?

- NAD^+ is present in higher amounts than NADH + H^+ while NADPH occurs in higher amounts than NADP in cells in physiological conditions.
- The main function of NAD^+ is biological oxidation in ETC and the main purpose of NADPH is in various reductive reactions e.g. 'de Novo' synthesis of long chain FA (palmitic acid), cholesterol biosynthesis, etc.

Q.82. What are the sources of niacin?

Both nicotinamide and coenzyme forms are widely distributed in plants and animals.

- ***Vegetable sources:*** legumes (Peas, beans, lentils), nuts, certain green vegetables, germ and pericarp (bran in cereal grains), coffee and tea and yeasts.
- ***Animal Sources:*** liver, kidney, meat, fish, eggs.

Q.83. What is the chief metabolite of niacin excreted in urine?

Major urinary metabolite is a **methylated derivate,** N-methyl nicotinamide. The methylation occurs in liver by the enzyme ***nicotinamide methyl transferase***, CH_3 group is donated by "S-adenosyl methionine" (active methionine).

Q.84. State the daily requirement of niacin.

- Adults = 16 to 20 mg
- Children = 6 to 14 mg.
- Pregnancy and lactation = 16 to 20 mg.

Q.85. Name one fat soluble vitamin and one water soluble Vitamin synthesized in the body.

- ***Fat soluble vitamin:*** Vitamin D_3 (cholecalciferol) is synthesized from 7-dehydrocholesterol present in human skin by sun rays (UV irradiation).
- ***Water soluble vitamin:*** Niacin synthesized from amino acid tryptophan in liver, B_6-PO_4 is required as a coenzyme.

Q.86. How much niacin is synthesized from tryptophan?

60 mg of amino acid tryptophan can synthesize 1 mg of niacin.

Q.87. Name some vitamins, fat soluble/water soluble, synthesized in intestine by bacterial flora.

- Fat soluble vitamin: Vitamin K_2
- Water soluble vitamin: B_1, B_2, niacin, pyridoxine (B_6), Pantothenic acid (B_5), Biotin, Folic acid, vitamin B_{12}.

Q.88. Why niacin requirement in diet decreases with good dietary protein intake?

Because niacin can be synthesized from tryptophan present in good quality proteins in the diet. Hence dietary requirement decreases.

Q.89. What is the deficiency disease produced by niacin deficiency?

Niacin deficiency produces the disease called **pellagra** (pelle = skin; agra = rough). Clinical features is described as "3 D's".

- ***Dermatitis:*** bronzing and thickening of skin.
- ***Diarrhea:*** with angular stomatitis and glossitis.
- ***Dementia***.

Q.90 What is the principal manifestation of niacin deficiency in dogs?

- Produces ***canine black tongue.***

Q.91. Why pellagra is more common in staple maize eaters?

- Maize contains protein called ***zein.*** It is an incomplete protein, lacks amino acid tryptophan. Hence body synthesis of niacin from tryptophan does not occur.
- Requirement of dietary niacin increases with high corn diet/and in chronic maize eaters.

Q.92. Name another protein which lacks tryptophan.

- Gelatin.

Q.93. What is pyridoxine?

Pyridoxine is also called vitamin B_6, a watersoluble vitamin B-complex group. Chemically it is 2-methyl-3-OH-4, 5 dihydroxy methyl pyridine. It is also called as ***pyridoxol.***

Q.94. What are the different forms of vitamin B_6?

- ***Pyridoxine*** (pyridoxol), ***pyridoxal*** (aldehyde form), and ***pyridoxamine*** (amine form) are the three forms of vitamin B_6. They all possess vitamin B_6 activity and are interconvertible.

Q.95. What are the biological active forms of vitamin B_6?
Biological active forms are:
- Pyridoxal - PO_4 and
- Pyridoxamine - PO_4.

Q.96. What is the function of the active forms of B_6?
Both act as coenzyme and principally involved with metabolism of amino acids.

Q.97. Name four important metabolic reactions in which pyridoxal-P acts as a coenzyme.
- ***Transamination reaction (co-transaminase).***
- ***Decarboxylation*** of amino acids to form biogenic amines (co-decarboxylase).
- As coenzyme for the enzyme *kynureninase* in tryptophan metabolism—required for niacin synthesis from amino-acid tryptophan.
  Note: In B_6-deficiency niacin synthesis from tryptophan does not take place.
- Required in transulfuration reaction and as a coenzyme for ***desulfhydrases*.**

Q.98. Enumerate other biochemical functions of vitamin B_6.
- Required for synthesis of sphingomyelin.
- Required in intramitochondrial FA synthesis for chain elongation.
- In porphyrin synthesis: required as a coenzyme in conversion of α-amino-β-keto adipic acid to δ ALA.
- In interconversion of glycine and serine.
- Required as a coenzyme in the biosynthesis of arachidonic acid from linoleic acid.
- Required for active transport of L-amino acids through cell membranes.
- As a constituent of muscle phosphorylase.

Q.99. State sources of vitamin B_6.
- ***Animal sources:*** Egg-yolk, fish and meat are good sources. **Milk is a poor source.** Highest concentration occurs in royal jelly (Bee).
- ***Vegetable sources:*** Vegetables like cabbage, legumes, yeast, germinating seeds, cereal grains, etc.

Q.100. What is the daily requirement of vitamin B_6?

- Adults: 2 mg/day
- Infants: 0.3 to 0.4 mg/day.
- Pregnancy and lactation: 2.5 mg/day.

Q.101. What is the relation of vitamin B_6 intake with diet?

Requirement of vitamin B_6 is increased with increased intake of dietary proteins as it is involved as a coenzyme in many metabolic reactions of protein metabolism.

Q.102. State the disease produced by vitamin B_6 deficiency.

No deficiency disease has been described. But B_6 deficiency can produce:

- ***Epileptiform convulsions in infants:*** In B_6 deficiency, glutamic acid cannot be converted to **γ**-amino butyric acid (GABA). This leads to lowering of 'GABA' concentration producing convulsions.
- ***Pyridoxine responsive anaemia:*** A hypochromic microcytic anaemia with high serum Fe level and haemosiderosis of liver, spleen, and bone marrow may occur. In B_6 deficiency, heme synthesis suffers and Fe cannot be utilized.

Q.103. Why vitamin B_6 deficiency with neuropathies occur in treatment of tuberculosis with isonicotinic acid hydrazide or isoniazid (INH) therapy?

Isoniazid, the antituberculous drug produces vitamin B_6 deficiency as isoniazid forms ***hydrazone complex*** with pyridoxine resulting in incomplete activation of the enzyme.

Q.104. What is the biochemical indicator that points to vitamin B_6 deficiency?

In B_6 deficiency, **kynurenine** and **3-OH kynurenine** levels increase due to non-conversion of 3-OH-Kynurenine to 3-OH-anthranilic acid and they are ***converted to "xanthurenic acid"*** in extra-hepatic tissues and is excreted in increased quantities in urine. Hence, ***xanthurenic acid index*** has been taken as a reliable biochemical indicator of vitamin B_6 deficiency.

Q.105. What is lipoic acid?

Lipoic acid is a sulphur containing fatty acid, also called as **"thioctic acid"**, a member of watersoluble B-complex group. Chemically it is "6, 8 dithio-octanoic acid".

Q.106. What is the biological active form of lipoic acid? State the metabolic role in the body.

There is ***no biological "active"*** form of lipoic acid. It itself serves as coenzyme in the following metabolic reactions:

- As a coenzyme with ***pyruvate dehydrogenase complex (PDH)*** for oxidative decarboxylation of pyruvic acid to acetyl CoA.
- Similarly acts as a coenzyme for conversion of α-oxoglutarate to succinyl CoA.
- Required as co-enzyme with the enzyme ***sulfite oxidase*** for conversion of SO_2^-(sulfite) → to SO_4 (sulfate), hypoxanthine also is required.

Q.107. What is pantothenic acid?

Pantothenic acid is a water soluble vitamin B-complex. Also called Vitamin B_5. Chemically pantothenic acid consists of **β**-alanine in peptide linkage with a "di-OH-di-methyl butyric acid called pantoic acid."

β-alanine + Pantoic acid → Pantothenic acid.

Q.108. What is the biological active form of pantothenic acid?

- Coenzyme A.

Q.109. How do you write coenzyme A in short form?

- Co A-SH.

Q.110. What is the active or reactive group in coenzyme A?

The terminal -SH group (or thiol group) of β-mercaptoethanolamine is the reactive site of the coenzyme A molecule.

Q.111. What is the metabolic role of pantothenic acid?

- Only demonstrated metabolic role of pantothenic acid is as a constituent of CoA-SH, which is essential for various metabolic processes involving carbohydrates, lipids, proteins and various physiologically important biomolecules in the body.
- CoA-SH is required for formation of:
  - Acetyl CoA ("active" acetate)
  - Succinyl CoA ("active" succinate)
  - Activation of FA to form Acyl CoA.

Q.112. State the important dietary sources of pantothenic acid.

- ***Animal sources:*** egg-yolk, kidney, liver, milk, fish, etc.
- ***Vegetable sources:*** cereals, legumes, cabbage, sweet potatoes, molasses and yeast cells. Richest known sources is royal jelly.

Q.113. What is the daily requirement of pantothenic acid?

The human requirement is not known exactly as the vitamin is widely present in almost all food stuffs. ***An ordinary mixed diet provides about 5 mg which is more than sufficient*** for an adult.

Q.114. What is the deficiency disease produced by pantothenic acid deficiency?

- No deficiency disease recognized in man.
- ***Burning feet syndrome*** (also called ***Gopalan's syndrome***) observed in certain malnourished individuals in India responds well to pantothenic acid. ***A synergistic effect is seen with thiamine, riboflavin and niacin.***

Q.115. What is biotin (vitamin H)?

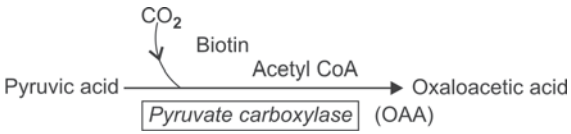
Biotin is a ***sulphur containing water soluble*** vitamin B-complex. Chemically it is a heterocyclic monocarboxylic acid.

Q.116. What is the biological active form of biotin? What is CO_2 fixation reaction?

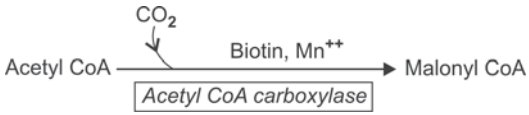
No biological active form has been described. But biotin is linked to the ε-NH_2 group of lysine of the apoenzyme of various ***carboxylases*** enzymes. CO_2 is then added to the biotin 'nitrogen' of the enzyme as a whole, to form ***N-carboxy-biotin*** which brings about addition of CO_2 to the substrate called as ***CO_2 fixation reaction***.

Q.117. Give three typical examples of CO_2-fixation reaction?

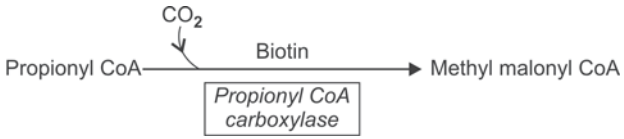
- ***Conversion of PA to OAA:***



- ***Conversion of acetyl CoA to malonyl CoA:***



- ***Conversion of propionyl CoA to methylmalonyl CoA:***



Q.118. What are the dietary sources of biotin?

- ***Animal sources:*** Liver, kidney, milk and milk products, egg-yolk, etc.
- ***Vegetable sources:*** Green leafy vegetables, legumes, cereal grains, molasses.

Q.119. What is the daily requirement of Biotin?

- Adults: 25 to 50 mg daily.
- Children: 20 to 40 mg daily.

Q.120. Feeding of raw egg-white produces biotin deficiency, why?

Raw egg-white contains an antivitamin, a heatlabile protein called ***avidin*** which destroys dietary biotin and produces deficiency of the vitamin.

Q.121. What is the deficiency disease produced by biotin deficiency?

No deficiency disease has been described in humans.

Q.122. How biotin deficiency can be produced in experimental animals?

- By inclusion of large amounts of raw egg-white in the diet.
- By using sulphonamides or broad spectrum oral antibiotics for prolonged periods so that intestinal bacterial flora is destroyed.

Q.123. State the deficiency mainfestations in animals produced experimentally.

- Dermatitis of the extremities.
- ***Spectacle-eyed*** appearance due to circumocular alopecia.
- Thinning or loss of fur/hairs.
- Paralysis of hindlegs.

Q.124. What is folic acid?

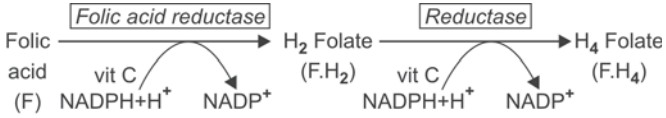
Folic acid is a water soluble B-vitamin. Chemically in its simple form is called ***"pteroyl glutamic acid" (PGA).*** It consists of:

- A "pteridine nucleus" (consisting a pyrimidine + pyrazine ring) +
- Para amino benzoic acid (PABA) +
- Glutamic acid.
  The number of glutamic acid residues varies—it can be one (monoglutamate), three (triglutamate) or seven (heptaglutamate).

Q. 125. What is the biological active form of folic acid?

- Tetrahydrofolate ($F.H_4$)

Q.126. How tetrahydrofolate, the active form is produced in the body?



- **$F.H_4$ acts as coenzyme,** Folic acid as such cannot function.

Q.127. What is folinic acid?

This is one of the active form of $F.H_4$ which is **formylated** at **position 5** ($f^5.FH_4$).

Q.128. What are other formylated active forms of $F.H_4$?

- $f^{10}.FH_4$ and
- $f^{5,10}\ FH_4$

Q.129. How f^{10} FH_4 is formed from folinic acid?

$f^5.FH_4$ can be converted to $f^{10}.FH_4$ by the action of an enzyme ***formyl FH_4 isomerase*** in presence of ATP. First $f^{5,10}$ FH_4 is formed, which by the action of ***cyclohydrase*** is converted to $f^{10}FH_4$.

Q.130. What is the metabolic role of folic acid?

The folic acid coenzymes are specifically concerned with metabolic reactions involving the **transfer and utilization of one carbon moiety.** One carbon moiety (C_1) may be methyl ($-CH_3$), formyl (-CHO), formate (HCOOH), forminino group (-CH=NH), or hydroxy methyl group ($-CH_2OH$)

Q.131. State the biochemical uses of folic acid in synthesis of biomolecules by accepting C_1-moiety.

- In incorporation of formyl carbon of purine ring (Position 2 and 8 of purine ring).
- Formation of "N-formyl methionine" of t-RNA (given by $f^{10}.FH_4$) required to initiate protein synthesis in prokaryotes.
- Glycine → to serine conversion (formation of **β**-carbon of serine).
- Homocysteine → to form methionine.
- Uracil → to form thymine.

Q.132. Name two important Folic acid antagonists.

- Aminopterine.
- Amethopterin (methotrexate)

Q.133. How do the folic acid antagonists act?

They inhibit the enzyme ***folic acid reductase*** and thus prevents formation of active forms of folic acid (FH_2 and FH_4). Thus they block synthesis of nucleic acids and inhibits cell division.

Q.134. What is the clinical use of folic acid antagonists?

On account of their ability to inhibit cell division and multiplication they have been used in treatment of:

- Leukaemias
- Erythraemias
- Malignant growth
- Recently in weekly small doses methotrexate has been used in treatment of rheumatoid arthritis.

Q.135. What is the deficiency manifestation of folic acid?
Folic acid deficiency produces macrocytic anaemia with megaloblastic bone marrow changes. Unlike vitamin B_{12} deficiency it does not produce neurological manifestations.

Q.136. Name the clinical types of macrocytic anemias due to folic acid deficiency with probable cause/or defect.
- Nutritional macrocytic anemia (dietary deficiency)
- Megaloblastic anemia of infancy (dietary deficiency)
- Megaloblastic anemia of pregnancy (probably relative deficiency)
- Macrocytic anemia in liver diseases (may be inadequate storage/conversion).
- Megaloblastic anemia in Celiac disease and tropical sprue (inadequate absorption)
- Macrocytic anemia after extensive intestinal resection (inadequate absorption).
- A congenital familial (inherited) type of macrocytic anemia (due to absence of ***folic acid reductase enzyme***).

Q.137. How will you determine the folate status of the body?
Red cell folate status is a reliable indicator of folate status of the body, as folate incorporated into RB cells during erythropoiesis is retained during entire life span of red blood cells. Average red cell folate is 300 ng/ml of whole blood on a PCV of 45% (range 160 to 640 ng/ml).

Q.138. What is the biochemical indicator of folate deficiency?
"FIGLU" test is a biochemical indicator of folate deficiency.

Q.139. What is the basis of FIGLU test?
In the metabolism of amino acid histidine there is a folic acid dependent step in which ***"Forminoglutamic acid" (FIGLU)*** is converted to glutamic acid (GA). In folic acid deficiency, this reaction cannot be carried out, as a result "FIGLU" accumulates in the blood and excreted in urine.

Thus ***"FIGLU" excretion in urine is a reliable biochemical index of folic acid deficiency.***

Q.140. What is the daily requirement of folic acid?
- Adult = 400 to 500 µg/daily
- Children = 100 to 300 µg/daily
- Pregnancy and lactation: 600 to 800 µg/daily.

Q.141. How much folic acid is required in treatment of macrocytic anemias?
A daily dose of 200 mg of folic acid is given orally or 10 to 20 mg may be required IV in severe cases.

Q.142. What is vitamin B_{12}? What is the chemical name?
Vitamin B_{12} is a water soluble B-vitamin, chemically it is called as ***cyanocobalamine***, in this cyanide group is bound to cobalt atom.

143. What are the other varieties of vitamin B_{12}?
If cyanide group is replaced by -OH group or NO_2 or Cl^- or SO_4, different forms are produced. They are respectively as follows:

- ***Hydroxy cobalamine or hydroxo cobalamine ($B_{12}a$)***
- ***Nitrito-Cobalamine ($B_{12}c$)***
- ***Chlorocobalamine***
- ***Sulphato cobalamine.***

Biologic action of all these varieties are similar.

144. Which type of vitamin B_{12} is superior therapeutically and why?
Hydroxocobalamine ($B_{12}a$) is superior to other varieties due to:

- It is more active in enzyme systems.
- It binds firmly to plasma proteins.
- It is retained longer in the body for its action when given orally.

Hence, $B_{12}a$ is more useful for therapeutic administration of B_{12} by mouth.

Q.145. What are R-proteins?
R-Proteins are cobalamine binding proteins, secreted by the salivary glands and the stomach and bind the dietary cobalamines at the acid pH. R-Proteins are normally degraded by pancreatic proteases and releases cobalamine.

Q.146. Why vitamin B_{12} is called as extrinsic factor of Castle?
Castle observed that for absorption of dietary vitamin B_{12} an "intrinsic" factor produced by the parietal cells of gastric mucosa is necessary. So Castle named dietary vitamin B_{12} as "extrinsic factor".

Q.147. What is the site of absorption of vitamin B_{12}?

- Ileum.

Q.148. Name the factors required for dietary vit B_{12} absorption. State their functions.

- Presence of HCl in stomach (acid pH)—to bind B_{12} with R-Proteins.
- *Proteases* in pancreatic juice to release B_{12} from bound form with R-proteins.
- Intrinsic factor of Castle.
- Ca^{++} ions, and
- a relasing factor in ileum.

} Both are required to B_{12} free from intrinsic factor for absorption across ileal mucosa.

Q.149. What is the nature of "Intrinsic factor" of Castle? Where is it produced?

Intrinsic factor of Castle (IF) is a low molecular weight, a specific glycoprotein, non-dialyzable and thermolabile. It is secreted by the parietal cells of gastric mucosa of cardiac end and fundus of stomach, but not the pylorus.

Q.150. What is the function of Intrinsic factor of Castle?

Intrinsic factor (IF) binds B_{12}, released by the *protease* action from R-proteins combination, and carries B_{12} through the intestinal lumen to the highly ***specific absorption site*** in the ileum. In presence of Ca^{++} ions and a releasing factor (RF) secreted by duodenum, B_{12} is separated from IF combination and absorbed.

Q.151. How vitamin B_{12} is transported in the blood?

- Vitamin B_{12} is transported in blood in association with specific binding proteins called ***transcobalamine I*** and ***Transcobalamine II***. Transcobalamine III has also been described.
- ***Physiologically transcobalamine II is most important.*** Transcobalamine I exists in plasma as well as in Liver, where it functions as "storage form" of B_{12} which is unique for water soluble B vitamins.

Q.152. What is the storage capacity and site of storage of vitamin B_{12}?

Vitamin B_{12} is stored in liver in combination with transcobalamine I. An adult man on normal non-vegetarian diet can store several mg (4 mg). As storage capacity of the vitamin is high, development of deficiency state take long time.

Q.153. Why people who are strictly on vegetarian diets are more prone to develop B_{12} deficiency?

Vitamin B_{12} is mainly obtained from animal sources like liver, meat, fish, eggs, milk and milk products. Vitamin B_{12} is absent from foods of vegetable sources. Hence a person who is strict vegetarian, even not taking eggs/milk and milk products is more prone to develop B_{12} deficiency.

Q.154. What is the major circulating form of vitamin B_{12}?

- Methyl cobalamine.

Q.155. What is the normal serum level of B_{12}?

Normal serum level varies from 0.008 to 0.42 μg/dl (Average = 0.02 μg/dl)

Q.156. What are the biological active forms of vitamin B_{12}?

"Biological active" forms of B_{12} are called ***cobamide coenzymes***, which act as coenzyme with various enzyme systems.

Q.157. How the cobamide coenzyme is formed?

Vitamin B_{12} is normally cobalamine containing a "cyanide group" attached to cobalt (Co); but in its conversion to cobamide coenzyme form, the ***cyanide group is substituted by "Adenosyl moiety"*** or $-CH_3$ group which is directly attached to cobalt (Co).

Q.158. Name the different types of cobamide coenzymes.

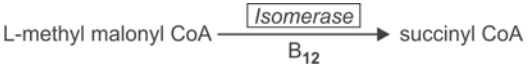
So far at least **4 varieties** have been described. They are:

- **DBC:** 5, 6, dimethyl benzimidazole
- **BC:** benzimidazole cobamide.
- **AC:** adenyl cobamide.
- **MC:** methyl cobamide.

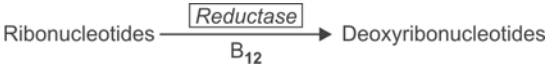
These cobamide coenzymes do not contain the cyanide group and are called ***"Corrinoid coenzymes"***.

Q.159. Name at least four important metabolic role of cobamide coenzymes.

- L-methyl malonyl CoA to succinyl CoA.



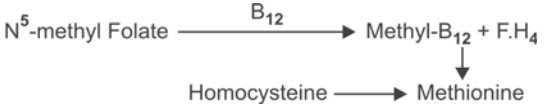
- Methylation of homocysteine to methionine.
- Conversion of ribonucleotides to deoxyribonucleotides which is necessary for DNA synthesis.



- Methylation of pyrimidine ring to form thymine.

Q.160. What is meant by folate trap?

- B_{12} is necessary as coenzyme for conversion of N^5-methyl $F.H_4$ to form methyl-B_{12}, which is required for conversion of homocysteine to methionine.



- In B_{12} deficiency, the above reactions cannot take place and folate is permanently trapped as N^5-methyl FH_4 and is, therefore, not available for C_1-transfer. It is called as ***folate trap***. This ***results in diminished synthesis of thymidylate and DNA.***

Q.161. What is the daily requirement of vitamin B_{12}?

- Adults: 3.0 mg/day
- Children: 1 to 2 mg/day
- Pregnancy and lactation: 4 mg/day.

Q.162. What is the deficiency disease produced by B_{12} deficiency?

- ***Pernicious anaemia,*** a macrocytic megaloblastic anaemia + subacute combined degeneration of the cord.

Q.163. What other clinical features found associated in B_{12} deficiency?

- Mucosal atrophy of stomach ***(IF is not produced)***
- Absence of HCl ***(achlorhydria)***

- Inflammation of tongue ***(glossitis)***
- Inflammation of mouth ***(stomatitis)***
- Degenerative changes of posterior and lateral columns of spinal cord.

Q.164. What advice you will give to strict vegetarians to prevent development of B_{12} deficiency?

Strict vegeterians should take milk/milk products and at least one egg twice a week to prevent development of B_{12} deficiency.

Q.165. What is the biochemical indicator of B_{12} deficiency?

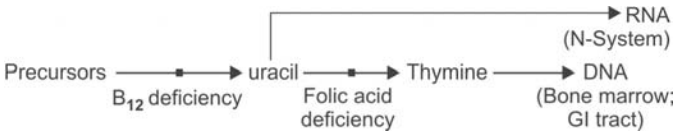
- ***Methyl malonic aciduria.***
- In B_{12} deficiency, L-methyl malonic acid cannot be converted to succinyl CoA, hence it accumulates in blood and tissues and excreted in urine.
  Thus, increased urinary excretion of methyl malonic acid in urine is a reliable biochemical index of B_{12} deficiency.

Q.166. Can methyl malonic aciduria be caused as inherited disorder?

Inherited deficiency of ***isomerase*** enzyme can cause methyl malonic aciduria. In this, vitamin B_{12} deficiency is not there.

Q.167. Why vitamin B_{12} deficiency is associated with neurological manifestations, which is not seen in folic acid deficiency?

- Explained by **Nieweg's hypothesis:**



It is postulated that folic acid is concerned with DNA metabolism, would explain how folic acid deficiency produces GI tract and bone marrow abnormalities unaccompanied by neurologic lesions.

On the other hand, B_{12} deficiency cause neurological lesions as a result of RNA deficiency as well as changes in bone marrow and GI tract from DNA deficiency.

- ***Other aggravating factors:***
  - Accumulation of methyl malonic acid
  - Also due to deficiency of methionine required for transmethylation for the metabolism of myelin sheath.

Q.168. Name one parasitic infection of gut which causes B_{12} deficiency.

A megaloblastic anemia responsive to B_{12} therapy occurs with infestation of the gut with fish tapeworm called ***diphyllobothrium latum,*** which eats up large amounts of B_{12} from the gut producing B_{12} deficiency to the host.

Q.169. Why acute pernicious anemia and Juvenile form are called autoimmune diseases?

- Antibodies to IF/and/or parietal cells have been demonstrated (auto antibodies).
- Type I IF antibody blocks attachment of B_{12}
  Type II IF antibody attaches to complex.

Q.170. State four important biochemical functions of inositol.

- ***Structural function:*** Phosphatidyl inositol forms biomembranes of cells and cell organelles.
- Some hormones react with membrane phophatidyl inositol and release "myoinositol 1, 4, 5-triphosphate" which acts as a ***messenger*** and liberates Ca^{++} from bound form.
- has a ***lipotropic*** function.
- has been incriminated in forming a complex with vitamin E required for proper storage of creatine-P in muscles.

Q.171. What are the important functions of choline in the body?

- Functions as a **"lipotropic"** agent and prevents fatty infiltration of liver.
- Occurs as a constituent of phospholipids (PL) like lecithins and sphingomyelins.
- Choline and its oxidation product betaine act as a methylating agent and involved in one carbon (C_1) transfer.

Q.172. Under what conditions the need for vitamin B-complex increase in the body?

- In pregnancy and lactation.
- During prolonged administration of broad spectrum oral antibiotics.

- Increased alcohol intake.
- In anoxia-shock and hemorrhage.
- In malnourished individuals.
- In serious illness, injury, burns and early convalescence.
- Increased carbohydrates/protein intake.
- Increased calorie expenditure like fever, hyperthyroidism.

Q.173. State the diseases in which vitamin B_6 has been used empirically.

- Nausea and vomiting of pregnancy (morning sickness).
- Radiation sickness.
- Muscular dystrophies.
- Treatment of hyperoxaluria and recurring oxalate stones of kidney.

Q.174. What is vitamin C? What is the chemical name?

Vitamin C is a water soluble vitamin other than the vitamin B complex group. It is known as L-ascorbic acid.

Q.175. Vitamin C has a strong reducing property, How?

Reducing property of vitamin C depends on the liberation of H-atoms from the enediol -OH groups on C_2 and C_3. In this action the ascorbic acid is oxidized to dehydroascorbic acid.

Q.176. Why vitamin C is not synthesized in humans?

- Lower animals can synthesize vitamin C from Glucose by uronic acid pathway.
- ***Man***, ***monkey*** and ***guinea pigs*** cannot synthesize vitamin C because they lack the enzyme that is required for conversion of keto-gulonolactone to ascorbic acid. Hence entire human requirement must be supplied by the diet.

Q.177. In which forms vitamin C exists in the blood and tissues?

Vitamin C exists in the body largely in the reduced L-ascorbic acid form, with a relatively small amount of oxidized form "dehydroascorbic acid". Both the forms exists in reversible equilibrium and both are active physiologically and metabolically.

Q.178. What are the chief metabolites of vitamin C in humans?

- Oxalic acid
- Diketogulonic acid, and
- L-threonic acid.

Conversion of vitamin C to oxalates in humans account for the major part of the endogenous urinary oxalate.

Q.179. What is the biological active form of vitamin C?

There is ***no biological active form*** of the vitamin. Ascorbic acid (vitamin C) itself acts as a coenzyme in various metabolic reactions.

Q.180. State three important metabolic role of vitamin C.

- ***Role in cellular oxidation-reduction*** serving as hydrogen transport agent.
- ***Role in collagen synthesis:*** Hydroxyproline and hydroxy lysine are important constituents of mature collagen fibres. Precollagen molecules contain the amino acids proline and lysine which are hydroxylated by ***hydroxylases*** in presence of vit. C, Fe^{++} and molecular O_2. In vitamin C deficiency, the collagen synthesis is defective.
- ***Absorption of Fe:*** Vitamin C helps in conversion of Fe^{+++} (ic) to Fe^{++} (ous) form for absorption.

Q.181. State a few other biochemical functions of vitamin C?

- ***Role in tryptophan metabolism:*** vitamin C is required as co-factor for hydroxylation of tryptophan to 5-OH derivative in the pathway of biosynthesis of serotonin (5-HT).
- ***Role in tyrosine metabolism:*** vitamin C is required as a co-factor with the enzyme ***"p-OH-phenyl pyruvate hydroxylase"*** required for conversion of p-OH phenyl pyruvate to homogentisic acid.
- ***Role in formation of carnitine:*** Formation of carnitine in Liver by hydroxylation of γ-butyrobetaine requires vitamin C and Fe^{++}.
- ***Role in formation of catecholamines:*** Vitamin C is required as a coenzyme with the enzyme dopamine hydroxylase, which catalyzes the conversion of dopamine → norepinephrine.
- Role in steroidogenesis.

Q.182. What are the sources of vitamin C?

- ***Mainly vegetable sources:***

  Good sources are **citrous fruits**—oranges/lemons, other fruits like papaya, guava, pineapple, banana, strawberry.

  Amongst vegetables—cabbage and cauliflower, Germinating seeds, green peas, potatoes, **amla**, tomatoes, etc.

Q.183. Which is the richest source of vitamin C?
- **Amla is the richest source.**

Q.184. What is the daily requirement of vitamin C?
- Adults: 60 to 75 mg daily.
- Children: 30 to 40 mg daily.
- Pregnancy and lactation: 100 to 150 mg daily.

Q.185. What is the deficiency disease produced by vitamin C deficiency?
Vitamin C deficiency produces the disease called ***scurvy***.

Q.186. What is the basic biochemical defect in scurvy?
The main basic defect is a failure to deposit intercellular cement substance and defective collagen synthesis.

Q.187. State the chief clinical manifestations of scurvy?
- Capillaries are fragile and there is tendency to hemorrhage. Petechial, subcutaneous, sub-periosteal and even internal hemorrhage.
- Wound healing is delayed due to deficient formation of collagen.
- Gums are swollen and spongy and bleeds on slightest pressure.
- Poor dentine formation in children, leads to poor teeth formation.
- Osteoid of bone is poorly laid and mineralization of bone is poor, bones are weak and readily fractures.
- ***Anemia:*** hypochromic microcytic type due to Fe deficiency due to poor absorption.

Q.188. What are the effects of excessive intake of vitamin C?
- Administration of large amounts of vitamin C is not known to produce any harmful effects in humans.
- But in rats, dehydroascorbic acid in large doses (1.5 gm/kg body wt) produces permanent diabetes, similar to that produced by the glycoside alloxan.

Q.189. State some diseases in which vitamin C is used empirically for treatment.
- Has been found to control and alleviate infectious diseases ***when used as adjunct*** e.g. tuberculosis, streptococcal infection, to cut short paroxysmal attack of whooping cough, etc.

- Help in wound healing: used empirically in treatment of traumatic ulcer, burns, gastric/and duodenal ulcers, corneal ulcerations, etc.
- Used in allergic conditions, common cold, and coryza.
- In very large dosage 4.0 gm/daily used in rheumatoid arthritis/rheumatic fever.
- In methemoglobinaemia—for reversal of methemoglobin to normal Hb.

Q.190. Name the vitamins/or precursors which have antioxidant and anticancer activity.

- Vitamin A and its precursors carotenoids **β-Carotene**.
- **Lycopene**, a carotenoid present in ripe tomatoes.
- **Vitamin E** (Tocopherols)
- **Vitamin C** (Ascorbic acid)

Q.191. Name the water soluble vitamins observed to prevent birth defects.

- Folic acid.
- Inositol in folic acid resistant cases.

CHAPTER

10 Heme—Synthesis and Catabolism

Q.1. What is heme?
Heme is the prosthetic group of certain conjugated proteins. It is a tetra-pyrrole contaning a metal ion in the center usually Fe.

Q.2. Name five examples of conjugated proteins in which heme is a prosthetic group.
- Hemoglobin of mammalian erythrocytes.
- Myoglobin in muscles.
- Cytochromes-respiratory enzymes (in ETC).
- Catalase enzyme.
- Peroxidase enzyme.

Q.3. What are porphyrins?
Porphyrins are complex structures consisting of 4 pyrrole rings united by methylidene (or methyne) bridges.

Q.4. Where are porphyrins synthesized in humans?
Porphyrins are synthesized partly in the mitochondria and partly in the cytosol of aerobic cells like liver and developing erythrocytes.

Q.5. What are the starting materials for synthesis of heme?
- Succinyl CoA, and
- Glycine.

Q.6. What is δ-ALA?.
- **δ**-ALA (amino levulinic acid) is the first compound formed in the biosynthetic pathway by condensation of succinyl CoA and glycine.
- First **α**-amino-**β**-keto adipic acid is formed which undergoes **spontaneous decarboxylation** to form **δ**-ALA.

- Both the reactions are catalyzed by the enzyme ***"δ-ALA synthetase"*** which requires B_6-P and $Mg.^{++}$ Synthesis occurs in mitochondria.

Q.7. Which is the precursor of pyrrole rings?

- Porphobilinogen.

Q.8. How porphobilinogen is formed?

δ-ALA after its formation comes out of mitochondrion to cytosol. Two molecules of **δ**-ALA condense to form a molecule of porphobilinogen, the reaction is catalyzed by the Zn-containing enzyme ***δ-ALA dehydratase*** and requires Cu^{++} as a cofactor.

Q.9. From which series heme is synthesized?

Four molecules of porphobilinogen under the influence of the enzymes ***deaminase*** and ***isomerase*** forms the major series ***uroporphyrinogen III***, which ultimately leads to the formation of heme. This occurs in cytosol.

Q.10. How protoporphyrin IX is formed?

Protoporphyrin IX is formed form coproporphyrinogen III in mitochondrion by an ***oxidative decarboxylase enzyme system*** containing flavins as coenzymes. First protoporphyrinogen IX is formed which is converted to protoporphyrin IX by another ***oxidase***.

Q.11. How is heme formed from protoporphyrin IX?

An atom of Fe^{++} is inserted into central position of protoporphyrin IX to form the heme. It is catalyzed by the enzyme ***heme synthetase (ferrochelatase)*** which for optimal functions requires relative anaerobiosis and GSH. Fe^{++} is brought by ***transferrin*** (Siderophillin). It operates inside mitochondria.

Q.12. What are the key regulatory enzymes in heme synthesis?

- ***δ-ALA synthetase***
- ***δ-ALA dehydratase***

Both are main rate-limiting enzymes in the synthetic pathway. End product heme inhibits both the enzymes by "feed-back" inhibition.

Q.13. What is the role of oxygen in the heme synthesis?

Role of oxygen in heme synthesis is rather complex:

- ***In vivo*** stimulated by low O_2 tension (e.g.living in high altitudes)

- ***In vitro*,** conversion of porphobilinogen to uroporphyrinogen III and protoporphyrin to heme, are both inhibited by O_2.
- But ***O_2 is required for oxidative decarboxylation of coproporphyrinogen*** III and oxidation of protoporphyrinogen IX to protoporphyrin IX.

Q.14. What are porphyrias?

When the blood levels of uroporphyrins and coproporphyrins are increased above normal level and excreted in urine/feces, the condition is called as porphyria.

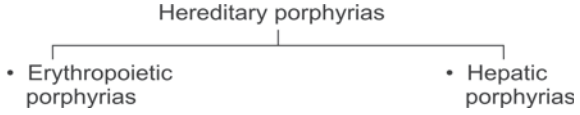
Q.15. What are the two main types of porphyrias?

- Hereditary (congenital/familial) porphyrias.
- Acquired porphyrias.

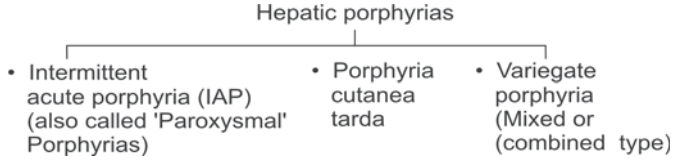
Q.16. How congenital or hereditary type is further classified?

- Hereditary or congenital porphyrias can be divided into **2 main groups** based on the porphyrin and its precursors content in bone-marrow (erythropoietic) and in liver (hepatic).

  Thus,



Q.17. How hepatic porphyrias are further classified?



Q.18. What is the enzyme deficiency in congenital erythropoietic porphyrias?

Due to preponderance of type I porphyrins, both uroporphyrin I and coproporphyrin I (minor series), the enzyme deficiencies suggested as follows:

- Deficiency of ***isomerase*** enzyme, or
- Relative preponderance of ***deaminase*** activity with low or absent ***isomerase*** activity.

Q.19. What is the clinical presentation and biochemical findings in hereditary erythropoietic porphyria?

- Abnormal photosensitivity to light and development of skin lesions.
- Biochemically blood contains increased amounts of porphyrins of type 1 (**minor series**) and precursors porphobilinogen and **δ**-ALA.
- Urine is ***port-wine*** or red in color containing type I series in oxidized forms, uroporphyrin I and coproporphyrin I (both red pigments).

Q.20. What are the salient features of hepatic porphyrias?

- Principal organ affected is liver.
- Abnormal and excessive production of porphyrins type III **(major series)** and their precursors **δ**-ALA and porphobilinogen which are excreted in urine.

Q.21. What is the enzyme deficiency in intermittent acute porphyria (IAP)?

Partial deficiency of ***deaminase*** enzyme.

Q.22. What is the clinical presentation and biochemical finding in IAP?

- Photosensitivity of skin is absent.
- Patient presents with GI symptoms: acute attacks of abdominal pain, nausea and vomiting, CV abnormalities and neuropsychiatric symptoms and signs
- Excretes large quantities of porphobilinogen and **δ**-ALA. Freshly passed urine is normal but on exposure of air turns ***port-wine or red color***.

Q.23. What are types of acquired porphyrias? Mention a few important causes.

- Coproporphyrin type III formed in excessive quantities and excereted in urine.

  Causes:
  - Exposure to certain toxic chemicals and heavy metals e.g. lead (Pb) and arsenic.
  - Acute alcoholism and cirrhosis liver in chronic alcoholics.
- Coproporphyrin type I formed in excessive quantities and excerted in urine.

Causes:
- Obstructive jaundice
- Cirrhosis in nonalcoholics
- Blood dyscrasias e.g. leukemias, hemolytic anemia.

Q.24. What is porphyrinuria?
Excretion of porphyrins including its precursors in excess amount than normal in urine is called porphyrinuria.
Note: Porphyrins excreted in urine in hepatic porphyrias as **zinc-complexes**, whereas those of erythropoietic porphyrias are in the **"free"** form.

Q.25. What is normal excretion of porphyrins in urine?
Normally, only small amounts of coproporphyrins type I and III, 60 to 280 mg in the ratio of 70% type I and 30% of type III are excreted in urine per day in normal health. Uroporphyrins are excreted only in negligible amounts of 15 to 30 mg per day.

Q.26. What are bile pigments?
Bile pigments are:
- **Biliverdin**, and
- **Bilirubin**.

The color of the bile is due primarily to these pigments. Normally there is slight traces of biliverdin in human bile, and ***bilirubin is the principal bile pigment.***

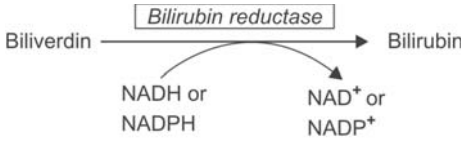
Q.27. What is the turnover of Hb in a normal adult?
In a normal adult human of 70 kg body wt approximately 6.25 gm of Hb (90 mg per kg) is synthesized and degraded daily, producing approximately 210 to 250 mg of bilirubin which are excreted daily by the liver in the bile.
Note: ***Degradation of one gram of Hb yields*** *approximately* ***35 mg of bilirubin.***

Q.28. What is principal source of bilirubin?
Principal source is from **"heme"** of **"effete" erythrocytes.** Approximately 85% of bilirubin is derived from senescent erythrocytes by conversion of "heme" of Hb to biliverdin and bilirubin within RE cells.

Q.29. What are the principal sites of formation of bile pigments?
Bone marrow, spleen, and liver: the bone marrow appears to be the most active site.

Q.30. Which bile pigment is formed first in RE cells?
The bile pigment biliverdin is first formed in RE cells and then converted to bilirubin whithin RE cells.

Q.31. State how biliverdin is converted to bilirubin?
Biliverdin is converted to bilirubin in RE cells by a specific enzyme *bilirubin reductase* which utilizes either NADPH or NADH as hydrogen donor.



Q.32. State the other sources of circulating bilirubin.
15% of newly synthesized bilirubin is derived from sources other than maturing circulating RB cells. Possible other sources are:
- Heme formed from Hb-synthesis.
- Destruction of immature erythrocytes in the bone marrow.
- Degradation of Hb within erythrocyte precursors.
- Breakdown of other hemecontaining pigments. Such as:
  - Cytochromes
  - Catalase
  - Myoglobin etc.

Q.33. What is meant by shunt hyperbilirubinemia?
Excessive production of bilirubin from "heme" of erythrocyte precursors in bone marrow or direct synthesis in marrow, gives rise to hyperbilirubinemia and jaundice. Such a condition is called as ***"shunt hyperbilirubinemia".***

Q.34. Which enzyme is responsible for break down of Hb?
- The enzyme which breaks down Hb is ***heme-a methenyl oxygenase,*** present in microsomal fraction of RE cells which brings about oxidative scission of the "Fe-porphyrin ring". The opening of porphyrin ring labilizes the removal of Fe which goes to "iron pool" and reutilized.
- Whether globin is seperated first or after ring opening is controversial. Globin separated is reutilized.

Q.35. What is the biochemical indicator to measure rate of heme catabolism?

- Oxidative scission takes place at the α-methane bridge between pyrrole ring I and II, which ***results in loss of one carbon as CO (Carbon monoxide).***
- ***Rate of elimination of CO in expired air has been used biochemically as an index of rate of heme catabolism.***

Q.36. State the nature of bilirubin first formed in RE cells.

- Bilirubin first formed in RE cells from breakdown of Hb is called ***unconjugated bilirubin***.
- It is insoluble in water and highly lipid soluble.
- It is carried in the blood being bound to albumin and is taken to liver for conjugation.

Q.37. Why unconjugated bilirubin is not excreted in urine?

As it is bound to colloidal protein albumin it has large molecular size, hence cannot be filtered by glomerulus and cannot be excreted in urine.

Note: Hence no bile pigments found in urine in unconjugated hyperbilirubinemias including hemolytic anemia.

Q.38. What is bilirubin encephalopathy (kernicterus)?

- Bilirubin encephalopathy (kernicterus) is a serious condition developing in "unconjugated hyper-bilirubinemias". It is ***characteristically seen in "hemolytic disease of the new born (HDN)".***
- Unconjugated bilirubin bound to albumin is lipid soluble and can enter in brain and gets deposited in neurons of basal ganglia, hippocampus, cerebellum and medulla causing necrosis of nerve cells probably by interferring with cellular respiration.

Q.39. Which drug administration increases risk of kernicterus in jaundiced neonates?

Administration of sulphonamides to pregnant women in terminal stage and neonates increases the risk of "kernicterus", as sulphonamides are also bound to albumin, hence the binding capacity of unconjugated bilirubin is modified.

Q.40. Where and how conjugation of bilirubin takes place?
Unconjugated bilirubin brought to liver is taken up selectively by liver cells and conjugation takes place with the help of the enzyme ***glucuronyl transferase*** and UDP-glucuronic acid.

Q.41. What are the products of conjugation of unconjugated bilirubin?
- Bilirubin monoglucuronide and
- Bilirubin diglucuronide (major portion).

Q.42. What is the function of the enzyme dismutase?
Two molecules of bilirubin monoglucuronides can form one molecule of bilirubin diglucuronide by the enzyme ***dismutase*** present in liver cells.

Q.43. State the nature of conjugated bilirubin.
- Conjugated bilirubin is water soluble.
- Smaller in molecular size as they are not bound to albumin.
- Conjugated bilirubin can pass through glomerular filter and can appear in urine (Bilirubinuria).
  Note: ***Bilirubinuria is found in obstructive jaundice and hepatocellular jaundice.***

Q.44. What is the normal fate of conjugated bilirubin?
Conjugated bilirubin is led to bile and excreted in GI tract. In the lower portion of the intestine specially in cecum and colon, the bilirubin is released from the glucuronides by the help of the enzyme *β-glucuronidase* produced by bacterial flora. The released bilirubin undergoes a series of reductive changes and ultimately forms L-urobilinogen (L-stercobilinogen) and excreted in feces, which gets oxidized to L-stercobilin in presence of air giving normal golden-yellow color to feces.
Note: L-urobilinogen and L-stercobilinogen are structurally similar.

Q.45. What is the enterohepatic circulation of bile pigments?
Products of progressive reduction of conjugated bilirubin may in part be absorbed from the intestine and returned to

liver for its re-excretion called as ***enterohepatic circulation*** and excreted in urine which normally contains traces of urobilinogen.

Q.46. Name the drugs which increase the activity of the enzyme glucuronyl transferase.

- Hepatic ***glucuronyl transferase*** activity is increased after administration of certain drugs viz. benzpyrene, aminoquinolines, chlorcyclizine and phenobarbitones to normal adults and neonates. Administration of these drugs ***results in proliferation of smooth endoplasmic reticulum*** and increases the synthesis of the enzyme and its activity.
- Most commonly clinically used drug is the phenobarbitone.

Q.47. Name three inherited disorders which produce unconjugated hyperbilirubinemia and jaundice.

- Gilbert's disease or syndrome.
- Crigler-Najjar syndrome-Type I and Type II.
- Lucey-Driscoll syndrome.

Q.48. What is Gilbert's disease?

- The disease is characterized by low grade chronic **unconjugated hyperbilirubinemia** and jaundice.
- Age group affected 18 to 25 years. Detected suddenly during routine examination. Patient usually complaints of fatigue, weakness and abdominal pain.
- Serum bilirubin level in 85% cases is usually less than 3 mg/dl.
- ***Defects:***
- Due to a defect in hepatic clearance of bilirubin possibly due to defect in uptake of bilirubin by liver cells.
- Also due to reduced ***glucuronyl transferase*** activity.

Q.49. What is Crigler-Najjar syndrome? Differentiate Type I and Type II.

- Both types are inherited disorder of bilirubin metabolism characterized by congenital non-hemolytic unconjugated hyperbilirubinemia and jaundice.

- Differentiating features of two types are:

Type-I	*Type-II*
• More severe	• Less severe
• Serum bilirubin exceeds 20 mg/dl when untreated.	• Serum bilirubin is usually less then 20 mg/dl.
• Risk of Kernicterus	• No risk of kernicterus.
• Usually fatal	• Less fatal
• ***Defect:*** Inherited absence of ***glucuronyl transferase*** enzyme	• Bilirubin monoglucuronide can be formed but cannot add the second glucuronyl group.
• Responds well to Phototherapy	• Responds well to therapy with phenobarbitone

Q.50. What is Lucey-Driscoll syndrome?

- A transient familial neonatal non-hemolytic **unconjugated hyperbilirubinemia** and jaundice.
- Healthy looking women can give birth to infants with jaundice and with risk or kernicterus.
- ***Defects:***
  Probably a progestational steroid derivative present in mother's blood which can inhibit the ***glucuronyl transferase*** activity.

Q.51. Name one inherited disorder which produces conjugated hyperbilirubinemia and jaundice.

- Dubin-Johnson syndrome.

Q.52. What is Dubin-Johnson syndrome? Mention some salient features.

- An autosomal recessive disorder.
- Characterized by ***conjugated hyperbilirubinemia*** and jaundice in childhood and during adult life.
- ***Defects:***
  Conjugation of bilirubin takes place but there is defect in hepatic secretion of conjugated bilirubin in bile.
- ***BSP test:*** shows secondary rise in plasma concentration due to reflux of the conjugated BSP ***(pathognomonic for diagnosis).***

Q.53. What is neonatal physiological jaundice?

- Most common cause of ***unconjugated hyperbilirubinemia*** and jaundice in neonates.
- ***Defects:*** Probably multiple causes:
- Accelerated haemolysis
  - Due to immature hepatic system for uptake, conjugation and secretion of bilirubin.
  - ***Glucuronyl transferase*** activity is reduced.

Responds well to:

- Phenobarbitone administration, and
- Phototherapy.

CHAPTER

11 Digestion and Absorption

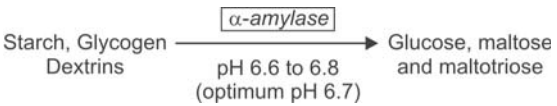
Q.1. What are the dietary sources of carbohydrates?
Dietary carbohydrates principally consist of:

- ***Polysaccharides:*** starch and glycogen (rice, wheat barley, liver, etc.)
- ***Disaccharides:***
- Sucrose (cane sugar / table sugar)
- Lactose (milk sugar), and
- Maltose (malt sugar)
- And small quantities of ***monosccharides*** like fructose and pentoses.

Q.2. What are the enzymes present in saliva?
Saliva contains only enzyme ***amylase*** (also called ***ptyalin***), a carbohydrate splitting enzyme. Chemically it is **α**-amylase.

Q.3. Name the activators of the enzyme amylase.
Activators are Cl^- and Br^-.

Q.4. What is the action of salivary amylase?
The salivary ***amylase*** hydrolyzes **α** 1 → 4 glucosidic linkages at random deep inside polysaccharide molecule like starch, glycogen and dextrins producing smaller molecules glucose, maltose and maltotriose. Ptyalin action stops in stomach when pH falls to 3.0.



Q.5. What is the carbohydrate splitting enzyme present in Gastric juice?

- No carbohydrate splitting enzyme is present in Gastric juice.
- Some dietary sucrose may be hydrolyzed to equimolar amounts of glucose and fructose by HCl in gastric juice.

Q.6. What are the carbohydrate splitting enzymes present in pancreatic juice?

The only carbohydrate splitting enzyme is pancreatic ***amylase***. Action and products are similar to ptyalin. Optimum pH is 7.1 and it also requires Cl^- or Br^- for activation.

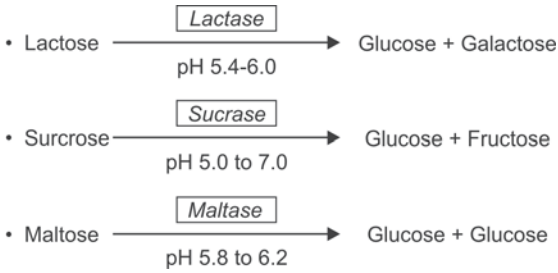
Q.7. Name the carbohydrate splitting enzymes present in intestinal juice.

- Intestinal ***amylase***
- Disaccharide splitting enzymes:
- ***Lactase***
- ***Sucrase***
- ***Maltase*** and ***Isomaltases.***

Q.8. What is the action of intestinal amylase?

The enzyme hydrolyzes terminal $\alpha 1 \rightarrow 4$ glycosidic linkage in polysaccharides and oligosaccharide molecules liberating "free" glucose molecules.

Q.9. State the action of disaccharide-splitting enzymes of intestinal juice.



Q.10. What is the action of isomaltase?

The enzyme ***isomaltase*** catalyzes hydrolysis of $\alpha 1 \rightarrow 6$ glucosidic linkage thus splitting α-limit-dextrin at the branching points and produces maltose and glucose.

Q.11. How many types of maltases identified in intestinal epithelial cells?

Five types of maltases have been identified so far in intestinal epithelial cells.

- ***Maltase V*** can act as ***isomaltase*** over and above its action on maltose.
- ***Maltase III*** and ***maltase IV*** also possess *sucrase* activity.

Q.12. What are the end-products of digestion of carbohydrates?

Complex dietary carbohydrates are broken down during its passage in GI tract. The end-products of digestion are:

- ***Monosaccharides:*** glucose, fructose, galactose, mannose.
- ***Pentose Sugars:*** xylose, arabinose, etc.

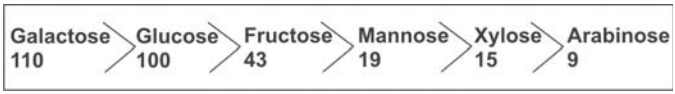
Q.13. What is the site of absorption of carbohydrates?

All monosaccharides, products of digestion of dietary carbohydrates, are practically completely absorbed almost entirely from the small intestine. Rate of absorption diminishes from above downwards, proximal jejunum three times grater than that of distal ileum.

Q.14. Is the rate of absorption same for all monosaccharides? If not, state briefly about the rate of absorption of different monosaccharides.

Rate of absorption of various monosaccharides and pentose sugars are not same. They are:

- Glucose nad galactose are absorbed very rapidly. Galactose is absorbed more rapidly than glucose.
- Fructose and mannose have intermediate rate.
- Pentose sugars are absorbed slowly.
- Taking glucose absorption as 100, comparative rate of absorption of sugars are as follows:



Q.15. Why glucose and galactose are absorbed rapidly?

- All monosaccharides are absorbed to some extent by simple diffusion due to concentration gradient.
- But glucose and galactose are absorbed rapidly due to **"active" transport.**

Q.16. What are the structural features required for active transport?

Wilson and **Craine** suggested that to be actively tranported sugar must have the following chemical configuration:

- Must have a six membered ring.
- Must have one or more carbon atoms attached to C_5,
- Must have a-OH group at C_2.

The above chemical configuration is satisfied by glucose and galactose only.

Q.17. What is the active transport?

Glucose and galactose are absorbed in presence of $\mathbf{Na^+}$, using a ***carrier protein*** (transport protein), which is present in the "brush" border of intestinal epithelial cells. It is a ***Co-transport*** (symport) mechanism. Glucose and galactose have their own specific carrier proteins. Requires energy which is provided by ATP.

Q.18. State the nature of carrier protein.

The carrier protein has the following characteristices:

- Has two binding sites—one for Na^+ and the other one for the sugar-glucose/or galactose
- It is specific for glucose/galactose.
- It is mobile, it can carry the sugar to the other side and after delivery of sugar can return to "brushborder" again.
- It is sodium dependent, and
- It is energy dependent.

Q.19. What is the function of sodium in glucose absorption?

Sodium-binding by the "carrier Protein" is a prerequisite for glucose/galactose absorption. Sodium binding changes the conformation of the ***carrier protein*** molecule so that it enables the binding of sugar to take place.

Q.20. How does ORS help in diarrhea of children?

The basic concept of "cotransport" of glucose and sodium (Na^+) has clinical application of treatment of diarrhea in

children. To correct dehydration, electrolyte loss and to provide nutrient, glucose and salt mixture is given orally to children having diarrhea. Availability of Na^+ helps in glucose absorption rapidly. Sodium also will carry water so that dehydration and electrolyte deficit is corrected. Glucose provides energy.

Q.21. Why fructose is not absorbed by active transport?
Fructose does not have a-OH group on C_2.

Q.22. How is fructose absorbed? Why its rate of absorption is greater than pentose sugars but less than glucose/ galactose?

- Furctose is absorbed to some extent by simple diffusion by concentration gradient like the pentose sugars.
- But is absorbed more rapidly than pentose sugars, as it is absorbed by ***facilitated transport*** which requires the presence of "carrier protein" but does not require energy.

Q.23. How the Pentose sugars are absorbed?
Pentose sugars are absorbed ***passively by simple diffusion*** which depends on the concentration gradient. Neither "carrier proteins" nor energy is required.

Q.24. State the factors that influence the rate of absorption of carbohydrates from intestine.

- ***State of intestinal mucosa:*** absorption suffers when mucosa is not healthy.
- ***Length of contact:*** less absorption in hurried bowel.
- ***Hormones:***
  - ***Thyroid hormones:*** increases absorption by action directly on intestinal mucosa.
  - ***Adrenocortical hormones:*** absorption decreases in adreno-cortical deficiency due to decreased concentration of Na^+ in body fluids.
  - ***Anterior pituitary hormones:*** hyperactivity induces thyroid overactivity and increases absorption.
  - ***Insulin:*** has no effect on intestinal absorption.
- ***Vitamins:*** Absorption is diminished in vit B deficiency viz. thiamine, pyridoxine, pantothenic acid.
- ***Inherited enzyme deficiencies*** like ***sucrase*** and ***lactase*** can interfere with hydrolysis of corresponding disaccharides and their absorption.

Q.25. What does happen in inherited lactase and sucrase deficiencies?

- Due to inherited deficiency of the enzymes, the disaccharides cannot be hydrolyzed, resulting to accumulation of lactose/ or sucrose in intestinal tract; they are ***osmotically active*** and hold water producing diarrhea.
- The accumulated lactose/or sucrose are fermented by intestinal bacteria producing gases which develop abdominal distension, flatulence and abdominal cramps.

Q.26. What is disaccharidurıa?

- The inherited deficiency of ***disaccharidase*** causes increased excretion of disaccharides.
- Patients with intestinal damage viz. sprue and celiac disease, may excrete more than 300 mg of disaccharides.

Q.27. What are the dietary sources of fats?

- ***Animal sources:*** Dairy products like milk, butter, ghee etc. meat and fish specially pork, eggs.
- ***Vegetable sources:*** Various cooking oils from various seeds viz. sunflower oil, groundnut oil, cotton seed oil, mustard oil etc. and fats from other vegetables.

Q.28. What are the problems faced by fat digestion and how it is overcome?

The degestion of fats and other lipids poses special problem becuase of:

- The insolubility of fats in water, and
- Because lipolytic enzymes are soluble in aqueous medium.

The above problem is overcome in the gut by emulsification of fats by bile salts present in bile and phospholipids (PL). The breaking of large fat particles into smaller fine particles by emulsification ***(micelles)***, increases the surface exposed to interaction with ***lipases;*** thus the rate of digestion of fats is proportionally increased.

Q.29. What is Lingual lipase? What is its action?

Lingual lipase is secreted by the Ebner's gland present in the dorsal surface of the tongue. Optimum pH of activity is 4.0 to 4.5. ***Best substrates for action are short chain*** and

medium chain TG. Thus milk fats are the best substrates for this enzyme. The released short-chain and medium chain FA are absorbed directly from the stomach wall and enter the portal blood.

Q.30. What is gastric lipase? State its action.

- Small amount of gastric lipase is present in the gastric juice. The over-all digestion of fats by gastric lipase is negligible because:
  - No emulsification of fats occur in stomach.
  - The enzyme is secreted in small amount.
  - pH of gastric juice is highly acidic and not conducive for lipase activity.
- Gastric lipase activity requires presence of Ca^{++}
- Whatever mimimal action of gastric lipase is there, it is confined to highly emulsified fats viz. those of milk fats and fats present in egg-yolk which are already in emulsified form.

Q.31. Name the lipolytic enzymes present in the pancreatic juice.

- ***Pancreatic lipase*** (also called "steapsin")
- ***Phospholipase A_2*** (lecithinase)
- ***Cholesterol esterase.***

Q.32. What is the peculiarity of pancreatic lipase activity?

Pancreatic lipase is peculiar in that is is virtually specific for the hydrolysis of *primary ester* bond at 1 and 3 position. It **cannot readily hydrolyze the "ester linkage" of position 2.**

Q.33. How the ester linkage at position 2 is hydrolyzed?

An ***"isomerase"*** enzyme converts the **β**-monoglyceride to **α**-monoglyceride, which is then acted upon by pancreatic lipase to produce complete hydrolysis of TG molecule.

Q.34. State the products of TG hydrolysis by pancreatic lipase formed in intestinal lumen.

- **α, β**-diglyceride-not absorbed
- **β**-monoglyceride (72%)
- **α**-monoglyceride (6%)
- Three molecules of FFA
- Glycerol (22%)

Q.35. What is the pH of pancreatic lipase activity?

- pH = 7.0 to 8.8

Q.35. State the role of bile salts in pancreatic lipase activity. What is *co-lipase*?

- Bile salts are necessary for emulsification of fats to form ***micelles***, thus giving more surface area for pancreatic lipase activity.
- Bile salts also help in combining the pancreatic *lipase* enzyme with two molecules of a small protein called ***"co-lipase"*** (mol wt = 10,000) in the intestinal lumen. This combination of lipase with co-lipase has **two effects:**
  - enhances the lipase activity in the intestinal pH.
  - also protects the enzyme.

Q.37. What is the role of Ca^{++} in pancreatic lipase activity?

In the presence of Ca^{++} in the intestine, the FFA are immediately precipitated as "soaps" (insoluble Ca-soaps) and are thereby prevented from inhibiting further lipase action. Thus calcium facilitates lipase action.

Q.38. What is meant by enterohepatic circulation of bile salts?

Bile salts of the "micelles" are not absorbed immediately. They are re-dissolved in other emulsified particles and reabsorbed later in the lower part of small intestine and returned to the Liver via portal vein for resecretion in bile. This is known as ***"enterohepatic circulation of bile salts"***.

Q.39. What happens to glycerol formed in the intestinal lumen of TG hydrolysis by pancreatic lipase?

Twenty two percent of **"Free" Glycerol** formed in intestinal lumen is absorbed directly to portal blood. ***This glycerol is not utilized in re-synthesis of TG in intestinal ephithelial cells.***

Q.40. What happens to FFA formed in the intestinal lumen by TG hydrolysis?

Fate of FFA depends on their carbon contents. Thus,

- Short chain FA and medium chain FA less than 10°C and some unsaturated FA are absorbed directly to portal blood and taken to liver.
- Long chain FA absorbed in intestinal epithelial cells are activated to "acyl CoA" and utilized for resynthesis of TG.

Q.41. What happens to absorbed α-monoglyceride and β-monoglyceride?

- In the intestinal epithelial cells, absorbed **α**-monoglyceride (6%) are acted upon by ***intestinal lipase*** enzyme and forms glycerol and FFA, both are utilized for re-synthesis of TG.
- **β**-monoglyceride (72%) are absorbed in intestinal epithelial cells, which take up one "acyl CoA" and forms TG.

Q.42. What are the sources of α-glycero-P in intestinal epithelial cells required for resyntheis of TG?

- Glycerol formed from α-monoglyceride is converted to α-glycero-P by the enzyme ***glycero-kinase*** and ATP.
- Some amount of α-glycero-P is contributed from glycolysis operating in intestinal epithelial cells. Both are utilized for resynthesis of TG.

Q.43. What is the fate of resynthesized TG in intestinal epithelial cells?

Re-synthesized TG is insoluble in water (hydrophobic) and cannot pass to lacteals. Hence it is converted to lipo-protein complex called ***chylomicrons*** which is hydrophilic and water soluble and are thrown into the lacteals.

Q.44. How chylomicrons are formed from TG in the intestinal epithelial cells?

TG gets covered with a layer of hydrophilic phospholipids (PL), cholesterol/cholesterolesters and incorporation of a specific apo-protein ***apo-B_{48}***. Addition of these "polar" substances convert the water insoluble TG to soluble "chylomicrons".

Q.45. What happens to dietary phospholipids?

- Due to its ***Polar structure*** and hydrophilic properties some of PL are ***absorbed directly*** to portal blood.
- Some PL is incorporated in chylomicrons and VLDL synthesis in intestinal mucosal cells and carried in lymphatics.
- Pancreatic juice contains an enzyme called ***"phospholipase A_2"*** (lecithinase). In presence of Ca^{++} and bile-salts, ***"phospholipase A_2"*** *phospholipase* A_2 hydrolyzes PL to form free FA and Lysophospholipid which are absorbed.

Q.46. What happens to dietary cholesterol?

- Cholesterol appears to be absorbed from the intestine almost entirely in "free" form.
- Pancreatic juice contains an enzyme ***"cholesterol esterase"***, which may either catalyze the esterification of free cholesterol with FA or it may catalyze also hydrolysis of cholesterol esters.
- Both free and ester cholesterol are also incoporated in formation of chylomicrons and VLDL.

Q.47. What is chyluria?

It is an abnormality in which the patient excretes ***milky urine*** because of presence of abnormal connection of urinary tract and lymphatic drainage system of intestine ***(chylous fistula).***

Q.48. What is chylothorax?

An abnormal connection between pleural and the lymphatic drainage of small intestine results in accumulation of lymph in pleural cavity producing "milky" pleural effusion called ***chylothorax***.

Q.49. What is the effect of feeding short and medium chain TG in chyluria and chylothorax?

Feeding of short and medium chain TG less than 10 C removes the milky appearance. This is due to the fact that short and medium chain TG can be absorbed directly to portal blood system, by-passing the lacteals.

Q.50. Name the sources of dietary proteins.

- ***Animal sources***: Milk and dairy products, meat, fish, eggs, liver, pork, etc.
- ***Vegetable sources***: Cereals, pulses and legumes, peas, beans, nuts, etc.

Q.51. What is the proteolytic enzyme present in mouth?

No proteolytic enzyme is present in mouth (in saliva).

Q.52. Name the proteolytic enzymes present in gastric juice.

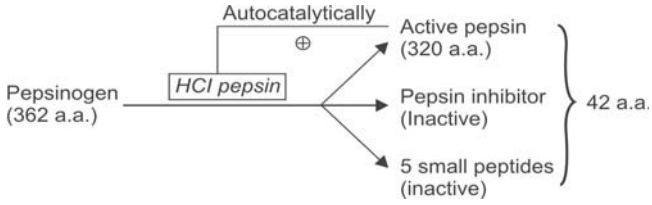
- *Pepsin*-most potent proteolytic enzyme.
- *Rennin.*
- *Gastriscin* and
- *Gelatinase*

Q.53. How pepsin is secreted?

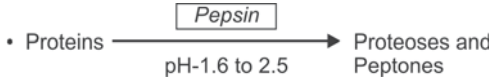
Pepsin is not secreted as such. It is secreted as inactive zymogen form ***pepsinogen*** which is synthesized by "chief cells" of stomach.

Q.54. How active pepsin is formed?

Inactive zymogen form "pepsinogen" (mol. wt. 42, 500) is hydrolyzed in the stomach with the help of HCl and subsequently by pepsin itself (autocatalytically) to form the "active pepsin" (mol wt. 34, 500)

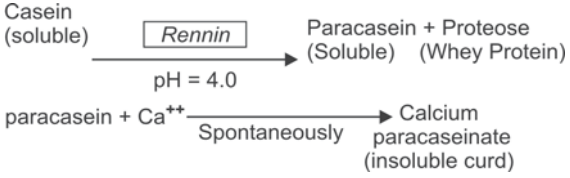


Q.55. What is the action of pepsin on dietary proteins?

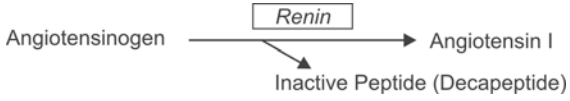


Q.56. Differentiate Rennin and Renin.

- ***Rennin*:** a proteolytic enzyme present in gastric juice of babies/infants. It is absent in adults. It acts on milk protein casein, a phosphoprotein.



- ***Renin:*** a proteolytic enzyme produced by JG cells of kidney which **produces "angiotensin I"**



Q.57. What other proteolytic enzymes can act on milk protein casein?

- ***Pepsin,*** and
- ***α-Chymotrypsin***

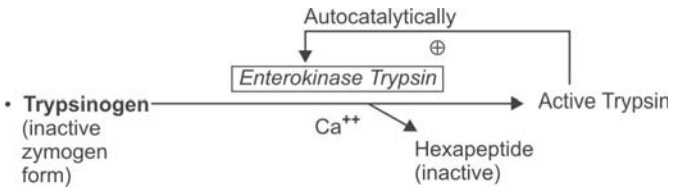
Q.58. Name the proteolytic enzymes present in pancreatic juice.

- ***Trypsin*—**most potent Proteolytic enzyme.
- ***Chymotrypsin***
- ***Carboxypeptidases A and B.***
- ***Elastases*** and
- ***Collagenases***

Q.59. How Trypsin is secreted?

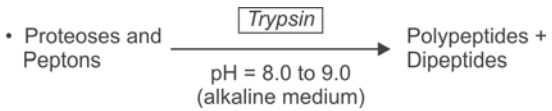
Trypsin is secreted as an inactive zymogen form ***trypsinogen*** which is activated to form active Trypsin.

Q.60. How active trypsin is formed?



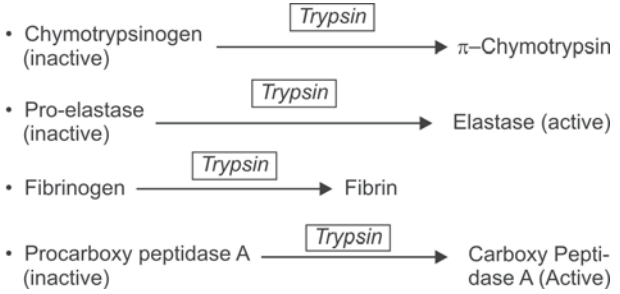
- ***Enterokinase,*** a glycoprotein enzyme present in intestinal juice

Q.61. What is the action of trypsin on proteins?



Q. 62. State the other proteolytic functions of trypsin.

Trypsin can bring about activation of other zymogen froms of proteolytic enzymes as follows:



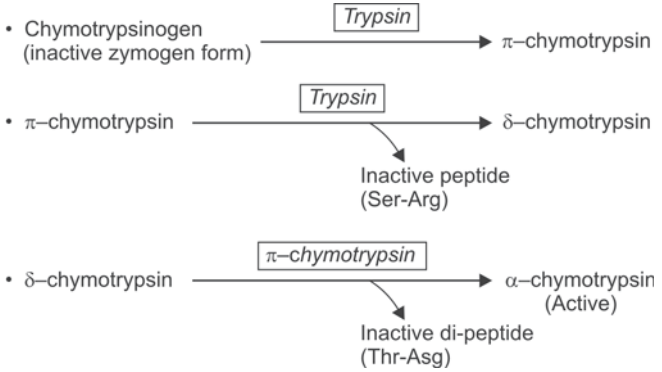
Q.63. Name some trypsin inhibitors.

- Raw egg white contains watersoluble mucoprotein, a very potent trypsin inhibitor.
- Human and bovine colostrum and raw soy beans also contain trypsin inhibitor.
- Trypsin inhibitors have been reported from lung tissue and blood recently.
- Chemical Di-isopropyl fluoro-phosphate (DFP) is also atrypsin inhibitor.

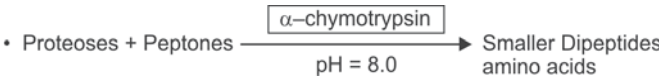
Q.64. What is the active form of chymotrypsin?

- **α**-chymotrypsin.

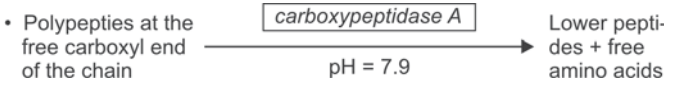
Q.65. How α-chymotrypsin is formed?



Q.66. What is the action of active α-chymotrysin on proteins?



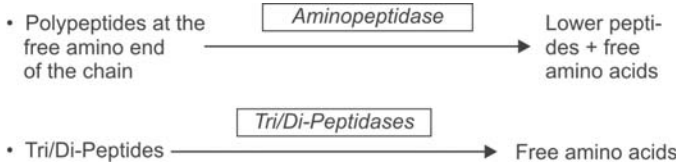
Q.67. What is the action of carboxy peptidase A?



Q.68. Name the proteolytic enzymes present in intestinal juice.

- ***Enterokinase*** activates trypsinogen to form active trypsin.
- ***Aminopeptidases*** e.g. leucine aminopeptidase (LAP)
- ***Prolidase*** and
- ***Tripeptidase****/and* ***Di-peptidase***

Q.69. What is the action of aminopeptidase and tri/dipeptidases on proteins?



Q.70. What is the action of prolidase?

- It is an exopeptidase.
- Can hydrolyze a proline peptide of collagen molecule and liberates proline.

Q.71. Name the proteolytic enzymes which are endo-pepdtidases.

- *Pepsin*
- Trypsin
- *α-chymotrypsin*

Q.72. Name the proteolytic enzymes which are exo-peptidases.

- *Amino peptidases*
- *Carboxy peptidases A and B*
- *Prolidase*

Q.73. Enumerate the functions of HCl in stomach.

- Activation and conversion of pepsinogen to "active" pepsin.

- Provides suitable acid pH (1.5 to 2.5) for the activity of pepsin.
- Helps swelling and denaturation of proteins for easy enzyme action.
- Hydrolysis of disaccharides like sucrose.
- Helps conversion of Fe^{+++} (ic) to Fe^{+++} (ous) for absorption.
- Stimulation of the ***secretion of secretin*** in the duodenum.

Q.74. What is the site of absorption of amino acids?
Amino acids are absorbed from ileum and distal jejunum. Oligopeptides like di- and tri-peptides are absorbed from duodenum and proximal jejunum.

Q.75. How L-amino acids are absorbed from intestine?
L-amino acids are absorbed rapidly from small intestine by "active" transport. Mechanism of absorption is similar to glucose. Requires sodium dependent ***carrier protein*** and energy is provided by ATP. Pyridoxal-P is probably also required for the process.

Q.76. How D-amino acids are absorbed?
D-amino acids are absorbed slowly. They are absorbed by simple passive diffusion which is dependant on concentration gradient.

Q.77. What is the role of glutathione in amino acid absorption?
Meister proposed that glutathione participates in an "active group translocation" of L-amino acids (execpt L-Proline) into the cells of small intestine, kidneys, brain, etc. He proposed a "cyclic pathway" in which glutathione is regenerated again. This is called as ***"γ-glutamyl cycle"***.

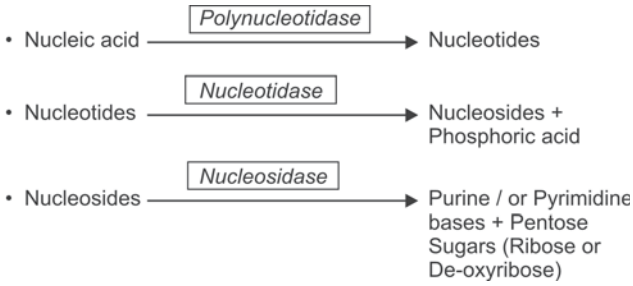
Q.78. State the salient features of γ-glutamyl cycle.

- Glutathione combines with L-amino acid in presence of Na^+ and the enzyme ***γ-glutamyl transferase*** and forms γ-glutamyl-amino acid complex and cysteinyl-glycine which breaks up into L-cysteine and glycine.
- "γ-glutamyl amino acid complex" in presence of the enzyme ***γ-glutamyl cyclo-transferase*** forms 5-oxo-proline and L-amino acid which is absorbed from the intestine.

- 5-oxo-proline in presence of the enzyme ***5-oxo-prolinase*** and ATP forms L-glutamic acid.
- L-Glutamic acid combines with L-cysteine and glycine to regenerate glutathione again to repeat the cycle again.

Q.79. How are dietary nucleic acids digested?

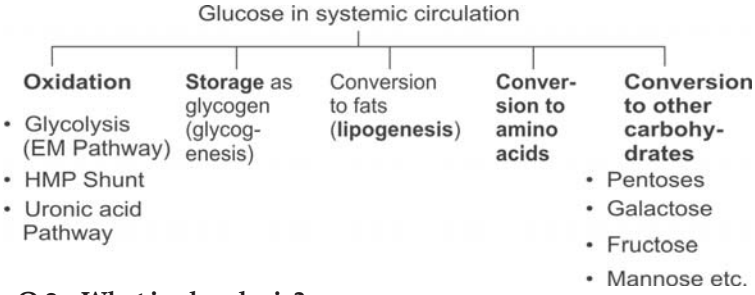
In the small intestine, following enzymatic actions take place:



CHAPTER

12 Metabolism of Carbohydrates

Q.1. What are the fates of glucose in the body?
Fates of Glucose in the body are shown below:



Q.2. What is glycolysis?
Oxidation of glucose or glycogen to pyruvate and lactate is called glycolysis. It is also called **Embden-Meyerhof pathway.** It occurs virtually in **all tissues**.

Q.3. What is the role of O_2 in glycolysis?
The glycolytic pathway is unique in the sense that it can occur in presence of O_2 if available ***("aerobic" phase)*** and it can function also in absence of O_2 ***("anaerobic" phase)***.

Q.4. In which part of the cell glycolysis occurs and where the enzymes are located?
Glycolysis occurs in cytosol and enzymes involved are cytosolic (extramitochondrial).

Q.5. State the biomedical importance of glycolysis.
- Provides energy.
- Cardiac muscle has poor glycolytic activity and poor survival under conditions of ischemia.

- Rate of glycolysis is very high in fast growing cancer cells. Enhanced glycolysis produces more pyruvic acid than TCA cycle can handle. Hence pyruvic acid accumulates and forms lactic acid producing ***local lactic acidosis*** which is congenial for certain cancer therapy.
- Inherited enzyme deficiencies like ***hexokinase*** and ***pyruvate kinase*** produce hemolytic anemia.

Q.6. Name the steps of glycolysis where ATP is consumed.

ATP is utilized for phosphorylations:

- For conversion of glucose → glucose-6-P
- For conversion of fructose-6-P → fructose 1,6-bi-P.

Body spends two ATP molecules (-2 ATP)

Q.7. Which enzymes are required for phosphorylations?

- For phosphorylation of glucose: ***Hexokinase***/and/or ***glucokinase*** enzymes.
- For phosphorylation of Fructose-6-P: ***Phosphofructokinase*** enzyme.

Q.8. State Five differences between hexokinase and Glucokinase?

Hexokinase	Glucokinase
• Found in all tissues	• Found only in Liver
• More stable	• More labile
• Non-specific, can phosphorylate any of the hexoses	• Specific only for glucose.
• Km is low, hence high affinity for glucose.	• Km is high, hence low affinity for glucose.
• **Main function** to make available glucose to tissues for oxidation at lower blood glucose level.	• **Main function** to clear glucose from blood after meals and at blood levels greater than 100 mg per dl.

Q.9. Which is the most energy-yielding step in glycolytic pathway?

Oxidation of glyceraldehyde-3-P, in presence of O_2, by the enzyme ***glyceraldehyde-3-P dehydrogenase*** which is NAD^+ dependent. 2 NADH when oxidized in ETC gives 6 ATP.

Q.10. What is substrate-level phosphorylation?

It is the formation of ATP at substrate level without participation of ETC.

Q.11. Give two examples of substrate level phosphorylation in glycolytic pathway.

- Conversion of 1, 3-biphosphoglycerate to → 3-phosphoglycerate by the enzyme ***Phosphoglycerate kinase***.
- Conversion of phosphoenol pyruvate to "Enol pyruvate" by the enzyme "Pyruvate kinase."

In the above two reactions ADP is converted to ATP at the substrate level (+ 4 ATP).

Q.12. What it the end product of glucose oxidation by glycolysis?

- Pyruvic acid.

Q.13. What is the further fate of pyruvic acid formed by glycolysis?

Further fate of PA **depends** on the **redox state** of the tissues:

- **If O_2 available,** PA is oxidatively decarboxylated to two carbon unit "acetyl CoA" ("active acetate") which is further metabobized in TCA cycle.
- **In absence of O_2,** PA is converted to lactic acid (LA) by reduction by the enzyme ***lactate dehydrogenase***. H is given by NADH + H^+ which is converted to NAD^+, so that anaerobic glycolysis continues: ***No ATP is formed.***

Q.14. Name the inhibitors of glycolysis.

- ***Iodoacetate and iodoacetic acid***: inhibits ***Glyceraldehyde-3-P-dehydrogenase***.
- ***Arsenite***: inhibits ***phosphoglycerate kinase*** indirectly and no ATP is formed at substrate level.
- ***Fluoride***: inhibits the enzyme ***enolase***.

Q.15. State clinical importance of use of fluoride.

Sodium fluoride is used alongwith K-oxalate in collection of blood for glucose estimation. **Sodium fluoride inhibits the enzyme *enolase* and prevents *in vitro* glycolysis**.

Q.16. Name the tissues which solely depends for energy from glycolysis.

- Red blood cells, and
- Brain and nervous tissue.

Q.17. How many ATPs are produced in glycolysis in presence of O_2 (aerobic phase)? Explain.

- ***Produces 8 (eight) ATP.***
- Details as follows:

a. Loss:	• Phosphorylation of Glucose	= – 1 ATP
	• Phosphorylation of Fructose-6-P	= – 1 ATP
		– 2 ATP
b. Gain:	• Oxisation of Glyceraldehyde-3-P	= + 6 ATP
	• Phosphoglycerate kinase reaction (Substrate level)	= + 2 ATP
	• Pyruvate kinase reaction (substrate level)	= + 2 ATP
		+ 10 ATP

* **Net gain** = 10 ATP – 2 ATP
= 8 ATP

Q.18. How many ATPs are produced in glycolysis in absence of O_2 (anaerobic phase)?

- In absence of O_2, NADH + H^+ produced by oxidation of glyceraldehyde -3-P, cannot be oxidized in ETC NADH is converted to NAD^+ in reduction of pyruvate to lactate. Hence 6 ATP is not produced.
- In **anaerobic phase**, per molecule of glucose oxidized,

4 ATP – 2 ATP = 2 ATP will only be produced

Q.19. What are the rate-limiting and key enzymes of glycolysis?

The rate limiting and key enzymes are:

- *Glucokinase/Hexokinase*
- *Phosphofructokinase*, and
- *Pyruvate kinase*.

Q.20. Enumerate the sources of pyruvic acid (PA) in the body.

- By glycolysis—principal source.
- Oxidation of LA → PA in presence of O_2.
- Deamination of alanine.
- Other pyruvic acid forming amino acids e.g. Metabolism of glycine, serine, cysteine/cystine, threonine.
- Decarboxylation of oxalo-acetic acid (OAA)
- From malic acid by malic enzyme.

Q.21. Enumerate the fate of pyruvic acid (PA) in the body.

- Oxidative decarboxylation of PA to form acetyl CoA in presence of O_2.
- Reduction of PA $\rightarrow$ LA in absence of O_2.
- Amination to form alanine.
- Conversion to glucose (gluconeogenesis)
- Conversion to malic acid.
- Formation of oxaloacetate (OAA) by "CO_2- fixation reaction".

Q.22. State the irreversible steps in glycolysis.

- Glucose-6-P $\rightarrow$ Glucose.
- Fructose-1, 6-bi-P $\rightarrow$ Fructose-6-P
- Phophoenol pyruvate $\rightarrow$ Enol-pyruvate.

Q.23. What is pasteur effect?

The phenomenon of inhibition of glycolysis by O_2 is called pasteur effect.

Q.24. What is Carb-tree effect?

This is opposite of Pasteur effect, which represents decreased respiration of cellular systems caused by high concentration of glucose.

Q.25. What is anaplerotic reaction or anaplerosis?

- A sudden influx of PA or acetyl CoA to the TCA cycle might seriously deplete the supplies of OAA required for the ***citrate synthase*** reaction.
- **Two reactions** that are auxiliary to TCA cycle operate to prevent this situation. These are called ***anaplerotic reactions*** (or "filling-up" reactions), and the phenomenon is called ***anaplerosis.***

Q.26. Name the two anaplerotic reactions.

- Conversion of PA to OAA by ***CO_2-fixation reaction*** by the enzyme ***pyruvate carboxylase*** which requires biotin, ATP, Mg^{++} and acetyl CoA. ***Acetyl CoA acts as a +ve modifier***; it helps the enzyme to maintain "active" conformation.
- Conversion of PA to OAA through malic acid formation.

Q.27. Name the inhibitor of *lactate dehydrogenase* enzyme (LDH).

- ***Oxamate:*** It competitively inhibits ***lactate dehydrogenase*** **(LDH)** and prevent re-oxidation of NADH.

Q.28. What is Rapaport-Leubering cycle or shunt (RLC or RLS)?

- RLC/or RLS is a ***diversion in glycolytic pathway in Red blood cells***. Conversion of 1, 3-BPG to 3 PG does not occur and ATP is not formed at substratre level. **It forms 2, 3-BPG**.
- It is calculated to deplete and waste the energy needed by the RB cells.

Q.29. What are the functions of 2, 3 BPG?

- ***Role in Hb:*** In adults Hb-A, the concentration of 2, 3-BPG is high↑, affinity of O_2 is less↓ and unloading/ dissociation of O_2 is more↑. In foetal Hb (Hb-F): reverse occurs.
- ***Role in hypoxia:*** Hypoxia favors an increase in 2,3-BPG level↑ in RB cells, thus enhancing unloading of O_2 to tissues.

Q.30. What is the enzyme involved in oxidative decarboxylation of PA to acetyl CoA?

- Enzyme required is ***pyruvate dehydrogenase complex***, a multienzyme complex which can exist as "active" and "inactive" forms.
- The enzyme complex consists of:
  - 29 molecules of pyruvate dehydrogenase (PD)
    + 8 molecules of FP-containing dihydrolipoyl dehydrogenase, and
    + 1 molecule of dihydrolipoyl transacetylase.

Q.31. Name the coenzymes/cofactors required for PDH complex.

At least **six conenzymes/cofactors** are required by PDH complex. They are:

- Thiamine pyrophoshate (TPP)
- Lipoic acid
- CoA-SH
- FAD
- NAD^+ and
- Mg^{++}

Q.32. What is the active form of PDH?

"Active" forms of PDH is the dephosphorylated form. ***Insulin stimulates phosphatase enzyme and converts* 'inactive'** → to 'active' form by dephosphorylation.

Q.33. What is the inactive form of the PDH enzyme?
"Inactive" form is the Phosphorylated form catalyzed by the enzyme ***"PDH kinase"***. Following favors the formation of "inactive" form:
- Rise in ATP/ADP ratio↑
- Rise in NADH/NAD^+ ratio↑
- Acetyl CoA/CoA. SH ratio↑
- Increased cyclic AMP level in cells↑

Q.34. Pyruvic acid is formed in cytosol by glycolysis but oxidative decarboxylation takes place in mitochondrion. Pyruvic acid is impermeable to mitochondrial membrane. How it is done?
Pyruvic acid formed in cytosol is not permeable to mitochondrial membrane, it is transported to mitochondrion by a specific ***transport*** protein.

Q.35. What is TCA cycle?
TCA cycle is the final common pathway for metabolism of carbohydrates, lipids, and proteins (III Phase of metabolism). It is a cyclic process, and involves a sequence of compounds interrelated by oxidation-reduction and other reactions which finally produces CO_2 and H_2O.

Q.36. Why TCA cycle is also called Krebs cycle or citric acid cycle?
- **Krebs** first discovered this cycle hence also called as "Krebs cycle".
- First compound formed by combination of OAA and acetyl CoA is citric acid, hence called as citric acid cycle.

Q.37. Where and under what conditions does TCA cycle function?
- Mitrochondria of the cells.
- Under aerobic conditions-requires presence of O_2.

Q.38. Can TCA cycle function in absence of O_2?
TCA cycle cannot function in absence of O_2.

Q.39. Where are the enzymes of TCA cycle located?
Enzymes of TCA cycle are located in mitochondrial matrix, either free or attached to the inner surface of the inner mitochondrial membrane, which facilitates the transfer of reducing equivalents to the adjacent enzymes of ETC.

Q.40. Succinyl CoA is an intermediate in TCA cycle. How it is formed?

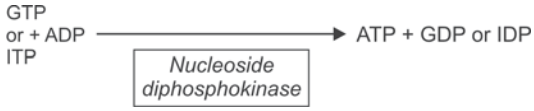
Succinyl CoA is formed by oxidative decarboxylation of α-oxoglutarate by ***"α-oxo-glutarate dehydrogenase complex"*** which requires TPP, Lipoic acid, CoA-SH, FAD, NAD^+ and Mg^{++} ions as coenzymes/cofactors.

Q.41. State the biomedical importance of TCA cycle.

- Final common pathway for metabolism of carbohydrates, lipids and proteins through acetyl CoA.
- One acetyl CoA is oxidized to CO_2 and H_2O giving out energy (12 ATPs/mole of acetyl CoA oxidized).
- Intermediates of TCA cycle play major role information of glucose, synthesis of heme, fatty acid, cholesterol, steroid and non-essential a.a.

Q.42. Give an example of substrate level phosphorylation in TCA cycle.

Conversion of succinyl CoA → succinic acid produces ATP at substrate level without participation of ETC. The reaction requires GTP or ITP.



Q.43. State the inhibitors of TCA cycle.

- ***Fluoroacetate*:** Inhibitor of ***"aconitase"*** and allows citrate to accumulate.
- ***Arsentie:*** inhibits ***"α-oxoglutarate dehydrogenase"*** enzyme complex and allows accumulation of α-oxoglutarate (α-Keto glutarate)
- ***Malonate/OAA:*** inhibits ***succinate dehydrogenase*** by competitive inhibition and allows accumulation of succinate.

Q.44. What is the importance of OAA in TCA cycle?

- It is required to start the cycle
- A small quantity is necessary.
- At the end of the cycle, OAA is regenerated by oxidation of malate by ***matate dehydrogenase.***
- Thus OAA acts ***catalytically*** to restart the cycle again.

Q.45. What will happen to TCA cycle if OAA is not available?
In absence of OAA, TCA cycle will not operate. Acetyl CoA will accumulate and will be diverted to form ketone bodies and biosyntheis of FA and cholesterol.

Q.46. Why TCA cycle is said to be amphibolic in nature?
TCA cycle has **dual role:**

- ***Catalytic role:*** The acetyl CoA produced by metabolism of carbohydrates, lipids and proteins are completely oxidized to produce CO_2, H_2O and energy.
- ***Anabolic role*** (Synthetic role): Intermediates of TCA cycle are utilized for synthesis of various biologically important compounds in the body e.g.
- Synthesis of non-essential a.a.
- Formation of glucose (gluconeogenesis)
- FA synthesis
- Synthesis of cholesterol and steroids.
- Heme synthesis.

Q.47. State the over-all bioenergetics in complete oxidation of glucose/glycogen in glycolysis-cum-TCA cycle in presence of O_2.

A. *Glycolysis*	*ATP yield per hexose unit*
• Glycogen → F-1, 6-bi-P	–1 ATP
• Glucose → F-1, 6-bi-P	–2 ATP
• Glyceraldehyde-3-P Dehydrogenase (2 NADH → 2 NAD^+)	+6 ATP
• Substrate level phosphorylation:	
– Phosphoglycerate kinase	+2 ATP
– Pyruvate kinase	+2 ATP
Net gain in glycolysis:	
• For Glucose =	+8 ATP
• For Glycogen =	+9 ATP
B. ***Oxidative decarboxylation of P.A.:***	
• PDH complex (2 NADH → 2NAD^+)	+6 ATP
C. ***TCA cycle:***	
• Isocitrate dehydrogenase (2 NADH → 2 NAD^+)	+6 ATP
• α-oxoglutarate dehydrogenase (2 NADH → 2 NAD^+)	+6 ATP
• Substrate level phosphorylation: Succinate thiokinase 2 (GTP or ITP) → 2 ATP	+2 ATP

- Succinate dehydrogenase
  2 FAD. $H_2 \rightarrow$ FAD + 4 ATP.
- Malate dehydrogenase
  2 NADH $\rightarrow$ 2 NAD^+ + 6 ATP

Total = + 24 ATP

Total energetics:
- Per mole of Glucose = 24 + 6 + 8 ATP = 38 ATP
- Per mole of Glycogen = 24 + 6 + 9 ATP = 39 ATP

Note: Under anaerobic conditions (in absence of O_2):
- Glucose = + 2 ATP
- Glycogen = + 3 ATP

Q.48. State the efficiency of complete oxidation of glucose.
- One mole of glucose after complete oxidation produces = 38 ATP
- Total energy captured in ATP per mole of glucose oxidized=
  7600 × 38 = 2,88,800 calories.
- Oxidation of one molecule of glucose "in vitro" produces = 6,86,000 calories

$$\therefore \text{ Hence efficiency} = \frac{2,88,800}{6,86,000} \quad 100 = 42\%$$

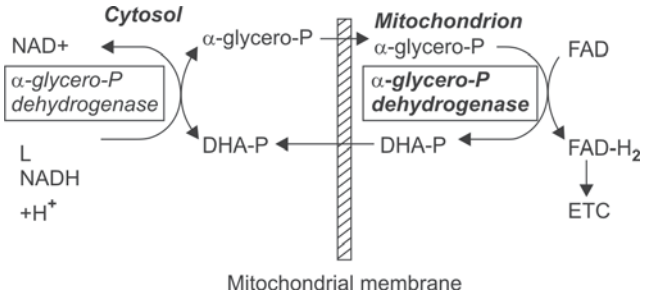
Q.49. Acetyl CoA is formed inside mitochondria but the fatty acid synthesis ('de novo') from acetyl CoA occurs in cytosol. Acetyl CoA is not permeable to mitochondrial membrane. How acetyl CoA made available in cytosol?
- Acetyl CoA is transported out in the form of citrate, an intermediate of TCA cycle, to cytosol, as citrate is readily permeable to mitochondrial membrane.
- In the cytosol, citrate is cleaved by the enzyme ***citrate cleavage enzyme*** **(ATP-citrate lyase)** to acetyl CoA and OAA, So that acetyl CoA can be used for FA synthesis.

Q.50. In glycolysis, NADH is produced in cytosol, but it is oxidized in ETC in mitochondria to produce ATP. NADH is not permeable to mitochondrial membrane. Explain how it is achieved?
- NADH produced in cytosol by glycolysis transfer the reducing equivalents through the mitochondrial

membrane via substrate pairs linked by suitable dehydrogenases by "shuttle systems". Two such shuttle systems are:

- Glycerophosphate shuttle.
- Malate shuttle.

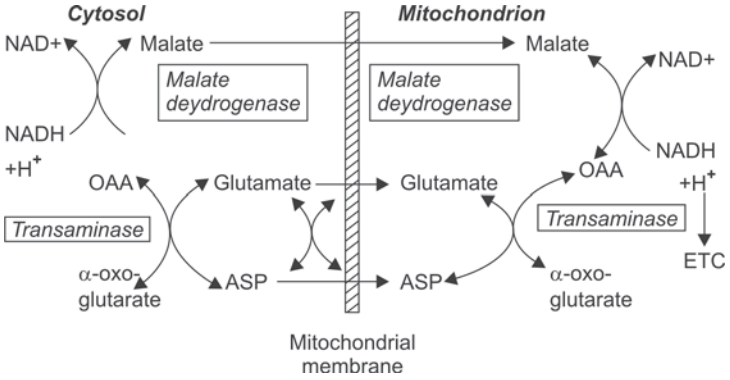
Q.51. Show schematically glycerophosphate shuttle.



***Note*: α-glycero-P-dehydrogenase in mitochondrion is Fp-dependant.**

Hence produces 2 ATP per mole of glucose oxidized. Hence in this 36 ATP is produced per mole of glucose oxidized.

Q.52. Show schematically malate shuttle.



Note:

- ***Malate shuttle is commonly used by the body.***
- ***Use of malate shuttle forms 38 ATP.***

Q.53. Enumerate the sources of succinyl-CoA ("active" succinate) in the body.

- Oxidative decarboxylation of α-oxoglutarate.
- Formed from propionyl CoA via methyl-malonyl CoA.
- Activation of succinic acid by thiokinase, ATP and Mg^{++}.
- Formed from valine through L-methyl-malonyl CoA.

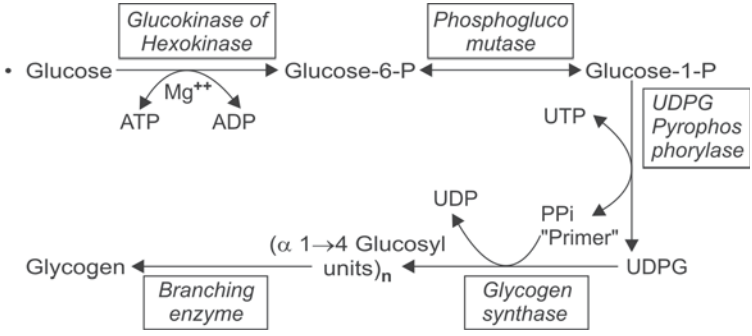
Q.54. Enumerate the fate of succinyl CoA in the body.

- Heme synthesis.
- Activation of acetoacetate by the enzyme ***"thiophorase" ("CoA transferase")***
- Formation of succinate in TCA cycle by ***succinate thiokinase*** (Substrate level phosphorylation).

Q.55. What is glycogenesis?

It is the formation of glycogen from glucose in the body.

Q.56. Show schematically the steps of glycogenesis.



Q.57. What happens to liberated UDP by glycogen synthase action?

UDP is converted to UTP again by the enzyme ***nucleoside diphosphokinase*** and is reutilized again for UDPG formation.

Q.58. What is the primer? How the first primer formed?

- The ***first "primer"*** originally supposed to be synthesized on a protein backbone which is a process similar to synthesis of other glycoproteins.
- ***A Pre-existing glycogen molecule or "primer" is a*** must, so that UDPG can add the glucose molecule to the outer end of a chain.

Q.59. How much ATP is spent by the body to add one glucose unit to outerchain?

- In each addition of glucose unit, 2 ATP molecules are expended by the body:
  - One ATP is utilized in phosphorylation of glucose → Glucose-6-P.
  - Another ATP is used to convert UDP to UTP again.

Q.60. What is key and ratelimiting enzyme in glycogenesis?

- Glycogen synthase enzyme.

Q.61. What are the active and inactive forms of the enzyme glycogen synthase?

- Glycogen synthase enzyme occurs as 'active' GS 'a' or 'inactive' GS 'b' forms and both are interconvertible.
- GS 'a' → GS 'b' by **phosphorylation** which is modulated by ***cyclic-AMP dependent protein kinase*** and glycogenesis is stopped.
- GS 'b' → GS 'a' is formed ***by dephosphorylation*** catalyzed by the enzyme ***"protein phosphatase-1"*** when glycogenesis starts.

Q.62. What is the role of branching enzyme?

Branching enzyme establishes a branch point by $\alpha 1 \rightarrow 6$ linkage in the growing glycogen molecule. When a particular chain has lengthened to minimum of 11 glucose residues by addition of glucose units by ***glycogen synthase*** enzyme, the branching enzyme comes to action and transfers a part of $\alpha\ 1 \rightarrow 4$ chain, ***minimum of 6 glucose residues to a*** neighboring chain to form $\alpha\ 1 \rightarrow 6$ linkage ***establishing a branch point.***

Q.63. What is UDPG? What are its functions?

- UDPG is uridine diphosphate glucose. It is an intermediate in glycogenesis. It is activated glucose which adds one glucose unit to a chain in 'primer' molecule, by $\alpha\ 1 \rightarrow 4$ glycosidic linkage.
- **Other functions:**
  - It is formed as an intermediate in uronic acid pathway required for formation of D-glucuronic acid.
  - It is also required for synthesis of lactose from galactose in lactating mammary gland.

Q.64. State the factors that bring about stimulation and inhibition of glycogenesis.

- ***Stimulators:***
  - Insulin
  - Glucocorticoids
  - High concentration of glucose.
- ***Inhibitors:***
- Increase concentration of Glycogen (by "feedback" inhibition).
- Increased cyclic AMP ↑ in the cells, which can be brought about by hormones viz.,
  - Epinephrine,
  - Norepinephrine,
  - Glucagon,
  - Thyroid hormones.

Q.65. How insulin increases glycogenesis?

Insulin directly stimulates the enzyme ***protein phosphatase-1*** thus brings about dephosphorylation of *glycogen synthase* and forms "active" glycogen synthase, GS 'a', and increases glycogenesis.

Q.66. How does increased cyclic AMP level in cells inhibit glycogenesis?

- Increased cyclic AMP in the cells converts *inactive* ***protein kinase*** (C_2R_2) → *active protein kinase* (C_2)
- Active protein kinase (C_2) has following two effects:
  - Brings about **phosphorylation of 'GS'** enzyme with the help of ATP and thus GS 'a' → GS 'b' inhibiting glycogenesis.
  - Also converts a protein factor ***inhibitor 1*** (inactive) and phosphorylates it to form "active inhibitor 1-P", which in turn inhibits *Protein Phosphatase-1*, so that conversion of inactive GS 'b' → to active GS 'a' does not occur thus inhibiting glycogenesis.

Q.67. What is the clinical importance of gycogenesis with K^+ influx?

- ***In treatment of hyperkalaemia:*** Insulin and Glucose is administered in hyperkalaemia. By this glycogenesis is enhanced so that K^+ goes into the cells which reduces hyperkalaemia.

- ***Treatment of Diabetic Ketoacidosis with insulin and Glucose:*** Glycogenesis is enhanced so that K^+ goes into the cells so danger of "hypokalaemia" to precipitate.
  Note: The patient should be monitored for potassium level in blood.

Q.68. What is glycogenolysis?

Breakdown of glycogen to glucose is called as glycogenolysis.

Q.69. Which is the key and rate limiting enzyme in glycogenolysis?

- ***Phosphorylase*** enzyme.

Q.70. What are the active and inactive forms of liver phosphorylase?

- Active phosphorylase is the **phosphorylated form** "active" phosphophosphorylase 'a'.
- Inactive phosphorylase is the **dephosphorylated form** "inactive" dephosphophosphorylase 'b'.

Q.71. What are the active and inactive forms of muscle phosphorylase?

- Active muscle phosphorylase 'a' is a **tetramer**, with molecular wt = 500,000 and ***contains 4 mols of pyridoxal-P*** per molecule of the enzyme.
- Inactive muscle phosphorylase 'b' is a **"dimer"**, with molecular wt = 250,000 and ***contains 2 mols of pyridoxal-p*** per molecule of the enzyme.

Q.72. What are the basic differences between liver and muscle phosphorylase?

- There is no cleavage of structure with liver phosphorylase as compared to muscle phosphorylase.
- Four molecules of pyridoxal-P is required for activity of muscle phosphorylase, not so with liver phosphorylase.
- Muscle phosphorylase is not affected by glucagon ***(no receptor on muscle).***

Q.73. What are the effects of increased cyclic AMP level in cells on glycogenolysis?

- Increased cyclic AMP level in cells activates ***protein kinase***. Active protein kinase (C_2) converts "inactive" phorphorylase kinase 'b' to "active" phosphorylase kinase 'a' by Phosphorylation.

- Active phosphorylase kinase 'a' with the help of ATP ***phosphorylates*** "inactive" dephosphophosphorylase 'b' and converts to "active" phosphophosphorylase 'a' which brings about glycogen breakdown.

Q.74. What is the role of protein factor inhibitor-1?
Active protein kinase (C_2) at the same time Phosphorylates the protein factor "inhibitor-1" (inactive) and converts to inhibitor-1-P (active) which inhibits ***protein phosphatase-1*** which inturn inhibits conversion of phosphorylase kinase 'a'→ 'b'. Phosphorylase kinase 'a' inturn phosphorylates dephosphophosphorylase 'b' to active phosphophosphorylase 'a' which brings about glycogen breakdown.

Q.75. Name the hormones which bring about glycogenolysis through increased cyclic AMP levels in cells.
- ***Catecholamines***-Epinephrine and Nor-epinephrine
- ***Glucagon***, and
- ***Thyroid hormones***.

Q.76. What is the product of phosphorylase activity on glycogen molecule?
Active phosphorylase in presence of inorganic Pi brings about **phosphorolytic cleavage** of α 1→4 glycosidic bond from outermost chain of glycogen molecule and ***glucose is released as glucose-1-P*** and ***not free glucose***.

Q.77. What is the action of debranching enzyme?
Debranching enzyme acts on a 1→6 linkage at the branch point and releases one molecule of ***free glucose***.

Q.78. What is the fate of glucose-1-P released by Phosphorylase activity.
- Glucose-1-P is converted to glucose-6-P by the enzyme **phosphoglucomutase**.
- In liver and kidney (**NOT in muscle**), glucose-6-P is acted upon by the enzyme ***glucose-6-phosphatase*** and free glucose is formed.

Q.79. Why glucose is not formed in muscle from glycogen breakdown?
In muscle, ***glucose-6-phosphatase*** enzyme is ***absent,*** hence glucose-6-P enters the glycolytic cycle and forms pyruvates and lactates.

Q.80. How does glucagon action differ from catecholamines in glycogenolysis?

- Catecholamines cause breakdown of liver as well as muscle glycogen.
- But glucagon breaks down only liver glycogen and ***not muscle glycogen (as receptor for glucagon is not present in muscle)***.

Q.81. What is calmodulin?

Calmodulin is a Ca^{++} -dependent regulatory protein which is specific for calcium. It is a flexible protein, with 4 binding sites for Ca distributed in 4 domains.

Q.82. What are glycogen storage diseases (GSDs)?

These are a group of **inherited disorders** associated with glycogen metabolism, familial in incidence, and ***characterized by deposition of normal or abnormal type and quantity of glycogen in various tissues***.

Q.83. Name the six classical types of GSDs and indicate the enzyme deficiencies.

Type/Name	*Enzyme deficiency*
• **Type I-von Gierke disease**	Glucose-6-Pase
• **Type II-Pompe's disease**	Acid maltase
• **Type III-Forbe's disease**	Debranching enzyme (Limit dextrinosis)
• **Type IV-Andersen's disease**	Branching enzyme. (Amylopectinosis)
• **Type V-McArdle's disease**	Muscle phosphorylase
• **Type VI-Her's disease**	Liver phosphorylase

Q.84. State the salient clinical features with biochemical correlation found in von Gierke disease (Type I-GSD).

- As ***glucose-6-phosphatase*** enzyme is absent, glucose is not formed from breakdown of Liver glycogen resulting to ***hypoglycaemia***.
- ***Fat is oxidized to derive energy***. More acetyl CoA- is formed which is diverted to ***ketone bodies*** formation and **cholesterol bio-synthesis**↑ which can form **xanthomas**.
- Persistent hypoglycaemia inhibits insulin↓ secretion, which in turn ***inhibits protein synthesis***↓ causing ***stunted growth*** and ***dwarfism***.

- Hypoglycaemia also stimulates secretion of cetecholamines↑ and glucagon↑ which increase glycogenolysis in muscles producing lactic acid ***(lactic acidosis).***
- Increased lactic acid in blood competes with uric acid excretion by kidneys leading to **increased blood uric acid**↑ level producing ***Gout***.
- Normal type of glycogen accumulates in large quantities in liver, kidney and intestinal mucosal cells inhibiting their functions.

Q.85. What is hexose monophosphate pathway or shunt?
It is an alternate pathway of glucose oxidation which takes place in certain special tissues to serve certain special functions.

Q.86. How does HMP shunt differ from EM Pathway?
Essential differentiating points are:

HMP Shunt	*EM Pathway*
• Occurs in certain special tissues only	• Occurs in all tissues
• A multicyclic Process	• Not so
• $NADP^+$ acts as H-acceptor	• NAD^+ acts as H-acceptor
• ATP is not produced Not meant for energy.	• Energy producing pathway ATP is produced.
• CO_2 is formed	• CO_2 is never formed.

Q.87. In which part of the cell HMP shunt operates?
- In the cytosol.

Q.88. Name some of the tissues in which HMP shunt operates.
HMP shunt operates in certain special tissues to serve specific functions e.g. liver, adipose tissue, RB cells, lens of the eye, adrenal cortex, gonads, cornea, and lactating mammary gland.

Q.89. Why HMP shunt is called as a multicyclic process and what are the products.
It is called a multicyclic process, as 3 mols of glucose-6-P enter the cycle, producing 3 mols of CO_2↑, 6 mols of NADHP and 3 mols of 5-C residues which rearrange to give 2 mols of glucose-6-P (re-enters the cycle) and one mol of glyceraldehyde-3-P.

Q.90. Which is the key and rate-limiting enzyme in HMP shunt?

- Glucose-6-phosphate dehydrogenase (G-6-PD).

Q.91. What is the role of thiamine in HMP shunt?

TPP the active form of thiamine is required as a coenzyme with ***"transketolase"*** enzyme for two transketolation reactions.

Q.92. Name the tetrose sugar formed in HMP shunt.

- Erythrose-4-P.

Q.93. How does $NADP^+$/NADPH ratio regulates HMP shunt?

- If **the ratio is high**, it enhances the rate-limiting reaction and shunt pathway.
- If **the ratio is low**, inhibits G-6-PD and 6-Phospho-gluconate dehydrogenase enzymes and decreases shunt pathway.

Q.94. State the hormones that regulate HMP shunt.

Insulin: It induces the synthesis of dehydrogenase enzymes and enhances the activity of the pathway.

Thyroid hormones: Stimulates the activity of G-6-PD enzyme of the pathway.

Q.95. State the metabolic role of HMP shunt.

- ***Does not produce energy***.
- ***Provides NADPH:*** required for various reductive synthesis.
- ***Provides pentoses:*** required for nucleic acid synthesis.
- ***Role in lens metabolism:*** Provides reduced glutathione (G-SH) necessary for maintenance of lens Proteins.
- ***Role in RB Cells:*** Provides reduced Glutathione (G-SH) necessary for RB cells membrane integrity.
- ***CO_2 is produced*** which is used in CO_2 fixation reaction.

Q.96. Mention some metabolic reactions where NADPH is used for reductive processes.

NADPH is used in a number of metabolic processes where it ***provides hydrogen*** for ***reductive synthesis*** e.g.

- Extramitochondrial *de novo* fatty acid synthesis.
- In synthesis of cholesterol.
- In synthesis of steroids.
- In conversion of oxidized glutathione to reduced glutathione.

- For conversion of phenylalanine to tyrosine.
- For conversion of methaemoglobin→Hb.
- In synthesis of sphingolipids.
- In "microsomal" chain elongation of FA.
- In uronic acid pathway.

Q.97. Muscle tissues contain very small amounts of the dehydrogenases enzymes but still skeletal muscle is capable of synthesizing pentose sugars. How?

Probably this is achieved by ***reversal of shunt Pathway*** utilizing fructose-6-P, glyceraldehyde-3-P and the enzymes ***transketolase*** and ***transaldolase*** (by non-oxdative Pathway).

Q.98. What happens to persons having inherited deficiency of G-6-PD?

Red blood cells get hemolyzed when the susceptible individuals with inherited G-6-PD deficiency are subjected to **antimalarial drugs** like primaquine or pamaquine and **other oxidant drugs** like sulphonamides, Sulphones, nitrofurans and analgesics producing hemolytic anemia.

Q.99. What is favism?

Acute hemolytic anemia with hemoglobinuria may occur in G-6-PD deficient persons who are sensitive to fava beans (*Vicia fava*) either on ingestion of uncooked or lightly cooked beans or inhalation of pollens.

Q.100. What is the relation of G-6-PD deficiency with *Plasmodium falciparum* infection and with color blindness.

- G-6-PD deficiency found to ***confer some protection against Plasmodium falciparum infection***. It may lessen the severity of malarial infections in young children and infants.
- The disease shows a close linkage with color blindness.

Q.101. What is Wernicke-Korsakoff syndrome?

- A genetically variant form of ***transketolase*** occurs which cannot bind TPP thus affecting transketolation reaction.
- The patient shows severe neuro-psychiatric symptoms characterized by lesions and hemorrhages near III ventricle.

- The patient shows deranged mental function, loss of memory, depression, disorientation and mental confusions.

Q.102. What is gluconeogenesis (or neoglucogenesis)?

The formation of glucose or glycogen **from noncarbohydrate sources** is called gluconeogenesis.

Q.103. Name the substrates used for gluconeogenesis?

- Glucogenic amino acids.
- Lactates and pyruvates.
- Glycerol obtained from lipolysis of fats.
- Propionyl-CoA.

Q.104. Why gluconeogenesis is necessary for the body?

- To maintain ***basal level*** of glucose in carbohydrate deprivation/or when carbohydrates are not available in sufficient amounts for nutrition to brain/RB cells.
- To clear the blood from certain metabolic products e.g.
  - Lactic acid produced from muscles and RB cells.
  - Glycerol produced from lipolysis in adipose tissues.

Q.105. Name the rate-limiting enzymes of gluconeogenesis.

- **Four rate limiting enzymes** are:
  - Pyruvate carboxylase (mitochondrial).
  - Phosphoenol pyruvate carboxykinase (cytosol).
  - Fructose-1, 6-biphosphatase (cytosol).
  - Glucose-6-phosphatase (cytosol).

Q.106. Name the irreversible steps in gluconeogenesis and enzymes used to circumvent the irreversible steps.

Irreversible steps (Energy barrier)	*Enzymes used to circumvent*
• Pyruvate → phosphoenol pyruvate.	• Pyruvate carboxylase (Mitochondrial)—conversion of PA to OAA by CO_2 fixation reaction. • Phosphoenol pyruvate carboxykinase (cytosol) converts OAA to phosphoenol pyruvate.
• Fructose-1-5-bi-P → fructose-6-P	• Fructose-1, 6-bi-phosphatase (cytosol).
• Glucose-6-P → glucose.	• Glucose-6-phosphatase (cytosol).

Q.107. Name the tissues where gluconeogenesis occur and name one disease and one condition in which gluconeogenesis is significantly enhanced.

- Principally occurs in **liver (85%)** and **kidney (15%)**.
- Uncontrolled diabetes mellitus and prolonged starvation.

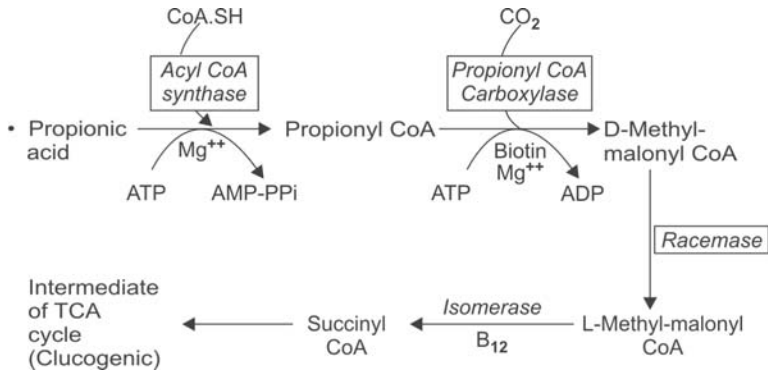
Q.108. State how glucose is formed from glycerol.

- Glycerol is phosphorylated in presence of the enzyme ***"Glycerokinase"*** and ATP to form ***α-Glycero P***.
- α-Glycero-P is converted to Di-OH-acetone-P by dehydrogenase and NAD^+.
- Di-OH-acetone-P and glyceraldehyde-3-P forms fructose, 1-6, bi phosphate which by reversal of glycolysis form glucose.

Q.109. Mention the sources of propionyl-CoA in humans.

- From catabolism of L-methionine.
- Catabolism of Isoleucine.
- Oxidation of odd-chain F.A.
- Synthesis of bile acids.
- Non-oxidative deamination of L-threonine.

Q.110. Show schematically how glucose is formed from propionic acid.

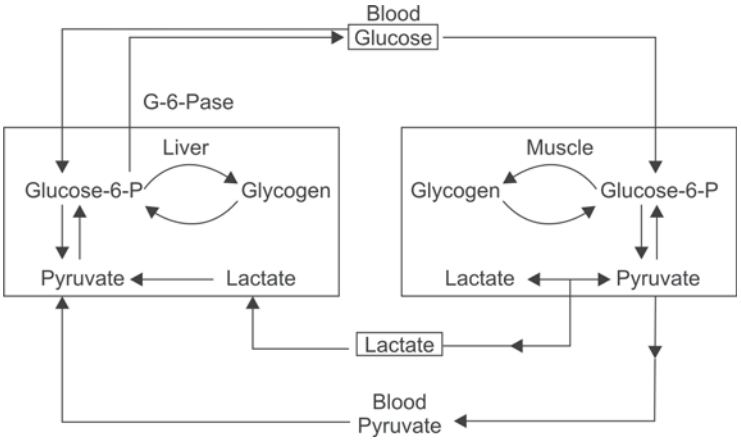


Q.111. What is the role of hormones in gluconeogenesis?

- **Glucagon:** increases gluconeogenesis from lactic acid (LA) and amino acids.

- **Gluco-corticoids:** stimulate gluconeogenesis by increasing protein catabolism in the peripheral tissues and increasing hepatic uptake of amino acids. It increases the activity of transaminases and other key enzymes concerned in gluconeogenesis.

Q.112. Show schematically "Cori cycle" (lactic acid cycle).



Q.113. What is lactate-propanediol pathway?

- Lactic acid (LA) is first reduced to form lactaldehyde.
- Lactaldehyde is converted to Acetol.
- Acetol in turn is reduced in presence of NADH to form 1, 2-propanediol which is glucogenic.

Q.114. What is uronic acid pathway?

It is an alternative pathway for glucose oxidation. This pathway also does not produce energy.

Q.115. What is the metabolic significance of uronic acid pathway.

- **Formation of D-glucuronic acid** which is used for detoxication.
- Produces vitamin C in lower animals, But cannot synthesize vitamine C in man, and other primates including G. pigs due to absence of enzymatic machinery.
- Inherited deficiency of the enzyme ***"L-xylitol dehydrogenase"*** produces essential pentosuria.
- Excessive xylitol or parenteral administration of xylitol may lead to oxalosis.

Q.116. What are the metabolic functions of D-Glucuronic acid produced in uronic acid pathway?

- ***Detoxication:*** Detoxicates drugs, chemicals, antibiotics, hormones, etc. convert them to corresponding soluble glucuronides which are excreted.

  Examples:
- ***Aromatic acid*** like benzoic acid.
- ***Phenol*** and ***secondary/tertiary aliphatic alchohols***.
- ***Drugs*** and ***other xenobiotics***—they are first hydroxylated by mono-oxygenase cyt.P_{450} system and then conjugated with-D-glucuronic acid.
- ***Antibiotics*** like chloramphenicol.
- ***Steroid hormones*** and thyroid hormones.
- ***Bilepigments:*** unconjugated bilirubin is conjugated with UDP-glucuronic acid and converted to soluble mono and diglucuronides.
- Synthesis of heteroglycans containing D-glucuronic acid e.g. Heparin, Hyaluronic-acid, chondroitin SO_4.

Q.117. Name some drugs which increase the formation of D-Glucuronic acid by uronic acid pathway.

- Barbiturates
- Amino Pyrine and
- Antipyrine

Q.118. Name one principal dietary source of galactose.

- Milk sugar or Lactose.

Q.119. What happens to the absorbed dietary galactose?

- Galactose is carried to liver by portal blood where it is mostly, converted to D-Glucose.
- Galactose used by tissues is synthesized from glucose.

Q.120. Enumerate the metabolic importance of galactose.

- Galactose is required in lactating mammary gland for ***synthesis of lactose*** of breast milk.
- Galactose is utilized in ***brain*** and ***nerve tissues*** for ***synthesis of galactolipids***.
- Galactose is required for ***synthesis of chondromucoids*** and ***mucoproteins***.
- Inherited deficiency of enzymes in pathway of galactose metabolism produces inherited disorder ***galactosemia***.

Q.121. Name the specific enzyme which phosphorylates galactose and mention the product.

- **Enzyme:** ***Galactokinase***
- **Product:** Galactose-1-P.

Q.122. How the milk sugar lactose is synthesized in lactating mammary gland?

- UDP-glucose is converted to UDP-galactose by the enzyme ***epimerase.***
- UDP-galactose is also formed from D-galactose.
- UDP-galactose so formed condenses with one molecule of glucose to form lactose. The reaction is catalyzed by the enzyme ***lactose synthetase"*** (also called ***galactosyl transferase.***

Q.123. How biosynthesis of lactose is regulated?

- ***Lactose synthetase*** enzyme has **two subunits:**
  - Catalytic unit called *galactosyl transferase.*
  - Modifier unit called **α**-lactalbumin.
- In the lactating mammary gland, binding of **α**-lactalbumin, the modifier unit to the catalytic subunit brings about the transfer of "galactosyl moiety" to D-glucose to form lactose.
- **α**-lactalbumin is under the hormonal control.

Q.124. State the role of hormones on lactose synthesis.

- ***Prolactin:*** increases the rate of synthesis of lactose by increasing the synthesis of both subunits of the enzyme.
- ***Progesterone:*** inhibits synthesis of **α**-lactalbumin↓ thus decreasing the synthesis of lactose.
- At parturition, level of progesterone decreases↓ and **α**-lactalbumin synthesis↑ increases.

Q.125. What is galactosemia?

Galactosemia is an **inherited disorder** in which there is inability to convert galactose to glucose in normal manner due to inherited deficiency of the enzymes.

Q.126. What are the enzyme dificiency in galactosemia?

- ***In classical type:*** Deficiency of enzyme ***galactose-1-P-uridyl transferase.***
- ***In minor type:***
  - ***Galactokinase*** deficiency
  - Rarely deficiency of the enzyme ***epimerase***.

Q.127. State the salient clinical features in galactosemia.

- ***Infants*** appear normal at birth but later:
  - fails to thrive
  - becomes lethargic and may vomit.
  - develops hypoglycemia and
  - may develop jaundice.
- ***After 2 to 3 months:***
- develops **cataracts** both eyes.
- **mental retardation** due to accumulation of galactose and galactose-1-P in cerebral cortex.
- liver may have **fatty infiltration** and produces **cirrhosis liver**.

Q.128. What is the cause of hypoglycemia in galactosemia?

- Due to enzyme deficiencies galactose cannot be converted to glucose.
- Increased galactose level ↑ increases insulin secretion↑ which lowers blood sugar ↓
- Galactose-1-P inhibits the enzyme ***Phosphoglucomutase.***

Q.129. What is the cause of cataracts in galactosemia?

- Excess of galactose in lens of the eye is reduced to ***"galactitol" (Dulcitol),*** an alcohol by the enzyme ***aldose reductase***. Galactitol cannot escape from lens cells. Osmotic effect of sugar alcohol contributes to injury to lens proteins producing cataracts.
- Excess of galactose inhibits the enzyme G-6-PD of HMP shunt leading to less NADPH which results to low reduced glutathione (G-SH) ↓.

Q.130. Name some dietary sources of fructose.

- Principal source is sucrose (Canesugar/table sugar), which on hydrolysis in intestine gives fructose.
- Other sources are fruit juices and honey.

Q.131. Name the specific enzyme that phosphorylates fructose and the product formed.

- Specific enzyme is ***fructokinase.***
- Product is fructose-1-P.

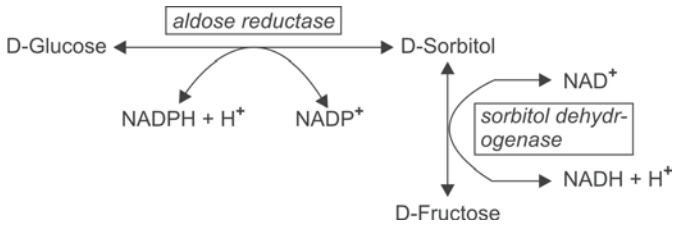
Q.132. State some metabolic importance of fructose.

- Frustose is easily metabolized and a good source of energy.

- ***Seminal fluid is rich in fructose*** and spermatozoa utilizes fructose for energy.
- Excess dietary fructose is harmful, leads to increased synthesis of TG.
- In diabetes mellitus, fructose metabolism through ***sorbitol pathway*** may account for development of cataracts and neuropathy.
- Inherited deficiency of the enzyme ***"aldolase-B"*** produces inherited disorder ***hereditary fructose intolerance.***

Q.133. How is glucose converted to fructose in seminiferous tubular epithelial cells?

- It is achieved by **sorbitol pathway**.
- Sorbitol pathway for conversion of glucose to fructose is shown schematically below:



Q.134. How do you explain biochemically the formation of cataract and neuropathies in diabetes mellitus?

- Formation of sorbitol from glucose by sorbitol pathway proceeds rapidly in the lens of the eye and Schwann cells of the nervous system.
- Elevated sorbitol concentration in these cells increase the osmotic pressure which may be responsible for the development of cataracts of lens of the eye and diabetic neuropathy.

Q.135. How does parenteral administration of fructose cause liver cell necrosis?

Fructose when administered parentally for nutrition can ***cause depletion of adenine nucleotides*** in liver producing liver cell necrosis.

Q.136. What is hereditary fructose intolerance?

- It is an ***inherited disorder,*** due to inerited deficiency of the enzyme ***aldolase B***. It leads to excessive rise of fructose↑ and fructose-1-P↑ in blood.

- Blood glucose falls leading to hypoglycaemia. The cause of hypoglycaemia is probably due to:
  - excessive insulin secretion↑ and
  - inhibition of ***phosphoglucomutase*** enzyme↓, by fructose-1-P
- Diets low in fructose and sucrose is beneficial.

Q.137. What is meant by true glucose value of blood?

"True" Glucose means estimation of glucose only and not the other reducing substances in the blood. This is achieved by *glucose oxidase* method.

Q.138. What are the normal fasting true glucose and blood sugar values?

	True glucose (By Glucose oxidase method	***Blood Sugar*** (By Folin and Wu's method)
Normal fasting level in blood:	60 to 100 mg%	60 to 120 mg%

Q.139. What are sources of blood glucose in the body?

- Absorption from GI tract (Dietary).
- Hepatic glycogenolysis.
- Gluconeogenesis in liver.
- Glucose obtained from other carbohydrates.

Q.140. How glucose is removed from the blood?

- Oxidation of glucose in tissues to provide energy.
- Hepatic glycogenesis.
- Glycogenesis in muscles and other tissues.
- Conversion to fat (lipogenesis) in adipose tissue mainly.
- For synthesis of lactose, fructose, ribose sugars, glycoproteins, glycolipids, MPS, etc.
- Excretion in urine when blood glucose exceeds renal threshold (abnormal condition).

Q.141. What is meant by the term renal threshold?

In normal individuals, glycosuria occurs when the venous blood exceeds 170 to 180 mg%. This level of venous blood glucose is termed as the ***renal threshold*** for glucose.

Q.142. What is TmG?

Rate of glucose absorption is expressed as TmG (Tubular maximum for Glucose) which is normally 350 mg/mt.

Q.143. What is meant by the term "post absorptive state"?
This is the fasting state, approximately 12 to 14 hours after last meal. There is practically no intestinal absorption. It is not prolonged starvation, as there are no metabolic abnormalities.

Q.144. What is meant by auto-regulation of blood glucose level?
- The blood glucose concentration in normal health regulates itself, hence called **"auto-regulation"**.
- It the ***ratio*** between insulin/antagonistic hormones adrenocorticoids and anterior pituitary hormone is of prime importance rather than their absolute amounts.

Q.145. What is the role of insulin on carbohydrate metabolism?
Net effect produces fall↓ in blood glucose concentration.
- Diminished supply of glucose to blood due to:
  - decrease hepatic glycogenolysis↓
  - increased hepatic glycogenesis ↑
  - Decreased gluconeogenesis↓
- Increase in the rate of utilization of glucose by tissue cells:
  - incresed uptake by tissues↑
  - increased oxidation for engery↑
  - increased lipogenesis↑

Q.146. Name the emergency hormones which increase blood glucose.
- **Catecholamines** viz. epinephrine and norepinephrine.
- **Glucagon**.

Q.147. What is hyperglycaemia?
- Increase in blood glucose level above normal is called hyperglycaemia.

Q.148. Enumerate some causes of hyperglycemia.
- Diabetes mellitus.
- Hyperactivity of thyroids (thyrotoxicosis), anterior pituitary (acromegaly/gigantism) and adrenal cortex (Cushing's syndrome).
- In diffuse diseases of pancreas e.g. in pancreatitis, carcinoma of pancreas.
- In sepsis and in some infectious diseases.
- In intracranial diseases e.g. meningitis, encephalitis, intracranial tumors, and hemorrhage.
- In emotional 'stress'.

Q.149. What is Hypoglycemia?

Decrease in blood glucose level below normal is called hypoglycemia.

Note: Hypoglycemia manifests clinically when the blood glucose is ***below < 40 mg% ("true" glucose)***.

Q.150. Enumerate some causes of hypoglycemia.

- **Overdosage of insulin in treatment of DM—most common cause** and clinically important.
- Hypoactivity of thyroids (myxedema, cretinism), anterior pituitary (Simmond's disease), and adrenal cortex (Addison's disease).
- Insulin secreting tumor (insulinoma)—rare cause.
- In severe liver diseases.
- Leucine sensitive hypoglycemia.
- Glycogen storage diseases (GSDs).
- Idiopathic hypoglycemia in children.
- Nonendocrine tumors like retroperitoneal fibrosarcoma can produce insulin-like hormones.

Q.151. What is mellituria?

Excretion of sugar when not specified is called mellituria.

Q.152. Is glucose excreted in a normal healthy individual?

In normal healthy individuals, a very small amount less then 0.5 gm of glucose may escape reabsorption by tubules and be excreted in urine. But this amount is not detected by Benedict's qualitative test.

Q.153. Define glycosuria.

Glycosuria is defined as the excretion of glucose in urine ***which is detectable*** by ***Benedict's qualitative test***.

Q.154. What are the types of glycosurias?

- **Two types:**
  - ***Hyperglycemic glycosuria:*** In this blood glucose is increased and crosses the renal threshold.
  - ***Renal glycosuria:*** In this blood glucose is normal but glucose appears in urine due to renal defect in tubular reabsorption.

Q.155. Give some examples of hyperglycemic glycosuria.

- Diabetes mellitus—clinical and experimental
- Hyperthyroidism,
- Increased secretion of catecholamines,

- Hyperactivity of adrenal cortex (Cushing's syndrome/ disease),
- Increased secretion of glucagon,
- Alimentary glycosuria (rare).

Q.156. What is Piqure glycosuria?

It is experimental hyperglycemic glycosuria. **Claude Bernard** found puncture of a particular spot in the floor of the IV ventricle in rabbits produces hyperglycemia and glycosuria temporarily. Also called ***"Puncture Diabetes"***.

Q.157. What is alloxan diabetes?

- It is also an experimental hyperglycemic glycosuria.
- Injection of ***alloxan***, a substance related chemically to pyrimidine bases, when injected to an experimental animal like dog, produces permanent diabetes characterized by hyperglycemia and glycosuria. The condition is called **"alloxan"** diabetes.
- Alloxan acts specifically on **β**-cells of islets of Langerhans and produces degeneration, necrosis, and resorption of **β**-cells.

Q.158. Give some causes of renal glycosuria.

- ***Hereditary:***
  - Probably absence of "carrier proteins"
- ***Acquired:***
  - Diseases of renal tubules.
  - Heavy metal poisioning like lead (Pb), cadmium (Cd), mercury (Hg), etc.
- ***Physiological:***
  - In pregnancy due to lowering of renal threshold.

Q.159. How will you differentiate glycosuria in a pregnant lady whether it is physiological or actually due to associated DM?

Can be differentiated by performing a fasting blood glucose level. In former it will be normal but in later condition the fasting blood glucose will be high.

Q.160. What is phlorhizin glycosuria?

- It is an experimental renal glycosuria.
- **Phlorhizin** is a glycoside, when given to dogs 1 gm/day s.c., it produces intense renal type of glycosuria.

- Phlorhizin displaces Na^+ from sodium binding site of "carrier" protein, hence glucose cannot be bound to glucose binding site and not reabsorbed.

Q.161. What is diabetes mellitus?

A chronic disease due primarily to a disorder of carbohydrate metabolism, cause of which is ***deficiency or diminished effectiveness of insulin***, resulting in hyperglycemia and glycosuria. Secondary changes may occur in the metabolism of proteins, fats, water and electrolytes and in tissues/and organs sometimes with grave consequences if not treated.

Q.162. What are the etiological types of DM?

- ***Primary (or idiopathic) type:*** Constitute major group. Exact cause is not known. Metabolic defect is insufficient insulin which may be absolute or relative.
- ***Secondary type:*** Constitute minor group where it is secondary to some disease processes.

Q.163. What are the clinical types of primary (idiopathic) DM?

- ***Juvenile-onset diabetes:*** also called type I or Insulin dependent (IDDM).
- ***Maturity-onset diabetes:*** also called type II or non-insulin dependent (NIDDM).

Q.164. Name possible factors incriminated for Type I and Type II primary DM.

Juvenile-onset DM (Type-I)	*Maturity-onset DM (Type-II)*
• Occurs in young age.	• Occurs in middle aged individuals-more in women.
• ***Heredity:*** Genetic factor is important, susceptibility is associated with particular HLA phenotype	• Obesity. • 'Stress' like pregnancy may precipitate.
• Autoimmunity.	• Diet: Over eating and underactivity predisposing factor.
• Certain viral infections may precipitate.	
• Deficiency of Insulin.	• Plasma insulin normal but insulin not utilized -"insulin antagonism" receptor deficiency.

Q.165. What are the causes of secondary DM?

- ***Pancreatic diseases:*** Pancreatitis, hemochromatosis ("bronzed" diabetes), malignancy of pancreas.
- ***Hyperactivity of antagonistic hormones:*** Hyperthyroidism/Cushing's syndrome/disease, hyperpituitarism, increased glucagon activity.
- ***Iatrogenic:*** In genetically susceptibles, it can be precipitated by prolonged therapy with corticosteroids, thiazide diuretics.

Q.166. State briefly the changes in carbohydrate, lipids and protein metabolism that occur in DM.

- ***Carbohydrate metabolism:***
  - Decreased and impaired uptake and transport of glucose by muscle/and adipose tissue.
  - Diminished oxidation of glucose.
  - Promotes gluconeogenesis in liver.
- ***Lipid metabolism:***
  - Fats are oxidized for energy producing increased amounts of acetyl CoA, which form more of ketonebodies and cholesterol.
  - Increased FFA inhibits *acetyl CoA carboxylase* ↓ and inhibit FA synthesis ↓.
- ***Protein metabolism:***
  - Increased breakdown of tissue proteins producing negative N-balance.
  - More deamination of amino acids leads to more urea formation and increased urea excretion.
  - Protein synthesis is decreased↓ in all tissues due to absolute or relative deficiency of insulin.

Q.167. State important biochemical changes that occur in an untreated uncontrolled DM.

- ***Hyperglycemia:*** increase in blood glucose↑.
- ***Glycosuria:*** excretion of glucose in urine.
- ***Hypercholesterolemia:*** increase in blood cholesterol↑.
- ***Ketonemia:*** increased ketone bodies in blood-acctoacetic acid, β OH-butyric acid and acetone.
- **Increased urea** and non protein nitrogen in blood.
- ***Dehydration.***

- ***Acidosis*:** Lowered pH↓, hyperventilation-(Metabolic) and Kussmaul breathing.
- ***Lowered HCO_3*** and alkali reserve ↓.
- ***Lowered sodium*↓** in blood-hyponatraemia and disturbance in fluid and electrolyte balance.

Q.168. What is meant by glucose tolerance?

The ability of the body to utilize glucose is ascertained by measuring its glucose tolerance. It is indicated by the nature of blood glucose curve following the administration of a standard dose of glucose.

Q.169. Name some conditions where you find decreased glucose tolerance.

- Diabetes mellitus.
- Hyperthyroidism.
- Hyperactivity of adrenal cortex and anterior pituitary.

Q.170. Name some conditions where you find increased glucose tolerance.

- Hypothyroidism,
- Hypofunction of adrenal cortex (Addison's disease),
- Hypopituitarism,
- Hyperinsulinism,
- Decreased absorption of glucose like sprue/celiac disease, etc.

Q.171. What are the types of glucose tolerance test?

Two types:

- Standard oral glucose tolerance test (OGTT).
- IV glucose tolerance test.

Q.172. State some indications for performing a standard oral glucose tolerance test.

GTT is indicated:

- In patients with transient or sustained glycosuria who have no clinical symptoms of diabetes with normal fasting or PP blood glucose.
- In patients with symptoms of diabetes but with no glycosuria and normal fasting blood glucose.
- In persons with strong family history but no overt symptoms.

- In patients with glycosuria associated with thyrotoxicosis, infections/sepsis, liver diseases, pregnancy.
- In woman with characteristically large babies 9 pounds or individuals who were large babies at birth.
- In patients with retinopathies or neuropathies.
- In patients with or without symptoms of DM showing one abnormal value.

Q.173. What precautions you will take before performing a standard oral glucose tolerance test?

- Patient should be in complete physical/mental rest prior to test.
- ***No smoking*** before the test.
- ***Should be on normal carbohydrate diets at least for 3 days prior to test*** (approximately 300 gm/day), otherwise false high curve may be obtained.
- The individual should take usual supper at about 2000 hrs previous night and does not eat or drink anything after that.
- On the day of test, early morning, a cup of tea/coffee may be given without sugar or milk if the individual so desires. No other food or drink is permitted till the test is over.
- All samples of blood should be venous preferably.

Q.174. How will you perform a standard glucose tolerance test (GTT).

- A 'fasting' sample of venous blood is collected in fluoride bottle **("fasting" sample of blood)**.
- The bladder is completely emptied and urine is collected for qualitative test for glucose and ketone bodies **("fasting" urine)**.
- The individual is given 75 gm of glucose dissolved approximately in 250 ml of water to drink orally. Time of oral glucose administration is noted.
- A total of **five** specimens of venous blood and urine are collected every ½ hr, after the oral glucose (½ hr, 1 hr, 2 hr, and 2½ hr samples of blood and urine).
- Glucose content of all six (including fasting sample) samples of blood are estimated. Corresponding urine

samples are tested qualitatively for glucose and ketone bodies.
- The results of blood glucose values are plotted as a graph against time. The curve thus obtained is called ***glucose tolerance curve***.

Q.175. Describe a typical normal glucose tolerance curve (GTC).
- Fasting glucose is within normal limits of 60 to 100 mg % ("true" glucose).
- The highest peak value is reached within one hour.
- The highest value does not exceed the renal threshold i.e. 170 to 180 mg %.
- At 2 hr, there is a ***hypoglycemic dip*** (10 to 15 mg lower than fasting value).
- The fasting level is reached by 2½ hr.
- No glucose or ketone bodies are defected in any specimens of urine.

Q.176. Describe a diabetic type of GTC.
- Fasting blood glucose is definitely raised > 110 mg% ("true" glucose),
- The highest vlaue is usually reached after 1 to 1½ hr.
- The ***highest value exceeds the normal renal threshold.*** The ***blood glucose does not return to the fasting level within 2½ hrs***, remains much higher than the fasting value. This is the ***most characteristic feature of DM.***
- Urine samples show presence of glucose. Urine may or may not contain ketone bodies depending on the type of DM and its severity.
  - According to severity, the GTC may be:
  - Mild diabetic curve.
  - Moderately severe diabetic curve.
  - Servere diabetic curve.

Q.177. What is the characteristic of renal glycosuria curve?
- The blood glucose levels remain below renal threshold in all the samples.
- Glucose appears in urine almost in all the samples even when blood glucose is much below 170 mg %. Patients who show no glycosuria when fasting may have glycosuira when blood glucose is raised.

Q.178. What is a lag curve (or oxyhyperglycemic curve)?

- Fasting blood glucose is normal, but it rises rapidly in the next ½ to 1 hr. and exceeds the renal threshold so that the corresponding urine specimens show the presence of glucose.
- The return to normal value is rapid and complete.

Q.179. In which conditions you get a lag curve?

- In hyperthyroidism.
- After gastroenterostomy.
- During pregnancy.
- Also in "early" diabetes.

Note: A patient showing lag curve should be reviewed from time to time after every six months.

Q.180. State the indications for performing IV GTT.

- IV GTT is preferred when an abnormality in glucose absorption is suspected. Thus IV GTT is indicated:
  - In hypothyridism,
  - In sprue,
  - Caeliac disease, etc.

Q.181. What is the indication of cortisone stressed GTT? What is the basis of the test?

- ***Used*** for ***detecting "latent" diabetes*** or ***"pre-diabetes"***.
- It is based on the fact that while a large dose of ACTH or suitable corticosteroid produces a raised GTC and glycosuria in normal healthy persons, smaller doses will do so in "latent" or "pre-diabetics".

Q.182. What is the dose of corticosteroids used for cortisone stressed GTT?

- Two doses of 50 mg cortisone orally. First dose is given 8½ hours before GTT and second dose 2 hrs before GTT.
- Alternatively, two doses of prednisolone 0.4 mg/kg body wt, ½ the dose at midnight and ½ at 6 AM before carrying out GTT at 8 AM.

Q.183. What is extended GTT?

Instead of ending at 2½ hrs after taking glucose orally, ½ hrly blood glucose are done for periods up to 4 to 5 hours.

Q.184. What is the usefulness of extended GTT?

- ***Extended GTT is useful:***
  - To differentiate transient attacks of hypoglycemia from those due to insulin secreting tumors of islet of Langerhans of the pancreas (insulinomas) and also other abnormal endocrine conditions such as Simmond's disease, which cause hypoglycemia.
  - In the endocrine disorders, fall in blood glucose at the end of tolerance last tends to be progressive.

CHAPTER

13 Metabolism of Lipids

Q.1. Name the principal plasma lipids. Mention their range and mean average value.

Principal plasma Lipids		*Range (mg %)*	*Mean average (mg %)*
• Triacylglycerol (TG)	—	80 to 180	150
• Total phospholipids (PL)	—	125 to 390	120
• Cholesterol Total	—	150 to 250	200
Nonesterified (Free)	—	25 to 105	55
Ester	—	125 to 145	—
• Free Fatty acids (FFA) or unesterified FA (NEFA)	—	6 to 16	10
Total Lipids	—	360-820	560

Q.2. Plasma lipids are water insoluble (hydrophopic), how are they carried in the aqueous medium of blood?

TG (water ***Insoluble)*** + Coated with "polar" lipids like PL and Cholesterol and its esters + water soluble apo-proteins → Froms lipoprotein complex (water soluble)

Q.3. How resynthesized TG in intestinal epithelial cells is carried in the blood?

Carried as lipoprotein complex ***chylomicrons***. Thus chylomicrons are responsible for transport of ***exogenous*** dietary TG.

Q.4. How TG synthesized in liver is carried in blood?
Carried as lipoprotein complex called ***Very Low density lipoprotein (VLDL)***. Hence VLDL is responsible for transport of ***endogenous*** TG.

Q.5 How FFA/non-esterified FA (NEFA) is carried in the blood?
In circulation, FFA/NEFA combines with albumin and carried as ***albumin-FFA complex***. Some 25 to 30 mols of FFA are carried in blood in combination with one mol of albumin.

Q.6. How Plasma lipids are separated?
- By electrophoresis
- By ultra centrifugation.

Q.7. Name the fractions separated by ultracentrifugation.
Separation by ultracentrifugation depends on density changes assessed by Sf units (Svedberg Floatation units).

Lipoproteins	*Density*	*Sf units*
• Chylomicrons	0.96	> 400
• Very low density lipoproteins (VLDL)	0.96-1.006	20-400
• Low density lipoproteins (LDL)	1.006-1.063	2-20
• High density lipoproteins (HDL)	1.063-1.21	0-2

Q.8. Name the fractions of lipids separated by electrophoresis.
- ***Chylomicrons:*** slowest moving and remains near to origin.
- ***Pre-β lipoproteins:*** equivalent to VLDL
- ***β-lipoproteins:*** equivalent to LDL
- ***α-lipoproteins:*** equivalent to HDL—moves fastest and occupies position of α-globulin.

Q.9. What is Friedwald formula? What for it is used?
Friedwald formula is used for calculation of serum LDL cholesterol. The formula is as follows:

$$\text{Total cholesterol} - \text{HDL cholesterol} - \frac{\text{TG}}{5}$$

Q.10. State the normal values of serum TG chylomicrons, VLDL, HDL, LDL and LDL cholesterol.

Lipids		*Normal serum level in mg%*	
		In males	*In females*
• Serum TG	—	60 to 165	40 to 140
• Serum chylomicrons	—	up to 28 mg% (in postabsorptive state)	
• Serum VLDL	—	up to 240	up to 210
• Serum HDL	—	35 to 60	40 to 70
• Serum LDL	—	up to 550 mg%	
• Serum LDL-cholesterol	—	up to 190 mg%	

Q.11. What is meant by element constant?

- Cytoplasm and cell membranes of all organs are composed of ***element constant***. It is composed chiefly of PL alongwith smaller amounts of other lipids including cholesterol and its ester.
- Fat content of "element constant" is not diminished by starvation.

Q.12. What is meant by element variable?

Fats present in fat depots is called ***element variable***. They are subcutaneous fatty tissues, retroperitoneal fatty tissues, intermuscular fatty tissues, etc. It is mainly composed of triacylglycerol (TG), also called neutral fats (NF). The amount fluctuates with dietary intake. It is diminished in starvation.

Q.13. Is adipose tissue is a static lump of fats? or it is in dynamic equilibrium?

- Adipose tissue is not just a static lump of fats. It is in ***dynamic state***. Breakdown of fats (lipolysis) and esterification of fats (lipogenesis) take place all the time.
- The ***two processes are not forward and reverse processes*** of the same reaction. They are entirely different pathways. ***Resultant of these two processes determine the level of FFA in the circulating blood.***

Q.14. Name the substrates required in adipose tissue for TG synthesis.

Two substances are required:

- **α**-glycero-P and
- Acyl CoA (Activated FFA).

Q.15. What are the sources of FFA in the adipose tissue?

- Dietary FFA
- Synthesis of FA (palmitic acid) from acetyl CoA by extramitochondrial "deNovo" synthesis.
- FFA obtained from lipolysis in adipose tissue itself.
- FFA obtained from delipidation of TG of circulating chylomicrons and VLDL by the enzyme ***lipoprotein lipase***.

Q.16. How α-glycero-P is formed in the body?

- Conversion of glycerol to **α**-glycero-P by the enzyme ***glycerokinase*** and ATP.
- Di-hydroxy acetone-P obtained from glycolysis can be converted to **α**-glycero-P.
- Also from catabolism of lecithin in the body.

Q.17. Can adipose tissue utilize the glycerol formed by lipolysis in synthesis of TG?

Glycerol produced by lipolysis **cannot be utilized** by adipose tissue in synthesis of TG as the enzyme ***glycerokinase*** is **absent** in adipose tissue. Hence glycerol produced by lipolysis passes to blood.

Q.18. What is the source of α-glycero-P in adipose tissue for synthesis of TG?

For provision of **α**-glycero-P in adipose tissue for TG synthesis, the tissue is dependent on a proper supply of glucose and glycoysis. ***Di-hydroxy acetone-P formed in glycolysis is converted to α-glycero-P.***

Q.19. Name the enzymes required for breakdown of TG (lipolysis).

Three enzymes are required:

- ***Triacyl glycerol lipase***-Hormone sensitive and key rate limiting enzyme. The enzyme can exist in "active" or "inactive" state.
- ***Diacyl glycerol lipase*** } Both are non-
- ***Monoacyl glycerol lipase*** } hormone sensitive.

Q.20. How the action of *TG lipase* is regulated?

- ***TG lipase*** can exist in active "a" and inactive "b" forms.
- Increased Cyclic AMP↑ level in the cells converts "inactive" Cyclic AMP dependent protein kinase (C_2R_2) to "active" protein kinase (C_2), which in turn phosphorylates "inactive" hormonesensitive TG lipase "b" to "active" TG lipase "a" which breaks down TG to form DG + FFA.
- Active TG lipase "a" is converted to "inactive" TG lipase "b" by dephosphorylation.

Q.21. List the hormones that increase TG synthesis.

- ***Insulin:*** The principal hormone
- ***Prolactin:*** Effective only when given in large doses.

Q.22. What is the action of insulin on adipose tissue?

- Insulin increases esterification (TG formation) as it enhances the glucose uptake by adipose tissue cells. Also increases glucose oxidation which provides **α**-glycero-P through di-OH-acetone-P.
- Insulin inhibits lipolysis. This is brought about by decreasing the levels of Cyclic AMP↓ in the cells. This is achieved by:
  - Inhibiting ***adenyl cyclase*** enzyme↓ and
  - Increasing the ***phosphodiesterase*** activity↑. Lowered cyclic AMP↓ brings about dephosphorylation of TG lipase "a" → to form TG lipase "b" (inactive) through protein kinase.

Q.23. What is the net effect of insulin on plasma FFA level?

Net result of insulin on adipose tissue is to inhibit the release of FFA from adipose tissue which results in ***fall of circulating plasma FFA level↓***.

Q.24. List the hormones that increase the rate of breakdown of TG (lipolysis) in adipose tissue.

- ***Catecholamines:***
  - Epinephrine and Norepinephrine are the principal lipolytic hormones.
- ***Other hormones are:***
  - ***Glucagon,***
  - ***Growth hormones (GH),***
  - ***Gluco-corticoids (GC),***
  - ***Thyroid hormones,***
  - ***ACTH, α and β MSH, TSH and vasopressin.***

Q.25. How catecholamines increase lipolysis?

- Stimulate the activity of ***adenyl cyclase*** and increasing Cyclic AMP level↑ in the cells.
- Cyclic AMP inturn activates TG lipase "b"→"a" through cyclic AMP dependent *proteinkinase*.

Q.26. How does gluco-corticoids and thyroid hormones help in lipolysis?

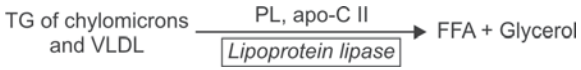
For an optimal effect most of the lipolytic hormones ***require the presence of gluco-corticoids (GC)*** and ***thyroid hormones in minimal amount***. They act in a "facilitatory" or "permissive" capacity with other lipolytic hormones.

Q.27. How the adipose tissue metabolism is affected in diabetes mellitus (DM) and starvation?

In diabetes mellitus (DM) and starvation, availability of glucose in adipose tissue is grossly reduced, resulting in lack of α-glycero-P. Thus, **rate of re-esterification is decreased**↓. Lipolysis is greater than re-esterification resulting to accumulation of FFA and **increase in Plasma FFA level**↑.

Q.28. What is *Lipoprotein lipase*? Where it is found? What is the action of this enzyme?

- The enzyme ***lipoprotein lipase*** is located in the walls of the blood capillaries in various organs.
- TG of circulating chylomicrons and VLDL is acted upon by *lipoprotein lipase* which brings about progressive delipidation. The enzyme requires PL and apo-C II as cofactors



Q.29. What is Clearing factor?

Heparin stimulates and release ***lipoprotein lipase*** from vascular capillary walls by deanchoring from binding site to circulating blood. By progressive delipidation of TG of chylomicrons and VLDL produces clearing of lipaemia. Hence heparin is called as ***clearing factor.***

Q.30. What is Brown adipose tissue? Where is it located?

- Brown adipose tissue is involved in metabolism particularly when heat generation is necessary.
- The tissue is extremely active:
  - in arousal from hibernation
  - in animals exposed to cold and
  - in heat production in newborns.
- **Location site:** It is present particularly in the thoracic region.

Q.31. State the characteristic features of Brown adipose tissue.

- A high content of mitochondria.
- A high content of cytochromes which gives it the brown colour.
- Relatively rich in carnitine.
- Well-developed blood supply.
- O_2-consumption is high.
- Possesses the enzyme ***glycerokinase*** (cf white adipose tissue-glycerokinase is absent).
- Low *ATP-synthase* activity.

Q.32. What is the function of Brown adipose tissue?

It plays a **role in heat production** for vital organs serving as a sort of "heating pad" for local application of heat to the vital organs of the thorax, the upper spinal cord, and the autonomic sympathetic chain.

Q.33. What is thermogenin? What is its role in Brown adipose tissue?

- In terms of chemiosmotic theory, it appears that the proton gradient normally present across the inner mitochondrial membrane of coupled mitochondria is continuously dissipated in brown adipose tissue, by a thermogenic protein, called **"thermogenin"** which acts as a proton conductance pathway through the membrane.
- ***Oxidation and phosphorylation are not coupled in mitochondria of brown adipose tissue*** which accounts for the heat.

Q.34. How fatty acids are oxidized?

- **β**-oxidation: Principal method of oxidation of FA.
- **α**-oxidation
- **ω**-oxidation
- Peroxismal FA oxidation.

Q.35. What is β-oxidation?
β carbon atom of a fatty acid is oxidized to -COOH by the removal of 2 carbon atoms at a time in the form of acetyl CoA.

Q.36. Who discovered β-oxidation of FA?
The classical theory of β-oxidation was the outcome of the work of **Knoop**.

Q.37. Name the tissues in which β-oxidation occurs.
Circulating FA are taken up by tissues like liver, kidney, muscle, brain, lungs, testes and adipose tissue. These tissues are able to oxidize long chain acyl CoA. In cardiac muscle, fatty acids are important fuel, 80% of energy is derived by FA oxidation.

Q.38. What is the site of β-oxidation of FA in a cell? Mention the enzyme responsible.
- ***Site*** In mitochondrion of the cell.
- ***Enzyme*** Serveral enzymes known collectively as ***FA oxidase enzyme system*** are found in the mitochondrial matrix, adjacent to the respiratory chain (ETC). These enzymes catalyze **β**-oxidation of a long chain FA to acetyl CoA.

Q.39. Activation of Long chain FA occurs in cytosol, but β-oxidation occurs in mitochondrial matrix. Activated long chain FA (acyl CoA) is impermeable to mitochondrial membrane. Explain how acyl CoA reach mitochondria?
Acyl CoA penetrate to the inner mitochondrial matrix only in combination with a substance called ***carnitine*** present in mitochondrial membrane.

Q.40. What is carnitine?
- Carnitine is chemically **"β-OH-γ-trimethyl ammonium butyrate"**. It is widely distributed in liver, and other tissues, particularly in large quantities in muscles.
- Carnitine level of tissues is considerably influenced by dietary methionine and choline levels.
- It is synthesized from lysine and methionine principally in liver and in kidney.

Q.41. What are the functions of carnitine?

- Carnitine does not penetrate the mitochondrial membrane. It is considered as a ***carrier molecule*** and ***acts as a ferry boat*** to transport long chain Acyl CoA across mitochondrial membrane for its oxidation in mitochondrial matrix.
- Also facilitates exit of acetyl-CoA and acetoacetyl CoA from mitochondria to cytosol where FA synthesis occurs.

Q.42. State the enzymes involved in transportation of acyl CoA to mitochondrial matrix by carnitine.

- ***Carnitine-Palmitoyl transferase I***
- ***Carnitine-acyl carnitine translocase***
- ***Carnitine-Palmitoyl transferase II.***

Q.43. Name the steps of β-oxidation.

Once acyl CoA is transported by carnitine in the mitochondrial matrix, it undergoes **β**-oxidation by the enzyme complex ***fatty acid oxidase system***. The steps of **β**-oxidation are:

- ***Dehydrogenation*** by the enzyme ***acyl CoA dehydrogenase***. H-acceptor is FAD.
- ***Hydration*** by the enzyme ***enoyl hydrolase***, addition of one molecule of H_2O.
- ***Dehydrogenation*** by the enzyme ***3-OH acyl CoA dehydrogenase***. H-acceptor is NAD^+.
- ***Thiolytic cleavage*** by the enzyme ***thiolase*** and CoA SH.

Q.44. How many ATPs are formed by one turn of β-oxidation of FA?

Five ATP:

- Two from oxidation of reduced FAD in ETC.
- Three from oxidation of reduced NAD in ETC.

Q.45. What are the endproducts of thiolytic cleavage in each turn?

Thiolytic cleavage in each turn results in formation of:

- One molecule of acetyl CoA.
- And an acyl CoA molecule containing 2 carbons less than the original molecule, which again enters the cycle.

Q.46. How many acetyl CoA are produced from β-oxidation of one molecule of palmitic acid?

- Palmitic acid is $C_{15}H_{31}$ COOH.
- For complete oxidation by **β**-oxidation, it will **undergo 7 cycles** producing 7 acetyl CoA in each turn + one acetyl CoA extra will be produced in last cycle.

∴ β-oxidation of one molecule of palmitic acid will from 8 acetyl CoA.

Q.47. How much energy will be produced by β-oxidation of palmitic acid? What is the efficiency?

• Each cycle produces 5 ATP, hence 7 cycles will produce	7 × 5 = 35~P
• Total 8 molecules of acetyl CoA when oxidized in TCA cycle will form (12 × 8)	96~P
	Total = 131~P
• In initial activation of palmitic acid ~ P bonds utilized.	= – 2~P
	Total = 129~P
∴ Energy production =	129 × 7.6 = 980 KC
• Caloric value of palmitic acid	= 2340 KC/mole
∴ Efficiency = $\frac{980}{2340} \times 100$	= 41% of total energy of combusion of palmitic acid.

Q.48. What are the products of β-oxidation of FA with odd number of C-atoms?

- Acetyl CoA in each cycle + 3 carbon residue propionyl CoA in the last cycle.
- Propionyl CoA from odd-chain FA is converted to succinyl CoA, an intermediate of TCA cycle through methyl malonyl CoA.

Q.49. What is α-oxidation?

- **α**-oxidation is another alternative pathway for oxidation of FA which involves decarboxylation of the -COOH group after hydroxylation and the formation of a FA containing an "odd-number" of carbon atoms which subsequently undergoes repeated **β**-oxidation producing $(\text{acetyl CoA})_n$ + propionyl CoA.
- It occurs in microsomes of brain and liver, an aerobic process requires molecular O_2.

Q.50. What is ω-oxidation?

In **ω**-oxidation, fatty acids undergo oxidation at **ω**-carbon, producing a dicarboxylic acid, which is then subjected to **β**-oxidation and cleavage to form successively smaller dicarboxylic acids.

Q.51. State the functions of α-oxidation.

- To synthesize **α**-OH fatty acids like ***cerebronic*** acid of brain cerebrosides and ***sulfatides***.
- To form odd carbon long chain FA required in brain sphingolipids.
- Help to oxidize phytanic acid of dietary phytols by the enzyme ***phytate α-oxidase*** to produce CO_2 and odd C-chain FA ***pristanic acid*** which is further completely oxidized by **β**-oxidation.

Q.52. What is Refsum's disease?

An inherited disorder due to deficiency of the enzyme ***phytate α-oxidase***, as a result phytanic acid cannot be converted to pristanic acid which accumulates in blood and tissues. Principal manifestation is neurological-polyneuropathy with muscle atrophy.

Q.53. What is peroxismal FA oxidation?

A modified form of **β**-oxidation in peroxisomes in which very long chain FA (C_{20} to C_{38}) take place. End products are octanoyl CoA and acetyl CoA which are removed from peroxisomes to mitochondria with the help of carnitine for further oxidation.

Q.54. What is Zellweger's syndrome (hepatorenal syndrome)?

A rare inherited disorder in which there is ***inherited absence of peroxisomes*** in all tissues. Due to absence of peroxisomes and its enzymes fail to oxidize long chain FA in peroxisomes, resulting to accumulation of long-chain FA (C_{26} to C_{38}) in brain and other tissues like liver/kidney.

Q.55. What happens in carnitine deficiency?

- ***Produces hypoglycaemia:*** episodic periods of hypoglycemia owing to reduced gluconeogenesis resulting from impaired FA oxidation.
- Muscular weakness
- Accumulation of lipids
- Impaired ketogenesis in the presence of raised FFA.

Q.56. Name the methods available in the body for FA synthesis and chain-elongation of FA.

- **Three systems** available:
  - Cytoplasmic (extramitochondrial) "de Novo" synthesis of FA from acetyl CoA.
- **For chain elongation:**
  - Mitochondrial system
  - Microsomal system.

Q.57. What is starting material and end product in extramitochondrial "de Novo" FA synthesis?

- Starting material is **Acetyl CoA.**
- End product is always **palmitic acid.**

Q.58. State the materials required in extra-mitochondrial "de Novo" FA synthesis.

- **Materials required are:**
  - Acetyl CoA
  - Malonyl CoA—formed from acetyl CoA by CO_2-fixation reaction.
- **Enzymes:**
  - ***Fatty acid synthetase*** A multienzyme complex
  - ***Acetyl CoA carboxylase*** Required for formation of malonyl CoA.
- **Coenzymes and cofactors:**
  - Biotin, NADPH and Mn^{++}
  - CO_2—source is HCO_3^-
  - ATP—for energy.

Q.59. What is the enzyme required in lower organisms?

It is done by **"acyl carrier protein" (ACP)**. Six enzymes are associated in ACP whose functional units are 4-phosphopantetheine (-SH) and cysteine (-SH).

Q.60. What is the enzyme system present in higher animals including mammals for "de Novo" synthesis?

In higher animals, the synthesis is carried out by a multienzyme complex called ***fatty acid synthase***, which also **incorporates ACP.** This multienzyme complex is a **dimer**, i.e. made up of two identical monomeric units (I and II) aligned "head to foot" on either side. One end has **the**

4-phosphopantetheinyl group of ACP **(Pan-SH), while the other** end has cysteinyl SH **(cys-SH).** Each monomeric unit has seven enzymes required for total synthesis of fatty acid palmitic acid from acetyl CoA.

Q.61. Name the tissues in which "de Novo" synthesis of FA take place.

FA synthesis occurs in adipose tissue, liver, brain kidney, mammary gland, and lungs in which the multienzyme complex have been found in soluble cytosolic fraction of these tissues.

Q.62. What is the first reaction in the "de Novo" synthesis of FA and what is the rare limiting key enzyme?

The first reaction is the ***conversion of acetyl CoA to form malonyl CoA.*** The reaction is catalyzed by the enzyme ***acetyl CoA carboxylase*** in presence of Biotin, CO_2, Mn^{++} and ATP ("CO_2-fixation reaction"). ***Acetyl CoA carboxylase*** is the **rate-limiting** and **key enzyme** of lipogenesis.

Q.63. Name the reductive steps in the biosynthesis of FA which require NADPH.

- Reductive enzymes which require NADPH are:
- ***β-keto acyl reductase***
- ***Enoyl reductase.***

Q.64. What are the sources of NADPH in FA synthesis?

- HMP-shunt is the Principal source of NADPH,
- Other alternative sources are:
  - Isocitrate dehydrogenase
  - Malic enzyme.

Q.65. State the role of hormones in lipogenesis.

- ***Glucagon:*** Inhibits FA synthesis by inhibiting the key enzyme ***acetyl CoA carboxylase***.
- ***Insulin:*** Increases FA synthesis- in several ways:
- By decreasing lipolysis↓
- Activation of **protein phosphatase**↑
- Stimulating synthesis and activation of *citrate lyase*.
- Enhancing formation of acetyl CoA by stimulating glycolysis.

Q.66. Differentiate mitochondrial and microsomal chain elongation system. Mention the salient points.

Microsomal system	*Mitochondrial system*
• Usual common pathway.	• Not a common pathway
• Operates in endoplasmic reticulum (ER) of microsomes	• Operates in mitochondria
• Acyl CoA of saturated C_{10} to C_{16} FA are the starting material.	• Palmityl CoA is usually the starting material.
• End product is next higher homologue	• Stearic acid is produced.
• Requires O_2 (aerobic)	• Operates under relative anaerobiosis. High NADH/ NAD^+ ratio favours.
• Acetyl CoA is added through malonyl CoA	• Acetyl CoA is directly incorporated in palmityl CoA.
• Pyridoxal-P is not required.	• Pyridoxal-P is required.
• NADPH is required.	• NADPH is required.

Q.67. What is the enzyme that degrades lecithin?
- Phospholipase A_2.

Q.68. What are the end-products of lecithin catabolism?
- **α**-glycero-P and
- Nitrogenous base choline.

Q.69. Name the substrates required for synthesis of lecithin/ cephalin.
- **α, β**-diacyl glycerol
- Choline for lecithin and ethanolamine for cephalin.

Q.70. What are the substrates required for synthesis of sphingosine (sphingol)?
- Palmityl CoA and
- Serine.

Q.71. How Arachidonic acid is synthesized from linoleic acid? Involves 3 steps reactions:
- Linoleic acid is activated to form Linoleyl-CoA which is dehydrogenated to form γ-linolenyl-CoA.
- γ-linolenyl CoA is converted to "dihomo-γ-linolenyl CoA by addition of one molecule of acetyl CoA in the microsomal system of chain elongation.
- Dihomo-γ-linolenyl CoA forms arachidonic acid by another dehydrogenation.
- Pyridoxal-P is required as coenzyme in second step.

Q.72. Name the ketone bodies.

- Acetoacetic acid
- β-OH butyric acid
- Acetone

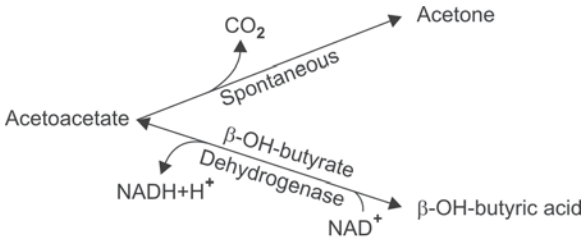
Q.73. Which is the first ketone body formed?

- Acetoacetate.

Q.74. What is the site of ketone body formation and the enzymes?

- ***Liver is the only organ*** where ketone bodies are formed.
- Enzymes are mitochondrial.

Q.75. Show schematically the inter-relationship of three ketone bodies.



Q.76. Which ketone body out of the three exists in highest concentration?

- β-OH butyric acid.

Q.77. What is the normal concentration of ketone bodies in the blood and the urine?

- Blood = 1 mg/100 ml
- Urine = less than 1 mg/24 hours urine.

Q.78. What is ketonaemia and ketonuria?

- ***Ketonaemia:*** Rise of ketone bodies in blood above normal is called ketonaemia.
- ***Ketonuria:*** When the blood level of ketone bodies rises above renal threshold, approximately 70 mg%, they are excreted in urine and is called as ketonuria.

Q.79. What is ketosis?

Accumulation of abnormal amount of ketone bodies in blood/and tissue fluid is termed ketosis.

Q.80. What are the causes of ketosis?

- Prolonged starvation—The simplest way by which ketosis can occur.
- Diabetes mellitus—severe form.
- In some types of alkalosis.
- Some of the glycogen storage disease (GSDs), e.g. in Type I-von Gierk disease
- Toxemia of pregnancy
- In prolonged ether anesthesia.
- After severe exercise in the post-absorptive state.
- Injections of anterior pituitary extracts.

Q.81. How ketoacidosis is produced?

Acetoacetic acid and β-OH butyric acid are moderately strong acids, they are buffered in blood and tissues entailing some loss of buffer cations, which progressively depletes the alkali reserve↓ causing ketoacidosis.

Q.82. What is meant by the term ketogenesis?

The formation of ketone bodies in the liver is called as ketogenesis.

Q.83. What is meant by ketolysis?

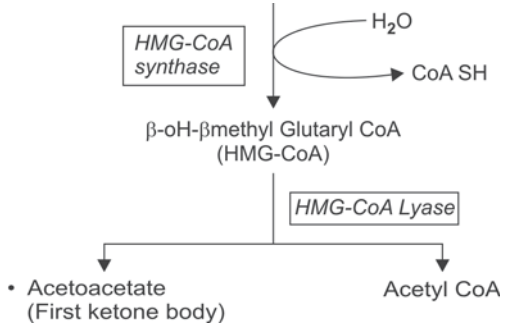
The breakdown and utilization of ketone bodies by extrahepatic tissues is called ketolysis.

Q.84. Why ketone bodies are not degraded in liver?

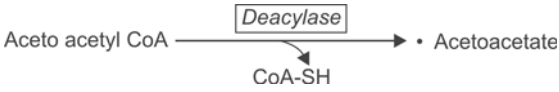
As the enzymatic machinery for degradation like ***thiophorase*** and ***thiolase*** are absent in liver.

Q.85. How ketone bodies are formed in the liver?

- ***Principal Pathway*** is by HMG-CoA formation:
  - Acetyl CoA + Acetyl CoA → Acetoacetyl CoA.
  - Acetoacetyl CoA + Acetyl CoA

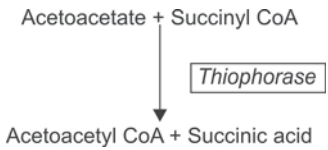


- ***Minor pathway:*** Acetoacetate can also be formed by deacylation.



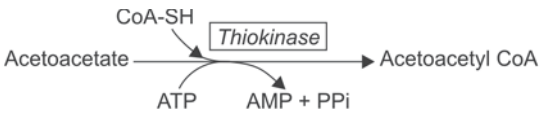
Q.86. How are ketone bodies utilized by extrahepatic tissues?
Ketone bodies once formed in the liver flow to the blood from where ketone bodies are taken up by extrahepatic tissues, and **utilized as "fuel".**

- ***Main Pathway:*** uses succinyl CoA



Note: Thiophorase **enzyme also called as** ***CoA transferase.***

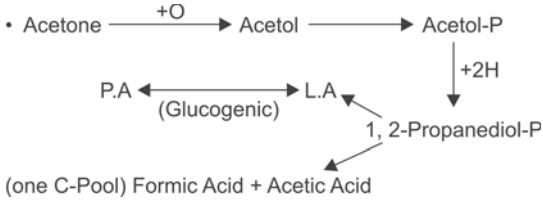
- ***Minor Pathway:***



Q.87. What happens to Acetone?
Excess of acetone is breathed out in breath and also excreted in urine. This gives a "fruity" smell in breath and in urine.

Q.88. Can acetone be glucogenic?

A ***propanediol*** **pathway** of acetone metabolism has been proposed which is glucogenic.



Q.89. How esterification of TG acts as antiketogenic mechanism?

Once FFA derived from lipolysis of TG in adipose tissue are esterified in liver to form TG, they become negligible source of ketone bodies. Hence esterification can be regarded as a significant antiketogenic mechanism.

Q.90. Name the ketogenic substances.

- All FFA (90% of food fats)
- Ketogenic amino acids (40%).

Q.91. Name the antiketogenic substances.

- All carbohydrates
- Insulin
- 60% of proteins—glucogenic amino acids
- Glycerol part of TG lipolysis which is glucogenic (10%).

Q.92. What diet you will recommend to prevent ketone bodies formation.

Clinical rule is that the total fat content (F) of the diet must not exceed the sum of twice the carbohydrates (C) and 1/2 of the proteins (P) i.e. F = or < (2C + 1/2 P).

Q.93. How will you reduce ketosis in diabetic ketosis?

- By giving insulin parenterally + IV Glucose saline.
- Antiketogenic substances like carbohydrate diet, and glucogenic amino acids (like aspartate).

Q.94. How will you reduce ketosis in prolonged starvation?

- Carbohydrate diet +
- Glucogenic amino acids (like aspartate).

Q.95. Name the tissues that can synthesize cholesterol.
Liver, skin, small intestine, gonads, adrenal cortex, kidney, brain of newborns.

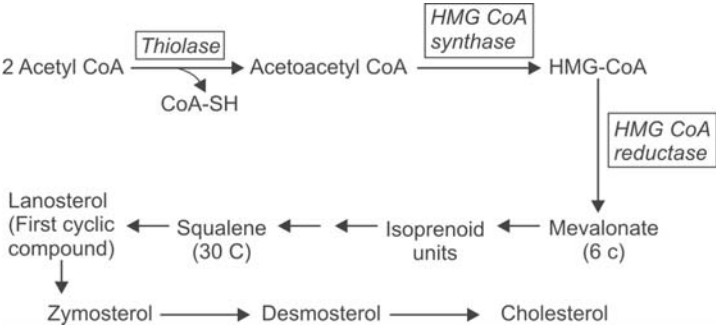
Q.96. Can adult brain synthesize cholesterol?
Adult brain cannot synthesize cholesterol.

Q.97. Where the enzyme system for cholesterol biosynthesis located?
- Enzyme system of cholesterol biosynthesis are associated with:
  - Cytoplasmic particles (microsomes)
  - Soluble fraction of cytosol.

Q.98. What is the starting material for cholesterol biosynthesis?
- Acetyl CoA ("active" acetate).

Q.99. How many carbons are there in cholesterol and what are the sources?
There are **27 carbons** in cholesterol, the entire carbon skeleton is derived from acetyl CoA.
- 15 carbons are derived from -CH_3 group
- 12 carbons from -COOH group.

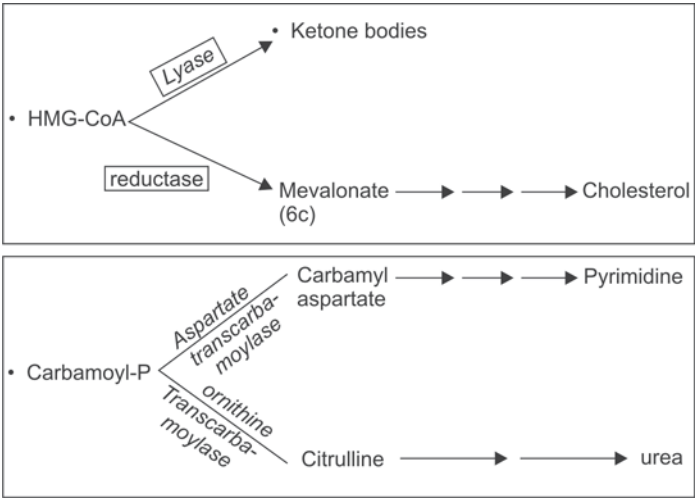
Q.100. Show schematically the steps of cholesterol biosynthesis.



Q.101. What is the key and rate limiting enzyme in cholesterol biosynthesis?
HMG-CoA reductase which converts HMG-CoA to mevalonate.

Q.102. What is a committed step in a metabolic pathway?
Committed step in a metabolic pathway is a branch point substrate from which different metabolic pathways emanate and exerts independent regulation at the levels of enzymes in branch pathway.

Q.103. Give two examples of committed step.



Q.104. How cholesterol biosynthesis is regulated by HMG-CoA reductase?

- Cholesterol itself inhibits the enzyme ***HMG-CoA reductase*** by "feed-back" inhibition.
- Fasting/Starvation inhibits the enzyme and decreases cholesterol synthesis.
- Increased dietary intake reduces the endogenous hepatic biosynthesis of cholesterol by reducing the activity of ***HMG-CoA reductase.***
- ***Hormones:***
  - **Insulin** and **thyroid hormones** increases HMG-CoA reductase activity↑
  - **Glucagon** and **corticosteroids** decreases↓ the activity of the enzyme.

Q.105. What is the role of Cyclic AMP on cholesterol synthesis?
HMG-CoA reductase exist in "active" and "inactive" forms which is reversibly modified by phosphorylation and

dephosphorylation which is mediated through Cyclic AMP dependent ***protein kinase***.

- Increased Cyclic AMP in cells inhibit cholesterol synthesis by converting "HMG-CoA reductase" to inactive form.

Q.106. How much cholesterol is present in an egg?

- Fresh whole egg = approx 468 mg%
- Fresh yolk of egg = approx 2000 mg%.

Q.107. State some factors that affect cholesterol level in blood.

- Dietary fats containing high saturated FA increases blood cholesterol level; consumption of polyunsaturated FA decreases.
- Excessive amounts of sucrose consumption increases blood cholesterol level.
- Intake of excess calories increases blood cholesterol level.
- Large amounts of nicotinic acids cause lowering of blood cholesterol.
- Increased dietary fibres lowers cholesterol level, by increasing excretion of cholesterol in bile acids.
- Physical exercise lowers cholesterol level.
- Pyridoxine deficiency increases blood cholesterol level.

Q.108. Name three hypolipidaemic drugs mentioning probable mode of action.

Clofibrate: inhibits hepatic cholesterol synthesis and inhibits secretion of VLDL.

Oestrogen: lowers cholesterol level and increases HDL.

Lovostatin: reduces LDL cholesterol level (mevastatin).

Q.109. What is meant by hypercholesterolemia?

Increase in serum cholesterol level above normal is called hypercholesterolemia.

Q.110. Name Six conditions where hypercholesterolemia occur.

- Diabetes mellitus
- Nephrotic syndrome (Type II nephritis)
- Obstructive jaundice
- Myxedema
- Xanthomatous biliary cirrhosis
- Idiopathic hypercholesterolemia.

Q.111. What is hypocholesterolaemia?

Decrease in blood cholesterol level below normal is called hypocholesterolemia.

Q.112. Name five conditions where hypocholesterolaemia occur.

- Thyrotoxicosis
- Malabsorption syndrome
- Acute infections
- Pernicious anemia and other anemias
- Wasting diseases like malignancies.

Q.113. What are bile acids?

Bile acids are formed by degradation of cholesterol.

They are of **2 types:**

- ***Primary bile acids:*** Synthesized in liver from cholesterol. They are:
  - Cholic acid-largest fraction
  - Chenodeoxycholic acid.
- ***Secondary bile acids:*** Produced in intestine from primary bile acids by the action of intestinal bacteria. They are:
  - Deoxy cholic acid
  - Lithocholic acid.

Q.114. What is the quantity of bile secreted per day?

Approximately 500 to 1000 ml of bile is secreted by liver per day.

Q.115. Why the colour of the bile is yellow?

Due to presence of bilirubin.

Q.116. Name one enzyme present in bile.

- ***Alkaline phosphatase.***

Q.117. How does hepatic bile differ from gallbladder bile? State some essential differences.

Hepatic bile	*Gall bladder bile*
• pH = 7.0 to 8.2	6.0 to 7.0
• Sp.gr. = 1.010	1.040
• Water = 97.2%	88%
• Bile acids = 1.2%	6.2%
• Total lipids = 0.3%	2.5%
• Cholesterol = 0.08%	0.5%
• Inorganic salts = 0.84%	0.75%

Q.118. Name the bile salts.

They are:

- Sodium/or potassium glycocholate.
- Sodium/or potassium taurocholate.

Q.119. State at least five important functions of bile salts.

- ***Lowering of surface tension:*** They aid in emulsification of fats.
- They help in absorption of fat soluble vitamins like A, D, E, and K.
- Keeps cholesterol in solution.
- ***Choleretic action:*** Bile salts are absorbed from intestine, taken by portal blood to liver, and stimulate secretion of bile (enterohepatic circulation of bile salts).
- Bile salts accelerate the action of ***pancreatic lipase*****.**

Q.120. What are gallstones?

Bile salts keep cholesterol in solution in gallbladder bile. If bile salts content is decreased due to any cause, the phospholipid (PL) also decreases because secretion of PL in bile depends on availability of conjugated bile salts. Solubility of cholesterol depends on the ratio of cholesterol with the conjugated bile salts + PL. Due to decrease in bile salts and PL, leads to an imbalance of the ratio, as a result solubility of cholesterol is hampered leading to crystallization of cholesterol which grow to form stones.

Q.121. What are the types of gallstones?

Mainly 3 types:

- ***Cholesterol stones:*** Single or multiple, mulberry shaped, mainly formed of cholesterol, not radiopaque.
- ***Pigment stones:*** Small multiple stones, consist of bile pigments + Ca + other organic substances, Dark green or black in color, not radiopaque.
- ***Mixed stones:*** Mixture of cholesterol + bile pigments + calcium and other organic substances, dark brown and faceted, may be radiopaque.

Q.122. What are lipoproteins?

Lipoproteins are conjugated proteins. Lipid part is the prosthetic group and lipid free proteins are specific and are called **"apo-lipoproteins" (apo-proteins)**. Lipoproteins, serve as carrier of lipids in plasma.

Q.123. What is the composition of chylomicrons?
Chylomicron is composed largely of TG (87 to 88%) + about 8% PL, 3% free and esterified cholesterol and 0.5 to 2% of specific apoprotein called ***apo-B_{48}***.

Q.124. Where chylomicrons are synthesized? What happens after synthesis?
Chylomicrons are synthesized only in the intestinal walls. Size ranges from 0.075 to 1 mm (average 0.5 mμ in diameter). Chylomicrons pass out through the cell membrane of bases and lateral walls of intestinal epithelial cells, and moves through extracellular spaces of these cells to enter lymphatic vessels. Later goes to systemic circulation through the thoracic duct.

Q.125. What is the function of chylomicrons?
Chylomicrons are the principal carrier of ***exogenous*** TG obtained from diet.

Q.126. How does "nascent" chylomicrons differ from the circulating chylomicrons?
Nascent chylomicrons contain **"apo-B_{48}"** principally but may also have **"apo-A"**. During circulation, chylomicrons interact with HDL and take up **apo-C** and **apo-E**.

Q.127. What is lipoprotein lipase?
- ***Lipoprotein lipase (LPL)*** is an enzyme present on the walls of blood capillaries of many tissues like adipose tissue, lungs, heart, spleen, etc. where it is attached to capillary wall membrane with proteoglycan chains of heparan SO_4.
- Heparin and insulin activates LPL and it requires apo-C I and II, and PL for activity of the enzyme.

Q.128. Where very low density Lipoprotein (VLDL) is formed?
VLDL is synthesized principally **in liver**. A small amount is also formed in intestinal mucosal cells.

Q.129. What is the composition and function of VLDL?
- VLDL contains 90 to 93% of total lipids with approximately 50 to 56% of TG, 19% PL and 19% of free and esterified cholesterol and contains apoprotein ***apo-B_{100}***
- ***Functions:***
  - VLDL is the principal carrier of ***endogenous*** TG synthesized by liver cells.
  - **VLDL by degradation produces IDL and LDL.**

Q.130. How does nascent VLDL differ from circulating VLDL?
The ***nascent*** VLDL contains principally **apo-B_{100}** but circulating VLDL interacts with HDL and receives **"apo-C"** and **"apo-E"**.

Q.131. How chylomicrons are degraded?
Circulating chylomicrons while passing through different organs are acted upon by the enzyme ***lipoprotein lipase***. The enzyme hydrolyzes TG of chylomicrons and continued delipidation produces smaller particles rich in cholesterol and cholesterol esters called ***chylomicron remnants***. They are cleared by liver cells.

Q.132. How is VLDL degraded?
- Circulating VLDL while passing through different organs are also acted upon by the same enzyme ***lipoprotein lipase*** **(LPL)**, and hydrolyzes TG of VLDL. Delipidation of VLDL produces **smaller particles IDL** (Intermediate density lipoprotein), which corresponds to chylomicron remnants.
- IDL (VLDL remnant) is not taken up by liver cells. ***Further delipidation of IDL in circulation forms LDL.***

Q.133. How LDL is formed? Is it synthesized by liver/intestinal mucosal cells?
- LDL (Low density lipoproteins) are ***not synthesized by liver cells/or intestinal mucosal cells.***
- LDL are produced in circulation from VLDL. Continued delipidation of VLDL by ***lipoprotein lipase*** first produces IDL (VLDL remnants). Most of the IDL particles change into LDL particles by further delipidation of TG and by losing their "apo-E". This makes LDL richer in cholesterol (44 to 58% of cholesterol).

Q.134. What is the fate of circulating LDL? Why LDL-cholesterol is bad cholesterol?
- Circulating LDL principally ***catabolized in peripheral tissues viz., fibroblasts, hymphocytes, and arterial smooth muscle cells, in addition to liver.*** Plasma membrane of these tissues has specific "LDL-receptor-B_{100}", LDL rich in cholesterol interacts with the

"receptors" and deposits cholesterol ("Normal cholesterol transport"), apoprotein is hydrolyzed to amino acids.

- LDL cholesterol is ***bad cholesterol*** because it **deposits cholesterol** in smooth muscle cells of arteries and ***produces atherosclerosis. Increase LDL in blood is harmful and increases "risk" of myocardial infarction.***

Q.135. Where HDL is formed?

HDL is synthesized mainly in liver but also to some extent in intestinal mucosal cells.

Q.136. How does nascent hepatic HDL differs from nascent intestinal HDL?

- ***Nascent intestinal HDL*** contains only "apo-A". But while circulating it acquires apo-C and apo-E.
- On the other hand, ***nascent hepatic HDL*** contains both apo-A and apo-C.

Note: Apo-C and apo-E are only synthesized in liver and not in intestinal epithelial cells.

Q.137. What is meant by scavenging action of HDL? Why HDL-Cholesterol is called as good cholesterol?

- HDL plays a major role in the removal of cholesterol from peripheral extra-hepatic tissues and transport the cholesterol to the liver where it is metabolized to form bile acids. This action has been called as ***scavenging action*** of cholesterol ***(reverse cholesterol transport)***.
- HDL by "Scavenging" action prevents atherosclerosis because it removes cholesterol from smooth muscle cells of arteries.
- HDL also contains 41% of cholesterol, but this cholesterol is called ***good cholesterol.*** **Thus high *HDL is beneficial for health and reduces the "risk" of myocardial infarction.***

Q.138. How HDL level in blood be increased?

- Regular physical exercise like brisk walking and jogging.
- A little quantity of alcohol intake daily.
- Increased estrogen level in blood.

Q.139. Why young ladies are protected against myocardial infarction?

Young females have higher levels of HDL. This is due to the effects of increased estrogen level in young ladies which protect them from myocardial infarction.

Note: After menopause, as estrogen level decreases, they become prone to myocardial infarction.

Q.140. How LDL-cholesterol level can be decreased?

- Avoid animal fats and hydrogenated saturated oils (like Dalda).
- To take vegetable seed oils containing polyunsaturated fatty acids like sunflower oil, groundnut oil, and gingilee oil.
- Dietary fiber through black gram or defatted gingilee brings down LDL.
- If needed, ingestion of desiccated thyroid (5 grains/ day).

Q.141. Which apoprotein is bad for health?

- Apo-B_{100} makes bad cholesterol and bad for health.

Q.142. Which apoprotein is good for health?

- Apo-A makes good cholesterol and good for health.

Q.143. What is hyperlipoproteinemias? What are the types?

Increase levels of lipoproteins above normal is called hyperlipoproteinemia.

They are of **2 types:**

- ***Primary (Familial/hereditary):*** They are genetic disorders characterized by distinct clinical syndromes.
- ***Secondary (Acquired):*** Due to underlying disease processes usually thyroid, liver and renal diseases.

Q.144. What is modified HDL?

- Hypercholesterolemic patients could have a modified HDL i.e. HDLc rich in cholesterol. HDLc has only apo-E. The apo-E secreted by macrophages which ingest modified LDL, may be the source of HDLc.
- HDLc may be a major vehicle for the transport of cholesterol from macrophages of the vascular wall and from the tissues to the liver.

Q.145. What is Lipoprotein (a) or LP(a)? What is the significance?
LP(a) is seen only in some persons. In 40% population, there is no detectable level of LP(a) in serum. Only in 20% of population the LP(a) concentration in blood is more than 30 mg/dl.

- When present it is associated with LDL and attached to apo-B_{100} by a S-S bond.
- **It is highly atherogenic** and is associated with myocardial infarction in younger age group 30 to 40 years.

Q.146. What is HDL_3? What is its function?
HDL_3 is the spherical HDL and contains apo-D and apo-AII.

- It functions as the ***"Cholesteryl-ester transfer protein"*** and transfers some cholesteryl esters from HDL to VLDL, LDL and chylomicrons in the plasma. These lipoproteins then transfer these 'cholesteryl esters' to liver for degradation.
- HDL_3 has been further fractionated into 3a, 3b and 3c.

Q.147. What is HDL-2? How it is formed?
Cholesterol released from chylomicrons and VLDL, during ***"Lipoprotein Lipase"*** activity is accepted by circulating HDL. With HDL-bound LCAT, the latter esterifies cholesterol into cholesteryl esters in HDL and thus **maintains a low concentration of free cholesterol in HDL particles** enabling the transfer of more cholesterol into the latter.

- Thus **HDL-3 is changed to HDL-2** which is **richer in cholesteryl esters** and **very low in free cholesterol. HDL-2 is "good cholesterol".** It contains apo A-I.
- It has been further fractionated to 2a and 2b. **HDL-2b is the main anti-atherogenic fraction.**

Q.148. What is apo-J (apo-lipoprotein J)? State its function.
It is a glycoprotein a dimer found in association with HDL-2. Its molecular weight is approx 50,000.

- Two monomeric units are α and β. **α subunit** consists of 205 a.a. and **β-subunit** has 222 a.a. It is found in atheromatous plaques.
- apo-J has been found to inhibit macrophage-mediated cell damage and thus it is **anti-atherogenic** and offers

protection to endothelial and smooth muscle cells from injury **(Protective function).**

Q.149. What is HDLc? Mention its role in the body.

HDL_c has been recently described which is found in the blood of diet-induced hyper, cholesterolemia.

- HDLc is rich in cholesterol and its sole apoprotein is apo-E. It is taken up by the liver via the apo-E "remnant" receptor and also by LDL–receptors.

Q.150. Name the Five types of primary hyperlipoproteinemias indicating the possible metabolic defect and main lipoprotein change.

Types	*Name*	*Metabolic Defects*	*Main Lipoprotein changes*
I	***Familial Lipoprotein lipase deficiency***	• Deficiency of *lipoprotein lipase*	• Chylomicrons^{+++} (Hyperchylomicronemia).
II	***Familial hypercholesterolemia (FHC)***	• increased synthesis of apo-B↑ • Defective catabolism of LDL (defective LDL receptor).	• Total cholesterol↑ and • LDL↑
III	***Familial dysbeta-Lipoproteinemia (Broad β-disease)***	• increased synthesis of apo-B↑ and apo-E↑	• VLDL↑ • IDL↑ • Broad **β**-lipoprotein (Floating **β**-band)
IV	***Familial hypertriglyceridemia (FHTG)***	• increase of TG↑	• VLDL ↑ (Pre-β-lipoprotein)
V	***Combined hyperlipidemias***	• Increase in TG↑ and cholesterol↑	• Both VLDL and chylomicrons↑ • α and β lipoproteins↑

Q.151. State one inherited condition in which HDL is absent. Describe briefly.

- Familial **α**-lipoprotein deficiency called as ***Tangier's disease.*** The disease is characterized by deficiency of **α**-lipoprotein (HDL)↓
- ***Metabolic defect:*** may be reduction in apo-AI and AII.
- Accumulation of cholesterol esters in different tissues. ***Clinically hyperplastic orange-yellow tonsils and adenoids.***
- Electrophoretically—**a broad β band** is seen.

Q.152. What is atherosclerosis?

This is a condition associated with thickening and hardening of arterial walls (sclerosis) due to deposition of lipids specially cholesterol and cholesterol esters in the smooth muscle cells of arterial walls. Alongwith lipids there is deposition of complex carbohydrates, blood and blood products, calcium deposits and fibrous tissues which hardens the wall and narrows the lumen.

Q.153. State the conditions where marked atherosclerosis occur.

Atherosclerosis can occur in diseases with elevated levels of cholesterol↑ and LDL↑ and lowered levels of HDL↑. The various diseases which could cause marked atherosclerosis are:

- Diabetes mellitus
- Hypothyroidism
- Nephrotic syndrome
- Familial hypercholesterolemia (FHC, Type II).

Q.154. What is the importance of LDL cholesterol/HDL cholesterol ratio?

- A ratio of LDL cholesterol/to HDL cholesterol is important.
- Normal ratio is 3.0 to 3.6
- If the ratio is greater than 3.6, the "risk" of myocardial infarction is more. If the ratio is less than 3.0, the "risk" is less.

Q.155. What is xanthomatosis?

It means accumulation of lipids, usually cholesterol in subcutaneous tissues with large "foam cells". It appears as raised soft nodules. Xanthomas develop in:

- Diabetes mellitus
- von Gierke disease (Type I GSD)
- Hyperlipoproteinemias.

Q.156. What is the normal fat content of liver?

Normal liver contains about 4% as total lipids, 3/4 of which is PL, and 1/4 as TG.

Q.157. State the sources of fat in the liver.

- Influx of dietary lipids.
- Synthesis of FA from carbohydrates/and proteins.
- Mobilization of FA from depots to liver.

Q.158. What is fatty liver? What are the consequences?

- If the fat content of liver, specially TG, increases above normal, it is called fatty liver. The increase may go up to 30% or more, mainly TG.
- When the condition becomes chronic, fibrotic changes and extensive necrosis in parenchyma occur leading to cirrhosis liver and hepatic failure.

Q.159. State the clinical conditions where fatty liver occurs.

- Alcohol abuse: most common cause in India.
- Malnutrition:
  - Protein calorie malnutrition
  - Also deficiency of EFA and lipotropic agents
- Diabetes mellitus
- Obesity
- Hepatotoxins and drugs.

Q.160. What are lipotropic agents? What is meant by lipotropism?

Agents such as:

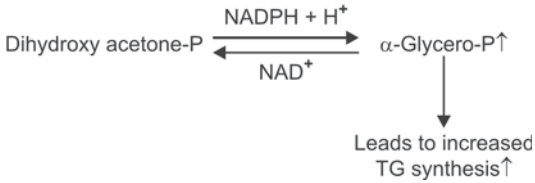
- Choline
- Methionine
- Betaine
- Inositol etc.

Which have the apparent effect of facilitating the removal of fat from liver and thus prevents accumulation of fats in Liver are called as ***lipotropic agents*** **(or "Lipotropins")**. The phenomenon is called as ***lipotropism***.

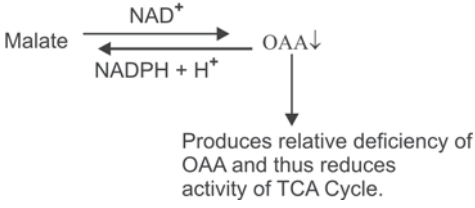
Q.161. Explain biochemically how excessive ethanol (alcohol) consumption produces fatty liver?

Due to ethanol oxidation in liver more NADH is produced. Hence ratio of $NADH + H^+/NAD^+$ increases↑. This leads to alterations in following metabolic reactions:

- ***Shift to the right*** of the following:



- ***Shift to Left*** of the following reaction:



Q.162. How carbon tetra chloride (CCl_4) produces fatty liver?

- Interferes with synthesis of apoprotein required to be incorporated in VLDL in liver
- Also affects sceretory mechanism of VLDL
- Can interfere with combination of TG with apoprotein
- Also mobilizes FA through release of catecholamines.

Q.163. State the sources of acetyl CoA in the body.

- Oxidative decarboxylation of pyruvic acid (PA).
- **β**-oxidation of FA
- Oxidation of ethanol (alcohol) by the enzyme ***alcohol dehydrogenase***.
- From catabolism of ketogenic amino acids, e.g. phenylalanine/tyrosine, tryptophan, leucine, isoleucine, lysine.
- Cleavage of citrate by ***citrate-cleavage*** enzyme ***(ATP-citrate lyase)***
- By Ketolysis acetoacetate + succinyl CoA, under the enzyme ***"thiophorase", (CoA transferase)*** produces acetoacetyl CoA, which splits to give acetyl CoA.

Q.164. State the fate of acetyl CoA in the body.

- Oxidation in TCA cycle to produce CO_2, H_2O and energy.
- Starting material for cholesterol biosynthesis.
- Starting material for formation of acetoacetate (ketone body) in liver (ketogenesis).
- Starting material for extramitochondrial "de Novo" synthesis of FA (palmitic acid).
- Mitochondrial chain elongation of FA, acetyl CoA, is directly added.
- Microsomal chain elongation of FA through malonyl CoA (acetyl CoA is added).
- Formation of acetyl choline.

- **Acetylation reaction (detoxication)** e.g.
  - Sulphonamides detoxicated to form N-acetyl derivative.
  - Bromobenzene detoxicated by acetyl CoA and cysteine.
- In melatonin synthesis: Formation of N-acetyl serotonin (melatonin) from serotonin.

Q.165. Name the tests used for detecting ketone bodies in urine.

- Rothera's test
- Gerhardt's test.

Q.166. What does Rothera's test detect?

Presence of:

- Acetoacetate and
- Acetone.

Q.167. Which test is more sensitive?

Rothera's test is more sensitive, positive (+ve) 1 in 40,000.

Q.168. What does Gerhardt's test detect?

- A positive test is given by acetoacetate only and ***not acetone.***
- It is less sensitive than Rothera's test, but it is simple to perform.

Q. 169. What is the fallacy with Gerhardt's test?

- If considerable phosphate is present in urine, a precipitate of ferric phosphates may be obtained. In that case, filter off and add a few drops of 10% ferric chloride again and observe for +ve purple color.
- **False +ve test is given by presence of salicylates in urine.** This can be differentiated from acetoacetate by their behaviour towards heat. On thoroughly boiling the urine, acetoacetate is converted to acetone by decarboxylation and the test becomes –ve. But in case of salicylates, it is not affected by heat and test shows +ve purple color.

Q.170. β-OH butyric acid is the prominent ketone body present. It is not detected by above two tests. How will you detect β-OH butyric acid in urine?

β-OH butyric acid is not detected by the above two tests. If it is desired to test the presence of **β**-OH butyric acid, it can be done after oxidizing to acetoacetate by H_2O_2. Rothera's test becomes positive.

CHAPTER

14 Metabolism of Proteins and Amino Acids

Q.1. What is meant by amino acid pool?

Amino acids in the blood are obtained from:

- ***From dietary proteins:*** absorption of amino acids from intestine.
- ***Breakdown of tissue proteins:*** both structural and functional.
- ***Synthesis of amino acids*** in liver mainly except essential amino acids.

Amino acids from all these sources get mixed up to constitute ***amino acid pool.*** It has no anatomical reality but represents an availability of amino acid building units. No functional distinction can be made between the fate of the amino acids derived from dietary source and those derived from tissue breakdown.

Q.2. What is meant by "circadian" changes in plasma amino acid levels?

The plasma levels of most amino acids do not remain constant throughout 24 hours period; but change by varying in a ***circadian*** rhythm, about a mean value. In general plasma amino acids levels are lowest at early morning and rise 15 to 35% by afternoon.

Q.3. What is the level of amino acids in blood?

- Blood amino acid level varies from 30 to 50 mg/dl in the postabsorptive state. In terms of amino acid N_2 it is 4 to 5 mg %.
- Following protein meal, the amino acid levels rise to 45 to 100 mg % (amino acid N_2 6 to 10 mg %).

Q.4. How tissues take up amino acids?

The amino acids are taken up by the tissues ***actively.*** Pyridoxal-P (B_6-PO_4) is required for the active transport.

Q.5. Name the hormones which favour tissue uptake of amino acids.

- Insulin
- Growth hormone (GH) and
- Testosterone favour the uptake of amino acids by tissues. Hence these hormones are called "anabolic" hormones.
- Estradiol stimulates selectively the uptake of a.a by uterus.
- Epinephrine and Glucocorticoids (GC) stimulate uptake of a.a by the liver only.

Q.6. What is meant by nitrogen balance or nitrogen equilibrium?

- In an adult healthy individual maintaining a constant weight, the amount of intake of 'N' in food (as dietary proteins mainly) will be balanced by an excretion of an equal amount of 'N' in urine (in the form of urea mainly, uric acid, creatine and to a small extent by amino acids) and
- In feces (mainly as unabsorbed N)
- Such an individual is then said to be in nitrogen balance or in nitrogen equilibrium.

Q.7. What is meant by positive nitrogen balance?

- If the amount of nitrogen consumed exceeds the amount of nitrogen lost, the person is said to be in ***positive nitrogen balance.*** This happens in:
  - Growing children
  - Pregnancy
  - Convalescence after illness/surgery, and
  - After administration of anabolic hormones.
- In positive nitrogen balance, the body puts on weight and N-intake will be greater than N-output since new cells are formed and N is retained as tissue proteins.

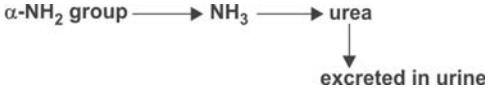
Q.8. What is meant by negative nitrogen balance?

- A subject whose intake of N is less than the output of N is said to have a ***negative nitrogen balance***. The person loses weight and the tissue proteins are catabolized to a

greater extent than they are formed. Such a situation occurs in:
- Old age
- Starvation
- Wasting diseases like tuberculosis
- Cancer
- Prolonged illness
- Postoperative conditions and burns.

Q.9. What non-protein nitrogenous substances are synthesized from amino acids? List them.
- ***Creatine*** from glycine, arginine and methionine.
- ***Heme*** from glycine and succinyl CoA.
- ***Melanins*** from tyrosine.
- ***Choline*** from serine through ethanolamine.
- ***Purine nucleotides*** from glycine with 5-Phosphoribosylamine.
- ***Pyrimidine nucleotides:*** from aspartate and carbamoyl phosphate.

Q.10. What happens to the N-Part of an amino acid?
In mammalian tissues, α-NH_2 group of amino acids, derived either from the diet or breakdown of tissue proteins, is first converted to NH_3 and then to urea and excreted in urine.



Humans are therefore called ***ureotelic.***

Q.11. What is the fate of α-NH_2 group of amino acid in other animals?
- Reptiles and birds produce uric acid in place of urea and they are called ***uricotelic*** organisms.
- Bony fishes produce NH_3 only and excretes it in urine. They are called ***ammonotelic*** organisms.

Q.12. What is transamination?
Transamination is a ***reversible reaction*** in which α-NH_2 group of one amino acid is transferred to an α-keto acid resulting in the formation of a new amino acid and a new ketoacid.

Q.13. Does formation of NH_3 take place in transamination reaction?

NH_3 formation does not take place in transamination reaction. The process represents only an ***"intermolecular" transfer of -NH_2 group without splitting out of N as NH_3.***

Q.14. Name the tissues in which transamination reaction occur.

Transamination takes place principally in

- Liver
- Kidney
- Heart
- Brain, but to a small extent transamination can take place in all tissues.

Q.15. Name the enzyme and co-enzyme required in transamination reaction.

- ***Enzyme: Transaminases*** (Now called as ***"aminotransferases)***
- ***Co-enzyme:*** Pyridoxal-P (B_6 - PO_4).

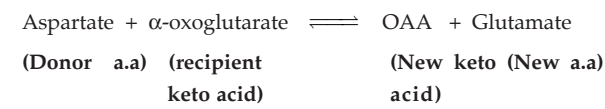
Q.16. Name the amino acids which do not participate in transmination reaction.

The amino acids which do not participate in transamination reaction are:

- Lysine
- Threonine
- Cyclic imino acids-Proline and OH-Proline.

Q.17. Name two specific transaminases which are of clinical importance. What are the substrates and end-products?

- ***Aspartate transaminase*** (or aspartate aminotransferase): Previously used to be called as "S-GOT"— serum glutamate oxaloacetate transaminase.



- ***Alanine transaminase*** (or Alanine aminotransferase): Previously used to be called as "S-GPT"— serum glutamate "pyruvate transaminase"

Alanine + α-oxoglutarate Pyruvic Acid + Glutamate

(Donor a.a) (recipient K.A) (New K.A) (New a.a)

Note: In both the cases, glutamic acid is produced as new amino acid.

Q.18. What are normal levels of S-GOT and S-GPT?

- **S-GOT** (aspartate transaminase): Normal serum activity is 4 to 7 I.u/L (7 to 35 Karmen units/ml).
- **S-GPT** (alanine transaminase): Normal serum activity is 3 to 15 I.u/L (6 to 32 Karmen units/ml).

Q.19. What is deamination? What are the types?

Deamination is the process by which N of amino acid is removedd as NH_3

2 types of demination:

- Oxidative deamination
- Non-oxidative deamination.

Q.20. What is the site of oxidative deamination?

- Liver and
- Kidney are the main organs where oxidative deamination takes place.

Q.21. What is the nature of enzyme and coenzyme that take part in oxidative deamination?

- ***Enzyme: L-amino acid oxidase*** which acts specifically on L-amino acids and oxidatively deaminate to form NH_3.
- ***Coenzyme:*** is Flavoprotein (FMN) which acts as H-acceptor.
- ***Nature of the enzyme:*** The ***L-amino acid oxidases*** are ***auto-oxidizable*** flavoproteins. The reduced flavoproteins FMN.H_2 are re-oxidized at substrate level directly by molecular O_2 **forming H_2O_2** which is converted to H_2O by the enzyme ***catalase***.

Q.22. What are the toxic substances that can form in oxidative deamination of L-amino acids by *"L-amino acid oxidase?*

- ***Superoxide anion O_2^-:*** This is scavenged by the enzyme ***superoxide dismutase.***
- ***H_2O_2:*** Scavenged by the enzyme ***catalase***.
  Both superoxide anion O_2^- and H_2O_2 are toxic and they can oxidize membrane proteins, lipids, -SH groups and methionine sulphur and produce harmful effects.

Q.23. What is the function of presence of *D-amino acid oxidase* in cells, when it is known there is absence of D-amino acids in cells?

There is no D-amino acids in the body tissues, hence it is not clear about the presence of ***D-amino acid oxidase*** in cells and its functions. Probably the dietary D-amino acids may be oxidized by ***D-amino acid oxidase*** and be isomerized through their keto acids to L-amino acids.

Q.24. Does oxidative deamination fulfil a major role in formation of NH_3 as:

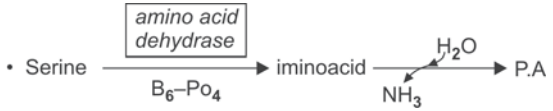
L-amino acid oxidase does not fulfil a major role in mammalian amino acid catabolism and formation of NH_3 as:

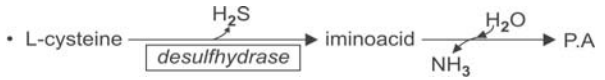
- It is restricted only to liver and kidney.
- Activity of the enzyme in these tissues is low.
- It does not have any effect on glycine or the L-isomers of the dicarboxylic acid or **β**-OH-**α**-amino acids.
- It does contribute to formation of NH_3 to some extent.

Q.25. What is Non-oxidative deamination? Give two examples.

Certain amino acids like OH-amino acids, histidine and S-containing a.a can be deaminated non-oxidatively **by specific enzymes** to form NH_3. They also do not fulfil major role in NH_3 formation.

- Two examples:





Q.26. State the principal method by which NH_3 is formed from L-amino acids.

- ***By transdeamination:*** It is combination of transamination + Deamination.
- The-NH_2 group of most L-amino acids are transferred to **α**-oxoglutarate by specific transaminases by the process

of transamination **forming L-glutamate** as end-product. L-glutamate is oxidatively deaminated by a specific Zn-containing enzyme ***glutamate dehydrogenase,*** of high activity and wide distribution, which requires NAD^+ or $NADP^+$ as coenzyme, to form NH_3.

- As the process involves first transamination and coupled with oxidative deamination, it is called as ***transdeamination***.

Q.27. List the sources of NH_3 in the body.

- Transdeamination—the major pathway.
- Oxidative deamination by ***L-amino acid oxidase***
- Non-oxidative deamination
- Absorption of NH_3 from gut produced by intestinal bacterial flora:
  - From dietary proteins
  - From urea present in fluids secreted into the gut
  - From uric acid secreted in bile (This assumes importance in intestinal obstruction and portocaval shunt)
- Pyrimidine catabolism (small fraction).

Q.28. What is the normal blood ammonia level?

- In man, normal blood level of NH_3 varies from 40 to 70 μg/dl.
- Free NH_4^+ (ammonium ion) concentration of fresh plasma is less than 20 μg/dl.

Q.29. Why NH_3 is toxic?

Exact cause is not known. The following associated biochemical changes are important:

- Increased NH_3 concentration enhnaces amination of **α**-oxoglutarate, thus decreasing the **α**-oxoglutarate, an intermediate of TCA cycle. This depresses TCA cycle affecting cellular respiration.
- Increased NH_3 concentration enhances glutamine formation from glutamate thus **reduces "brain cells pool" of glutamic acid,** which decreases formation of inhibitory neurotransmitter **"GABA"** (γ-amino butyric acid).
- Rise in brain glutamine level enhances the overflow of glutamine from brain which allows entry of tryptophan by the same "transporter". ***Hence tryptophan concentration in brain cells increases which leads to increased synthesis of serotonin.***

Q.30. What are the metabolic fate of NH_3 in the body?

- **Main fate:** toxic NH_3 is detoxicated to form nontoxic urea in liver by urea cycle and excreted in urine.
- **Formation of glutamine,** which acts as a reservoir of NH_3.
- **Amination** of **α**-keto acids to form **α**-amino acids.

Q.31. What is the other name of urea cycle?

- Krebs-Henseleit cycle.

Q.32. Why urea formation is called cyclic process?

One molecule of NH_3 and one molecule of CO_2 are converted to urea by Five sequential enzymatic reactions. In each turn of the cycle, Ornithine is regenerated in the last reaction which acts as a **catalytic agent** and repeats the cycle again. Hence it is a cyclic process.

Q.33. Which organ is associated with urea formation?

Liver is the only organ which can form urea and all the enzymes involved have been isolated from the liver tissue.

Q.34. Can other organs like kidney and brain synthesize urea?

- ***Kidney:*** **Can form upto arginine** but **cannot form urea** as enzyme ***"arginase"*** is absent in kidney tissues.
- ***Brain:*** Can synthesize urea if citrulline is available but lacks the enzyme ***ornithine transcarbamoylase*** which forms citrulline from ornithine.

Q.35. State the five steps of urea cycle indicating the enzymes involved and location of the enzyme.

		Enzyme	*Location*
• **Reaction 1 :**	Synthesis of Carbamoyl-P	Carbamoyl-P Synthetase I	Mitochondria
• **Reaction 2 :**	Synthesis of Citrulline	Ornithine transcarb-amoylase	Mitochondria
• **Reaction 3 :**	Synthesis of Arginino-succinate	Arginino-succinate Synthetase	Cytosol
• **Reaction 4 :**	Cleavage of Argininosuccinate	Arginino-Succinase	Cytosol
• **Reaction 5 :**	Cleavage of arginine to form urea and ornithine	Arginase	Cytosol

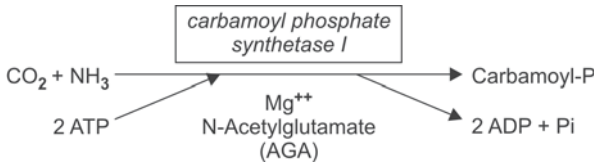
Q.36. What is the role of N-acetyl glutamate (AGA) in the first reaction in formation of carbamoyl-P?

N-acetyl glutamate (AGA) acts as an **allosteric activator** of the enzyme ***carbamoyl synthetase I,*** it brings about

conformational changes in the enzyme so that the second molecule of ATP can bind.

Q.37. Show schematically the first reaction, synthesis of carbamoyl-P. What are the starting materials? Is biotin necessary?

- **Starting materials:** CO_2 and NH_3
- **Biotin is not required.**



Q.38. How many amino acids take part in the urea cycle?

5 amino acids participate in urea cycle. They are:

- Ornithine
- Citrulline
- Glutamic acid
- Aspartic acid
- Arginine.

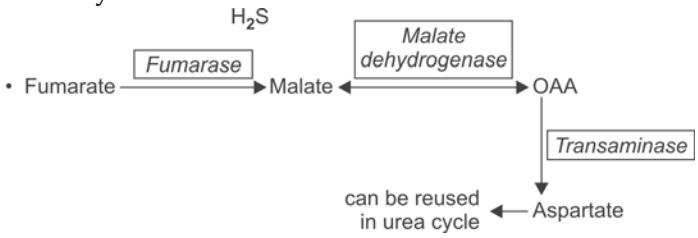
Q.39. What is the energy requirement in formation of one molecule of urea in urea cycle?

Three molecules of ATP are utilized for the formation of one molecule of urea. As one ATP is converted to AMP, it is equivalent to utilizing 4 high energy bonds.

Q.40. Urea contains two nitrogen (NH_2 groups), what are the sources of the two nitrogens?

- One nitrogen of NH_2 group is derived from the NH_4^+ ion (Reaction 1)
- Other nitrogen of NH_2 group is provided by aspartic acid (Reaction 3).

Q.41. How fumarate a by-product in fourth reaction can be converted to Aspartic acid required in the third reaction of urea cycle?



Q.42. What is the normal blood level of urea in the body?

- Normal level ranges from 20 to 40 mg%.
- Indians take less proteins in diet, hence in Indians normal level is taken as 15 to 40 mg%.

Q.43. In what conditions the blood level of urea increase:

Causes may be:

- Pre-renal
- Renal
- Postrenal

Q.44. Mention some pre-renal causes.

Conditions in which plasma volume/body fluid are reduced, e.g.:

- Salt and water depletion
- Severe and protracted vomiting as in pyloric/intestinal obstruction,
- Severe and prolonged diarrhea,
- Hemorrhage and shock,
- In burns.

Q.45. State some renal cuases:

- ***In acute glomerulonephritis.***
- In Type II nephrits (nephrotic syndrome)-in later stages.
- Chronic pyelonephritis.
- Malignant nephrosclerosis.
- Mercurial poisoning.

Q.46. Mention some post renal causes.

- Enlargement of prostate—benign and malignant
- Stone in urinary tract (urinary lithiasis)
- Stricture of urethra
- Tumors of the bladder affecting urinary flow.

Q.47. What is glutamine?

- Glutamine is chemically **δ**-amide of **α**-amino glutaric acid. Glutamine formation helps in removal of NH_3 which is toxic (detoxication of NH_3).
- Glutamine serves as an important reservoir of nitrogen in tissues which is readily available and can be drawn upon for various synthetic processes, when the body needs.

Q.48. How glutamine is synthesized in the body? Name the tissues involved in synthesis of glutamine.

Glutamine is synthesized in tissues like:

- Liver
- Kidney
- Brain and retina. It is formed from:
  - Glutamic acid and
  - NH_3 by the action of a mitochondrial enzyme ***"Glutamine synthetase"*** in presence of **ATP** and **Mg^{++}**.
- The reaction is **"irreversible"**, so that glutamine cannot reform glutamic acid and NH_3 by the same enzyme.

Q.49. What is the normal blood level of glutamine?

Normal blood level in a healthy adult ranges from 6 to 12 mg%.

Q.50. How NH_3 can be obtained from glutamine?

- Glutamine is **reservoir of NH_3** and **readily available source of NH_3.**
- Glutamine is hydrolyzed readily by a specific enzyme ***glutaminase***. The reaction is 'irreversible' and can occur in various tissues like liver, kidney, brain and retina.

Q.51. State some important functions of glutamine in the body.

- ***Transamidation:*** The amide N of glutamine can be transferred to keto-group of fructose-6-P to form glucosamine-P.
- ***Formation of guanylate (GMP).***
- ***Role of glutamine in kidney:*** in conservation of Na^+ ($NH_4 \rightarrow Na^+$ exchange)
- ***Role of glutamine in brain:*** Brain tissues cannot detoxicate NH_3 to form urea. Excess NH_3 entering brain, e.g. in hepatic failure is detoxicated by Glutamine formation.
- **Role of Glutamine in conjugation reaction** (Detoxication).
- **Role in cancer:** Certain tumors exihibit abnormally high requirement of glutamine/asparagine for their growth.

Q.52. What is the normal level of glutamine in CS fluid in health?

- Normal range of CSF glutamine is 6 to 14 mg%.

Q.53. What are the clinical manifestations of ammonia intoxication?

The symptoms of NH_3 intoxication include:

- A peculiar flapping tremor
- Slurring of speech
- Blurring of vision
- In severe cases lead to coma and death.

Q.54. How much urea is excreted daily in urine?

- 20 to 30 gm in 24 hours urine.

Q.55. Name the inherited disorders associated with urea cycle.

- Hyperammonemia Type I
- Hyperammonemia Type II, (also called ornithinemia)
- Citrullinemia
- Arginino-succinic aciduria
- Hyperargininemia.

Q.56. What are the fates of C-skeleton of amino acids after removal of NH_3 ?

The fates are:

- **Reamination** to form amino acid e.g. PA → Alanine, OAA → Aspartic acid, **α**-oxoglutarate → Glutamic acid.
- **Formation of** CO_2, H_2O and energy, after entering TCA cycle.
- **Formation of glucose** (glucogenic), or ketone bodies (ketogenic) or both.

Q.57. How much protein is potentially glucogenic?

It has been shown by experimental studies that 60 gm (approximately 58 gm) of glucose are formed and excreted in urine for 100 gm of proteins metabolized. Thus ***60% of proteins (amino acids) is potentially glucogenic.***

Q.58. Name at least ten amino acids which are glucogenic.

Glycine, alanine, serine, cysteine/cystine, threonine methionine, proline, valine, arginine, glutamate.

Q.59. Name the only amino acid which is ketogenic.

- L-leucine.

Q.60. Name at least three amino acids which are both glucogenic and ketogenic.

- Phenylalanine/tyrosine, tryptophan, isoleucine.

Q.61. What is decarboxylation reaction?

Decarboxylation is the reaction by which CO_2 is removed from the -COOH group of an amino acid resulting to formation of a biogenic amine. the reaction is catalyzed by the enzyme *decarboxylase* which requires pyridoxal-P (B_6-PO_4) as coenzyme.

Q.62. Name at least five biogenic amines of clinical importance. State the amino acid from which it is derived and mention biological importance in the body.

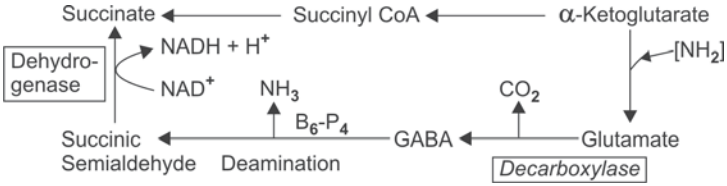
Biogenic amine	*Name of the amino acid*	*Biologic importance*
• **Histamine**	Histidine	• Vasodilator, B.P↓ • HCL↑ • Pepsin↑, • Liberated in anaphylactic reaction
• **γ-amino butyric acid (GABA)**	Glutamic acid	• Presynaptic inhibitor in brain • Forms a by-pass in TCA cycle (GABA shunt)
• **Taurine**	Cysteic acid (derived from cysteine)	• Constituent of bile acid (taurocholic acid)
• **Tryptamine**	Tryptophan	• Tissue hormone, a derivative of 5-OH tryptamine (serotonin) • Vasoconstriction, BP↑
• **Ethanolamine**	Serine	• Forms choline • Constituent of PL like cephalin.

Q.63. State the biogenic amines produced by putrefaction in the gut by intestinal bacterial action.

- ***Cadaverine:*** derived from lysine.
- ***Putrescine:*** derived from ornithine.
- ***Agmatine:*** derived from arginine.

Q.64. What is GABA shunt? Show schematically.

GABA by its conversion to succinic acid can form a **"by-pass" in TCA cycle** and this is called as **GABA shunt.**



Q.65. Give an example of decarboxylation which occurs spontaneously without an enzyme.

- Formation of acetone from acetoacetate

Q.66. Name the polyamines.

- Spermidine
- Spermine and
- Their precussor putrescine.

Q.67. Name the amino acids required in polyamine synthesis.

- ***Ornithine:*** The starting amino acid
- ***Methionine:*** Required as S-adenosyl methionine ("active" methionine).

Q.68. State clinical significance of polyamines.

- ***Increased urinary polyamine excretion claimed to be characteristic of malignant diseases.*** It has been reported to be increased in:
  - Leukemias
  - Carcinoma of ovaries, lungs, colon, rectum, prostate, GI tract, kidney, bladder and testes.
- ***Spermidine is the best "marker" of tumor cells destruction.***
- ***Putrescine is the best "marker" for cell proliferation.***

Q.69. What is carbamoyl-P synthetase II? What is its function?

Carbamoyl-P-synthetase II is an enzyme present in cytosol. It can form carbamoyl-P from CO_2 and amide-N of glutamine, which is used in pyrimidine synthesis.

Q.70. What are the aromatic amino acids? Name them, which is essential and which is not.

- Aromatic amino acids are:
  - Phenylalanine and
  - Tyrosine
- Phenylalanine is essential and must be provided in the diet
- Tyrosine is not essential as it can be formed from phenyl alanine in the Liver.

Q.71. What are the enzymes and coenzymes/cofactors required for conversion of phenylalanine to tyrosine?

- **Enzyme:** ***phenylalanine hydroxylase*****.**
- **Coenzymes/cofactors:**
  - Molecular O_2
  - NADPH
  - Fe^{++} and
  - FH_4 (Tetrahydrobiopterin).

Q.72. State how phenylalanine is converted to tyrosine in the body.

Reactions occur in two stages:

- ***Stage 1:*** Reduction of molecular O_2 to H_2O and conversion of Phenylalanine $\longrightarrow$ to tyrosine. Reduced from of folic acid $F.H_4$ acts as H-donor to molecular O_2 and is converted to $F.H_2$. In this stage, there is incorporation of one atom of molecular O_2 to p-position of phenyl alanine to form tyrosine, while other atom of O_2 is reduced to H_2O.
- ***Stage 2:*** Reduction of $F.H_2$ to reform $F.H_4$ takes place by NADPH, acting as a H-donor, catalyzed by the enzyme ***dihydrobiopterin reductase*****.**

Note: Both the reactions are irreversible.

Q.73. What is meant by sparing action?

The feeding of tyrosine/or cysteine decreases the need of phenylalanine/or methionine in the diet respectively. This is called "sparing" action. Phenylalanine/or methionine is spared from synthesis of tyrosine/or cysteine respectively.

Q.74. Tyrosine is a non-essential amino acid. In which condition it becomes an essential amino acid?

In phenylketonurias, where phenylalanine cannot be converted to tyrosine in the body due to inherited absence

of the enzyme ***phenylalanine hydroxylase***, tyrosine becomes an essential amino acid to the patient.

Q.75. Enumerate the metabolic role/or biomedical importance of tyrosine in the body.

Though tyrosine is a non-essential amino acid, it is of great metabolic importance as many ***"biologically" important compounds are formed from tyrosine.*** They are:

- ***Synthesis of thyroid hormones:*** Thyroxine (T_4), tri-iodo thyronine (T_3) and "reverse" T_3.
- ***Synthesis of catecholamines:*** Epinephrine and norepinephrine.
- ***Synthesis of melanin*** pigments in skin.
- ***Formation of biogenic amine*** tyramine.
- ***Formation of phenol and cresol.***
- ***Formation of tyrosine-o-sulphate,*** a constituent of fibrinogen molecule.

Q.76. What is phenylketonuria?

Phenylketonuria is an inherited disease due to inherited deficiency of the enzyme ***phenylalanine hydroxylase.***

- Due to the deficiency of the enzyme, phenylalanine cannot be converted to tyrosine resulting to accumulation of phenylalanine in blood and tissues. Phenylalanine undergoes transamination producing,
- Phenyl pyruvic acid
- Phenyl lactic acid
- Phenyl acetic acid

Which are excreted in urine, hence called as phenyl ketonuria.

Q.77. Why the urine of phenylketonuric patient has a mousy odour?

Phenyl acetic acid is conjugated with glutamine and excreted as ***phenyl acetyl glutamine*** **in urine which is responsible for the "mousy" odour.**

Q.78. What other biochemical changes occur due to accumulation of phenylalanine in phenylketonurics?

Accumulation of phenylalanine leads to:

- Defective serotonin formation
- Impaired melanin synthesis that is the reason for fair skin and hairs

- Excretion of phenylalanine into the intestine competes with tryptophan for absorption. Tryptophan becomes subject to action of intestinal bacteria resulting in formation of indole derivatives which are absorbed and excreted in urine.

Q.79. What are the clinical presentation of a phenylketonuric child?

Child is ***mentally retarded***. May have other features like, eczema, psychoses and seizures.

Q.80. What treatment you will suggest for a phenylketonuric child?

Child should be given diet with very low or absent phenylalanine. The diet can be terminated after six years of age, when high concentration of phenylalanine and its derivatives are not injurious to brain.

Q.81. A lady reports to you with the complaints her baby's napkin soiled with urine turns black on exposure to air. Which disease you will suspect?

• Alkaptonuria.

Q.82. What is alkaptonuria? What is the enzyme deficiency?

Alkaptonuria is an **inherited disorder** of phenylalanine/tyrosine metabolism in which homogentisic acid, a metabolite in the metabolic pathway, cannot be oxidized to Maleylacetoacetate due to the inherited deficiency of the enzyme ***homogentisate oxidase.***

Q.83. Why the urine becomes dark on standing in alkaptonuria?

Urine of alkaptonuric patient turns black gradually from top to down when exposed to air and on standing. Homogentisic acid excreted in urine gets oxidized to **black pigment (alkapton).**

Q.84. How will you produce alkaptonuria experimentally?

Experimental alkaptonuria can be produced by administering "**α-α'**-dipyridil", a chelating agent that binds strongly to Fe^{++} of the enzyme ***homogentisate oxidase*** and inhibits the reaction i.e. conversion of homogentisic acid to maleyl acetoacetate. Thus homogentisic acid accumulates and appears in urine.

Q.85. What is ochronosis?

In longstanding cases of alkaptonuria, ***homogentisic acid derivatives get deposited in cartilages*** of ears, nose and other exposed parts of the body leading to generalized pigmentation of connective tissues. Also gets deposited in joints leading to arthritis. This condition is called ochronosis.

Q.86. What is the mechanism of ochronosis?

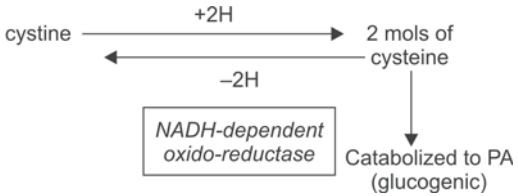
Exact mechanism is not known. Probably the homogentisic acid gets oxidized by ***polyphenol oxidase*** forming benzoquinone acetate, which polymerizes and binds to connective tissue macromolecules.

Q.87. What is albinism?

It is an **inherited disorder** of tyrosine metabolism in which the enzyme ***tyrosinase*** is lacking resulting to non-formation of melanin pigment for which the hair, skin and retina of eye are not black.

Q.88. What is the relationship between cysteine and cystine?

- Two molecules of L-cysteine oxidized to give one molecule of cystine which is joined by S–S–bond.
- One molecule of cystine is reduced to form two molecules of cysteine.



Q.89. What are the end-products of methionine degradation? What are the fate of end products?

End products are:

- ***Propionyl CoA:*** Converted to succinyl CoA through formation of Methyl malonyl CoA (glucogenic).
- ***α-amino butyric acid:*** Excreted in urine.

Q.90. What is active methionine? What is it known as chemically?

- **"Active methionine"** is an active compound in which CH_3 group is ***biologically labile*** and it acts as a methyl

(CH_3) group donor to a suitable "acceptor" so that it can form biologically important compounds.

- Chemically "active methionine" is ***S-adenosyl methionine***.

Q.91. How active menthionine is formed?

Activation of methionine occurs in presence of ATP, catalyzed by the enzyme called ***"L-methionine-adenosyl transferase"***. It requires presence of Mg^{++} and G-SH. In the process of activation, ATP donates the entire adenosine moiety to methionine and loses three molecules of PO_4, one as orthophosphate and two as pyrophosphates.

Q.92. Why methyl group in active methionine is labile and can be donated easily?

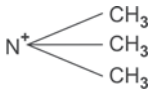
In **"active" methionine**, CH_3 group forms a ***"high energy" bond with sulphur (-S ~ CH_3)*** and ***not*** with carbon. This attributes to the lability of the CH_3 group which can be easily donated to a suitable acceptor.

Q.93. What is meant by transmethylation reaction?

Certain compounds of the body, with structures containing CH_3 group attached to an atom other than carbon can take part in enzymic reactions, whereby these CH_3 groups are transferred to a suitable "acceptor" which have no methyl group. Such reactions are called as ***transmethylation reaction***.

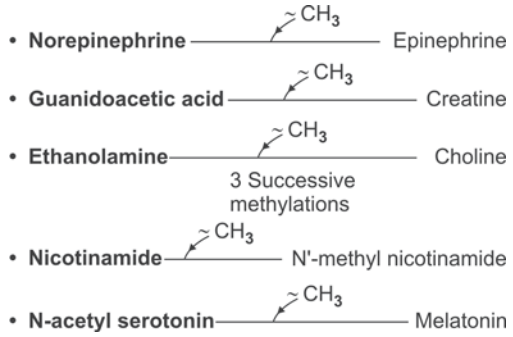
Q.94. Name the compounds which possess biologically labile methyl group.

- **Active methionine (S-adenosyl methionine):** containing -S ~ CH_3 group.
- **Choline:** containing -CH_3 groups attached to N as follows:



- **Betaine:** an oxidation product of choline.

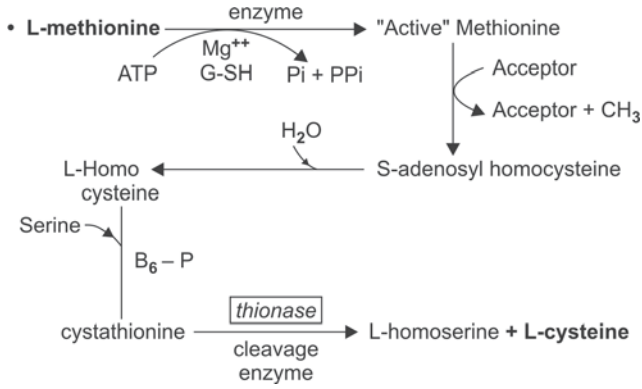
Q.95. Give Five examples of transmethylation reactions, where active methionine acts as methyl donor.



Q.96. Enumerate the metabolic role or biomedical importance of L-methionine.

- Methionine is **glucogenic**.
- Methionine is converted to L-cysteine in the body.
- ***Lipotropic function:*** provides choline, a lipotropic agent, by methylations of ethanolamine.
- **Transmethylation reactions:** by forming active methionine.
- **In polyamine synthesis.**
- **Formation of methyl mercaptan** in liver diseases, which accounts for foul odour in breath (foetor hepaticus).

Q.97. Show schematically how L-cysteine is formed from L-methionine.



Q.98. What contributes to C-skeleton and sulphur of L-cysteine?

- Sulphur of methionine is directly contributed to formation of L-cysteine.
- Carbon skeleton is derived from the amino acid serine.

Q.99. What is the end product of L-cysteine catabolism?

- Pyruvic acid (Thus it is glucogenic).

Q.100. Enumerate the metabolic role/or biomedical importance of cysteine.

- Cysteine is **glucogenic.**
- **Formation of glutathione.**
- **Formation of taurine** which is required for formation of taurocholic acid.
- **Formation of mercaptoethanolamine** by decarboxylation which is an important constituent of CoA-SH.
- **Role in detoxication:** involved in detoxication of aromatic compounds and forms mercapturic acids which are excreted in urine.
- Cysteine is an important constituents of **Scleroproteins.** It is present in proteins of nails, hairs, hoofs, and keratin of skin.

Q.101. Differentiate cystinuria and cystinosis.

Both are inherited disorders of cysteine metabolism.

Cystinuria	*Cystinosis*
• Due to renal transport defect.	• Also called as "cystine storage disease.
• Excretion of cystine in urine increases 20 to 30 times of normal.	• Defect: impaired conversion of cystine to cysteine (deficiency of ***"cystine reductase"***).
• Also there is increased excretion of dibasic amino acids: – Lysine • arginine, and – ornithine	• Widespread deposition of cystine in various tissues like liver, spleen, kidney, bone marrow, lymphnodes, and cornea.
• May form cystine stones.	• Cystine accumulates in lysosomes of cells of RE system.

Q.102. What is homocystinuria? What is the enzyme deficiency?

- An **inherited** disorder of metabolism of L-methionine or more specifically its metabolic intermediates homocysteine/or homocystine.

- ***Enzyme deficiency: cystathionine synthetase*** enzyme deficiency leads to accumulation of homocystine which is excreted in urine.

Q.103. State the salient clinical features of a case of homocystinuria.

- ***Mental retardation*** in children and surviving adults.
- Enlargement of liver ***(hepatomegaly)***.
- Affected individuals are extra-ordinarily tall, with long extremities frequently with flat feet with toes out ***("Charlie-Chaplin" gait).***
- Skeletal deformities involving spinal vertebrae (X-ray shows ***"cod-fish" appearance***)
- Dislocation of lens of the eye ***(Ectopia lentis).***
- ***Life threatening arterial/venous thrombosis.***

Q.104. State the metabolic role of glycine (aminoacetic acid).

Though glycine is the simplest and non-essential amino acid, but it is of great importance as it forms many biologically important compounds in the body. They are:

- **Synthesis of heme**
- **Synthesis of purine nucleus,** it contributes C_4, C_5 and N_7 of purine ring
- **Synthesis of glutathione**
- **Synthesis of creatine**
- ***Conjugation reaction:*** detoxicates benzoic acid to form hippuric acid which is excreted in urine.
- Formation of glycocholic acid **(bile acid).**
- ***Glucogenic:*** Glycine is converted to serine which is converted to PA (glucogenic).
- Source of **oxalate** and **formate** (one carbon metabolism).

Q.105. Name the histidine compounds present in the body.

- ***Histamine:*** a biogenic amine formed by decarboxylation.
- ***Ergothioniene:*** Present in RB cells and liver. Ruptured red cells liberate ergothioneine which can reduce Benediet's qualitative reagent.
- ***Carnosine:*** a dipeptide of histidine and **β**-alanine.
- ***Anserine:*** a methyl derivative of carnosine.

Q.106. What is glutathione?

Glutathione is a **tripeptide** of three amino acids: • Glutamic acid, • Cysteine and • Glycine (Glutamyl-cysteinyl-glycine).

Q.107. State five metabolic role/or biomedical importance of glutathione in the body.

- Reduced Glutathione (G-SH) is necessary for maintaining ***integrity of red cell membranes*** and ***lens protein.***
- It ***helps to destory*** H_2O_2 and other peroxides in cells. The reaction is catalyzed by selenium containing enzyme ***glutathione peroxidase.***
- ***Acts as coenzyme*** with liver enzyme ***glutathione-insulin transhydrogenase*** which helps in catabolism of Insulin.
- Glutathione takes part in **"γ-glutamyl cycle"** for absorption of amino acids from gut.
- Reduced glutathione (G-SH) is required as **coenzyme/ cofactor** for ***PG synthetase system*** in PG synthesis and also for formation of "active methionine".

Q.108. What is serotonin?

Serotonin is chemically 5-OH tryptamine (5-HT), and it is formed from amino acid tryptophan. It is present in blood and is produced in tissues like gastric mucosa, intestine, brain, mastcells and platelets. (Probably stored in Platelets?)

Q.109. State the cells that produce serotonin.

Serotonin is produced by special cells present in serotonin producing tissues, called as serotonin-producing cells" (also called ***argentaffin cells*** or ***Kultchitsky's cells***).

Q.110. How much tryptophan is converted to serotonin normally?

Only 1% of tryptophan is utilized for serotonin synthesis normally.

Q.111. How Serotonin is synthesized by the serotonin-producing cells in the body?

- Tryptophan is first hydroxylated to form 5-OH tryptophan in liver by ***hydroxylase*** enzyme in presence of O_2 and NADPH.
- 5-OH tryptophan is next **decarboxylated** by the enzyme ***decarboxylase*** to form 5-OH tryptamine (5-HT), called serotonin, the reaction requires B_6-P as coenzyme.

Q.112. What are the functions of Serotonin?

- It is a potent **vasoconstrictor,** increases B.P↑
- Produces **contraction of smooth muscles.**
- **Stimulates cerebral activity↑ (excitation).** Deficiency of serotonin in brain produces depressed activity (Depression).

Q.113. Name the enzyme which degrades serotonin. What is the degradation product?

- Enzyme is ***monoamine oxidase*** **(MAO)**
- Degradation product is 5-OH-Indole acetic acid (5-HIAA) which is excreted in urine.

Q.114. In which condition excessive amount of serotonin is produced in the body?

- Excessive amount of serotonin is produced in ***carcinoids***. a malignant tumour of serotonin-producing cells. It is also called as ***argentaffinoma***.
- The clinical features associated with it is called as ***carcinoid syndrome***.
- ***In carcinoids, 60% of tryptophan is utilized for serotonin formation*** as compared to 1% in normals.

Q.115. State the clinical features seen in carcinoid syndrome.

Symptoms are mainly due to excessive production of serotonin and its effects on smooth muscle. The clinical features are:

- Cutaneous vasomotor episodes of "flushing"
- Occasionally cyanotic appearance
- Chronic diarrhea
- Respiratory distress and bronchospasm
- Some may have right-sided heart failure. Serotonin passing through lungs is destroyed by "MAO" hence left side is not affected.

Q.116. What is melatonin?

- Chemically melatonin is "N-acetyl-5-methoxy serotonin". It is a hormone produced by the pineal gland and peripheral nerves of man and some other higher animals.
- The hormone lightens the color of melanocytes in the skin of frog and blocks the action of MSH (Melanocyte stimulating hormone) and ACTH.

Q.117. What is Hartnup's disease?

- It is an **inherited disorder** associated with the metabolism of tryptophan. The clinical symptoms are **mental retardation,** intermittent cerebellar ataxia and skin rash.
- Urine of patients with Hartnup's disease contains increased amounts of indoleacetic acid and tryptophan.

Q.118. Why it is called as Hartnup's disease?

The disease is ***named after the Hartnup family*** in which it was discovered.

Q.119. What is the metabolic defect in Hartnup's disease?

- An inherited deficiency of the enzyme ***tryptophan dioxygenase*** in liver.
- Could also be due to a defective indole transport and consequent impairment of tryptophan metabolism.

Q.120. What is the relationship between creatine-P and creatinine?

- Both are nonprotein nitrogenous compounds.
- Creatinine-P is chemically methyl guanidino acetic acid".
- Creatinine is ***anhydride of creatine***, the reaction is **irreversible** and **non-enzymatic.** Creatinine has a ring structure.

Q.121. In which tissues creatine is found and in what form?

- Creatine is a normal constituent of the body and is present principally **in muscles,** also found in brain, liver, testes and blood.
- It can occur in "free" from and mainly as "phosphorylated" form called creatine-P (also called as **phosphocreatine** or **phosphagen**).

Q.122. How much creatine is present in the body?

- Total amount in an adult human is approximately 120 gm.
- Of the total amount, 98% is present in muscles, of which 80% occurs in phosphorylated form, 1.3% in nervous tissues including brain and 0.5 to 0.7% in other tissues.

Q.123. In what form creatine is excreted in urine in normal health?

- As creatinine and NOT creatine.

Q.124. What is blood and plasma level of creatine?

- **In whole blood:** Creatine level varies from 2.0 to 7.0 mg%.
- **In plasma:** It is less than 1 mg%.
  - In males: It varies from 0.2 to 0.6 mg%.
  - In females: 0.35 to 0.9 mg%.

Q.125. What is the normal creatinine level in whole blood?

In whole blood creatinine level varies from 1.0 to 2.0 mg%. Creatinine is evenly distributed in blood and plasma.

Q.126. What is the urinary excretion of creatinine in normal health:

Urinary excretion of creatinine in normal health is as follows:

- In males: 1.5 to 2.0 gm in 24 hours urine.
- In females: 0.8 to 1.5 gm in 24 hours urine.

Q.127. Name the amino acids required for creatine synthesis in the body.

Three amino acids are required:

- Glycine
- Arginine
- Methionine.

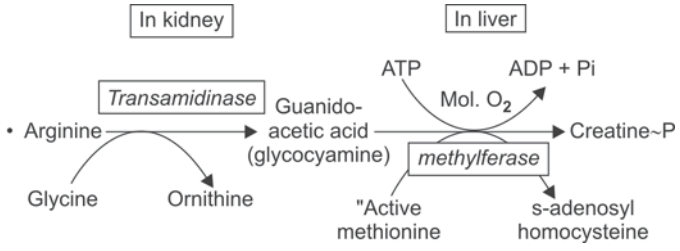
Q.128. Which are the starting amino acids?

- Arginine and
- Glycine.

Q.129. What are sites of synthesis of creatine in the body?

- First reaction occurs in **kidney**. The product formed is guanidoacetic acid (glycocyamine), which passes to blood.
- Second reaction occurs **in liver** where creatine-P is formed, which goes from liver to muscles and stored there.

Q.130. Show schematically the biosynthesis of creatine.



Q.131. Where does the formation of creatinine occur?

- In Liver and in Kidney.

Q.132. What is meant by transamidination?

Transfer of an amidine group $NH = \overset{NH_2}{\overset{|}{C}}$, from an amino acid to another amino acid, e.g. from arginine to glycine under the influence of an enzyme ***transamidinase*** to form guanidoacetic acid (also called glycocyamine).

Q.133. What is creatinuria? List the conditions where creatinuria occur?

Excretion of creatine in urine is called creatinuria.

Creatinuria can occur under following conditions:

- In children: Probably due to lack of ability to convert creatine to creatinine.
- Adult females: in pregnancy and maximum after parturition (2 to 3 weeks).
- **Thyrotoxicosis:** Probably due to associated thyrotoxic myopathy.
- Muscular dystrophies, in myositis and in myasthenia gravis
- Wasting diseases, e.g. in malignancies
- In febrile conditions
- Starvation
- Subjects lacking carbohydrates in diets and in diabetics.

Q.134. What is creatinine coefficient?

- It is the ratio of total creatinine in 24 hours urine in mg and total body weight in kg. Thus,

$$= \frac{\text{mg of creatinine in 24 hours urine}}{\text{body weight in kg}}$$

- The value is 20 to 26 for males and 14 to 22 in females.

Q.135. What is Jaffe's reaction?

- When serum is treated with alkaline picrate solution a red color develops. It is called as ***Jaffe's reaction.***
- The color is read against a "standard" similarly treated in a colorimeter and calculated to get the serum creatinine value.

Q.136. What is the fallacy with Jaffe's reaction?

Serum creatinine estimation by Jaffe's reaction ***does not give "true" creatinine value***. It measures also certain "non-creatinine chromogens", up to 20% in blood and 5% in urine.

Q.137. How will you exclude the chromogens from estimation and get "True" creatinine value?

For excluding the chromogens and to get "True" creatinine after precipitating proteins, creatinine is adsorbed on to Lloyd's reagent (Fuller's earth), a hydrated aluminium silicate, and then color developed with alkaline picrate and measured.

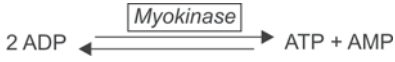
Q.138. What is Löhman reaction?

- Creatinine ~ P is the reservoir of energy in muscles. When muscles contract, energy is derived from breakdown of ATP to ADP and Pi. ATP must be reformed quickly to supply the energy.

- The high energy phosphate of creatine-P is transferred to ADP and ATP is reformed. This reaction is called as ***Löhman reaction*** and it takes place during muscle activity.

Q.139. What is myokinase reaction?

Two ADP molecules react to produce one molecule of ATP and AMP, the reaction is catalyzed by the enzyme ***myokinase (adenylate Kinase)***.



Q.140. What is meant by creatinine clearance test.

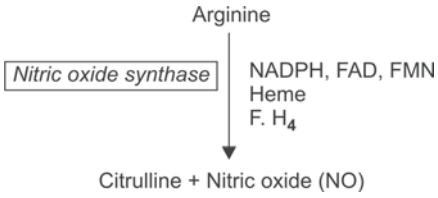
Endogenous creatinine clearance is used as a renal function test. At normal levels of cretinine in the blood, this metabolite is filtered at the glomerulus but neither secreted nor reabsorbed by the tubules. Hence its clearance measures the glomerular filtration rate (GFR).

Q.141. What is the normal endogenous clearance?
Normal values for creatinine clearance varies from 95 to 105 ml/mt.

Q.142. What are the biological importance and metabolic role of arginine?
- Semiessential amino acid required in growing children, in pregnancy and lactation
- Required for creatine synthesis
- Required in urea formation (an intermediate metabolite)
- Tissue protein formation
- Glucogenic amino acid.
- Formation of Nitric oxide (NO).

Q.143. Is nitric oxide found in our body?
Yes, it is found in our body. It is formed from the amino acid arginine.

Q.144. How nitric oxide (NO) is formed in the body?
Arginine is acted upon by an enzyme called ***"Nitrogen oxide synthase",*** a cytosolic enzyme which converts arginine do citrulline and nitric oxide.



Q.145. State the mechanism of the action of the enzyme.
- Nitric oxide synthase catalyzes a five electron oxidation of an amidine nitrogen of arginine.
- **Cofactors** required for the oxidation are NADPH, FAD, FMN, heme and $F.H_4$.

Q.146. Name the isoenzymes of Nitric oxide synthase enzyme (NOS).
Three isoenzymes have been isolated and described. They are:
- ***Endothelial NOS (eNOS)***
- ***Neuronal NOS (nNOS)***
- ***Macrophage NOS (iNOS)***

Q.147. Mention some important functions of Nitric Oxide (NO) in the body.

- Acts as a vasodilator and causes relaxation of smooth muscle.
- It has important role in the regulation of blood flow and maintenance of blood pressure.
- Inhibits adhesion, activation and aggregation of platelets.
- Acts as a neurotransmitter in the brain and peripheral autonomic nervous system.

Q.148. State the mechanism of action of Nitric oxide (NO).

Vasodilatation effect of Nitric oxide (NO) and inhibition of Platelets aggregation are mediated through increased cGMP↑ and protein kinase activity.

Q.149. What is EDRF? Has it any relation with Nitric oxide?

- EDRF is endothelium derived relaxing factor which produces vasodilatation.
- It is claimed that EDRF is same as Nitric oxide (NO).

Q.150. Mention some inhibitors of NO and NO synthase.

Inhibitors are as follows:

- Nitric oxide (NO) is inhibited by hemoglobin and other heme proteins which bind it tightly.
- **ADMA** (Asymmetric dimethyl arginine): an endogenous arginine analogue may function as a competitive inhibitor of NO synthase enzyme.

Q.151. State three clinical application of Nitric oxides.

- ***Nitroglycerine:*** The important coronary artery vasodilator used in Angina Pectoris acts to increase intracellular release of NO.
- ***In eclampsia and pre-eclampsia:*** The hypertension is due to increased production of NO due to probably formation of ADMA, which acts as competitive inhibtor of enzyme "NO synthase".
- ***In septic shock:*** Bacterial lipopolysaccharide present in blood causes uncontrolled production of NO leading to dilatation of blood vessels and lowering of BP.

Q.152. What are the metabolic role/or biomedical importance of threonine?

- An essential amino acid, must be provided in the diet.
- Required for tissue protein formation.

- By non-oxidative deamination forms **α**-keto butyric acid, which on oxidative decarboxylation gives Propionyl CoA (glucogenic).
- Cleaved by the enzyme ***threonine aldolase*** to form glycine and acetaldehyde.
- It can be converted to PA (thus glucogenic).
- It is a -OH amino acid like serine and acts as PO_4 carrier.

Q.153. What are the metabolic role/or biomedical importance of serine?

- Serine produces PA **(glucogenic)**.
- Tissue protein formation.
- "Carrier" of PO_4 group.
- Serine contributes to C-skeleton to form cysteine from methionine.
- Serine on decarboxylation **forms ethanolamine** which on methylations form choline (lipotropic agent).
- Serine and glycine are interconvertible.
- Serine is used for ***synthesis of sphingol***.
- **β**-carbon of Serine is used for **thymine formation**.
- -OH group of serine in an enzyme protein is phosphorylated/dephosphorylated ***to form active/inactive forms of the enzyme.***

Q.154. What is Proteinuria?

When proteins appear in urine in detectable quantities, it is called proteinuria. Albumin having the smallest size of the plasma proteins passes easily through damaged epithelia in kidney diseases. Hence, commonly called as "albuminuria".

Q.155. What are the types of proteinurias?

Two types:

- Functional proteinurias
- Organic proteinurias.

Q.156. State some causes of functional proteinuria.

- Violent exercise, long marches
- Pregnancy
- Orthostatic/postural proteinuria
- Alimentary proteinuria-rare.

Note: Usually temporary excretion of proteins in very small amounts less than 2%.

Q.157. State a few causes of organic proteinuria.

Found in many pathological conditions, grouped as:

- Prerenal
- Renal
- Post renal.

- ***Pre-renal causes:*** Cardiac diseases, liver diseases, abdominal tumors, malignancies.
- ***Renal causes:*** Acute and chronic glomerulonephritis, nephrotic syndrome (heavy albuminuria). In nephrosclerosis, TB of kidney and carcinoma of kidney.
- ***Postrenal causes:*** may be due to inflammatory, degenerative and traumatic lesions of ureter, bladder, prostate and urethra.

Q.158. What is Tamm-Horsfall protein? What is its function?

- Tamm-Horsfall protein, a glycoprotein, molecular wt—approximately 30,000 is found in relatively small amount in normal urine. It is secreted by the mucous glands of the normal genitourinary tract.
- ***Function:*** Probably it serves to protect the epithelia of the genitourinary tract.

Q.159. What is the most common bed-side test used for detecting albumin in urine?

- ***Heat test:*** Take 2/3 test-tube full of urine sample. Hold the test-tube from the bottom and heat the top portion of the tube. Add a few drops of 1% acetic acid drop by drop. Do not mix.
- Appearance of white turbidity/floccules in the heated portion indicate presence of albumin. If no turbidity/floccules occur, albumin is not detected.

Q.160. Why 1% acetic acid is added drop by drop after heating? What is the purpose?

It serves two purposes:

- If the urine is alkaline, on heating, phosphates may be precipitated which may be taken mistakenly as albumin. On acidification of heated urine, the phosphate if present disappears.
- By acidification of heated urine, the denatured albumin appears as floccules when PI of albumin is reached. ***Denatured albumin forms floccules at PI of the protein.***

Q.161. Does amino acids are excreted in urine?
Small amounts of amino acids, both free and combined, are excreted in urine. The free amino acid normally amounts to 1.4 mg/kg for men and 2.3 mg/kg for women.

Q.162. What is aminoaciduria?
Increased excretion of amino acids in urine than normal is called aminoaciduria.

Q.163. What are the types of aminoaciduria?
Amino acidurias can be divided into **two main groups:**
- Over-flow aminoaciduria.
- Renal amino aciduria.

Q.164. What is overflow amino aciduria?
In this, due to some metabolic defect/or other defects there occurs an increase in plasma level of one or more amino acids, which are excreted in urine, as it exceeds the capacity of normal renal tubules to reabsorb them.

Q.165. Give three examples of overflow amino aciduria.
- **In severe liver diseases,** e.g. acute yellow atrophy of liver and sometimes in cirrhosis liver, leucine and tyrosine are excreted in urine.
- Can occur in **wasting diseases.**
- **Metabolic defects affecting a single amino acid** or a small group, include inherited disorders, e.g. in phenylketonuria, histidinaemia with histidinuria, glycinuria, maple-syrup disease, etc.

Q.166. What is renal amino aciduria?
In this condition, plasma concentration of amino acids is normal, but because of defects in renal tubular reabsorption of amino acids, an increase amount of one, several or all amino acids escape in urine.

Q.167. Give some examples of renal amino aciduria.
The defect may be specific or non-specific.
- ***Specific defect:***
  - *Cystinuria:* due to common renal transport defect:
    - Cystine
    - Lysine
    - Arginine
    - Ornithine are excreted in urine.

- *Hartnup disease:* monoamino monocarboxylic acids are excreted.

- ***Non-specific defect:***
  - In Fanconi's syndrome
  - In Wilson's disease
  - In patients suffering from muscular dystrophies.
  - Heavy metal poisoning like Pb, Hg, Cd, etc.

Q.168. What are the sulphur compounds excreted in urine?

Sulphur is excreted in urine in **three forms:**

- **Inorganic SO_4 :** 80 to 85%.
- **Organic esters** or **ethereal SO_4 :** 5%
- **Neutral sulphur:** 15 to 20%.

Q.169. How much sulphur is excreted in urine?

Total urinary sulphur varies from 0.8 to 1.4 gm per 24 hours (average = 1.0 gm).

Q.170. What is the urinary N : S ratio?

Urinary N : S ratio = 13 to 16 on a high meat diet and during fasting.

Q.171. What are Ethereal SO_4 sulphur?

- It consists of Na and K salts of sulphuric acid esters of phenols such as indoxyl, skatoxyl, phenol and cresol. These ethereal SO_4 represent detoxication compounds of above and are formed in liver.
- Indoxyl and skatoxyl are formed by putrefactive decomposition of tryptophan in the gut, and phenol and cresol from tyrosine.

Q.172. What is indican?

K-salt of indoxyl sulphuric acid is called as indican and excreted in urine.

Q.173. What is the clinical significance of increased excretion of ethereal SO_4 and indican?

Excretion of Indican in urine is taken as a rough index of intestinal putrefaction. In acute intestinal obstruction, carcinoma of liver, cholera, typhus, excretion of ethereal SO_4 and Indican increases. In cholera and typhus sufficient indican is excreted to cause urine to assume a bluish tinge on standing.

Q.174. What is meant by glucose : nitrogen ratio (G : N ratio) or dextrose : nitrogen ratio (D : N ratio)?

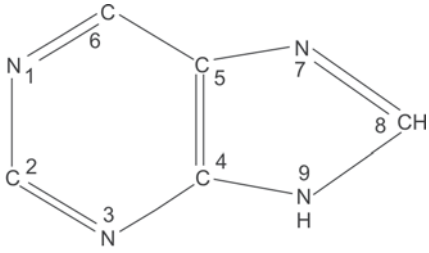
- It is the ratio of glucose and nitrogen excreted in urine. G : N ratio (also called D : N ratio) is important ***as it reflects the conversion of protein into glucose.***
- It is assumed that one gm of urinary nitrogen represents 6.25 gm of proteins.
- Normally, the G : N ratio = 3.65 : 1, 3.65 gm of glucose has come from 6.25 gm of protein.

$\therefore \quad \frac{3.65 \times 100}{6.25} = 58\%$ is the average conversion rate.

CHAPTER

15 Metabolism of Nucleoproteins

Q.1. What are the sources of nitrogen and carbon atoms of the purine ring?

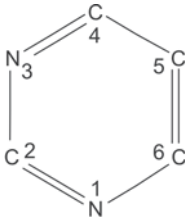


Purine ring

Sources:

- N_1 = amino nitrogen of aspartate
- C_2 and C_8 = For–mate via $F.H_4$ or β carbon of serine/or glycine
- N_3 and N_9 = Amide N_2 of glutamine
- C_4, C_5 and N_7 = From Glycine
- C_6 = from CO_2

Q.2. What are the sources of the nitrogen and carbon atoms of the pyrimidine ring?



Pyrimidine ring

Sources:

- C_2 = from CO_2
- N_3 = from amide N of glutamine
- N_1 and $C_{4,\ 5\ and\ 6}$ = from aspartic acid.

Q.3. What are the ingredients required for the synthesis of pyrimidines?

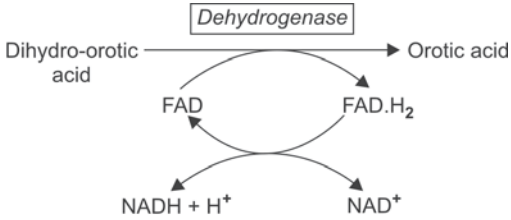
Ingredients required are:

- ***Carbamoyl phosphate:*** Synthesized from CO_2 and amide N of glutamine.
- **PRPP:** 5-phosphoribosyl-1-pyrophophate

- ***Enzymes:*** *Carbamonyl-P synthetase II* (cytosolic enzyme),
  - *Transcarbamoylase*
  - *Dihydro-orotase*
  - *Dehydrogenase*
  - *Transferease*
  - *Decarboxylase*
- **ATP:** For energy
- ***Amino acid:*** Aspartic acid
- ***Coenzymes/cofactors:*** FAD, NAD^+, Mg^{++}

Q.4. What is orotic acid?

- It is an intermediate metabolite in pyrimidine synthesis.



Q.5. What is the first Pyrimidine nucleotide formed?

- **Uridylic acid (UMP)** is the first pyrimidine nucleotide formed.
- Other pyrimidine nucleotides *viz.* UDP, UTP, CTP and d-UDP are synthesized from UMP.

Q.6. What is orotic aciduria?

An **inherited disorder** of pyrimidine synthesis. **Two types:**

- ***Type I orotic aciduria***
  - **Enzymes defect:** ***orotate phosphoribosyl transferase*** and ***orotate decarboxylase.***
  - Orotic acid is not converted to UMP. Results in accumulation of orotic acid in blood increasing its level. There is growth retardation and megaloblastic anemia.
- ***Type II orotic aciduira*:**
  - **Enzyme defect:** ***Orotidylate decarboxylase.*** It is also characterized by megaloblastic anemia and urinary excretion of orotic acid.

Q.7. State the end products of pyrimidine catabolism. Where pyrimidine catabolism takes place?

- **Liver** is the main site of catabolism of pyrimidines.

- **End products of catabolism are:**
  - CO_2 and NH_3—end products
  - **β**-alanine-side product from cytosine and uracil.
  - **β**-aminoisobutyrate: Side product formed from catabolism of thymine, it is excreted in urine.

Q.8. What is the fate of β-alanine formed in pyrimidine catabolism?

- **β**-alanine can be utilized for synthesis of
  - CoA-SH
  - Carnosine
  - Anserine
- Alternatively, **β**-alanine can be oxidized to acetate, NH_3 and CO_2.

Q.9. What is the clinical significance of β-amino-isobutyric acid?

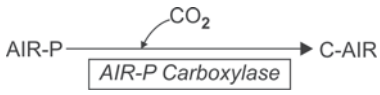
- **β**-amino isobutyric acid is excreted in large quantities in:
  - Leukemias and
  - When body is subjected to X-ray irradiation (**β**-amino isobutyric aciduria)
- **β**-amino isobutyrate can be converted to methyl malonic semialdehyde which, in turn, can form propionic acid which is converted to succinate (Thus it is glucogenic.).

Q.10. State the materials required for purine biosynthesis.

- **PRPP** (5-phosphoribosyl-1-pryophosphate): is the ***starting material***. It is formed from D-ribose-5'-P obtained from HMP shunt.
- ***Enzymes:*** Various enzymes ***synthetases, transferases, carboxylase,*** and ***hydroxylases***.
- ***Engery:*** Provided by ATP.
- ***Amino acids and derivatives:*** Glycine, aspartic acid and glutamine.
- **CO_2:** from HCO_3^-
- ***Coenzymes/and cofactors:*** $F.H_4$, $Mg.^{++}$

Q.11. Name the reaction which incorporates CO_2 to the substrate but it is not "CO_2-fixation reaction".

- Formation of 5-amino-imidazole-4-carboxylic acid ribotide (C-AIR)



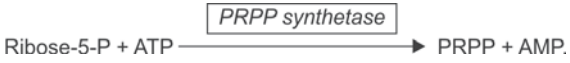
- The reaction uses CO_2 to carboxylate 5-amino-imidazole ribotide (AIR). It contributes to C_6 of the purine nucleus.
- ***The reaction is peculiar in that neither biotin nor ATP is required for this carboxylation reaction.***

Q.12. What is the first purine nucleotide formed in purine biosynthesis?

- ***First purine nucleotide formed is inosine monophosphate (IMP).***
- Once IMP is formed, it can form other purine nucleotides viz. AMP and GMP.

Q.13. How PRPP is formed?

- PRPP is formed from ribose-5-P and ATP catalyzed by the enzyme *PRPP synthetase*.



Q.14. What are various uses of PRPP?

PRPP is used for:

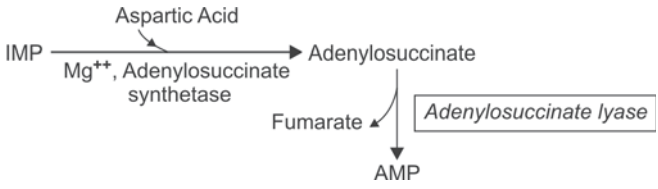
- Synthesis of both purine and pyrimidine nucleotides.
- Salvage pathways for both purine and pyrimidine bases.
- Biosynthesis of nucleotide coenzymes.

Q.15. How many high energy phosphate bonds are utilized in purine synthesis?

Expenditure of **six** high energy phosphate bonds are utilized in purine synthesis, ***thus it is energetically an expensive process.***

Q.16. How AMP is formed from IMP?

- Aspartic acid condenses with IMP to form adenylo-succinate, catalyzed by the enzyme ***"adenylosuccinate synthetase"*** and Mg^{++}. GTP provides the energy.
- Adenylosuccinate is then cleaved to form fumaric acid and AMP.



Q.17. How GMP is formed form IMP?

- IMP is first oxidized to xanthylic acid (xanthine monophosphate, XMP).
- Oxidation is catalyzed by a ***dehydrogenase,*** NAD^+ acting as H-acceptor.
- Glutamine gives the amide group to C_2 of XMP to form GMP.

Q.18. What is meant by salvage pathways for purine/ pyrimidine bases?

Many cells have pathways that can "salvage" the purine and pyrimidine bases to form the corresponding nucleotides and ***do not require expenditure of energy***. Such pathways are called **"salvage" pathways.**

Q.19. What are the salvage pathways by which purine bases form nucleotides?

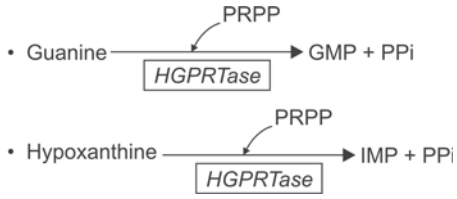
Two pathways are available for formation of nucleotides by salvage pathway from purine bases. They are:

- One step synthesis
- Two step synthesis.

Q.20. State one step synthesis of purine nucleotides by salvage pathway.

a. ***Formation of GMP and IMP*:**

- ***"Hypoxanthine-Guanine phosphoribosyl transferase"*** (HGPRTase) enzyme catalyzes one-setp formation of the nucleotides from either guanine or hypoxanthine, using PRPP as the donor of ribosyl moiety.

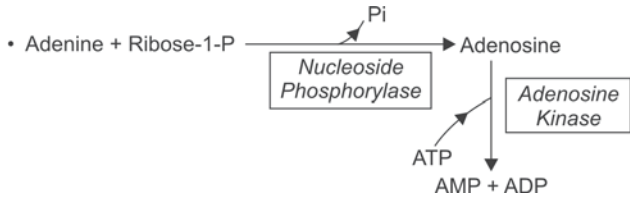


b. ***Formation of AMP***:

- The enzyme ***"adenine phosphoribosyl transferase" (APRTase)*** catalyzes the formation of AMP from adenine, ribosyl moiety is donated by PRPP.

Q.21. State the two-step synthesis of AMP?

- Also called as ***nucleoside phosphorylase-nucleoside kinase pathway.***



Q.22. Can GMP/IMP be formed by two-step synthesis?

Neither Guanosine nor inosine kinase have been detected in mammalian cells. So GMP/IMP cannot be formed by this pathway. Adenine is the only purine base that can be salvaged by two-step pathway.

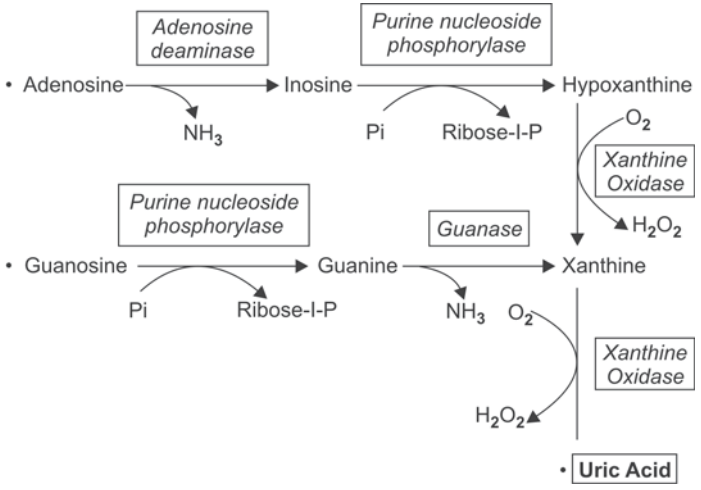
Q.23. Which tissues cannot synthesize the purine nucleotides?

Erythrocytes, neutrophils and ***brain cells*** are not capable of "de Novo" synthesis of purine nucleotides as they lack the enzyme ***"PRPP-amidotransferase"***.

Q.24. What is the end-product of catabolism of purines in humans?

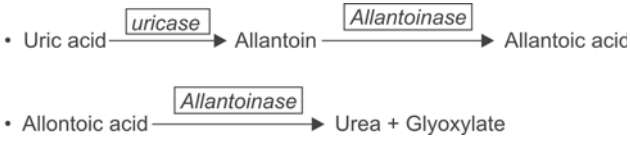
Uric acid is the chief end-product of purine catabolism in humans and primates.

Q.25. Show schematically how uric acid is formed in humans.



Q.26. Name the enzymes of uric acid metabolism which are absent in humans but present in lower animals. What is their function?

- ***Enzymes: uricase*** and ***allantoinase*** are absent in humans.
- In many non-primates, uric acid may be oxidized and decarboxylated by *uricase*, hepatic-Cu containing enzyme to form allantoin.
- Some fishes have *uricase*, as well as *allantoinase* which converts allantoin to allantoic acid.



Q.27. What is the nature of the enzyme *xanthine oxidase?*

- ***Xanthine oxidase*** is a metallo-enzyme and contains molybdenum (Mo), the trace element and also Fe^{3+}.
- It can act on hypoxanthine and converts it to xanthine. Also it can act on xanthine and converts it to uric acid.
- The enzyme requires FAD and molecular O_2 and produces H_2O_2 at substrate level.

Q.28. What is the normal uric acid level of blood?
- In males: 3.0 to 8.5 mg/dl.
- In females: 2.0 to 7.5 mg/dl.

Q.29. How much uric acid is excreted daily in urine?
- 400 to 600 mg/24 hrs urine.

Q.30. Which is the main organ where uric acid formation take place?
Liver is the principal organ. After formation uric acid is carried to kidney for its excretion.

Q.31. What is meant by miscible pool?
It is the quantity of uric acid present in body water. In a normal adult, an average of 1130 mg of uric acid is present.

Q.32. What is meant by the turnover of uric acid?
This indicates the rate at which the uric acid is synthesized and lost from the body. Normally 500 to 600 mg of uric acid is synthesized. Not all is excreted in urine, some uric acid is secreted in bile to reach the gut.

Q.33. What happens to uric acid in the gut secreted in bile?
By the action of intestinal bacteria, uric acid is **converted** to **urea** and **NH_3**.

Q.34. How uric acid is excreted?
- Uric acid in plasma is filtered by the glomeruli but is later partially reasorbed by the renal tubules.
- There is also now conclusive evidence for tubular secretion of uric acid by kidney.

Q.35. State the effects of hormones on uric acid excretion.
- Gluco-corticoids and
- ACTH increases the excretion of uric acid in urine.

Q.36. What are uricosuric drugs. Name them.
Uricosuric drugs are those that block reabsorption of uric acid by the renal tubules thus enhancing the excretion of uric acid in urine.
Examples:
- Salicylates
- Probenecid (Benemide)
- Halofenate

Q.37. Name the substances that can competitively inhibit the uric acid excretion.

Increased Lactic acid in blood as in Lactic acidosis competes with uric acid excretion resulting to retention of uric acid in blood thus increasing blood uric acid level.

Q.38. What is hyperuricemia?

Increase of blood uric acid level above normal is called hyperuricemia.

Q.39. What is hypouricemia? How it can happen and what are the effects?

- Decrease of blood uric acid level below normal is called hypouricemia.
- It can occur due to deficiency of ***xanthine oxidase*** so that uric acid is not formed, there is increased excretion of hypoxanthine and xanthine, due either genetic defect or to severe liver damage.
- In severe xanthine oxidase deficiency, patient may exihibit xanthinuria and xanthine lithiasis.

Note: Also can be produced by administering ***allo-purinol,*** a xanthine oxidase inhibitor.

Q.40. What do you mean by tophi?

Tophi are elevated nodules appearing in periarticular tissues of joints and cartilages of the ears. These are formed due to deposition of crystals of sodium urate in the articular and periarticular tissues of the joints.

Q.41. What is gout? What are the features?

Gout is a relatively rare chronic disease characterized by recurrent attacks of acute pain and swelling at first affecting only one joint, usually metatarso phalangeal joint of the big toe, later becoming polyarticular. The features are:

- Excess of uric acid in the blood (hyperuricemia).
- Deposition of sodium monourate crystals in periarticular tissues of joints and cartilages of ears producing "tophi".
- Recurring attacks of acute arthritis with swelling of joints.

Q.42. What are the types of Gout?

Two types:

- Primary gout

- Primary metabolic gout
- Primary renal gout

- Secondary gout:
  - Increase in purine catabolism *viz.* in leukemias, polycythemia, prolonged fasting.
  - Secondary renal gout: in renal failure.
  - In von Gierke disease (Type 1 GSD).

Q.43. What is the biochemical cause of gout in von Gierke disease (Type 1 GSD) ?

- Increased synthesis of purines followed by catabolism.
- ***Increased lactic acid in blood (lactic acidosis)*** competes with uric acid for excretion resulting to retention of uric acid, hyperuricemia and gout.

Q.44. How the blood uric acid level can be lowered in gout?

- By giving purine free diets.
- By increasing renal excretion of uric acid by suitable uricosuric drug like probenecid.
- By decreasing the synthesis of uric acid using ***xanthine oxidase inhibitor*** e.g. allopurinol (zyloprin) drug.
- By increasing oxidation of uric acid by administration of uric acid oxidase.

Q.45. How does allopurinol acts?

- Allopurinol has similar structure like hypoxanthine. Thus allopurinol **acts by competitive inhibition** of the enzyme ***xanthine oxidase*** and thus uric acid synthesis is inhibited.
- The durg causes a rapid fall in serum uric acid level.

Q.46. Outline briefly the line of treatment of a case of Gout?

Treatment of gout consists of:

- Palliative treatment
- Specific treatment
- ***Palliative treatment*:**
  - Bed rest in acute stage.
  - Diet: Purine free diet.

– Anti-inflammatory drugs to relieve pain. Drugs used are:
  - Colchicine in acute attack.
  - Non-steroid anti-inflammatory drugs (NSAIDs) e.g. Indomethacin, diclophenin, naproxen, brufen, pyroxicam, etc.

- ***Specific Treatment***

  Aims at lowering blood uric acid level.

  – Xanthine oxidase inhibitor: Allopurinol (zyloprin) drug of choice.
  – Using uricosuric drugs by enhancing renal excretion of uric acid e.g. probenecid (Benemide), halofenate.
  – By using drug to increase uric acid oxidations, e.g. urate oxidase.

Q.47. What is Lesch-Nyhan syndrome?

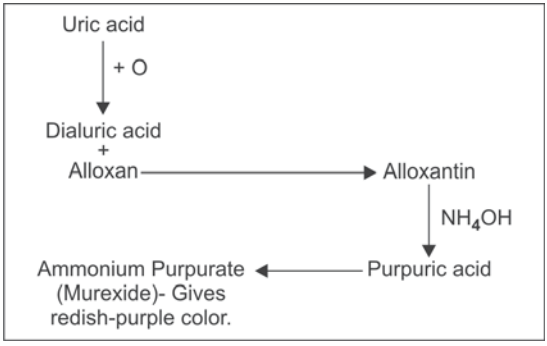
- It is an **X-linked recessive defect** of the enzyme ***hypoxanthine-guanine phosphoribosyl transferase*** **(HGPRTase).**
- Only males are affected.
- The enzyme is almost absent and leads to increased purine salvage pathway from PRPP.
- Clinically manifests as severe gout, poor growth, spasticity, **tendency to self mutilation** and renal failure.

Q.48. What is murexide test?

- Murexide test is used to detect uric acid in urine.
- To 5 to 8 drops of urine taken in a porcelain dish, 2 to 4 drops of conc. HNO_3 is added. Heat carefully till every trace of HNO_3 and H_2O removed. A ***reddish deposit of purpuric acid*** is formed.
- Dip a rod in dilute NH_3 and draw through the deposit. ***It turns reddish-violet*** due to formation of ***ammonium purpurate***. Add to the same deposit a drop of 5% NaOH, the deposit turns bluish-violet due to formation of Na-purpurate.

Q.49. State the chemistry of Murexide test.

The uric acid is ***oxidized to dialuric acid*** and ***alloxan***. These two substances condense to form ***alloxantin***. This alloxantin so formed reacts with ammonium hydroxide to form **purpuric acid**. The reddish-purple color is due to formation of ***ammonium purpurate (Murexide).***



CHAPTER

16 *Detoxication*

Q.1. What is detoxication?

The term detoxication refers to all those biochemical processes whereby the toxic substances present in foods, foreign molecules, drugs as well as toxic metabolites are rendered to water soluble non-toxic substances and excreted in the urine.

Q.2. Where is the site of detoxication taking place in the body?

- **Liver** is the major site of detoxication in the body.
- Hepatectomized animals do not have the capacity to detoxify.
- **Kidney** may also play a part to small extent.

Q.3. State the different mechanisms of detoxication.

The mechanism of detoxication involves reactions mainly of **4 types**. They are:

- Oxidation
- Reduction
- Hydrolysis
- Conjugation.

Q.4. What are the characteristics of conjugation?

- Conjugation mainly occurs in liver and to some extent in kidneys.
- Conjugation can take place independently or it is followed by oxidation, reduction or hydrolysis of a substance.
- Different conjugating agents are available in the body. Some of them are **amino acids**, peptide like **glutathione** and **D-glucuronic acid** synthesized in the body by uronic acid pathway.

- Conjugation can also occur by:
  - Methylation
  - Acetylation
  - "Active" SO_4 (PAPS).

Q.5. Name the amino acids which take part in conjugation reaction.

Amino acids that take part in conjugation reaction are:

- Glycine
- L-cysteine
- Glutamine—amide of glutamic acid.

Q.6. What are xenobiotics?

- Xenobiotics are foreign molecules which enter the body. ("Xenos" means stranger).
- They may be drugs, food additives, pollutants or carcinogens.
- Humans are constantly exposed to such xenobiotics in daily life.

Q.7. How xenobiotics are metabolized?

- Present concept is that the reaction with xenobiotics occur in **2 phases**.
  - ***Phase-1:*** This phase involves the hydroxylation, the major reaction, catalyzed by ***mono-oxygenases*** or ***cytochrome-P_{450}*** **species**. These enzymes can also catalyze reduction, deamination, desulfuration, epoxidation, etc.
  - ***Phase-2:*** The hydroxylated or other compounds produced in phase-1 are converted by specific enzymes to various water-soluble "Polar" metabolites by conjugation with various conjugating agents, *viz*, glutathione, D-glucuronic acid, active SO_4 (PAPS), methylation, acetylation, etc.
- The over-all purpose of these two phases is to increase their water solubility and thus facilitate their excretion from the body.

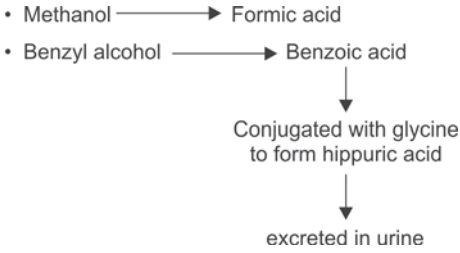
Q.8. How detoxication occurs by oxidation? Give three examples.

A large number of foreign substances are destroyed in the body **by oxidation**. Aliphatic as well as aromatic alcohols may be oxidized to corresponding acids via aldehyde

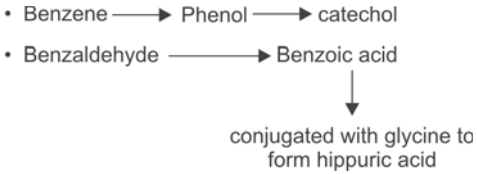
formation. In addition, certain amines, anilides and certain drugs also can undergo oxidation.

Three examples are:

- ***Oxidation of primary aliphatic and aromatic alcohols:***



- Oxidation of aromatic hydrocarbons and aldehydes:



- **Oxidation of anilides:**

Q.9. Name two drugs that are detoxicated by oxidation.

Certain drugs can be oxidized in the body and are excreted as-OH derivatives or salts.

Examples:

Q.10. How detoxication is carried out by reduction? Give examples.

- Reduction is ***usually not a common method*** and does not occur extensively in humans.

Examples:

- p-nitroben-zaldehyde —reduced→ p-amino-benzoic acid
- Picric acid (A nitrocompound). —reduced→ Picramic acid

Q.11. Name three drugs which are detoxicated in liver by hydrolysis.

- Digitalis (a cardiac glycoside) → sugar and aglycone
- Atropine (Tropyl tropate) → Tropic acid + Tropine
- Procaine → P-aminobenzoic acid.

Q.12. How conjugation takes place by methylation? Give examples.

Methylation is an important detoxication process by conjugation. It takes place in Liver and usual methyl donor is ***S-adenosyl methionine (active methionine)***.

Examples:

- Nicotinamide —(~CH_3)→ N-methylnicotinamide → excreted in urine.

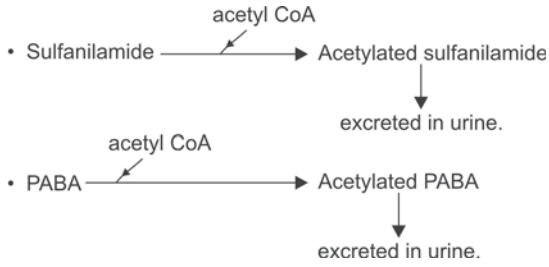
- O-methylation of hormone estrogen.
- O-methylation of certain naturally occurring amines with phenolic hydroxyl group e.g., Nor-epinephrine and epinephrine and their metabolites are methylated at the phenolic hydroxyl group.

Q.13. How conjugation takes place by acetylation? Give examples.

- Acetylation is done by **"active acetate" (acetyl CoA)**, conjugation with acetic acid occurs only with aromatic -NH_2 group. The reaction is catalyzed by the enzyme **acetyl transferase** present in the cytosol.

Examples:

- **Sulphadrugs:** In humans, sulphadrugs are conjugated by acetylation, 50% of sulphadrugs are excreted as acetylated derivatives.



Q.14. How antitubercular drug Isoniazid is detoxicated? What is the clinical importance?

- The drug "isoniazid" used in treatment of tuberculosis is detoxicated by acetylation.
- *Clinical importance:*
  - Polymorphic types of ***"acetyl transferases"*** exist, resulting in individuals who are classified as: (a) "Slow" acetylator and (b) "Fast acetylator.
    - ***Slow acetylators*** are more prone to certain toxic effects of isoniazid because the drug persists longer in these individuals due to slow acetylation.
    - ***Fast acetylators*** removes the drug by acetylation in a faster rate, hence less liable to toxic effects.

Q.15. How conjugation occurs with sulfuric acid? Give examples.

Sulfuric acid is used in humans for detoxication of various compounds having phenolic and/or hydroxyl groups. ***"Active" sulphate (PAPS) acts as the donor.***

Examples:

- Substances like **phenol, cresol, indole** and **skatole** formed in the gut by the action of the intestinal bacteria are absorbed and transported to liver where conjugated with "active" sulphate to form ***ethereal sulphates*** which are excreted in urine being less toxic and more acidic. ***K-salt of indoxyl sulfuric acid is indican.***

- Other compounds which are conjugated to form corresponding esters are:
  - **Tyrosine** forming tyrosine-O-SO_4 required for fibrinogen molecule.
  - The aminosugars, certain hormones like estrogens and androgens.

Q.16. List the various compounds that are conjugated with D-glucuronic acid.

- ***Bilirubin:*** to form bilirubin mono and di-glucuronide.
- ***Aromatic acids*** e.g. benzoid acid.
- ***Phenols*** and other secondary and tertiary aliphatic alcohols.
- ***Certain drugs:*** Morphine, menthol, pyramidon, acetanilide, sulfapyridine, etc.
- ***Antibiotics:*** Chloramphenicol.
- ***Hormones:*** Thyroid hormones, Derivative of steroids e.g. tetrahydroderivatives of cortisol, sex hormones metabolites, etc.

Formation of glucuronides play an important role in detoxication mechanisms of exogenous and endogenous compounds and their excretion as corresponding soluble glucuronides.

Q.17. How conjugation occurs with amino acid glycine? Give examples.

Glycine combines with potentially harmful substances mainly aromatic carboxylic acids in the body to form harmless derivatives which are excreted in the urine.

Examples:

- **Benzoic acid + Glycine** ⟶ Hippuric acid (Benzoyl Glycine)
- **Nicotinic acid + Glycine** ⟶ Nicotinuric acid.
- **Cholic acid** or **deoxy cholic acid** + Glycine ⟶ Glycocholic acid or glycodeoxycholic acid

Q.18. What are mercapturic acids? Which amino acid is involved?

In humans, few aromatic compounds are conjugated with the amino acid **L-cysteine** in presence of **acetic acid** to form ***mercapturic acids***. Coupling of cysteine with aromatic

compounds like bromobenzene, naphthalene are linked with acetylation.

Examples:

- Bromobenzene + L-cysteine + acetic acid ⟶ Bromophenyl mercapturic acid.
- Naphthalene + L-cysteine + acetic acid ⟶ Naphthyl mercapturic acid

Q.19. What is the role of glutamine in conjugation reaction?

In humans and in primates (Chimpanzee), glutamine conjugates phenyl acetic acid produced in phenyl ketonuric patients to form ***phenyl acetyl glutamine*** which is excreted in urine. This accounts for the ***mousy*** odour of the urine in phenyl ketonurics.

Q.20. What is the role of glutathione in detoxication of xenobiotics? What is the clinical importance?

- A number of potentially toxic electrophilic xenobiotics, e.g. certain carcinogens are conjugated to the nucleophilic G-SH, in reactions that can be represented as follows:

  R + G – SH ⟶ R – S – G, where "R" represents electrophilic xenobiotic.

- The reaction is catalyzed by the enzyme ***glutathione-S-transferase,*** the enzyme is present in high amounts in liver cytosol and in lower amounts in other tissues.

Clinical importance:

- If the potentially toxic xenobiotics are not conjugated with G-SH, they would be free to combine covalently with DNA, RNA or cell-proteins and can produce serious cell damage. G-SH is thus an important defence mechanism against certain toxic compounds such as some drugs and carcinogens.

Q.21. How selenium poisoning can be detoxified?

- Selenium poisoning develops due to high feeding of products obtained from the soil having high contents of selenium.

Reason of toxicity in selenium posioning:

Selenium replaces sulphur of cysteine and methionine in body tissues and interferes with the availability of these S-containing amino acids.

- ***Detoxication:*** The above can be cured by detoxification by giving ***P-bromobenzene***. Selenium containing species now forms "mercapturic acid" type of compounds with p-bromobenzene and thus excreted in urine.

Q.22. What is the role of BAL (British anti-Lewesite) in detoxication?

Detoxication of certain poisons like heavy metals is effected by "Brithish anti-Lewisite" (BAL) which is chemically 2, 3 mercaptopropanol. Heavy metals like arsenic (As), cadmium (Cd) and Mercury (Hg) are believed to bind the -SH groups of enzymes thereby inactivating them. BAL having a greater affinity of certain metals, when administered acts as an antidote by virtue of pulling away the metal ions from their enzyme combinations and forms a similar complex which is rather readily excreted.

Q.23. How cyanides are detoxicated in the body?

- Highly toxic cyanides are derived in the body in small amounts from certain fruits, proteins and tobacco smoke.
- Cyanides are conjugated by ***thiosulfates*** or in presence of colloidal sulphur. The reaction takes place in the liver and is catalyzed by the enzyme ***rhodanase*** (also called ***rhodanase* or *thiosulfate cyanide sulfur transferase.***

$$HCN + S \xrightarrow{\text{Rhodanase}} HCNS + Na_2SO_3$$

- Cyanide is also detoxicated by cysteine through **β**-mercapto-pyruvic acid.
- Traces of cyanide could be detoxified also by the formation of cyanocobalamine from other forms of vitamin B_{12}.

Q.24. What is the biomedical importance of cytochrome P_{448} system?

- Cytochrome P_{448} species have been found to be specific for metabolism of ***polycyclic aromatic hydrocarbons (PAHS).*** Hence this species has also been named as ***aromatic hydrocarbon hydroxylase*** **(AHH)**

Biomedical importance:

- **In the lungs of cigarette smokers** this enzyme may be involved in conversion of **"inactive PAHS" (Procarcinogens)** present in cigarette smoke to **"active carcinogens"** by **hydroxylation** reactions. Cigarette smokers were found to have higher levels of this enzyme in cells and tissues than non-smokers.
- Activity of this enzyme is increased (induced) in placentae of pregnant women who are cigarette smokers and thus foetus is exposed to potentially harmful metabolites (Carcinogens?).

CHAPTER

17 Metabolism of Minerals

Q.1. Name the principal mineral elements which are macronutrients.

Sodium, potassium, calcium, phosphorus, magnesium, sulphur, and chlorine. These are macronutrients or called as ***bulk elements.***

Q.2. How is sodium distributed in the body?

Sodium is the predominant cation of extracellular fluid (ECF) and present to the extent of 130 to 150 mEq/L (average 142 mEq/L). In the intracellular fluid (ICF). Sodium is less and it is approximately 5 mEq/L (average).

Q.3. What is the most common source of sodium?

- Table salt, used daily in cooking and seasoning.

Q.4. What are the other sources of sodium?

Animal foods are richer in sodium than plant foods.

- ***Animal sources:*** Egg, milk and milk products like cheese, butter, khoa, etc.
- ***Plant sources:*** Carrot, raddish, nuts, cauliflower, etc.

Q.5. What is the daily requirement of sodium in the body?

- Adults: 1.1 to 3.5 gm daily
- Children: 0.3 to 2.5 gm daily
- Infants: 0.1 to 0.5 gm daily.

Q.6. State the biomedical importance of sodium in the body.

- ***Maintaining fluid volume:*** contributes to crystalloid osmotic pressure and controls distribution of water in the body.
- ***Maintaining acid-base balance:*** $Na^+ - H^+$ exhange in renal tubules for conservation of Na^+ and acidity of urine.

- ***Buffer action:*** As $NaHCO_3$ it has buffer action (alkali reserve) and contributes to acid-base balance.
- Maintenance of ***viscosity*** of blood.
- ***Neuromuscular excitability.***
- ***Role in action potential:*** when Na^+ ions move inside a cell, action potential is set in.

Q.7. Name the hormones which affect sodium metabolism.

- Mineralocorticoids: Principally ***aldosterone,*** it causes sodium retention.
- Progesterone: also has similar effect.

Q.8. What is hypernatremia?

Increase of sodium level in blood above normal is called ***hypernatremia.***

Q.9. State a few conditions where hypernatraemia can occur?

- Adrenal cortical hyperfunction (Cushing's syndrome)
- Simple dehydration (Pure water depletion)
- Overzealous infusion of saline
- Diabetes insipidus
- Administration of ACTH
- ***Hypokalemia:*** Sodium and potassium levels have reciprocal relationship
- Edema.

Q.10. What is hyponatremia?

Decrease of plasma sodium level below normal is called ***hyponatremia***.

Q.11. State a few conditions where hyponatremia can occur.

- Medication of **diuretics**.
- ***Sweating:*** Excessive sweating associated with replacement by water but no salt (Pure salt depletion or secondary dehydration). ECF becomes hypotonic.
- Chronic renal diseases.
- Gastrointestinal loss: Prolonged diarrheas.
- ***Addison's disease:*** adrenal cortical insufficiency.
- Salt-losing syndrome.
- **Hyperkalaemia**
- Congestive heart failure.

Q.12. What is the distribution of potassium in the body?
Potassium is the ***principal cation*** of the intracellular fluid (ICF). Inside the cell, a part of it is in ionic form and the rest is bound to proteins.

Q.13. What is the normal potassium level in cell and plasma?
- Concentration of K^+ in the cellular fluid is 150 mEq/L (average).
- Plasma potassium level ranges from 3 to 5 mEq/L.

Q.14. What are the sources of potassium in the diet?
Good sources are chicken, banana, dates, almonds, beans, cabbage, Potato, etc.

Q.15. State the functions/biomedical importance of potassium in the body.
- Intracellular K^+ has a **role in acid-base homeostasis** and fluid and electrolyte balance.
- Extracellular K^+ **promotes relaxation** of **skeletal muscle**. It has also significant action on the depolarisation and contraction of the heart.
- **Resting membrane potential** is mainly due to the difference in K^+ diffusion in cell and is about -70 mv. During transmission of nerve inpulses, there is Na^+ influx and K^+ efflux and a reversal of polarization.
- **Role in sodium conservation:** In distal renal tubules K^+ - Na^+ exchange take place.
- K^+ ions are needed as a **cofactor** for maximum activity of the enzyme ***pyruvate kinase***.
- K^+ enters the cells with glucose in glycogenesis. ***Hence to correct hyperkalemia glucose and insulin are given.***

Q.16. What are the clinical manifestations of potassium deficiency?
- Deficiency of K^+ causes paralysis of skeletal muscle and aberrant conduction and abnormal activity of cardiac muscle.
- In K^+ deficiency, K^+ comes out of cells and H^+ goes inside the cells. ***As a result, it causes extracellular alkalosis*** and ***intracellular acidosis.*** In such a situation though there is extracellular alkalosis, the urine of the patient is acidic (called as ***paradoxic aciduria).***

Q.17. What is meant by hyperkalemia?
Increase of plasma K^+ level above normal is called **hyperkalemia**.

Q.18. State a few conditions where hyperkalemia occur.
- **Renal failure** and anuria,
- **Addison's disease**,
- Excessive intravenous administration of K-salts.
- **Tissue damage**, e.g. **crush injuries**.
- **Transfusion of stored blood** (more than two weeks old),
- ***Diabetes mellitus with ketoacidosis:*** In ketoacidosis there is substantial loss of intracellular K^+ to the ECF. This is partly due to increased activity of "sodium pump" which results from impaired glucose metabolism. Treatment with insulin allows resumption of "sodium pump" and movement of K^+ back into the cells which may ***precipitate hypokalemia. Frequent monitoring of plasma K^+ level is vital in treatment of diabetes mellitus with ketoacidosis.***

Q.19. What is meant by hypokalemia?
Decrease of plasma potassium level below normal is called **hypokalemia.**

Q.20. State a few conditions where hypokalemia can occur.
- Loss of K^+ in GI secretions:
  - Prolonged vomiting and severe diarrhea
  - **Cillous adenoma:** a mucous secreting tumor of GI tract, secretes large amount of K^+ into lumen of colon.
  - **Metabolic alkalosis.**
  - Chronic wasting diseases.
  - **In Cushing's syndrome,** adrenocortical hyperfunction hypokalemia is the rule. It is particularly severe if the process is due to production of ACTH by an "ectopic tumor".
  - **Other rare causes are:**
    - ***In Conn's tumour:*** Primary hyperaldosteronism characterized by increased loss of K^+ in urine.
    - ***Familial periodic paralysis:*** An inherited disorder in which sudden shift of K^+ into ICF occurs causing paralysis.
    - ***Renal tubular acidosis:*** Low serum K^+ is seen in renal tubular acidosis, in Bartter's syndrome and after administration of steroids.

Q.21. What is the daily requirement of potassium?
- Adults: 1.8 to 5.7 gm
- Children: 1.5 to 3.0 gm
- Infants: 500 to 1300 mg (0.5 to 1.3 gm).

Q.22. What is the relation of sodium and potassium entry/exit in cells?
- Na^+ entry in the cells is by passive diffusion but it is extruded out of the cells by "active" sodium pump and requires energy.
- K^+ entry on the other hand into the cells is active process by "sodium pump" and requires energy and its efflux from the cells is by passive diffusion.

Q.23. What is sodium pump?
Sodium pump is also called ***Na^+ -K^+ -ATPase***. It requires for its function ATP and Mg^{++}. It is a glycoprotein enzyme composed of **2 α** and 2 **β** chains. The enzyme hydrolyzes a high energy PO_4 bond of ATP and uses the energy thus released, **to transport three Na^{++} ions outside** and simultaneously **two K^+ ions inside** across the cell membrane.

Q.24. Name an inhibitor which inhibits sodium pump.
- Ouabain, a glycoside inhibits sodium pump.

Q.25. What is the distribution of calcium in the body?
- The human body contains approximately1 to 2 kg of Ca in a 70-kg adult. About 99% of it is in the bones and teeth.
- About 20 mg of Ca/100 gm tissue is present in the cells and body fluids and has a vital role to play.

Q.26. How much is the turnover of Ca in bones?
Approximately 700 mg of calcium enters and leaves the bones every day.

Q.27. What are the sources of calcium in the body?
It is widely distributed in food substances such as milk, cheese, egg-yolk, beans, lentils, nuts, figs, cabbage, etc.

Q.28. What is the daily requirement of calcium in the body?
- **Adult:** 800 mg daily (0.8 gm).
- **Children:** 800 to 1200 mg daily (0.8 to 1.2 gm).
- **Infants:** 360 to 540 mg daily (0.36 to 0.54 gm).

Q.29. What is the normal plasma level of Ca?
- Normal plasma Ca is 9 to 11 mg/dl.

Q.30. What are the different forms in which Calcium exist and indicate their level in plasma?

Plasma Ca exists in **three forms:**

- **Ionized Ca** (diffusible): 2 mg/dl.
- **Protein bound** (non diffusible): 5 mg/dl
- **Complexed form** (as citrate and phosphate): 2 mg/dl.

Q.31. What is the relation of calcium with albumin?

Protein bound Ca (non-diffusible) is mainly with albumin. Each gram of protein (through albumin) binds 0.84 mg of calcium.

> Hence Total Calcium = 0.84 × Serum Protein (grams) + Diffusible Calcium.

Q.32. How much calcium is excreted daily?

- **In urine:** 200 mg
- **In faeces:** 70 to 90% of total excreted Ca
- **In sweat:** 15 mg.

Q.33. What is the site of absorption of dietary calcium and what is the mechanism of absorption?

- About 40% of average daily dietary intake of Ca is absorbed from the gut. Calcium is absorbed mainly from the duodenum and first half of jejunum against electrical and concentration gradient.
- ***Mechanism***: ***Two mechanisms*** proposed. They are:
  - — Simple diffusion.
  - — An ***"active" transport*** process involving "Ca-Pump" and energy. Both the processes require 1, 25-di (OH) D_3 (Calcitriol) which regulates the synthesis of Ca-binding proteins and transport and also a Ca^{++}-dependent ATP-ase.

Q.34. State the factors that help in absorption of Ca.

Various factors which influence the absorption of Ca are:

- ***pH of intestinal milieu:*** An acidic pH favors absorption
- ***Composition of the diet:*** High protein diet favors absorption. Amino acids increase the solubility of Ca-salts and thus absorption.
- ***Sugars and organic acids*** like citric acid: increases the solubility of Ca-salts and absorption.

- ***Ca: P ratio:*** A ratio of food Ca to P not more than 1:2 and not less than 1:2 (ideal 1:1) is necessary for optimal absorption of Ca.
- ***Fatty acids, Phytic acid, oxalates:*** Form insoluble compounds and decreases calcium absorption.
- ***Fibers in diet:*** Excess dietary fibers decreases calcium absorption
- ***Role of vitamins:*** Vitamin D_3 as "calcitriol" increases absorption.
- ***Role of minerals:*** Excess of phosphates, high contents of Mg and Fe in the diet decreases absorption.
- ***Hormones:*** Parathormone (PTH) increases calcium absorption through "Calcitriol".

Q.35. Name the hormones which regulate Ca-level in blood.

- ***Calcitriol:*** It increases Ca-absorption.
- ***Parathormone (PTH):*** It increases Ca-absorption by stimulating ***1 α-hydroxylase*** and increases formation of calcitriol.
- ***Calcitonin:*** Lowers serum Ca level.

Q.36. State some important functions of calcium in the body.

- Required for growth and calcification of bones and teeth.
- Ca^{++} is required for neuromuscular transmission and normal excitability of heart which is Ca-dependent.
- Its binding with "troponin" is necessary for triggering muscular contraction.
- Plays a role of blood coagulation by forming Ca-fibrin complex and for bridging the phospholipids (PL) to the gla residues of prothrombin.
- Plays an important role as secondary/or tertiary messenger for some hormone actron.
- Calcium acts through calmodulin component of ***phosphorylase kinase*** and activates phosphorylase.
- Ca^{++} activates some enzymes e.g. ***lipase, ATP-ase, succinate dehydrogenase,*** etc.

Q.37. What is hypercalcemia?

Increase of serum calcium level above normal is called **hypercalcemia**.

Q.38. What is the most common cause of hypercalcemia in an out-patient department (OPD)?

- ***Primary hyperparathyroidism*** is the most common cause for out-patients (OPD cases).

Q.39. What is the most common cause of hypercalcemia in hospital in-patients?

- ***Malignancy*** is the most important cause for hospital in-patients.

Q.40. What are the causes of hypercalcemia in malignancy?

Hypercalcemia in malignancy may be due to:

- ***Humoral factors:*** Humoral hypercalcemia of malignancy (HMM) may be due to:
  - ***Production of PTHrP*** (PTH related protein).
  - ***Production of growth factors:*** like tumor-growth factor (TGF), Epidermal growth factor (EGF), platelets derived growth factor (PDGF).
- ***Direct skeletal involvement*** by tumors.
- ***Hematological malignancies:***
  - Production of cytokinase: Interleukin-1, tumor necrosis factor (TNF), lymphotoxins.
  - ***Excessive production of calcitriol,*** e.g. in lymphomas.

Q.41. State some other causes of hypercalcemia.

- ***Endocrine causes:*** Acromegaly, hyperthyroidism.
- ***Granulomatous diseases:*** TB, sarcoidosis.
- ***Overdosage of vitamins:*** Hypervitaminosis D, vit A intoxication.
- ***Iatrogenic (Drug induced):*** Milk alkali syndrome, thiazide diuretics, spironolactone.
- ***Miscellaneous causes:*** Idiopathic hypercalcemia of infancy (William's syndrome), increased serum proteins, renal failure.

Q.42. What is hypocalcemia?

Decrease of serum calcium level below normal is called **hypocalcemia.**

Q.43. State three important causes of hypocalcemia.

- ***Reduction of serum albumin*** (hypoalbuminemia): Most common cause. Can be due to:
- Malnutrition
- Malabsorption states
- Nephrotic syndrome
- Chronic Liver diseases (cirrhosis liver).

- ***Renal diseases and renal failure:*** Renal tubular dysfunction, acute tubular necrosis, chronic renal failure, etc.
- ***Hypoparathyroidism:*** May be surgically induced partial or complete, idiopathic (autoimmune), bioinactive PTH.

Q.44. How phosphorus is distributed in the body? What is the total body phosphate?

Total body phosphorus in an adult is approximately 0.7 to 1.0 kg. More than 85% is found in bones, 15% in soft tissues, about 5 gm in brain and 1% is found in EC fluid.

Q.45. How much is the daily requirement of P?

About 1.0 to 1.5 gm of phosphate is required to be taken in the diet daily.

Q.46. What is the normal serum level of P?

- **Adults:** Normal serum level is 3 to 4.6 mg/dl
- **Children:** 4 to 6 mg/dl.

Q.47. How much phosphorus is excreted in urine?

The urinary excretion of phosphate varies widely from 700 to 1200 mg per 24 hrs urine depending on the phosphate intake.

Q.48. Name the dietary sources of P.

Foods rich in phosphorus content are milk, cheese, egg-yolk, organ meats, fish and nuts.

Q.49. State some important functions of P in the body.

- Phosphate is important ***contituent of bones*** and teeth.
- Phosphates have an important ***role as high energy compounds*** e.g. ATP, UTP, CTP, creatine phosphate, GTP, etc.
- ***Role in acid-base balance:*** The buffer which is effectively handled by kindneys is phosphate buffer.
- Phosphate group is present in:
  - Phospholipids (PL),
  - Nucleic acids, and nucleotides
  - Cyclic AMP and GMP, and a large number of coenzymes like $NADP^+$, TPP, pyridoxal-P, in phosphoproteins like casein of milk and vitellin of egg-yolk
  - Phosphates are constituents of cell-membranes and nerve tissues.

– Phosphorylation/dephosphorylation is necessary for activation/inactivation of certain enzymes.

Q.50. State the relation of Ca and P in certain diseases.

- Primary hyperparathyroidism: $Ca\uparrow$, $PO_4\downarrow$
- Hypoparathyroidism: $Ca\downarrow$, $PO_4\uparrow$
- Vitamin D deficiency: $Ca\downarrow$, $PO_4\downarrow$
- Mallgancy: Tumor deposits in bones: $Ca\uparrow$, $PO_4\uparrow$.

Q.51. Enumerate some important metabolic role sulphur.

- ***Formation of "active SO_4" (PAPS)***
- Sulphur is involved in the ***formation of proteins*** such as keratin, chondroproteins, sulfolipids.
- Involved in ***formation of active-SH groups*** of enzymes like ACP and multienzyme complex of FA synthesis.
- Sulphur is important ***constituent of MPS*** like heparin, heparitin SO_4, chondroitin SO_4, keratin SO_4.
- **Iron-sulfur protein** (Fe: S) has important role in ETC.
- Sulphur is present in the proteins of biomembranes and cells.
- **Hormones:** Insulin, vasopressin, oxytocin contain sulphur.
- **Sulphur containing vitamins** such as thiamine, biotin, lipoic acid and CoA-SH are involved as coenzymes.
- **Constitute, S-S linkage** between two-SH groups of cysteine to form secondary and tertiary structure of proteins.
- **In S-adenosyl methionine** (active methionine), the CH_3 group is attached to S as high energy bond and thus becomes **"biologically labile".**
- Sulphated galactose occurs in **galactolipids.**
- **Role in detoxication:** Phenol, indole, skatole and certain steroids are detoxicated in liver by "active sulphate".
- **Glutathione** is an important S-containing tripeptide.

Q.52. What is the distribution of magnesium in the body?

Magnesium is the fourth most abundant and important cation in humans, approximately 2/3 of body Mg occurs in bones alongwith Ca and P, 1% occurs in ECF and remainder in soft tissues. Mg^{++} ions are present in all cells.

Q.53. How much is the total body Mg?

- Approximately 2400 mEq.

Q.54. What is the normal plasma level of magnesium?
Normal plasma level of Mg is 1.5 to 1.8 mEq/L. It is rigorously maintained within normal limits.

Q.55. What is the reliable indicator of Mg status in the body?
Erythrocyte levels of Magnesium are more reliable indicator of Mg status of the body.

Q.56. How much is the daily requirement of Mg?
- **Adult:** 200 to 300 mg
- **Children:** 150 to 200 mg
- **Infants:** 100 to 150 mg
- During pregnancy and lactation magnesium requirement is increased to 400 to 600 mg.

Q.57. What is the source of magnesium?
- Magnesium is widely distributed in vegetables, found in porphyrin group of chlorophyll of vegetable cells. Also found in almost all animal tissues.
- **Good sources are:** Cereals, beans, green vegetables, potatoes, almonds, milk and dairy products like cheese.

Q.58. State the factors that affect absorption of Mg?
- ***Amount of dietary intake.***
- ***Dietary Ca:*** Increased absorption in Ca-deficient diets.
- ***Motility and mucosal state:*** In hurried bowel and damaged mucosal state absorption is decreased.
- ***Vitamin D:*** helps in increased absorption.
- ***Hormones:*** Parathormone (PTH) and growth hormone (GH) increases absorption.
- ***Other dietary factors:***
  - High protein diet and neomycin therapy increases absorption.
  - Fatty acids (FA), phytates and phosphates decrease absorption.

Q.59. State some important functions of Mg.
- Magnesium is required ***as cofactor*** in many reaction in which ATP participates, as it is the ATP-Mg^{++} complex which acts as substrate.
- **Mg acts as an activator** and required as a cofactor in a wide spectrum of enzyme actions. Thus it is essential for glycolytic enzymes, ***peptidases, ribonucleases*** and co-carboxylation reaction.

- ***Neuromuscular irritability:*** High levels depress nerve conduction and low levels may produce hypomagnaesemic tetany.
- ***As a constituent of bones and teeth:*** About 70% of body Mg is present as apatite in bones, dental enamel and dentine.

Q.60. What is meant by hypermagnesemia?

Increased plasma level of Mg above normal is called **hypermagnesemia.**

Q.61. State some important conditions where hypermagnesemia occur.

- Uncontrolled DM
- Acute renal failure
- Adrenocortical insufficiency
- Hypothyroidism.

Q.62. What is hypomagnesemia?

Decreased plasma level of Mg below normal is called **hypomagnesemia.**

Q.63. State some conditions where hypomagnaesemia can occur.

- Malabsorption syndrome
- Chronic alcoholism
- Prolonged use of diuretics
- Hyperthyroidism
- Kwashiorkor
- Primary aldosteronism
- Renal diseases
- Prolonged gastric suction.

Q.64. What are "trace" elements? Name some of the important trace elements.

Trace elements are those elements which are required in very small amounts for nutritional and metabolic purposes.

Some important "trace" elements are:

- Iron (Fe)
- Zinc (Zn)
- Iodine (I_2)
- Cobalt (Co)
- Chromium (Cr.)
- Copper (Cu)
- Fluorine (F)
- Molybdenum (Mo)
- Manganese (Mn)
- Selenium (Se).

Q.65. What is the total iron content of the body?

- In normal adult of 70 kg total iron content varies from 2.3 to 3.8 grams.

- Average iron content of adult males is approximately 3.8 gm and in females 2.3 gm which may deplete in repeated pregnancies and menstrual loss.

Q.66. What are the types of iron present in the body?

Two types:

- ***Essential (or functional iron)***
- ***Storage iron.***

Q.67. Name some essential (or functional) iron.

- ***Heme Proteins:*** Hemoglobin, myoglobin, catalases, peroxidases, cytochromes.
- ***Iron requiring enzymes:***
  - *Xanthine oxidase*
  - *Cytochrome C reductase*
  - *Aconitase*
  - *Acyl CoA dehydrogenase*
  - *Succinate dehydrogenase*

Q.68. What are the storage iron? Name them.

Storage iron is present in **two major compounds.** They are:

- ***Ferritin and***
- ***Haemosiderin.***

Q.69. What is Ferritin?

- Free iron is toxic and catalyzes the conversion of O_2^- to hydroxy $OH^\bullet$ oxyradicals. Iron bound to ferritin is non-toxic and it is the storage protein of iron and found in blood, liver, spleen, bone marrow, and intestine (mucosal cells).
- **Apo-ferritin** is the apoprotein with a mol. wt. of 5,50,000. It takes up approximately 4500 iron (Fe^{3+}) atoms in a single molecule to form ferritin which is the primary and most easily available iron storage form.

Q.70. What is hemosiderin?

- Present evidence suggests that ***hemosiderin is derived from ferritin.*** It is ferritin with partially stripped shells. Hemosiderin is usually ***seen in states of iron overload,*** or when Fe is in excess.
- Fe in hemosiderin is available for formation of Hb but mobilization of Fe is much slower from hemosiderin than ferritin.

Q.71. What are the sources of Fe in the body?

Two sources:

- Exogenous and
- Endogenous.

a. ***Exogenous:*** Foods rich in Fe include:
   - ***Animal sources:*** Meat, fish, liver, red marrow are rich sources. Also found in shell fish.
   - ***Vegetable sources:*** Cereals (2.0 to 8.0 mg/100 gm) are the major rich source. Legumes, nuts, dates, amaranth leaves, molasses are other good sources.

b. ***Endogenous:***
   - Fe is utilized from ferritin of RE system and intestinal mucosal cells.
   - Fe obtained from ***effete*** red cells are also reutilized.

Q.72. What are the daily iron requirements?

Requirement of Fe varies according to age, sex, weight and state of health:

- An **adult male** requires approximately 10 mg/day.
- An **adult females:** 20 mg/day.
- **Children:** 10 to 15 mg/day.

- Requirement increases in pregnancy and lactation:
  - Pregnancy: 20 to 25 mg/day.
  - Lactating women: 25 to 30 mg/day.

Q.73. What is the demand of iron in a single pregnancy?

Approximately 1000 mg (1.0 gm) of iron which exceeds the normal iron stores.

Q.74. Under normal conditions, how much dietary Fe is absorbed?

Approximately 10% of dietary Fe is absorbed in normal individual. Out of 10 to 15 mg of daily intake, only 1.0 mg will be absorbed.

Q.75. What is mucosal block theory? or How Fe absorption from intestinal mucosa is regulated?

- Dietary Fe of food stuffs is converted to ***ferric*** (Fe^{3+}) iron by the HCl in gastric juice. It is reduced to ***ferrous*** (Fe^{2+}) by vitamin C and glutathione and absorbed as such.
- In intestinal mucosal cells, it is immediately oxidized to ***ferric*** (Fe^{3+}), principally by ceruloplasmin *(ferroxidase I)* and also to some extent by *Ferroxidase II,* which is taken

up by ***intracellular iron carrier (ICC).*** Some amount is delivered to mitochondria, the rest is distributed between apoferritin and apotransferrin respectively.

- The IIC holds Fe^{3+} iron either protein bound or chelated form which represents the ***carrier iron pool*** in the intestinal mucosal cells. Presence of sufficient amount of Fe in "carrier iron pool keeps the IIC nearly or totally saturated and reduces further Fe absorption.
- This is ***mucosal block*** theory proposed by **Garnick,** which regulates Fe absorption from the gut.

Q.76. State the other factors of iron absorption.

- ***Depends on source of Fe:***
  - Heme Fe mainly from animal source is absorbed efficiently (20 to 30%).
  - Non-heme Fe from Plant source is absorbed less (only 1 to 5%).
- ***Absorption of non-heme Fe is influenced by:***
- ***Composition of the diet:***
  - Alcohol favors Fe absorption.
  - Presence of vitamin C, glutathione helps absorption.
  - Foods that inhibit abosorption are: Tea, coffee, phytates, oxalates and excess of dietary fibers.
- ***pH of intestinal milieu:***
  - Acid pH of gastric juice liberates Fe^{3+} from the food stuffs.
  - pH of duodenum is most conducive for absorption.
- Copper deficiency causes decrease in Fe absorption.
- ***State of health of the individual:***
  - Healthy adults absorb 5 to 10% of dietary Fe which is approximately 1 to 2 mg
  - Iron-deficient adults absorb 10 to 20% of the dietary Fe equivalent to 3 to 6 mg of Fe.

Q.77. What is the normal serum Fe level?

- **In adult males:** 120 to 140 mg/dl
- **In adult females:** 90 to 120 mg/dl.

Q.78. What is TIBC (Total iron binding capacity)?

Total iron binding capacity (TIBC) is same in males and females and it varies from 300 to 369 mg/dl.

Q.79. What is the turn over of Fe?

The "turnover" of Fe in an adult in 24 hours has been calculated to be 35 to 40 mg.

Q.80. What is transferrin?

- Transferrin is a **non-heme Fe binding glycoproteins. Apo-transferrin** is the apoenzyme and Fe is its prosthetic group. It has a mol wt of 70,000 and it can **bind with two atoms of Fe** in the Fe^{3+} state synergistically in presence of HCO_3^- ion.
- It exists in plasma as β_1-globulin and is the **true carrier of Fe.** Also called as **siderophillin.**

Q.81. How much transferrin is saturated in normals?

In plasma, transferrin is saturated only to the extent of 30 to 33% with Fe.

Q.82. How Fe is bound to transferrin?

- For binding to transferrin, ferrous (Fe^{2+}) iron has to be oxidized to "ferric" (Fe^{3+}). This conversion takes place in presence of two Cu-containing enzymes, ***ceruloplasmin (ferroxidase I)*** and ***ferroxidase II.***
- It is also suggested that apoferritin itself may act as a *ferroxidase* and can oxidize $Fe^{2+} \rightarrow$ to Fe^{3+} form which binds tightly to ferritin.

Q.83. What is the result of Fe-deficiency?

Iron deficiency produces ***hypochromic microcytic anemia*** due to impaired synthesis of Hb.

Q.84. What is UIBC (unsaturated iron binding capacity)?

TIBC minus free Fe gives the unsaturated iron binding capacity (UIBC).

Q.85. List the biochemical parameters in iron deficiency anemia.

- Serum Fe is low ↓
- Total iron binding capacity (TIBC) rises ↑
- Serum ferritin level shows slow decline ↓
- Unsaturated Fe binding capacity (UIBC) is greater than normal.

Q.86. What is iron overload? How it can occur?

Iron overload is accumulation of excessive Fe in the body and is of clinical concern. Iron stores in the body increase. This may be due to:

- Excessive Fe absorption
- Parenteral Fe therapy.

- Repeated blood transfusions
- Cells both RE cells and parenchymal cells sequester Fe and cells start to fill with excess of hemosiderin.

Q.87. What are the types or Fe overload?

Two broad types:

- Hemosiderosis
- Hemochromatosis.

Q.88. What is the essential difference between the two types?

- When iron overload is ***associated with injury*** to cells, it is called ***hemochromatosis.***
- When iron overload is without damage to cells, it is called ***hemosiderosis.***

Q.89. What are the clinical manifestations of hemachromatosis?

Massive iron accumulation, mainly as ferritin and hemosiderin occurs in different organs *viz.* liver, pancreas, myocardium, skin, etc. producing cell injuries.

- ***Classical triad:***
  - Micronodular cirrhosis
  - Diabetes mellitus
  - Skin pigmentation (bronzed diabetes)

Deposition of Fe in myocardium can cause cardiomyopathy and heart failure.

Q.90. What is the risk of hemochromatosis?

- Can cause hepatocellular carcinoma.

Q.91. What is Bantu siderosis?

Bantus in Africa cook their foods in iron pots. Also keep alcohol in iron pots. This causes enhanced absorption of Fe leading to Bantu siderosis. Their PO_4 intake is usually low as they consume plenty of corns. Low PO_4 aggravates increased Fe absorption.

Q.92. What is the distribution of copper in the body?

- **Adult humans** contain approximately 100 to 150 mg of copper, out of which approximately 65 mg is found in muscles, 23 mg in bones and 18 mg in Liver,
- **Fetal liver contains** approximately ***ten times more Cu than adult liver.***

Q.93. What are the different forms of Cu in tissues?

Copper occurs as:

- Erythrocuprein in RB cells.
- Hepatocuprein in liver cells.
- Cerebrocuprein in brain cells.

Q.94. What is the dietary source of copper?

- Average diet provides 2 to 4 mg/day.
- ***Good sources*** are: meat, shell fish, legumes, nuts and cereals.
- *Milk and milk products are poor sources.*

Q.95. What is the daily requirement of copper?

- **Adults:** 1.6 to 3.0 mg
- **Children:** 1.5 to 2.5 mg
- **Infants:** 0.6 to 0.8 mg.

Q.96. How copper is absorbed?

Although Cu^{2+} ions are insolube at intestinal pH, a low molecular eight substance present in saliva and gastric juice keeps the Cu in soluble form by complexing with it. This complex gets bound to a protein, ***metallothionein*** and gets absorbed from intestinal mucosal cells. Once absorbed, Cu is bound to albumin and reaches the liver.

Q.97. What is the role of liver in dealing with absorbed copper?

Liver processes absorbed Cu through **two routes:**

- Incorporation to a glycoprotein, ***apo-ceruloplasmin*** forming ceruloplasmin
- Secondly, Cu is excreted in the bile into GI tract from which it is not absorbed. ***Copper homeostasis is maintained almost exclusively by biliary excretion,*** the higher the load of absorbed Cu, more it is excreted through bile, finally through feces.

Q.98. What is the average serum and red blood cells copper level?

- Average serum copper level is 90 µg/dl
- In red blood cells: 93 to 115 µg/dl.

Q.99. Name some important enzymes which require Cu for activity.

Copper forms integral part of certain enzyme and help in enzyme activity. **Examples are:**

- ***Cytochrome oxidase*** **a^3/a**
- ***Monoamine oxidase*** **(MAO)**

- ***Superoxide dismutase*** **(cytosolic)**
- ***Tyrosinase***
- ***Catalase***
- ***Ascorbic acid oxidase***
- **δ-*ALA dehydratase.***

Q.100. State the biomedical functions of copper.

- Role in enzyme actions (as above)
- Role of Cu^{2+} in Iron metabolism (Discussed earlier).
- Role in maturation of elastin.
- Role in bone and myelin sheaths of nerve.

Q.101. What are the forms in which serum Cu exist?

Serum Cu exists in **two distinct forms:**

- ***Direct reacting Cu:*** Which is loosely bound to albumin and amino acid histidine, approximately in 4%. It reacts directly with diethyl dithiocarbamate.
- **Bound form:** which is bound to apoceruloplasmin and forms ceruloplasmin. Ninety six percent of Cu exist in ceruloplasmin.

Q.102. What is Wilson's disease?

- An **inherited disorder** of copper metabolism also known as hepatolenticular degeneration.
- ***Metabolic defect:*** two defects:
  - Defect in incorporation of Cu into apoceruloplasmin to form ceruloplasmin
  - Impaired excretion of Cu to bile.
- ***Blood: Serum*** Cu increases ↑ and ceruloplasmin level decreases↓
- ***Excess of Cu gets deposited in various tissues:***
  - ***Liver:*** Producing cirrhosis liver
  - ***Brain:*** Lenticular degeneration
  - ***Kidney:*** amino aciduria
  - ***Eyes:*** *Keyser-Fleischer ring*

Treatment: Removal of excess Cu can be achieved by administering Cu-chelating agent like "penicillamine".

Q.103. What is Menke disease?

- An **X-linked inherited disorder** of intestinal Cu absorption.
- ***Defect:*** Transport of Cu across the serosal aspect of intestinal mucosal cell membrane is defective.

- If not treated at birth, the disease produces:
  - Mental retardation
  - Temperature instability
  - Abnormal bone formation
  - Susceptibility to infection.

Q.104. What is the other name of Menke disease?

- Also called as ***kinky or steel hair syndrome.***

Q.105. What are the sources of fluorine in the body?

- **Main source:** drinking water.
- **Other sources:** Tea, and fishes like salmon, sardine, and mackerel contains small amounts.

Q.106. How much is the normal requirement of fluorine?

About one part of fluorine in one million parts of drinking water (one PPM) seems to serve daily requirement of fluorine in humans.

Q.107. Is fluorine toxic? What is the limit of daily requirement? What is the lethal dose in adult?

- Fluorine is a toxic trace element.
- ***Daily intake should not exceed 3 mg.***
- Lethal dose for an adult is 2.5 gm.

Q.108. State the important functions of fluorine.

Role in development of teeth:

- Fluorine is necessary in trace amount for tooth development, normal maintenance, hardening of dental enamel and preventing ***dental caries***.
- ***Role in development of bone:*** Trace amount of fluorine promotes normal bone development, increases retention of Ca^{2+} and PO_4 and prevent old age osteoporosis.

Q.109. What is fluoride toxicity? What is the disease called?

- Excess of fluoride in water/diet or inhalation is harmful and toxic.
- It produces the crippling disease called as ***Fluorosis***.

Q.110. What are the effects of excess fluorine on teeth and bone?

- ***Teeth:*** Increase fluoride content of the enamel and dentin may reduce Ca deposition in these tissues and may cause ***mottling*** of enamel. Produces discoloration, corrosion and stratification of enamel including formation of ***pits.***

- ***Bones:*** High fluoride content of bone stimulate osteoblastic activity and cause an abnormal rise in Ca deposition and increased density of bones.

Q.111. State the biochemical changes seen in fluorosis?

High fluoride concentration shows ***following biochemical changes:***

- Mitochondrial damage,
- Inhibits ***enolase*** and ***succinic dehydrogenase*** enzymes.
- **Inhibits protein synthesis** and steroid synthesis.
- ***Collagen synthesis:*** Collagen content is reduced, and its biosynthesis is adversely affected due to reduced proline uptake in collagen tissues.

Q.112. How fluorine content of water be reduced and fluorosis be prevented?

By removing fluorine of water by treatment with activated carbon.

Q.113. What are the dietary sources of zinc?

- ***Animal sources:*** Good sources are liver, eggs, and milk and dairy products.
- ***Vegetable sources:*** Good vegetable sources are cereals (unmilled), legumes, pulses, vegetables like spinach and lettuce, oil seeds and yeast cells.

Q.114. How much zinc is present in a human adult?

An adult human weighing 70 kg contains approximately 1.4 to 2.3 gm of zinc in the body.

Q.115. How dietary zinc is absorbed?

- Only a small percentage of dietary zinc is absorbed. Absorption occurs mainly from duodenum and ileum.
- A low molecular weight ***zinc binding factor*** is secreted by the pancreas which forms complex with zinc and helps in its absorption.

Q.116. What is the daily requirement of zinc?

- **Adult:** 15 to 20 mg
- **Children** and **infants:** 5 to 10 mg
- Pregnant and lactating women: 16 to 25 mg.

Q.117. What is the normal plasma level of zinc?

- Normal plasma level of zinc is 120 to 140 mg/dl.

Q.118. Name some enzymes which require Zn for activity.

Some of the important enzymes which contain Zn and requires for activity are:

- ***Carbonic anhydrase*—**present in RB cells, parietal cells and renal tubular epithelial cells
- ***Alcohol dehydrogenase***
- ***Glutamate dehydrogenase***
- ***Lactate dehydrogenase (LDH)***
- ***Alkaline Phosphatase (ALP)***
- ***Retinene reductase***
- ***Leucine amino peptidase (LAP)***
- ***δ-ALA dehydratase***
- ***Superoxide dismutase***
- ***Carboxypeptidase A***
- ***DNA and RNA polymerase.***

Q.119. State the biomedical functions of zinc in the body.

- ***Role in growth and reproduction:*** Zn deficiency may lead to dwarfism and hypogonadism.
- ***Role in wound healing***
- ***Role in insulin storage and secretion***
- Protamine-zinc insulin and Globin-zinc insulin: ***Long-acting insulin preparation***
- ***Role in vitamin A metabolism:*** Zinc has been claimed to stimulate the release of vitamin A from liver into the blood.
- ***Role in taste:*** Zinc containing protein ***gusten*** in saliva plays a role in taste.

Q.120. What is Acrodermatitis enteropathica?

- A rare **inherited disorder** in which primary defect is inability to absorb zinc.
- The disease is characterized by dermatologic, ophthalmologic, gastrointestinal and neuropsychiatric features alongwith growth retardation and hypogonadism.

Q.121. What is zinc-finger motif? What is its function?

- Protein TF III A, which is positive regulator of 5S- RNA transcription requires zinc for its activity. Each TF III A molecule contains 9 zinc ion in a repeating co-ordination complex.
- **Two types** of "zinc finger motif" are:
  - ***Cys-Cys zinc finger***, and
  - ***Cys-His zinc finger***.

Q.122. How manganese is transported in blood?
Manganese is transported in the plasma in combination with a β_1-globulin called as ***transmagnanin.***

Q.123. Name some enzymes which require manganese, for its activity.
Manganese **acts as a cofactor** or as an activator of many enzyme action. Examples are:
- ***Arginase***
- ***Iso citrate*** **dehydrogenase (ICD)**
- ***Superoxide*** **dismutase (mitochondrial form)**
- ***Phosphoglucomutase***
- ***Enolase***
- ***Lipoprotein*** **lipase**
- ***Leucine*** **aminopeptidase (LAP)**
- ***Pyruvate*** **carboxylase**
- ***Acetyl*** **CoA carboxylase.**

Q.124. State some biomedical functions of manganese.
- ***Role in bone formation:*** Plays a part in synthesis and deposition of MPS.
- ***Role in reproduction:*** Deficiency may cause sterility.
- ***Role in carbohydrate metabolism:*** In Mn deficiency, pancreatic hypoplasia, associated with diabetic type of GTT seen.
- ***Role in Hb synthesis:*** Mn^{++} required for ***δ-ALA synthetase*** activity.
- ***Lipotropic effect.***
- Also participates in ***proteoglycans and glycoprotein synthesis.***

Q.125. How we get chromium?
Dietary sources: Brewer's yeast is rich in chromium. Most grains and cereals contain significant quantities. Significant amount of chromium is obtained in the diet by cooking foods in stainless steel utensils.

Q.126. What is the role of chromium in carbohydrate metabolism?
Chromium is claimed to be a true potentiator of Insulin and hence is called as ***glucose tolerance factor (GTF).*** Trivalent chromium (Cr^{3+}) has been claimed to be a constituent of GTF.

Q.127. What is the occupational hazard with chromium?

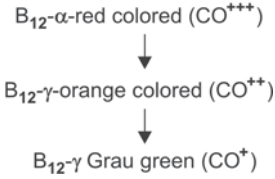
- Hexavalent chromium is toxic than trivalent chromium.
- Chronic occupational exposure to chromate is associated with ***increased risk of lung cancer***.

Q.128. Name one vitamin which contains cobalt.

- Vitamin B_{12} (Cyanocobalamine).

Q.129. What is the role of cobalt in formation of cobamide coenzyme?

Cobalt of vitamin B_{12} undergoes successive reduction catalyzed by the enzyme ***B_{12} reductase*** which requires NADH and FAD. Successive reductions that take place are as follows:



- B_{12}-γ(CO^{+}) reacts with ATP to form adenosyl coenzyme.

Q.130. Name the enzymes which require molybdenum (Mo) for activity.

The enzymes are:

- ***Xanthine oxidase***
- ***Aldehyde oxidase***
- ***Sulfite oxidase***
- ***NADH-nitrate reductase.***

Q.131. What is the source of selenium (Se)?

- Principal source of selenium is plant materials grown in selenium-containing soil.
- Selenium uptake in plant tissue is passive and is influenced by its concentration in the soil.
- A good correlation between selenium intake in food and blood levels has been found.

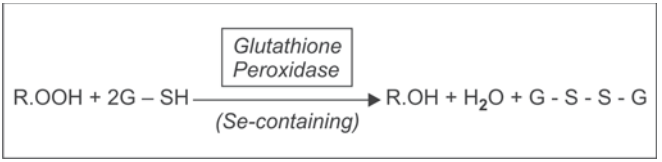
Q.132. What are the biological forms of selenium which occur in the body?

Biological forms of selenium which occur in animal body are selenium analogues of sulphur containing amino acids *viz.*

- **Seleno-methionine**
- **Seleno-cysteine**
- **Seleno-cystine**

Q.133. Name one selenium containing enzyme. What is its function?

- ***Glutathione peroxidase*** (Selenium-containing enzyme).
- ***Function:*** The enzyme is present in cell cytosol and mitochondria and functions to reduce hydroperoxides



Thus it ***acts as an intracellular anti-oxidant.***

- Selenium constitutes a second line of defence against peroxidation.
- Selenium and vitamin E has synergistic effect and prevents peroxidative damage to cellular and subcellular elements chiefly the membranes.

Q.134. What is the daily requirement of selenium?

- **Adults:** 60 to 100 µg
- **Children and infants:** 10 to 30 µg
- During pregnancy and lactation 65 to 120 µg.

Q.135. What are the features of selenium toxicity in humans?

- Excessive selenium intake can produce selenium toxicity.
- Human toxicity manifests as chronic dermatitis, loss of hair, and brittle nails. No hepatotoxicity is observed in humans.
- An early "hall-mark" of selenium toxicity is a ***garlicky smell*** in breath caused by ***exhalation of dimethyl selenide in breath.***
- Likely cause of selenium toxicity in humans is occupational exposure to electronics, glass and paint industries.

Q.136. How selenium toxicity can be treated?

Administration of halogenated aromatic hydrocarbons is believed to disengage from its association with sulphur-containing amino acids and thereby ameliorate the symptoms of such toxicity (See chapter on Detoxication).

Q.137. What is Keshan disease?

- It is selenium deficiency disease in humans.
- It manifests principally as ***cardiomyopathy,*** acute or chronic cardiac enlargement, arrhythmias and ECG changes.
- It has been reported from Keshan country of NE China where soil is deficient of selenium.
- It affects mainly children and younger women.

Q.138. What is Kaschinbeck disease?

It manifests as endemic human osteopathy (as osteo-arthritis). Seen in several parts of the eastern Asia and is ***characterized by degenerative osteoarthritis*** particularly affecting children between 5 and 13 years of age.

Q.139. What is the role of selenium in cancer?

- Selenium has been claimed to be a ***cancer protective agent.*** Areas having high selenium content in foods and high selenium levels in blood have lower incidence of cancers.
- In tissue cultures, selenium has been shown to decrease the mutagenic activity of several known carcinogens.

Q.140. What is the probable mechanism of anticancer activity of selenium?

Several mechanisms are considered for its anticancer acitivity:

- Probably selenium brings about changes in carcinogen metabolites.
- Protects from carcinogen-induced oxidant damage.
- Toxicity of selenium metabolites to tumor cells.

CHAPTER

18 Hormones: Chemistry and Functions

Q.1. Define hormones. Or what are hormones?
Hormones are chemical substances which are synthesized in the body in small quantities, enters circulation and carried to distant target organs and tissues where exert profound biochemical and regulation of metabolic events.

Q.2. How the word hormone derived?
The word hormone is derived from Greek word ***hormacin*** which means to excite.

Q.3. What are the similarities of hormones with enzymes?
Hormones act as body catalysts resembling enzymes. Like enzymes they are required in small quantities. They are not used up during the reaction.

Q.4. How hormones differ from enzymes?
- They are usually porduced in an organ other than that in which they ultimately perform their action.
- They are usually secreted in blood prior to use.
- Structurally, they are not always proteins.

Q.5. How will you classify hormones.
- Hormones can be classified in **two ways:**
  - – According to their chemical nature
  - – According to their mode of action.

Q.6. Classify hormones on their chemical nature.
Three main groups:
- ***Protein/Peptide hormones:*** They are either large proteins or small/medium size peptides, e.g. insulin, glucagon, parathormone (PTH), calcitonin, pituitary hormones, etc.

- ***Amino acid derivatives:*** They are derived from amino acid tyrosine, e.g. thyroid hormones, catecholamines (epinephrine and norepinephrine).
- ***Steroid hormones:*** Like corticosteroids, mineralocorticoids, androgens, estrogens, progesterone, etc.

Q.7. Classify hormones according to their mode of action.

- Hormones which ***act*** through the ***second messenger Cyclic AMP***, e.g. glucagon and most of the other protein hormones.
- Hormones which ***act at nuclear level (nuclear action):*** Hormones bind to high affinity "receptor" protein (cytosolic), move to the nucleus as a complex, interact with chromatin there and increase the production of m-RNA and corresponding proteins, e.g. steroid hormones.
- Hormones which ***act by binding to their "receptors" on the plasma membrane***, e.g. insulin.
- Hormones which ***directly reach nucleus*** and interact with specific receptor proteins and increase transcription and translation, e.g Tri-iodo thyronine (T_3).
- Hormones which ***increase the extent of translation*** without increasing transcription e.g. ACTH, Insulin.

Q.8. State the factors that regulate hormone action.

Action of a hormone at a target organ is regulated by ***four factors:***

- Rate of synthesis and secretion of hormone.
- Specific transport systems in plasma.
- Hormone-specific "receptors" in target cell membrane which differ from tissue to tissue.
- Ultimate degradation of the hormone usually by the liver or kidneys.

Q.9. Enumerate the different mechanisms of hormone action.

- Interaction with nuclear chromatin (nuclear action), e.g. steroids.
- By increasing/decreasing the Cyclic AMP level (second messenger) in the cells through ***"adenylate cyclase system"*** and GTP, e.g. most of the protein hormones.
- Acting on the "membrane receptors", e.g. insulin.
- Stimulation of enzyme synthesis at the ribosomal level.

- Direct activation at the enzyme level.
- By increasing/or decreasing the ionized Ca^{++} in the cells (third messenger) through phosphoinositides.

Q.10. What is the role of phosphoinositides in hormone action?
Certain hormones by binding to their receptors, enhances the breakdown of phosphoinositides by activating ***phospholipase C-phosphoinositol*** system, and produces "1, 4, 5 inositol triphosphate (ITP) and diacyl glycerol (DAG). Inositol triphosphate enhnaces the mobilization of Ca^{++} ions into the cytosol from mitochondrial intracellular Ca^{++} Pool and act as an important signal for hormone action **(third messenger)**.

Q.11. Name at least six important hypothalamic releasing or inhibiting factors.
- Corticotropin releasing hormone (CRH or CRF)
- Thyrotropin releasing hormone (TRH or TRF)
- Growth hormone releasing/inhibiting hormones (GHRH or GHRF and GHRIH/GHRIF).
- Gonadotropin releasing hormone (GnRH)—(includes LHRH and FSH-RH which affect LH and FSH secretion of anterior pituitary
- Prolactin release/inhibiting hormone (PRIH/PRIF). ***(It is claimed that PRIH is same as dopamine)***.
- Melanocyte stimulating hormone-releasing/inhibiting hormone (MSH-RH/MSH-RF or MSH-RIH/MSH/RIF).

Q.12. How does hypothalamic hormones act?
Most of the hypothalamic hormones exert their effects by a Ca^{++}-phosphoinositides mechanism and not through the mediation of Cyclic AMP.

Q.13. Name one hypothalamic hormone which is tri-peptide and acts through cyclic AMP.
- ***TRH (thyrotropin releasing hormone)***
- It is a neutral tripeptide and has no ionizable groups and chemically it is pyroglutamic acid-histidine-prolinamide.
- TRH increases c-AMP level in anterior pituitary cells, and releases TSH (thyroid stimulating hormone). TSH secretion inturn, increases thyroid hormones production.

- TRH also stimulates prolactin secretion.
- ***Estrogens act to increase sensitivity of anterior pituitary cells to TRH.***

Q.14. What are tropins? How they are regulated?

- A tropin or tropic hormone is the one which influences the activities of other endocrine gland, principally those involved in 'stress' and reproduction. These hormones are carried by the blood to other target glands.
- The pituitary tropins are under the positive and negative control of peptide factors from hypothalamus. Further the tropic hormones are usually subject to "feed-back" inhibition at the pituitary or hypothalamic level by the hormone product of the final target gland.

Q.15. Name the tropic hormones secreted by the anterior pituitary gland.

The tropic hormones produced by anterior pituitary are:

- **Thyroid stimulating hormone** (**TSH** or thyrotropin).
- **Adrenocorticotropic hormone** (**ACTH** or corticotropin).
- **Lactogenic hormone** (**LTR** or prolactin or luteotropin).
- **Gonadal hormones:**
  - Follicle stimulating hormone (FSH).
  - Luteinizing hormone (LH). Also called as interstitial cell-stimulating hormone (ICSH).

Q.16. Is growth hormone (somatotropin) produced by anterior pituitary is a tropic hormone?

Growth hormone (GH) does not act by influencing the activity of another endocrine gland. Hence it is **not a "tropic" hormone** as per definition of "tropins". The name somatotropin given to GH is a **"misnomer"**.

Q.17. Name the principal anabolic hormones.

- **Growth hormone (GH)**
- **Insulin**
- **Testosterone.**

All the above three hormones stimulate protein synthesis and brings about a positive nitrogen balance by retaining Nitrogen.

Q.18. What is the chemical nature of GH?

GH is a protein hormone consisting of a single polypeptide with a mol wt of 21,500. Human GH contains 191 amino acids.

Q.19. What is sulfation factor?

GH stimulates production of **somatomedins**, called as sulfation factors from liver and possibly kidneys which can produce many of the anabolic effects of GH.

Q.20. What is TSH?

- TSH is a tropic hormone (thyroid stimulating hormone or thyrotropic hormone).
- It is produced by basophil cells of anterior pituitary gland and is **glycoprotein** in nature. Its mol. wt. is approximately 30,000 and consists of **α** and **β** subunits. The biological specificity resides mainly in the **β**-subunit.

Q.21. Which tropic hormones share the α-subunit of TSH?

α-Subunit of TSH is nearly similar to LH, FSH and HCG.

Q.22. How does TSH act?

- There are glycoprotein receptors on the thyroid cells membrane which bind to the receptor-binding site on **β**-subunit of TSH. The complex then activates ***'adenyl cyclase'*** system increasing c-AMP level in the cells which act as "second messenger" and duplicates the functions of the hormone.
- TSH stimulates the synthesis of thyroid hormones at all stages such as "iodide trapping" (uptake), organification and coupling. It enhances release of stored thyroid hormones.
- It increases thyroidal growth and general metabolic activity including glucose oxidation.

Q.23. What is Cushing's disease? State the clinical features and biochemical alterations.

- The disease is due to overproduction of corticotropin (ACTH) because of tumor or hyperplasia of **β**-cells of anterior pituitary. This leads to hyperactivity of adrenal cortex leading to increased secretion of corticosteroids specially **glucocorticoids (GC)**.
- Produces hyperglycemia, glycosuria, muscle wasting, atrophy of skin, high NPN, negative nitrogen balance, high level of Free fatty acids (FFA), abnormal retention of fats giving ***moon face,*** buffalo neck, rentention of Na^+ and water and produces hypertension.

Q.24. What is Cushing's syndrome?

When the hyperactivity of adrenal cortex and increased secretion of Gluco-corticoids is ***due to tumor or hyperplasia of adrenal cortex primarily*** and does not involve anterior pituitary, the condition is called Cushing's syndrome. The clinical features and biochemical alterations are similar to Cushing's disease.

Q.25. What is the role of FSH in males?

- It stimulates seminal tubule and testicular growth.
- Plays an important role in maturation of spermatozoa. The conversion of primary spermatocytes into secondary spermatocytes in the seminiferous tubules is stimulated by FSH. ***In absence of FSH, spermatogenesis cannot proceed.***
- However, FSH by itself cannot cause complete formation of spermatozoa. For its completion ***testosterone is also required.***

Q.26. What is the role of LH in males?

Testosterone is produced by the interstitial cells of leydig only when the cells are stimulated by LH from anterior pituitary gland. The quantity of testosterone secreted varies approximately in proportion to the amount of LH available.

Q.27. What is β-lipotropin (β-LPH)?

- **β**-lipotropin is derived from the precursor molecule **"pro-opiomelanocortin peptide" (POMC)**. It is a single chain polypeptide containing 93 amino acids.
- **γ**-LPH containing 60 amino acids is a part of **β**-LPH

Function of β-LPH: **β**-LPH is the precursor for three types of endorphins **α**, **β** and **γ** and also for **β**-MSH.

Q.28. What are endorphins? What is their function?

Endorphins are a group of polypeptides which ***influence the transmission of nerve impulses.*** They are also known as ***opioids*** because they bind to those receptors which bind opiates like morphine and plays a role in pain perception.

- ***Types:*** There are **three types** of endorphins **α**, **β** and **γ**. The sequence of 31 amino acids at the C-terminus of **β**-LPH (obtained from POMC), i.e. a.a. 104 to 134 gives **β**-*endorphin*. **α**-*endorphin* (104 to 117) containing

17 a.a. less than the β from the C-terminus, and ***γ-endorphin*** (104 to 118) containing 16 a.a. less than the β from C-terminal end.

- ***Function:*** Endorphins bind to the same CNS receptors like the morphine opiates and they play a role in the endogenous control of pain perception. They have higher analgesic potency than morphine.

Q.29. What are encephalins?

- The opioides first discovered were two pentapeptides in the brain and were named encephalins. They are:
- Methionine-encephalin and
- Leucine-encephalin.

Q.30. Name the two octapeptide hormones of biomedical importance. Which part of the pituitary gland secretes them?

- **Vasopressin** (antidiuretic hormone).
- **Oxytocin.**

Both the hormones are ***stored and secreted from the posterior pituitary gland.***

Q.31. How does oxytocin and vasopressin differ structurally?

Oxytocin differs from vasopressin with respect of two amino acids only. Phenylalanine and arginine in vasopressin are replaced by isoleucine and leucine of oxytocin.

Q.32. What is the actual site of production of posterior pituitary hormones?

Posterior pituitary hormones are synthesized in neurosecretory neurons of hypothalamus. After synthesis they migrate through pituitary stalk to posterior pituitary gland.

Q.33 How vasopressin and oxytocin are stored in posterior pituitary gland?

Posterior pituitary hormones in posterior pituitary gland are stored in association with two proteins ***neurophysin I and II*** with molecular weight of 19000 to 21000 respectively.

Q.34 What are the functions of vasopressin?

- ***Antidiuretic effect:*** Principal action is antidiuretic effect, hence also called as "antidiuretic hormone" (ADH). It reabsorbs water from the kidneys by distal tubules and collecting tubules.

- ***Pressure effect:*** Stimulates contraction of smooth muscles and causes vasoconstriction by increasing cytosolic Ca^{++} concentration.
- ***Glycogenolytic effect:*** Produces glycogenolysis by increasing intracellular Ca^{++} concentration.
- ***Urea-retention effect.***

Q.35. How does vasopressin act on renal tubules?
By increasing Cyclic AMP in kidney tubules.

Q.36. What is diabetes insipidus?
It is **produced due to failure in secretion or action of vasopressin.** It is characterized by very high volumes of urine output, up to 20 to 30 liters per day with low sp.gr. and clinically by excessive thirst.

Q.37. What are the types of diabetes insipidus?
Two types:
- **Primary** (central or neurohypophyseal) diabetes insipidus: in this vasopressin secretion is poor.
- **Nephrogenic diabetes insipidus (secondary):** In this kidney connot respond to vasopressin due to renal damage. **The damage is common in psychiatric patients on lithium therapy.**

Q.38. What are the functions of oxytocin?
- Primary function of oxytocin is contraction of smooth muscle. This is mainly seen on mammary glands ***(galactobolic effect)*** and on uterus ***(uterine effect).***
- Estrogen increases the number of oxytocin receptors on these organs and progesterone decreases thus inhibiting the action.

Q.39. What is MSH?
- The hormones secreted by intermediate lobe or middle lobe of pituitary gland are called **"melanocyte stimulating hormones" or MSH.**
- The ACTH is cleaved to **β**-MSH which has 13.a.a There is also **α**-MSH which is present in larger quantities. Amino acids 11 to 17 of **β**-MSH are common to both **α**-MSH and ACTH.
- ***Hormonal Control:***
  - Hydrocortisone and cortisone inhibit the secretion of MSH.
  - Epinephrine and nor-epinephrine inhibit the action of MSH.

Q.40. What is the function of MSH? What is its role in Addison's disease?

- ***Function:*** MSH darkens the skin and is involved in ***skin pigmentation by deposition of melanin*** by melanocytes.
- ***Role in Addison's disease:*** In Addison's disease, there is deficiency of corticoids hence, **MSH production is in excess** which increases the synthesis of melanin resulting in brown pigmentation of skin in this condition.

Q.41. Mention the principal abnormalities associated with pituitary function.

Principal abnormalities are as follow:

- ***Hyperpituitarism:*** Excess production of GH (eosinophilic adenoma), e.g. gigantism, and acromegaly.
- Excess production of ACTH (basophilic adenoma), e.g. Cushing's disease.
- ***Hypopituitarism:*** Produces—dwarfism, pituitary myxedema.
- Panhypopituitarism.

Q.42. What is Fröhlick's syndrome?

A manifestation of hypopituitarism in childhood. Also called **adiposo-genital dystrophy**. Several pituitary functions are disturbed. The children are hypoplastic and there are diffuse deposits of fats.

Q.43. What is Lorain-Levy type?

- Also a manifestation of hypopituitarism in childhood.
- Mentally these patients are normal, general metabolism is unaffected. But skeletal growth ceases, and the secondary sexual characters do not appear.
- Causes may be ischemic necrosis/destructive lesion of pituitary.

Q.44. What are the hormones produced by the thyroid gland?

- **Follicular cells produce:**
  - Thyroxine (T_4)
  - Tri-iodothyronine (T_3)
  - "Reverse" T_3
- **Parafollicular cells (C-cells) produce:**
  - Calcitonin.

Q.45. What is the chemical nature of thyroid hormones?

Thyroid hormones are **iodinated amino acid tyrosine:**

- **T_4 (Thyroxine):** is 3, 5, 3', 5'-tetraiodothyronine.
- **T_3 (Tri-iodo thyronine):** is 3, 5, 3'-tri-iodo thyronine.
- **"Reverse"** T_3: is 3, 5', 3'-Tri-iodothyronine. Reverse T_3 has hormonal action. ***Presence of iodinations at 3 and 5 is needed for hormone action.***

Q.46. Name the raw materials required for biosynthesis of thyroid hormones.

Two raw materials (substrates) required by the thyroid gland to synthesize the thyroid hormones are:

- **Iodine (I_2),** and
- **Thyroglobulin—a glycoprotein.**

Q.47. What is the nature of thyroglobulin?

- Thyroglobulin is a **glycoprotein** present in colloid of thyroid follicles, a macroglobulin 19 S in type with a melocular weight of 666,000.
- ***Each molecule contains 115 tyrosine residues***. Iodination takes place with the tyrosine residues.

Q.48. What is meant by iodine "trapping"?

- The thyroid gland concentrates iodine by ***'actively'*** and ***'selectively'*** transporting it from the circulation. The transport mechanism is called as **"iodidetrapping"** or ***"iodide-pump"***.
- The iodide trapping is done:
  - **Against electrical gradient**
  - **Against concentration gradient (20:1).**

As it is actively taken in, it requires energy which is provided by ATP.

Q.49. How the iodide transporter pump acts?

- Iodide "transporter pump" is located in the basal plasma membrane in association with **Na^+-K^+ dependent ATP-ase** and requires a simultaneous activity of the "sodium-pump".
- Energy is provided by hydrolysis of ATP followed by a K^+ influx and Na^+ efflux.

Q.50. What is active iodine? How it is formed?

- The iodides before it is incorporated in tyrosine residues of thyroglobulin, is ***converted to "active" iodine.***

- At the colloid membrane interface the enzyme *thyroperoxidase* binds iodide (I^-) and thyroglobulin at distinct sites of its molecule and then in presence of H_2O_2, the enzyme oxidizes the iodide to "active" iodine which may be ***iodinium ion*** ***(I^+)*** or ***hypoiodite*** (HIO) or both.

Q.51. What is the nature of the enzyme thyroperoxidase.

- Oxidation of iodide to active iodine, iodination of tyrosine residues and coupling of iodotyrosines are catalyzed by a ***heme-containing*** particulate bound peroxidase called ***thyroperoxidase*** which requires H_2O_2 for its activity.
- Thyroperoxidase is a **tetramer**, having mol wt 90,000, H_2O_2 is produced by an NADPH-dependent enzyme system.

Q.52. How iodination of tyrosine residues occur?

- The process of iodination is called ***organification.***
- Catalyzed by the enzyme ***thyroperoxidases***.
- Active iodine produces iodination of tyrosine redisues:
  - Firstly at 3 position to form **mono-iodotyrosine (MIT)**
  - Next at 5 position to form **di-iodotyrosine (DIT).**

Q.53. How iodotyrosines are coupled?

- **Two molecules of DIT** undergo oxidative condensation under the influence of the enzyme ***thyroperoxidase*** to form T_4.
- Similarly **condensation of MIT with a molecule of DIT** produces T_3, and condensation of DIT with MIT forms **"reverse"** T_3.

Q.54. How much thyroid hormones secreted daily?

Approximately 80 to 90 mg of thyroid hormones is secreted daily under normal physiological conditions.

Q.55. How thyroid hormones are carried/transported in the blood?

T_4 and T_3 in plasma are carried by two specific plasma proteins:

- **Thyroxine binding globulin (TBG) and**
- **Thyroxine binding prealbumin (TBPA).**

When binding capacity of the above two carrier proteins is saturated, then they can be bound to serum albumin also.

Q.56. Which form of thyroid hormone is metabolically most active?

- Approximately 0.05% of the circulating thyroid hormones are in "free" unbound form.
- "Free" T_4 and T_3 are metabolically active hormones- **"Free" T_3 is more active than T_4.**

Q.57. State the conditions where TBG level increases.

- In pregnancy,
- After administration of estrogens,
- Women taking contraceptive pills.

Q.58. State the conditions in which TBG level decreases.

- In hypoproteinemic states, e.g. liver diseases, (cirrhosis Liver).
- In nephrosis,
- After treatment with androgenic/or anabolic hormones.

Q.59. How does T_3 and T_4 act on target tissues?

- The thyroid hormones "free" T_3 and T_4 bind to high affinity receptors in the "target" cell, T_3 ***having about ten times more binding affinity than T_4.***
- Approximately more than 80% of circulating T_4 is converted to T_3 in the peripheral tissues and thus the biological response is related to the final levels of "free" T_4 and T_3 that are bound to receptors.
- T_3 binds to the receptors in the nucleus, while T_4 first gets bound to cytoplasmic "core" receptors and then the complex gets translocated to the nucleus. In the nucleus, ***it exerts its effects in the transcription*** of m-RNA and translation of enzymes.

Q.60. State the metabolic effects of thyroid hormones.

- ***On carbohydrate metabolism:*** increases intestinal absorption of glucose, accelerate glucose oxidation, glycogenolysis and gluconeogenesis.
- ***On lipid metabolism:*** Increases lipolysis↑ in adipose tissue increasing plasma FFA↑, decrease in cholesterol and LDL in hyperthyroidism and increase in cholesterol and LDL in hypothyroidism.
- ***On protein metabolism:*** At physiological levels, thyroid hormones are protein anabolic by increasing transcription and translation. ***Above physiological levels, the hormones causes protein degradation (catabolic).***

Q.61. What is the effect of excess thyroid hormones on mitochondria?
Excess of thyroxine produces swelling of mitochondria and ***uncouples the oxidative phosphorylation*** producing heat in the body.

Q.62. How does antithyroid drugs act?
Most of the durgs that inhibit thyroid function act either by:
- Interferring with "iodide trapping".
- Inhibit conversion of iodide to "active iodine".
- Inhibits iodination and coupling.
- Inhibits hormonal release, or
- By inhibiting conversion of T_4 to T_3 at target tissues.

Q.63. Name some antithyroid drugs that inhibit "iodine trapping."
Chlorate, hypochlorite, periodate, perchlorate, pertechnate, etc.

Q.64. Give examples of antithyroid drugs that inhibit iodination and coupling.
- ***Thiocarbamides:*** Thiourea, thiouracil, propyl thiouracil, methimazole (tapazole), carbimazole, etc.
- ***Aminobenzenes:*** Sulphonamides, sulfonyl urea, tolbutamide, carbutamide, etc.

Q.65. Name some antithyroid drugs that inhibit release of thyroid hormones.
Colchicine, vinblastin, vincristine, cytochalasin.

Q.66. Name the antithyroid drugs that inhibit $T_4 \rightarrow T_3$ conversion in target tissues.
Propyl thiouracil, propanolol.

Q.67. What is Wolff-Chaikoff's effect?
At high serum concentrations of iodide exceeding 30 mg/dl, iodide exerts antithyroid effects and sometimes even produces goitre. This is called as ***Wolff-Chaikoff effect.***

Q.68. What are naturally occurring goitrogens?
- Vegetables of ***brassicacae*** family like cabbage, cauliflower, rutabages, mustard seeds, turnips, etc. contain ***thioglycosides*** which are called as ***pro-goitrin***.
- Pro-goitrin can be converted to ***goitrin***, an active antithyroid agent by colonic bacteria. Progoitrin activator present in vegetables is heat-labile.

Q.69. State the prinicipal abnormalities associated with thyroid function.

- ***Hyperthyroidism:***
- Exophthalmic goitre (Graves' disease)
- Toxic nodular goitre.
- ***Hypothyroidism:***
- Cretinism in children.
- Myxedema in adults.
- ***Simple Goitres:*** due to I_2 deficiency.
- ***Goitre due to inherited defects.***

Q.70. What is cretinism?

- Insufficient development of thyroid in embryonic life results in cretinism in a child.
- Cretinism is associated with ***slow growth***, ***dwarfism*** and ***mental retardation***. Dry skin, puffy lips, enlarged protruding tongue, scanty hair, pot bellies and vacant look are some of the clinical features of cretinism.

Q.71. What is myxedema?

- Myxedema is caused by hypothyroidism in adult (adult analogue of cretinism).
- ***Spontaneous adult myxoedema is now considered as auto-immune disease.***
- Patients complaints of undue sensitivity to cold, mentation is slow, body temperature is lowered. ***The disease is characterized by:***
  - Puffiness of face and extremities, dry coarse skin showing yellow tinge, falling of hairs specially of eye-brows, obesity, anemia, lowered BMR, ***hyper-cholesterolemia,*** and low serum PBI, T_3 and T_4 values.

Q.72. What is hyperthyroidism (thyrotoxicosis)?

Increased activity accompanied by excessive secretion of thyroid hormones produces Grave's disease (exophthalmic goitre). Hyperthyroidism may occur with or without goitre.

The disease is characterized by:

- Nervousness, irritability, weight loss, increased body temperature, fatigue, increased appetite (hyper-phagia), a fine tremor of the outstretched fingers, increased heart rate, protrusion of the eyeball (exophthalmos).
- ***Biochemically:*** High BMR, hypocholesterolemia, increased serum PBI, T_3 and T_4 levels.

Q.73. What is the normal serum PBI level?

- 3 to 7 mg/dl.

Q.74. What is the normal serum BEI?

- Normal serum BEI (butanol extractable iodine) is 2.0 to 6.5 mg/dl.
- The bound protein hormone is extractable with n-butanol. This fraction is measured as butanol-extractable iodine (BEI).

Q.75. What is LATS?

- LATS is long-acting thyroid stimulator.
- ***It is an IgG immunoglobulin*** produced in thyrotoxicosis by autoimmunization against thyroid components, probably a low molecular weight 4 S compound in thyroid cell-sap.
- Its effects on thyroid are much longer, it binds to "TSH receptors" on thyroid cell membrane and stimulates the action of TSH in stimulating the thyroid gland.

Q.76. What is the cause of exophthalmos?

A protrusion of the eyeballs (exophthalmos) ***results from mucoprotein deposition*** and edema in the retrobulbar tissue due to probably production of an ***exophthalmos producing IgG.***

Q.77. What is the simple endemic goitre?

Simple endemic goitre also called as colloid goitre is caused by deficiency of iodine in the diet. This occurs in regions, for away from the sea coast, where the soil and water are low in iodine.

Q.78. How can you prevent simple endemic goitre?

By advising **to take iodized salt**, containing 0.002% of iodide.

Q.79. What is chemical hyperthyroidism?

- In rare subjects, circulating level of bound and free T_4 is normal but the T_3 concentration is elevated and accounts for the thyrotoxic state, This is called as **chemical hyperthyroidism**.
- Probably in these patients T_3 is produced more from deiodination of T_4 at peripheral tissues.

Q.80. What is the clinical significance of iodine therapy by surgeons before surgery on thyroids?

Iodine therapy is sometimes resorted to by surgeons to hyperthyroid patients for a short period to prepare the patient for surgery (subtotal thyroidectomy).

Advantages of such iodine therapy are:

- Colloid accumulates and enhances firmness to the gland.
- Vascularity of the gland is decreased,
- Decreases blood thyroid hormones level,
- Reduces the chance of acute postoperative hyperthyroidism.

Q.81. Name the hormones involved in Ca and P metabolism.

- ***Parathormone (PTH),***
- ***Calcitonin, and***
- ***Calcitriol (1, 25 - di (OH)D_3)***

Q.82. What is the chemical nature of PTH?

It is a linear single chain polypeptide hormone consisting of 84 a.a., having an approximately mol wt of 9,500.

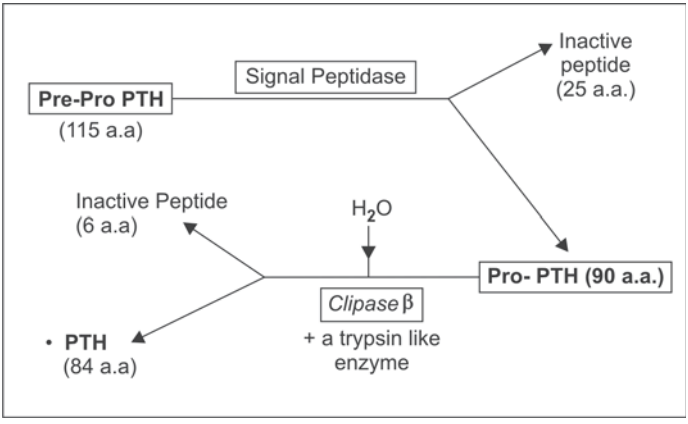
Q.83. Which cells of parathyroid glands produce PTH?

PTH is produced by the ***chief cells.***

Q.84. What are the precursors of PTH. Outline schematically the formation of PTH form the precursors.

Precursors of PTH are:

- Pre-pro PTH and
- Pro-PTH



Q.85. In which part of the peptide resides PTH activity?

- It has been claimed that **amino acid sequence 1 to 29 (or possibly 1 to 34)** from N-terminal end is essential for PTH activity.
- **Methionine** present in the peptide is an important a.a. and necessary for Ca-mobilizing effect.

Q.86. How does PTH act?

- Principally acts by increasing the cyclic AMP level↑
- Also increases ionic Ca^{++} in the cells.↑
- Increases citric acid and lactic acid in bone tissues which can solubilize the bone.
- Specifically inhibits osteoblasts and stimulates osteoclastic activity.

Q.87. State the effect of PTH on Ca and P metabolism.

- Increases serum Ca concentration↑
- Decreases serum inorganic PO_4 concentration↓
- Increases tubular reabsorption of Ca by the kidneys↑
- Decreases reabsorption of phosphates by the kidney↓
- Increases urinary PO_4 excretion↑

Q.88. Why PTH is called as a tropic hormone for calcitriol?

PTH stimulates the ***1-α-hydroxylase*** enzyme in renal tubules and increases conversion of 25-OH-D_3 to 1, 25-di-OH-D_3 **(calcitriol)** which increases intestinal absorption of Ca by increasing formation of ***Ca-binding protein.***

Q.89. What is von-Recklinghausen's disease?

- It is a manifestation of hyperparathyroidism on bone.
- Shows increased osteoclastic activity resulting to resorption of bones producting cyst like structures. It is called as ***osteitis fibrosa cystica*** **(von Recklinghausen's disease)**.

Q.90. What is tetany?

It is a manifestation of hypoparathyroidism. There is hypocalcaemia, specially ***decrease in ionic*** Ca↓ which produces hyperexcitability of muscular apparatus, fibrillation and twitchings of muscles, generalized clonic and tonic muscular spasms, resulting to epileptiform convulsions. The spasms of laryngeal muscles may cause asphyxia and death.

Q.91. What is Trousseau's sign?

It is seen in tetany. Application of pressure over arm with blood pressure cuff results in muscular spasm causing what is called as ***accoucheur hand*** **(carpopedal spasm)**.

Q.92. What is pseudo-hypoparathyroidism?

- It is a **congenital disorder** due to **inherited deficiency of GTP-dependent regulatory protein**.
- PTH secretion is normal.
- Though PTH is available, it cannot act on target cells, thus cyclic AMP is not increased to duplicate functions of PTH.
- **It is characterized by:**
  - Neuromuscular hyperexcitability
  - Stunted growth
  - Mental retardation
  - Short metacarpals and metatarsals.
- Biochemically, serum Ca↓ is low (hypocalcemia), hyperphosphatemia, **normal serum PTH**.

Q.93. What is $PTH_{\gamma}P$?

- It is a parathormone-related peptide produced in certain extraparathyroid tumors *viz.* Squamons cell carcinomas of lungs, esophagus, cervix and breast.
- Hence also called as humoral hypercalcaemic factor of malignancy (HHFM).
- $PTH_{\gamma}P$ can bind to parathormone receptor in target tissues like bones and kidney and can mimic the action of PTH producing hypercalcemia and hypophosphatemia.
- $PTH_{\gamma}P$ is produced by **a gene located in chromosome 12** which is distinct from PTH gene located on chromosome 11.

Q.94. What is the chemical nature of calcitonin?

Calcitonin is a single chain poplypeptide, contains 32 a.a., having a mol. wt. of 3600. An intrachain disulfide bridge joins two cysteine residues between position 1 and 7.

Q.95. What is the source of calcitonin?

Calcitonin is produced by **special cells called "C-cells"**.

Q.96. What are C-Cells? How are they formed?

- ***"C-Cells" constitute an endocrine system,*** derived from "neural crest" and are found in thyroids, parathyroids and thymus.
- It was initially thought that calcitonin is produced by parathyroid glands, but now it is confirmed that it is mainly produced by "C-Cells of thyroids" Hence it is also called as ***thyrocalcitonin.***

Q.97. What is the function of calcitonin?

- Calcitonin is a **Ca-lowering hormone**, its release is stimulated by high serum Ca level.
- Calcitonin acts both on bone and kidneys.
- It produces hypocalcaemia and hypophosphatemia. Calcitonin inhibits the resorption of bones by osteoclasts and thereby reduces mobilization of Ca and inorganic PO_4 from bones into the blood.

Q.98. State the condition in which excessive calcitonin is produced.

- **Medullary carcinoma of thyroid** is a tumor which arises from parafollicular C-cells of thyroid gland.
- Frequently patient with this tumor has been found to be associated with:
  - Cutaneous neuromas
  - Adrenal tumors and
  - Parathyroid enlargement.
- Patient also suffers from severe diarrhea.
- Excessive calcitonin is produced.

Q.99. State the therapeutic uses of calcitonin.

Calcitonin has been ***used in the following clinical conditions:***

- In Paget's disease
- In idiopathic hypercalcemia of infancy
- In hypercalcemia secondary to malignancies, hyperparathyroidism and vitamin D intoxication.

Q.100. What is the effect of calcitonin on calcitriol formation?

Calcitonin inhibits ***1-α-hydroxylase*** in renal tubules and inhibits production of calcitriol.

Q.101. Which part of pancreas functions as endocrine system?

- **Islets of Langerhans** function as endocrine system.
- They make up 1 to 2% of the weight of pancreas.

Q.102. State the cells present in islets of Langerhans and mention the hormones produced?

Three types of cells are present in islets of Langerhans. They are:

- ***α-Cells:*** Approximately 20%, produces the hormone glucagon.
- ***β-cells:*** Approximately 75%, produces the hormone insulin. Insulin molecules form polymers and also complexes with Zn^{++}
- ***δ-Cells:*** 1 to 8% of the cells. Responsible for formation of:
  - Somatostatin, and
  - A pancreatic polypeptide.

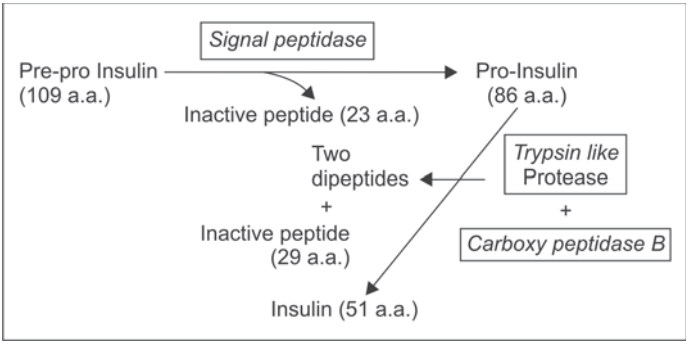
Q.103. What is the chemical nature of insulin?

- Insulin is a protein hormone, having mol. wt. 5734, composed of ***two polypeptide chains called "A" chain and "B" chain.***
- ***"A" chain*** contains 21 a.a and ***"B" chain*** contains 30 a.a. Total amino acids are 51.
- Both the chains are held together by two S-S linkages, cys 7 and cys 20 of "A" chains are joined to cys 7 and cys 19 of "B" chain. In addition, the "A" chain carriers an "intra-chain" S-S linkage between cys 6 and cys 11.

Q.104. What are the precursors of insulin? Outline the formation of insulin from the precursors.

Precursors are:

- ***Pre-pro insulin,*** and
- ***Pro-insulin.***



Q.105. State the factors which affect the secretion of insulin.

- ***Stimulation of Rt Vagus*** causes secretion of insulin.
- ***Hyperglycaemia:*** Directly stimulates **β**-cells increasing synthesis and release of insulin.
- ***Fructose and Mannose:*** Can stimulate insulin release.
- ***Fatty acids:*** Such as octanoic acid stimulate β-Cells to secrete insulin.
- ***Amino acids:*** Leucine and arginine can stimulate secretion insulin.
- ***Hormones;***
  - ***Glucagon:*** Potent stimulant of insulin secretion.
  - ***GH and glucocorticoids:*** Can produce increase in circulating insulin.
  - ***Epinephrine:*** Potent and highly effective inhibitor of insulin secretion.
  - ***GI hormones:*** Gastrin, CCK-PZ, and secretin can directly stimulate insulin secretion.
  - ***GIP*** stimulates insulin secretion in presence of hyperglycemia but not at normal blood glucose levels.

Q.106. How does insulin act on target tissues?

Insulin acts on target tissues by binding to specific ***"Insulin receptors"*** which are glycoproteins in nature. A high blood insulin level decreases the number of insulin receptors on target cell membrane, probably through internalization of the insulin-receptor complex into the cell and thus decreases the insulin-sensitivity of the target tissues.

Q.107. What is the net effect of insulin on carbohydrate metabolism?

- **Net effect is lowering of:**
  - Blood glucose level↓ and increases the glycogen store↑
- The above is achieved by:
  - Increase of glucose uptake↑, increasing glycolysis↑
  - Increasing conversion of PA to acctyl CoA
  - Stimulates glycogenesis↑
  - Decreases gluconeogenesis↓
  - Decreases glycogenolysis↓
  - Increases HMP shunt↑.

Q.108. What is the net effect of insulin on lipid metabolism?

- Net effect is
  - Lowering of blood FFA level↓ and
  - Increase of TG store (increases lipogenesis)↑
  - The above is achieved by:
    - Increasing FA synthesis↑
    - Increasing synthesis of TG↑
    - Decreasing lipolysis↓
    - Decreasing ketogenesis↓.

Q.109. What is the net effect of insulin on protein synthesis

- ***Net effect is insulin promotes protein synthesis↑. It is anabolic hormnone.*** Increases amino acids uptake↑ and protein synthesis. ***Adequate supply of insulin is necessary for protein anabolic effect of GH (Permissive effect).***

Q.110. How much Insulin is secreted daily under normal conditions?

Human pancreas secretes 40 to 50 units of insulin daily which is 15 to 20% of the hormone stored in the gland.

Q.111. What is the effect of insulin on mineral metabolism?

Decrease in concentration of K^+ and inorganic PO_4 in blood due to enhanced glycogenesis and phosphorylation of glucose. ***The insulin and glucose is used in treatment of hyperkalemia.***

Q.112. How Insulin is catabolized in the body? State the enzymes involved in degradation.

Major organs where insulin is catabolized are

- **Liver** and
- **Kidneys**, About 50% of insulin is degraded in its single passage through liver.

Enzymes involved: Glutathione-insulin transhydrogenase (also called ***insulinase***). The enzyme brings about reductive cleavage of S-S bond. After "A" and "B" chains are cleaved they are further hydrolyzed by proteolysis.

Q.113. What are insulin-like growth factors (IGFs)?

Two insulin like growth factors IGF-I (also called somatomedin C) and **IGF-II (multiplication stimulating activity, MSA)** have been found.

- They are not produced by pancreas but **formed by liver** and other tissues.

- IGF-I is a single chain polypeptide having 70 a.a. and IGF-II containing 67 a.a. They have approximately 50% of a.a. in common with insulin.
- While insulin is more potent as a hormone which controls metabolism, ***IGFs are more concerned with growth stimulation and cell proliferation.*** Both are carried by plasma proteins and each of them has unique receptors to act.

Q.114. Name some insulin preparations available for treatment of diabetics.

- ***Quick acting:*** Soluble insulin
- ***Retard insulin:*** For delayed and prolonged action
  - 'Lente' insulin (insulin-zinc-suspension)
  - Protamine insulin.
  - PZI (zinc and protamine insulin combined)
  - Globin insulin.
- ***For Quick and prolonged action:*** Regular soluble + One 'retard' insulin.

Q.115. Can Insulin be effective if administered orally?

Insulin being a protein hormone will be degraded by proteolytic enzymes present in the gut. Thus, it will be ineffective.

Q.116. What are oral hypoglycaemic agents? Name at least three such agents. How do they act?

These are drugs when taken orally produce hypoglycemia. They are not insulin preparations.

Examples are:

- ***Tolbutamide (orinase).***
- ***Chloropropamide (diabenase).***
- ***Biguanides like phenformin.***

These compounds are effective only if at least a part of pancreas is functional. Primary action of these drugs appear to stimulate insulin secretion. These drugs are widely used in treatment of maturity onset DM (Type-II).

Q.117. What is the chemical nature of glucagon?

It is a polypeptide containing 29 a.a., having mol wt approximately 3485, secreted by **α**-cells of islet of Langerhans. Unlike Insulin, it does not require Zn^{++} for its crystallization or storage.

Q.118. What is the precursor of glucagon?
It is first synthesized in **α**-cells as a prohormone called ***pro-glucagon.***

Q.119. State the factors controlling glucagon secretion.
- ***Hypoglycaemia:*** Is a potent stimulus and increases glucagon secretion.
- ***Fatty acids:*** Inhibit glucagon release.
- ***Amino acids:*** Most a.a. particularly arginine and alanine cause a rapid secretion.
- ***Calcium:*** Rise in serum calcium is a potent stimulus for glucagon secretion.
- ***Hormones:*** Growth hormone (GH) and CCK-PZ, stimulate the secretion.

Q.120. What are effects of glucagon on carbohydrate, lipid and protein metabolism?
- ***Carbohydrate metabolism:*** Net effect produces increase in blood glucose level↑ (hyperglycemia). This is achieved by:
  - Increasing glycogenolysis in liver↑ and
  - Increasing hepatic gluconeogenesis
- ***Lipid metabolism:***
  - Increases lipolysis in adipose tissue and liver producing rise in FFA↑ and glycerol↑.
  - Reduces FA synthesis↓.
- ***Protein metabolism:***
  - Reduces protein synthesis↓, and stimulates protein catabolism.↑

Q.121. What is the action of glucagon on muscle glycogen?
Cannot breakdown muscle glycogen ***as muscle cell membrane lacks the glucagon specific receptors.***

Q.122. What is the effect of glucagon on mineral metabolism?
- **K↑:** Glucagon increases K^+ release from Liver which is related to its hepatic glycogenolytic activity.
- **Ca↓:** Recently it has been shown that glucagon can release calcitonin from thyroid and thus it has calcium-lowering effect.

Q.123. What is the effect of Glucagon on heart muscle?
- Glucagon exerts of +ve ionotropic effect on heart without producing increasing myocardial irritability.

- ***Clinical importance:*** Glucagon has been used in treatment of heart diseases like cardiac failure and cardiogenic shock.

Q.124. State the clinical and therapeutic uses of glucagon.

- Most important use is in treatment of insulin-induced **hypoglycemia**.
- Long acting Zn-glucagon has been used in inoperable pancreatic cell tumors like **insulinomas**.
- Has been used in **heart failure** and **cardiogenic shock**.
- Recently it has been used in treatment of **acute pancreatitis** due to its inhibitory effect on exocrine secretions of pancreas.

Q.125. What is entero-glucagon?

A peptide isolated from gastric and duodenal mucosa. It is **produced by gastric/duodenal "A" cells and pancreatic α-cells**. It has action similar to pancreatic glucagon but it is less active in stimulating ***adenyl cyclase*** and therefore, cannot duplicate many of the functions of pancreatic glucagon.

Q.126. What is glicentin?

Other peptides with ***glucagon-like immunoreactivity (GLI)*** have been isolated form **"L"-Cells of the ileum and colon**. One of the major component of GLI is ***Glicentin*** which is a long polypeptide containing 100 a.a. Glicentin may mimic the actions of glucagon as it contains sequence of a.a. similar to pancreatic glucagon.

Q.127. What are the cells of adrenal medulla called?

Cells of adrenal medulla are large, ovoid columnar cells called ***pheochromocytes***. Cells are grouped in clumps around the blood vessels.

Q.128. State the hormones produced by adrenal medulla.

Hormones produced are called **catecholamines**, as they contain "catechol" nucleus and they are amines. They are:

- ***Epinephrine (adrenaline).***
- ***Norepinephrine (noradrenaline).***

In humans, adrenal medula contains 80% of epinephrine and 20% of Norepinephrine.

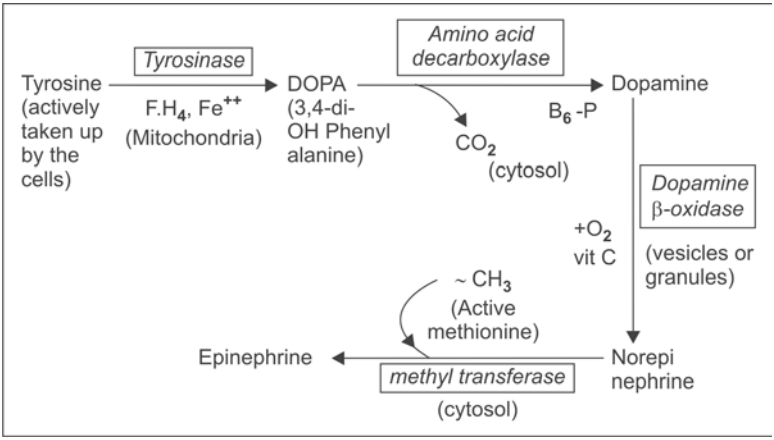
Q.129. What is the starting material for catecholamine synthesis?

- Amino acid tyrosine.

Q.130. Which is produced first in the synthetic pathway, epinephrine or norepinephrine?
Norepinephrine is produced first; then it is methylated to form epinephrine.

Q.131. What are the sites of catecholamine synthesis?
- Adrenal pheochromocytes and neuronal cells of sympathetic nervous system.
- In pheochromocytes of adrenal medulla, both norepinephrine and epinephrine are synthesized and stored.
- In neuronal cells, only norepinephrine is formed and stored.

Q.132. Outline the synthesis of catecholamines schematically.



Q.133. How catecholamines are stored?
Both the hormones are stored in the granules in the adrenal medulla and in adrenergic neurones as a complex containing ATP in ratio of 4 molecules of hormones: One mol of ATP and in combination with several incompletely characterized proteins like ***chromogenin A*** and ***chromomembrin B***.

Q.134. What are the effects of epinephrine on carbohydrate and lipid metabolism?
- ***Carbohydrate metabolism:***
  - *Glycogenolysis:* Epinephrine stimulates rapid breakdown of hepatic glycogen producing hypergly-

caemia. Also causes breakdown of muscle glycogen. In exercising muscle, this can result in increased LA↑
  - Gluconeogenesis ↑ is increased.
- ***Lipid metabolism:*** Lipolysis increases↑, produces increased FFA↑ and glycerol↑.

Q.135. How catecholamines are catabolized or degraded in the body? Name the enzymes involved?
- Five percent of epiephrine excreted as such in urine.
- Remaining part of hormones are **degraded by two enzymes** principally in liver/also other tissues:
  - ***Monoamine oxidase (MAO):*** A mitochondrial enzyme, brings about oxidation of side chain.
  - ***Catechol-O-methyl transferase (COMT):*** a cytosolic Mg^{++} dependent enzyme, brings about methylation of phenolic OH group at 3 position.

Q.136. What is the principal urinary metabolite of catecholamines?

VMA is the principal urinary metabolite. It is chemically 4-OH-3-methoxy mandelic acid. Also called as ***vanillylmandelic acid (VMA),*** constitutes 41% of total metabolites.

Q.137. What is pheochromocytoma?
- It is the tumor of the chromaffin tissue of adrenal medulla.
- The **disease is characterized by:**
- Abnormal rise in BP↑ (hypertension) which may be "paroxysmal" or sustained.
- Hyperglycaemia/and glycosuria.
- Increased plasma FFA↑ and
- Increased BMR
- Plasma catecholamines are increased↑ markedly and urinary excretion of VMA increases↑.

Q.138. What is phentolamine or regitine test?
- Used as a test for pheochromocytoma.
- Phentolamine is a specific antagonist to norepinephrine. In the presence of sustained hypertension due to pheochromocytoma, the rapid IV injection of phentolamine produces a sustained fall of BP↓ within 2 to 5 minutes, which is characteristically seen in pheochromocytoma.

Q.139. State the type of hormones produced by adrenal cortex.
Adrenal cortex produces a number of steroid hormones. **Functionally,** they can be divided into **three groups:**

- ***Glucocorticoids:*** Primarily affect the metabolism of carbohydrates, lipids and proteins and relatively minor effects on electrolytes and water metabolism.
  - ***Mineralocorticoids:*** Primarily affect the reabsorption of Na^+ and excretion of K^+ and distribution of water in tissues.
  - ***Sex hormones:*** Small amounts of androgens and oestrogens are produced.

Q.140. Histologically what are the layers of adrenal cortex?
In adrenal cortex, **three zones/layers** can be differentiated histologically. They are:

- ***Zona glomerulosa:*** Outermost layer.
- ***Zona fasciculata:*** Middle layer.
- ***Zona reticularis:*** Innermost layer.

Q.141. Name three glucocorticoids. Which is the major glucocorticoid in circulating blood?
Three glucocorticoids are:

- ***Cortisol*** (17-OH corticosterone or compound F).
- ***Cortisone*** (Compound E).
- ***Corticosterone*** (Compound B).

Cortisol is the major free circulating glucocorticoid in human plasma.

Q.142. Name three hormones which have mineralocorticoid activity. Which one is the major mineralocorticoid in circulating blood?
Three mineralocorticoids are:

- ***Aldosterone,***
- ***11-deoxy cortisol, and***
- ***11-deoxy corticosterone (DOC)***

Aldosterone is the principal circulating mineralocorticoid in humans.

Q.143. Which layers of adrenal cortex are involved in formation of steroid hormones?

- Cells of all three layers can form the steroid hormone ***corticosterone.***

- Cells of inner two zones can form ***cortisol*** and the sex hormones.
- ***Cells of zona glomerulosa can form only aldosterone.***

Q.144. Why aldosterone is formed by cells of zona glomerulosa only and not by cells of other two layers?

Aldosterone can be synthesized only by cells of zona glomerulosa because the cells of zona glomerulosa have the enzymes ***18-hydroxylase*** and ***18-hydroxysteroid dehydrogenase*** which are lacking in other two layers.

Q.145. Structurally how aldosterone differs from cortisol?

Both are C_{21} steroids and have -OH group at C_{11}. But **aldosterone has an aldehyde group** (-CHO gr.) at C_{18} instead of $-CH_3$ gr. of cortisol.

Q.146. How much glucocorticoid is secreted daily?

- During a 24-hour period, a normal adult human secretes about 5 to 30 mg of cortisol and 1 to 6 mg of corticosterone.
- Cortisol secretion has a **diurnal rhythm**, peak secretion occurs in early morning 4 AM and decrease at night.

Q.147. How glucocorticoid secretion is regulated?

Glucocorticoid secretion is stimulated by the anterior pituitary corticotropin ACTH which is in turn regulated by hypothalamic "Corticotropin-releasing hormone" (CRH or CRF) and by negative "feed back inhibition', long loop/ short loop, by glucocorticoid level in the circulating blood.

Q.148. What is the starting material for synthesis of corticosteroids?

All corticosteroids are synthesized by a common pathway from **cholesterol**, the starting material in the adrenal cortex.

Q.149. What are the normal plasma levels of glucocorticoids?

In the resting state:

- Plasma contains 5 to 15 μg/dl of cortisol (average 12 μg/dl).
- Plasma corticosterone level varies from 0.04 to 2 mg/dl (average 1.0 mg/dl).

Q.150. How cortisol is transported in the blood?

- 10% (approximately 0.5 to 0.8 mg) is in **"free" active state.**
- **90% is protein bound,** out of this 30% is bound to albumin and 60% (approximately 10 to 15 mg) bound to

a specific cortisol-binding protein, an α_2-globulin called ***transcortin***.

Q.151. Glucocorticoids are catabolic to peripheral tissues but anabolic to liver: Justify.

- **Action on peripheral tissues** like muscles, adipose tissues, lymphoid tissue is **'catabolic'** (spares glucose). Thus
  - Glucose uptake decreased↓ and
  - glycolysis↓
  - Lipolysis↑, increasing FFA↑ and glycerol↑ in plasma, TG formation↓.
  - Protein breakdown↑, plasma amino acids↑, protein synthesis↓
- **Action on Liver is "anabolic":**
  - Amino acids uptake by liver cells↑, protein synthesis in liver cells enhanced↑.
- Gluconeogenesis from amino acids and glycerol is increased↑.

Q.152. State the other physiological functions of cortisol and its related clinical significance if any.

Functions	*Clinical significance*
• ***Antiinflammatory action***	• Used for treatment of rheumatoid arthritis, rheumatic fever and acute glomerulonephritis.
• ***Immunosuppressive effect***	• Used in organ transplantation, and • for treatment of bronchial asthma and status asthmaticus.
• ***Effect on exocrine secretions:*** • Increased HCl↑. • Increased secretion of Pepsinogen↑ and trypsinogen↑	• Prolonged treatment with GC may produce GI ulcers.
• ***Effect on bones:*** reduces osteoid matrix, favours osteoporosis, may be excessive loss of Ca.	• Prolonged treatment with GC may produce osteoporosis and fracture of bone.
• ***Hematological effects:***	
• Destruction of lymphocytes and shifts of lymphocytes to lymphoid tissues.	• Produces lymphopenia. Also reduction of circulating monocytes and eosinophils.
• ***Surfactant action:*** Increases production of pulmonary surfactant.	• Prevents respiratory distress syndrome (RDS).

Q.153. What is Addison's disease?

Hypoactivity of adrenal cortex produces Addison's disease. The disease results from failure of adrenal cortex to produce adrenocortical hormones.

Q.154. How Addison's disease be caused?

- Primary atrophy of adrenal cortex **(Idiopathic type)**-usually ***autoimmune disorder.*** Greater than 53% cases show circulating auto-antibodies.
- **Other causes** can be destruction of adrenal cortex by diseases: TB, malignancy, fungus infection, amyloidosis, hemosiderosis, leukemic infiltrations.

Q.155. What are the clinical features and biochemical changes in Addison's disease?

The clinical features and biochemical changes are:

- Decrease Na^+ reabsorption↓, decreased ECF volume↓, development of acidosis, reduced urinary NPN↓ and K^+↓ hyponatremia and hyperkalemia.
- A characteristic clinical feature: Patient develops abnormal bronze pigmentation of skin and mucous membranes due to overactivity of MSH.

Q.156. How does aldosterone occurs in blood?

In circulating blood, aldosterone can **exist** both in ***"aldehyde"*** and ***"hemi-acetal" forms.***

Q.157. How much aldosterone is secreted per day in a normal healthy adult?

Approximately 30 to 75 μg of aldosterone is secreted per 24 hours by the adrenal cortex.

Q.158. What is the normal plasma level of aldosterone?

Approximately 0.03 to 0.08 μg of aldosterone/dl of plasma. (average 0.05 μg). It is carried in the blood bound to serum albumin.

Q.159. What are the main effect of aldosterone on metabolism?

- Increases rate of tubular reabsorption of Na^+ and Cl^-.
- Increases renal excretion of K^+.
- Increased secretion of aldosterone promotes **alkalosis**.
- Increase in ECF volume.

Q.160. State the primary factors that stimulate aldosterone secretion.

Primary factors that stimulate the rate of aldosterone secretion are:

- Decreased sodium concentration of plasma.
- Increased K^+ concentration of plasma.
- Decreased ECF volume, hypovolemia and hypotension.

Q.161. What is Renin-Angiotensin system?

- **Renin** is a **proteolytic enzyme**, having a molecular weight of 35,000, produced from juxtaglomerular cells of afferent arteriole of nephron (vascular pole?).
- Renin acts on a plasma substrate, an α_2 globulin called ***angiotensionogen*** (or hypertensinogen) and forms ***angiotensin I,*** a **decapeptide**, having a mol wt of 1296.
- While circulating, angiotensin I is acted upon by a protease enzyme called ***converting enzyme*** in lungs capillaries and forms ***angiotensin II,*** an **octapeptide**, having mol wt 1046.
- **Angiotensin I is the "active" component** of the system, which acts on zona glomerulosa cells which increases the syntheses and release of the hormone aldosterone.

Q.162. How renin is inactivated?

- By "Feed-back inhibition" by aldosterone level itself.
- Also destroyed by a cephalin derivative in plasma.
- Also ***inhibited by a lysophospholipid***, liberated by the action of ***phospholipase A_2.***

Q.163. What is Conn's syndrome?

Conn's syndrome due to increased secretion of aldosterone (primary aldosteronism), due to tumors of adrenal cortex, usually an adenoma called **"aldosteronoma"—**hyperactivity is confined to excess production of aldosterone.

Q.164. What are the salient clinical and biochemical features of Conn's syndrome?

- **Salient features** are:
  - Increased Na^+ retention (hypernatraemia),
  - Severe K^+ depletion (hypokalaemia) and
  - Alkalosis.

- Sodium retention causes:
  - Hypertension which may result in congestive cardiac failure without edema,
- K^+ depletion and alkalosis give rise to periodic muscle weakness and intermittent "tetany".
- Failure of renal tubules to respond to ADH causes polyuria and polydypsia.

Q.165. Name some conditions which cause secondary hyperaldosteronism.

- Cirrhosis Liver
- Nephrotic syndrome
- Congestive heart failure.

Q.166. Name the androgens produced by adrenal cortex.

Main androgens produced by adrenal cortex are:

- ***Dehydroepiandrosterone (DHEA),***
- ***DHEA-SO_4,***
- ***Δ^4- androstenedione, and***
- ***11-β-OH androstenedione.***

Q.167. Enumerate the types of sex hormones. Where are they formed?

- Sex hormones are of **3 types:**
  - **Androgens** or male hormones
  - **Estrogens** or Female hormones
  - **Gestogens** or **progestational** hormones
- The sex hormones (gonadal hormones) are produced by the testes, ovary and corpus luteum.

Q.168. Name the naturally occurring androgens in humans.

They are:

- Testosterone (Main hormone),
- Epiandrosterone (3-β androsterone),
- Androsterone, and
- Dehydroepiandrosterone (DHEA).

Q.169. State the sites of androgen production.

- Androgens are produced in ***testes (Leydig cells), adrenal cortex, ovary*** and ***placenta (?).***
- They are formed from either active acetate or cholesterol. Pregnenolone being an important intermediate.

Q.170. Name the immediate precursor of testosterone.
Androstenedione is the immediate precursor of testosterone.

Q.171. What is the biologically active form of testosterone?
Testosterone is converted to ***biologically active*** and potent form ***dihydrotestosterone (DHI)*** in the testes and extra testicular tissues like prostate, seminal vesicles and target tissues.

Q.172. Is testosterone produced in females?
Small amount of testosterone is produced in females, approximately 0.03 μg/dl, which is formed mainly from peripheral conversion of androstenedione to testosterone by the ovary.

Q.173. How much testosterone is secreted per day in males? What is the normal plasma level in males?
- In normal healthy adult male, approximately 4 to 12 mg of testosterone is secreted per day.
- Normal plasma level in males is approximately 0.6 μg/dl.

Q.174. How testosterone is transported in the blood?
- About 10% is bound to a β-globulin, called ***sex hormone-binding globulin (SHBG)***, also called as **"testosterone-estrogen binding globulin" (TEBG).**
- Approximately 87 to 89% is carried being bound to serum albumin.
- Remaining approximately 1 to 3% circulates in active "free" form.

Q.175. How testosterone secretion is regulated?
- It is regulated by luteinizing hormone (LH) of the anterior pituitary.
- A high blood level of testosterone exerts ***feed-back inhibition*** of LH secretion.

Q.176. State the metabolic role of androgens.
- Both testosterone and dihydrotestosterone (DHT) are protein anabolic and growth promoting hormones
- It promotes protein synthesis- in male accessory glands
- Stimulates growth of bones before the closure of epiphyseal cartilages
- Increase in body weight, chiefly due to increase in skeletal muscle

- Produces decrease in urinary N_2 (urea)↓, without an increase in blood NPN
- Increases fructose production↑ by seminal vesicles and utilization of spermatozoa.

Q.177. What are 17-Keto steroids (17-oxo-steroids)? What is the source in males and females?

- Androgens are excreted in urine as 17-keto steroids (also called 17-oxo-steroids).
- ***In males:*** 17-Ketosteroids arise from testes (1/3 of total) while the major amount arises from the adrenal cortex (2/3 of the total).
- ***In females:*** 17-Ketosteroids are almost entirely arises form adrenal cortex.

Q.178. What is the normal values of urinary 17-Keto-steroids?

- Normal adult males excrete 9 to 24 mg of neutral 17-keto steroids in 24 hours.
- Normal adult females excrete 5 to 17 mg.

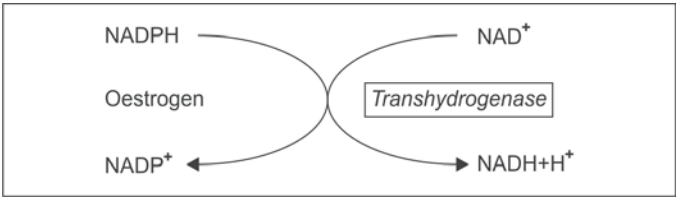
Q.179. Name the naturally occurring estrogens in humans.

- ***β-Estradiol,***
- ***Estrone, and***
- ***Estriol.***

The principal estrogenic hormone in circulation and the ***most active from of the oestrogen*** is **β-estradiol,** which is in metabolic equilibrium with estrone. β-estradiol is 10 times more potent than estrone and 300 times more potent than estriol.

Q.180. What is transhydrogenation reaction? What is the role of estrogen?

Estrogens may **act as a cofactor** in transhydrogenation reaction in which H^+ ions and electrons are transferred from reduced $NADP^+$ and forms NADH.



Q.181. What is progesterone?

Progesterone is the hormone produced principally by the corpus luteum and also by adrenal cortex and testes. Also formed by placenta during later part of pregnancy.

Q.182. What is the immediate precursor of progesterone?

Pregnenolone is the immediate precursor of progesterone.

Q.183. How progesterone is transported in the blood?

- In contrast to estrogens/and androgens, about 40% of Progesterone is carried by ***corticosteroid binding globulin (CBG)***, a **β-globulin.**
- About 1 to 5% of total progesterone is transported in active "free" form.
- Remaining is carried loosely bound to albumin, and to a small extent by other plasma proteins and ***oroso-mucoid.***

Q.184. What are the hormones produced by placenta?

Human placenta produces and secretes following hormones:

- ***Peptide hormones:***
  - Human chorionic gonadotropin hormone (hCG).
  - Chorionic somatomammotropin (CS)—also called placental lactogen.
- ***Ovarian steroid hormones:***
  - Progestins, and
  - Estrogens chiefly estriol.

Q.185. State the chemical nature of HCG?

It is a glycoprotein consisting of two subunits **α** and **β**.

- **α**-chain is made up of 92 a.a. and is identical to human FSH, LH and TSH.

Carbohydrate moieties are present as follows:

- **α**-chain carries two asparagine-linked oligosaccharides.
- **β**-chain has more carbohydrates and contains two asparagine-linked oligosaccharides and four Seine-linked oligosaccharides.

Q.186. State the action of HCG.

- ***Luteotrophic effect:*** Produces enlargement of corpus luteum and stimulates its secretion. It maintains a secretory corpus luteum in first three months of pregnancy.

- ***Testosterone secretion:*** Like LH, the hormone stimulates the growth of interstitial cells (Leydig cells) of embryonic testes and produces testosterone. This helps in virilization of reproductive system of male embryo.

Q.187. What is somatostatin? What is its chemical nature?

- **Somatostatin** is a peptide consisting of 14 a.a. There is an interchain S-S linkage joining cysteine at position 3 and cysteine at position 14
- It was fast isolated from hypothalamus and was called as GH release inhibitory factor.

Q.188. What are the sources of somatostatin?

Three sources:

- Hypothalamus,
- **δ**-Cells of islets of Langerhans of pancreas,
- D-cells of antral mucosa of stomach and duodenal mucosa.

Q.189. State the actions of somatostatin.

- ***Hypothalamic somatostatin:*** Inhibits GH release.
- ***Pancreatic somatostatin:*** Inhibits both insulin and glucagon secretion and ***act as an intra-islet regulator*** of secretion of these hormones.
- ***GI somatostatin:*** Inhibits secretion of Gastrin, CCK-PZ, GIP and motilin (GI hormones).

Also inhibits gastric acid secretion, pancreatic HCO_3^- and enzyme, gastric emptying and gallbladder contraction.

Q.190. What is relaxin? State its nature and function.

- Relaxin is a hormone concerned with the relaxation of pelvic tissues and cavity in conjuction with other factors.
- Chemically it is a dipeptide consisting of 2 chains, having 22 and 26 a.a. and mol wt of 9000.
- It is produced during pregnancy by corpus luteum and also by placenta and its production is stimulated by progesterone.
- It causes dilatation and softening of uterine cervix, facilitating child birth.

Q.191. Mention the important GI hormones.

- ***Gastrin family:***
  - Gastrin
  - CCK-PZ.

- *Secretin family:*
  - Secretin
  - Enteroglucagon including glicentin
  - GIP (gastric inhibitory peptide),
  - VIP (vasoactive intestinal polypeptide).
- *Neurocrine peptides:*
  - Neurotensin
  - Bombesin-like peptide (BLP)
  - Substance P
  - Somatostatin.

Q.192. What is meant by paracrine function and autocrine function?

- ***Paracrine function:*** Hormones which act on adjacent cells in a given tissue is called as "paracrine" function.
- ***Autocrine function:*** Hormones when act on the cells in which they are synthesized is called as "autocrine function".

Q.193. List the different GI hormones, their site of origin and major actions.

Hormones	*Localization*	*Major actions*
• ***Gastrin***	• Antral mucosa of stomach and duodenal mucosa. • Special type of cells called **'G' cells**.	• Increases Gastric motility↑ • Stimulates secretion of enzymes↑ from stomach and pancreas
• ***CCK-PZ*** (Called CCK only)	• Duodenojejunal portion • Special cells called '**I'-cells**	• Contraction of gall-bladder (due to CCK) • Secretion of enzymes rich in pancreatic juice (due to PZ)
• ***Secretin***	• Duodenum and upper jejunum • Special cells Called **'S' cells**	• Stimulates secretion of water↑ and bicarbonates↑ from pancreas • Inhibits gastric motility↓
• ***GIP* (Gastric inhibitory peptide)**	• Duodenal and jejunal mucosa • Special cells '**K' cells**	• Inhibits gastric acid secretion↓ and gastric motility • Enhances glucose mediated insulin release

Contd...

Contd....

• ***VIP* (Vasoactive intestinal Poypeptide)**	• Upper intestinal wall, pancreas	• A weak stimulation of pancreatic volume↑ flow, but not enzyme secretion
	• Special Cells: **'D_1' Cells**	• Inhibits gastric acid↓ and pepsin↓
• ***Motilin***.	• Small intestinal mucosa	• Stimulates intestinal smooth muscle contraction↑ • Stimulates acid↑ and pepsin secretion↑
• ***Substance P***	• Gut and brain	• Produces smooth muscle contraction in intestine
• ***Somatostatin***	• Hypothalamus • Islets of Langerhans of pancreas • GI tract-'D' cells	• Over-all inhibitory effects • Decreases gastric acid secretion↓, pancreatic enzyme secretion↓ • Stimulates gastric and pancreatic secretion↑
• ***Bombesin***	• Stomach and duodenum	• Increases motility of gallbladder and intestine↑
• ***Entero-Glucagon and GLI (Glucagon like immunoreactivity)***	• Gastric and duodenal 'A' cells • GLI: 'L' cells of ileum and colon	• Glicentin, a major component of GLI mimic the actions of glucagon
• ***Pancreatic Polypeptide (PP)***	• Pancreas. • Cells-'D'cells	• Inhibits pancreatic bicarbonate↓ and protein enzyme secretion↓
• ***Chymodenin***	• mucosa of small intestine	• Specific stimulation of chymotrypsin↑ secretion of the pancreas

CHAPTER

19 Protein Synthesis, DNA Replication and DNA Recombinant Technology

Q.1. What is transcription?

Transcription is the process by which m-RNA is formed which is identical in sequence with one of the strands of duplex DNA.

Q.2. What is the enzyme responsible for transcription?

- ***RNA Polymerase*** is the key enzyme in transcription.
- A single type of ***RNA polymerase*** is responsible for synthesis of m-RNA, r-RNA and t-RNA in bacteria.
- In eukaryotes, several different enzymes are required to synthesize the different types of RNA. They are called as ***RNA polymerase I, RNA polymerase II,*** *and* ***RNA polymerase III****.*

Q.3. What is pribnow box or TATA box?

- The Pribnow box is a particular sequence in DNA contained with the promoter region. It is located 5 to 10 bases to the left, i.e. upstream the first four bases that will be copied into RNA. It orients ***RNA polymerase*** as to the direction and start of synthesis.
- All Pribnow boxes are variants of TATA ATG sequences and sometimes referred to as TATA box.

Q.4. What are the precursors of m-RNA?

- hn RNA, and
- Pre-m-RNA.

Q.5. What is hn RNA?

- hn RNA is a large molecular size RNA, contains 500 to 4000 nucleotides, it is bound to a protein called **"informofers"**, ½ life is short approximately 20 minutes.
- Only a few can give rise to Pre-m-RNA.

Q.6. What is Pre-m-RNA? What is its function?

- **Pre-m-RNA:** It is formed from hn-RNA.
- It has **two regions** called:
  - ***Exons***—active regions ***used for "coding"*** and ***Introns***—intervening regions not required for translation.
  - The "introns" transcripts are excised and "exons" are spliced together to give the proper m-RNA required for translation.

Q.7. What is the nature of fully formed m-RNA?

Fully formed m-RNA ready for protein synthesis in cytosol has following:

- ***In its 5'-end,*** a ***cap of guanosine*** triphosphate with methyl group in position 7 (GmTP).
- Also a poly-A tail either obtained from precursor or added in cytosol.

Q.8. What is the purpose of GmTP capping of m-RNA?

Required for translocation of the m-RNA and its binding to 40 S ribosome in eukaryotes.

Q.9. What is the function of Poly-A tail of m-RNA?

Necessary for intracellular stability of m-RNA.

Q.10. Name two inhibitors of transcription.

- ***Rifamycin:*** binds with β-subunit of the ***RNA Polymerase*** and blocks the initiation of transcription.
- ***Actinomycin D:*** forms a complex with the double stranded DNA and prevents the movement of core enzyme and inhibits the process of chain elongation.

Q.11. Where is the site of protein synthesis in a cell?

The site of protein synthesis inside the cell is the **polysome** of the rough endoplasmic reticulum.

Q.12. What is genetic code?

The 64 combinations of three bases responsible for coding amino acids, initiating and stopping protein synthesis are arranged in the form of a table which is generally known as ***genetic code.***

Q.13. What is a codon?

- The bases A, G, T and C found in DNA are organized into 3-letter code words called ***codon.***

- The cistrons of DNA give the required information to the m-RNA synthesized by DNA during transcription. m-RNA gets the instruction imprinted in the codons.
- Each amino acid must be having a definite codon in m-RNA. The codons are all a sequence of three nucleotides.

Q.14. What are the characteristics of genetic code?

Characteristics of **genetic code** are:

- Degeneracy
- Unambiguity
- Universality
- Colinearity of gene and product
- Non-overlapping
- Commalessness.

Q.15. What is meant by Wobbling Phenomenon?

Often the base of the third position is insignificant because the four codons differing only in the third base represent the same amino acid. The reduced specificity at the last position is known as ***third base degeneracy*** or ***Wobbling Phenomenon.***

Q.16. What do you mean by non-sense triplets?

Three of the 64 triplets e.g. ***UAA, UGA,*** and ***UAG,*** do not code for any amino acid. They function rather as a signal to terminate a Peptide chain at that point. Hence they are called as ***non-sense*** codons or preferably called as ***chain terminating triplets or codons.***

Q.17. What are the ingredients required for protein synthesis?

Materials or ingredients required for protein synthesis are:

- Amino acids-20 amino acids
- DNA and three RNA's-m-RNA, t-RNA and r-RNA
- Polyribosomes (polysomes).
- ***Enzymes:***
  - *Aminoacyl t-RNA synthetase* enzyme required for activation of amino acids
  - *Peptide synthetase (peptidyl transferase).*
- ***Factors:***
  - **Initiation factors:** eIF-1, eIF-2, eIF-3, eIF-4A, eIF-4B, eIF-4G, eIF-4E, eIF-5
  - Elongation factors: EF_1, and EF_2
  - Release factors: RF_1, RF_2, RF_3

- ***Conenzyme and cofactors:***
  - $F.H_4$: required in prokaryotes only for formylation of methionine.
  - Mg^{++}
- ***Energy:*** ATP and GTP.

Q.18. What is the nature of ribosomes in eukaryotes?

- It is in a combination of ribosomes (polysomes) that the biosynthesis of protein take place.
- In eukaryotes, each ribosome is 80 S made of two pieces, one 40 S and other 60 S. There is an 18 S portion of 40 S in which binding is initiated.
- The ribosomes have the ribosomal RNA and the ***interference factors*** which offer specificity to them. They also take up ***initiation factors*** (Proteins IF_1, IF_2, and IF_3).
- The 60 S piece of eukaryote ribosomes are separate at the begining of peptide synthesis.
- The **60 S piece** of **eukaryote ribosome** has **got 2 sites:**
- One called ***aminoacyl site (A site),*** and
- The other ***peptidyl site (P site).*** The two pieces of ribosomes are separate at the beginning of peptide synthesis.

Q.19. Name two amino acids which have a single codon.

- Methionine and
- Tryptophan.

Q.20. What is anticodon?

It consists of a triplet present in the ***anticodon arm*** of t-RNA molecule and it relates to the codon of the amino acid on m-RNA to be carried. Actual amino acid to be transferred to ribosome is decided by the anticodon.

Q.21. What is the function of DHU loop of t-RNA?

Recongnition of the t-RNA by the ***aminoacyl t-RNA synthetase*** rests in the dihydrouracil (DHU) loop of t-RNA.

Q.22. What is the function of TψC loop of t-RNA?

The thymine-pseudouridine-cytosine (TψC) loop of t-RNA ***binds t-RNA-AA to ribosomal surface.***

Q.23. What are the steps of protein synthesis?

The process of protein synthesis after transcription and formation of m-RNA take place can be divided in the following steps:

- Activation of amino acids,
- Intitiation,

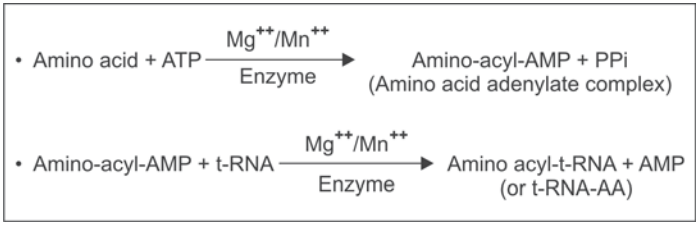
- Elongation, and
- Termination.

Q.24. How amino acids are activated?

Before any molecule of amino acid could be used for protein synthesis it has to be activated and converted to t-TNA-amino acid. Each of 20 amino acids must have a definite t-RNA for the formation of "t-RNA-AA".

Q.25. What is the enzyme required for activation of amino acid? Give the steps.

- ***Enzyme:*** Is ***amino acid-t-RNA synthetase.***
- ***Steps:*** The reactions occur in **two steps** and both the reactions are catalyzed by the same enzyme ***amino acid-t-RNA synthetase*** which requires Mg^{++}/Mn^{++} as cofactor.
- Steps of activation are:



- In the aminoacyl t-RNA (t-RNA-AA), the α-COOH group of the amino acid remains esterified with the 3'-OH group of the 3'-terminal adenosine on the "acceptor arm" of t-RNA.

Q.26. What is the role of ATP in activation of amino acid?

Two high energy bonds of ATP are used in the formation of "t-RNA-AA" which is equivalent to utilization of 2 ATP molecules.

Q.27. Which amino acid is bound first to ribosome?

- **In prokaryotes,** and in the mitochondria of the eukaryotes, **N-formyl methionine** is the first amino acid derivative to be bound to ribosome. For formylation, folate as $F.H_4$ is required.

- ***In eukaryotes,*** the first codon is **AUG** and it codes for methionine which is the first amino acid to be taken up. ***It is not formylated derivative.***

Q.28. What is initiation ? Mention the steps of initiation.

The initiation may be divided arbitrarily into **4 steps:**

- Dissociation of the ribosome 80s into 60s and 40s subunits
- Formation of 43s pre-initiation complex
- Formation of initiation complex
- Formation of 80s initiation complex

Q.29. How dissociation of ribosome takes place? Mention the factors required and its function.

- Before initiation process start, 80s ribosome dissociates into 60s and 40s subunits.
- Two initiation factors, eIF-3 and eIF-1A binds to the newly dissociated 40s subunit

Function:

- This binding of initiation factors ***prevent reassociation*** of 60s and 40s.
- It allows the other translation initiation factors to associate with 40s subunit and prepares it for formation of 80s initiation complex.

Q.30 What is 43s pre-initiation complex? How it is formed?

- Formation of 43s pre-initiation complex ***involves the binding of GTP with eIF-2 and*** forms a binary complex.
- The binary complex then binds to Met-RNA and forms a ternary complex.
- The ternary complex binds to 40s ribosomal subunit and **forms the 43s preinitiation complex. This complex is stabilized by association with eIF-3 and eIF-1A.**

Q.31. What is the importance of eIF-2? Describe the structure.

- In eukaryotes**, the eIF-2 is the controlling factor in protein synthesis initiation.**
- **Structurally, eIF-2 is a heterotrimer** and consists of 3 subunits α, β and γ. Subunit α most important, eIF-2 α is phosphorylated by at least 4 different protein kinases viz. **HCR, PKR, PERK** and **GCN-2.** The kinases are activated when a cell is under stress, e.g. virus infection, heat shock, carbohydrate and protein deprivation.

Q.32. How initiation complex is formed?

Binding of m-RNA with 43s pre-initiation complex is necessary to form the "initiation complex". In all eukaryotic cells, 5′ terminals of m-RNA are **"capped,"** **which is methyl guanosyl triphosphate.**

Q. 33. How binding of m-RNA with 43s pre-initiation complex is facilitated?

- The binding is facilitated by 5′ methylated cap which requires a **"cap-binding protein complex."**
- Cap-binding protein complex consists of **eIF-4F=eIF-4E and eIF-4G+eIF-4A**. This complex binds to the cap through eIF-4E protein.
- eIF-4A and elF-4B bind and reduce the complex secondary structure of the 5′-end of m-RNA through ***hydrolysis of ATP by helicase activities providing energy.***

Q.34. What is 48s initiation complex? What is its function?

The association of m-RNA with the 43s preinitiation complex **produces the 48s initiation complex.**

Function:

After formation of 48s initiation complex, it ***searches for precise initiation codon AUG*** for methionine. Which is determined by ***"Kozak consensus sequence*** in eukaryotes.

Q.35. What is the importance of eIF-4E factor?

eIF-4E is the most important factor because ***it recognizes the cap of 5′-methylated end of m-RNA*** and is the ***rate limiting step*** in protein synthesis.

- ***Insulin, IGF-1, PDGF, interleukin-2*** and ***angiotensin II*** phosphorylate eIF-4E and increases protein synthesis.

Q.36. State the role of 3′-poly(A) Tail in initiation.

3'-poly(A) Tail has a binding protein "Pab IP". This complex helps in the initiation acting synergistically with cap.

- Pab IP bound to the poly (A) tail interacts with eIF-4G, which in turn binds to eIF-4E, that is bound to the cap structure, which probably help to direct the 40s ribosomal subunit to the 5′ end of the m-RNA.

Q.37. How 80s initiation complex formed? State its function.

The 48s initiation complex now binds to ***60s which was free after ribosomal dissociation, forms 80s initiation complex.***

This requires ***hydrolysis of GTP for energy.*** The eIF-2 carries GTP and "eIF-5 GTPase" activity, both interacts to bring about hydrolysis.

- This reaction then results in release of all the initiation factors bound to the 48s initiation complex, which are the then recycled.
- There occurs rapid association of 40s and 60s sybunit to form the 80s ribosomal complex ready for protein synthesis.

Q.38. State reception sites on 80s initiation complex.

The 80s complex has ***two receptor sites.***

- ***'P' site on peptidyl site.*** At this point the met-tRNA is on the 'P' site, on this site, the growing peptide chain will grow.
- ***'A' site or aminoacyl site:*** At this point it is free, the new incoming t-RNA with the amino acid to be added next is taken up at this site.
- The t-RNA binds with ribosome through the pseudouridine arm.

Q.39. What is elongation?

- Elongation is a **cyclic process** on the ribosome in which one amino acid is added to the nascent peptide chain.
- The peptide sequence is determined by the codons present in the m-RNA.
- It ***requires elongation factors-EF-IA, EF-2.***

Q.40. Mention the steps involved in elongation.

The ***steps are mainly three:***

- The binding of new aminoacyl-tRNA to 'A' site.
- Peptide bond formation
- Translocation process.

Q.41. State how amino-acyl tRNA is bound to the 'A' site .

In the 80s ribosome initiation complex, the 'P' site is occupied by met-tRNA and ***'A' site is free. The fidelity of protein synthesis depends on having correct, aminoacyl-tRNA in the 'A' site as per codon reading.***

- ***Elongation factor EF-IA forms a ternary complex with GTP*** and the entering amino acyl-tRNA (A1). This complex allows the aminoacyl-tRNA to enter the 'A' site.

- ***GTP is hydrolyzed to give energy*** and this is catalyzed by an active site on the ribosome. This releases the EF-1A-GDP and Pi.
- The EF-1A-GDP is converted again to EF-1A-GTP by other soluble protein factors and GTP. It is further recycled.

Q.42. State briefly how a peptide bound is formed.

The α-NH_2 group of the new aminoacyl-tRNA (A_1) in the 'A'site combines with the –COOH group of Met-tRNA occupying the 'P' site.

- The reaction is catalyzed by the enzyme ***"peptidyl transferase"***, a component of the 28s RNA of 60s ribosomal subunit.
- Because the amino acid on the ***aminoacyl-tRNA is already "activated" the reaction does not require any further energy.*** The reaction results in formation of a peptide to the t-RNA in the 'A' site.
- Now the growing peptide chain is occupying 'A' site and naked free t-RNA at 'P' site.

Q.43. What is translocation? What is the role of EF-2?

The t-RNA is fixed at the 'P' site, attached by its anticodon and having no amino acid ***(free and naked t-RNA)*** by the open CCA tail it is bound to an exit site ('E' site) on the large ribosomal subunit.

- At this point, elongation factor 2 (EF-27) binds to and displaces the peptide-tRNA from the 'A' site to the 'P' site and at the same time the deacylated free t-RNA is on the 'E-site' from which the t-RNA leaves the ribosome.
- Now, EF-2-GT- complex is hydrolyzed to EF-2-GDP, ***the energy from hydrolysis moves the m-RNA forward by one codon,*** leaving the 'A' site free to receive another ternary complex of a new amino acid (A_2) as per codon, and repeat the cycle of elongation.

Q.44. How chain termination is brought about? What is the role of release factors RF-1 and RF-3?

After multiple cycles of elongation process, it results to formation of a polypeptide chain. Where the desired protein molecule is synthesized, a ***'step-codon' or terminating codon appears in the 'A'*** site of m-RNA.

- The stop codons are **UAA, UAG,** or **UGA**. There is no t-RNA with an anticodon capable of recognizing such a termination signal.

  Role of release factors RF-1 releasing factor recognizes that a stop codon has come in the 'A' site.
- This protein factor RF-1 is a complex ***consisting of another releasing factor RF-3 with bound GTP.***
- This complex with the help of ***'peptidyl transferase'*** brings about hydrolysis of the bond between the peptide and the t-RNA occupying the 'P' site.
- ***The energy is provided by GTP → GDP+ Pi conversion.*** The hydrolysis releases the synthesized peptide chain, m-RNA and t-RNA from the 'P' site.
- The 80s ribosome now dissociates into 60s and 40s subunits which are then recycled.

Q.45. What are polyribosomes?

In eukaryotic cell, a single ribosome is capable of synthesizing 400 peptide bounds each minute.

Many ribosomes can work on the same m-RNA molecule simultaneously and these aggregate are called poly-ribosomes or polysomes. In such cases, each ribosome may be 80 to 100 nucleotides apart on the m-RNA (Minimum 35 nucleotides).

Q.46. What is the role of ATP in protein synthesis? How many high energy bounds of ATP are utilized?

- ***ATP is required for the activation of amino acids*** and formation of t-RNA-amino acid complex. In this reaction one ATP is converted to AMP ***(two high energy bounds are utilized).***
- One ATP is required for ***formation of "initiation complex".***
- One ATP is required in ***formation of 48s initiation complex.*** The hydrolysis of ATP by 'helicase' activity for associating 5'-end of m-RNA with binding of eIF-4A and elF-4B.

Total high energy bounds of ATP utilized = 4.

Q.47. What is the role of GTP in protein synthesis? How many GTP required in protein synthesis?

GTP is required as follows:

- ***GTP is required for binding with eIF-2*** for forming a binary complex required in formation of 43s pre-initiation complex.
- ***GTP hydrolysis provides energy for formation of 80s initiation complex.***
  eIF-2 carries GTP and eIF-5 GTPase activity.
- GTP is required for ***binding aminoacyl t-RNA to 'A' site.***
- EF-2 (elongation factor-2)-GTP complex is hydrolyzed to give ***energy for translocation, movement of m-RNA forward by one codon.***
- ***RF-3 with bound GTP is required for termination process.***
- ***Total requirement of GTP = 5.***

Q.48. What is the role of hormones in protein synthesis?

- Anabolic hormones which increase protein synthesis are:
  - Insulin
  - Growth hormone (GH)
  - Androgens
- Insulin stimulates protein synthesis by increasing incorporation of amino acids into protein at the level of translation by RNA.
- Growth hormone (GH) increases protein synthesis by stimulating the synthesis of DNA.

Q.49. State at least seven inhibitors of protein synthesis and indicate their mode of action.

- ***Puromycin:*** Inhibits protein synthesis by releasing nascent polypeptide chains before their synthesis is complete. It binds to 'A' site and inhibits entry of aminoacyl-t-RNA.
- ***Tetracyclines:*** Prevent binding of "t-RNA-AA" to ribosome and thus inhibits initiation process.
- ***Chloromycetin (Chloramphenicol):*** Inhibits ***peptidyl transferase*** activity and prevents in the peptide bond formation.
- ***Streptomycin:*** binds to 50S subunits resulting to decreased rate of protein synthesis.

- ***Erythromycin:*** binds to 50S subunit and inhibits translocation.
- ***Neomycin B:*** alters combination of t-RNA-ribosome complex.
- ***Cycloheximide:*** inhibits ***peptidyl transferase*** activity and also elongation process.

Q.50. What is the action of diphtheria toxin on protein synthesis?

Diphtheria toxin is a lethal protein toxin. It binds with EF-2 and blocks its capacity to carry out translocation.

Q.51. What is operon?

One or more structural genes together with their operator gene and regulator gene on DNA constitute the genetic structure known as operon.

Q.52. What is structural gene?

The structural gene DNA directs the synthesis of specific enzyme and other proteins through the m-RNA template.

Q.53. What is an operator gene?

The operator gene exerts control over the adjacent structural genes located on the same chromosomes, since formation of m-RNA can be effected only at a point on DNA where the operator occurs.

Q.54. What is meant by repressor?

- A third gene called regulator gene controls the activity of the operator gene. This regulator gene does so by its activity to induce the synthesis of protein macromolecules called ***"repressors"***.
- The repressor substance is usually a macromolecule, usually protein in nature (may be nucleic acid or nucleoprotein). It may require some small molecules as **co-repressors** before effecting repression.

Q.55. What is derepression?

Some other small molecules can combine with repressor macromolecules and change their nature. In that case, the repressor loses its ability to repress gene expression. This phenomenon is called "derepression". Inducers bring about derepression.

Q.56. What is Lac operon?

- Lactose metabolism in *E.coli* is effected by the **"Lac operon"**.
- Lac operon consists of an ***"i" gene*** (regulator gene), an operator gene and three structural genes:
  - ***"Z" gene*** responsible for formation of **β-*galactosidase,***
  - ***"Y" gene*** for the enzyme ***permease*** and
  - ***"A" gene*** for formation of ***acetylase***.

Q.57. What is DNA replication?

- DNA molecule duplicates itself and it is designed primarily to allow for hereditary transmission by the formation of exact copies of the original.
- DNA is a double-helix, one strand of DNA is complement of the other. Upon unwinding of the double helix, each strand acts as a template for the formation of new strand. This process is called DNA replication.
- ***Genetic information must be scrupulously transmitted to the progeny and hence the replication of DNA should be carried out with high fidelity to maintain genetic stability.***

Q.58. State the mechanisms by which DNA replication take place.

Theoretically, DNA replication can take place as follows:

- Conservative
- Dispersive
- Semiconservative
- Theta replication.

Q.59. What is conservative replication?

Conservative replication means the double helical DNA of the parent cell is just copied as an intact DNA. The two new strands formed are different from those of the parent DNA.

Q.60. What is dispersive replication?

Dispersive replication means the parent DNA breaks into fragments and these fragments are amalgamated with the new ones.

Q.61. What is theta replication?

Most prokaryotic DNA replicates when it is in a circular form, such replication is called as theta replication.

Q.62. What is semiconservative replication?

- This is the method of replication with human DNA. The parent molecule unwinds at the locations of replication, the remaining portions being intact.
- Each unwound strand acts as a "template" and a new daughter strand is formed following the rules of base-pairing.
- Finally, the daughter molecule is released. In the daughter, the one strand is only new, the other is from the parent. Hence, the mode of replication is called ***semi conservative. In this method one strand of parent DNA gets incorporated in each molecule.***

Q.63. Who proved semi conservative replication?

Meselson and **Stahl** proved semi conservative replication experimentally in *E.coli.*

Q.64. State the steps of DNA replication.

Steps of DNA replication are as follows:

- Unwinding of parental DNA.
- Formation of ***replication fork***.
- ***Synthesis of RNA "Primer"***, complementary of DNA template, the enzyme required is ***RNA primase***.
- Leading strand is synthesized in the 5' to 3' direction by the enzyme ***DNA polymerase.***
- Lagging strand is synthesized as ***okazaki*** fragments.
- RNA pieces are removed when polymerization is complete.
- The gaps are filled by deoxynucleotides and the pieces are joined by the enzyme ***DNA ligase.***

Q.65. What are the types of DNA polymerase in pro-karyotes?

In prokaryotes, there are **three enzymes.** They are:

- ***DNA polymerase I (Pol I)***
- ***DNA polymerase II (Pol II)***
- ***DNA polymerase III (Pol III).***

Q.66. What are the types of DNA polymerase in eukaryotes?

There are **three DNA polymerases** in eukaryotes. They are:

- ***Polymerase α (maxi polymerase)***
- ***Polymerase β (mini polymerase)***
- ***Polymerase γ***

- **α** form has high molecular weight as compared to **β**, and is involved in DNA replication.
- **β** is for repair, while **'γ** is mitochondrial.

Q.67. What are okazaki fragments?

DNA synthesis requires the formation of short RNA strands. The **new DNA synthesized will be a continuation of these short RNA strands.** In eukaryotes, the short RNA strand is about 10 nucleotides in length. The RNA and short DNA (about 150 bases) combination is known as ***okazaki*** fragments.

Q.68. What is the role of the enzyme DNA ligase?

- *DNA ligase* joins the ends of two pieces of DNA strands by synthesizing a phosphodiester bond between 3'-OH at the end of one chain and 5'-phosphate at the end of the other. The enzyme is a protein polypeptide having mol wt of 77,000.
- In eukaryotes, ATP provides the energy. In case of *E. coli*, NAD^+ is used for energy from the cleavage of pyrophosphate bond.

Q.69. State the other functions of DNA ligase.

In addition to its function in DNA replication, the *DNA ligase* also serves the following in eukaryotes:

- To repair single stranded *nicks* in duplex DNA.
- To join segments of DNA in recombinant DNA.
- To link the ends of linear DNA duplex to yield circular DNA.

Q.70. What is meant by "S-phase" of DNA replication?

- DNA synthesis occurs during the ***S-phase*** of the cell cycle. This period is referred as the synthetic or "S" phase. This is usually separated temporarily from the mitotic phase by non-synthetic period referred to as ***gap 1 (G-1)*** and ***gap-2 (G-2)*** occurring before and after the S-phase respectively.
- During 'S'-phase of the cell-cycle, mammalian cells contain greater quantity of ***"DNA polymerase α"*** than during non-synthetic phase.

Q.71. Name at least four inhibitors of DNA and RNA synthesis and their mode of action.

- ***Actinomycin D:*** inhibits the enzyme ***DNA dependent RNA polymerase*** and thereby the synthesis of RNA
- ***Rifampicin (Refampin):*** inhibits RNA initiation
- ***Mitomycin C:*** inhibits synthesis of DNA and also causes extensive fragmentation of DNA
- ***Anthramycin:*** inhibits RNA and DNA synthesis.

Q.72. How DNA damage can occur during DNA replication?

DNA damage during DNA replication can occur as follows:

- Misincorporation of deoxynucleotides during replication.
- By spontaneous deamination of bases during normal genetic functions, e.g. ***spontaneous deamination of cytosine to uracil.***
- From X-radiation that cause "nicks" in DNA.
- From UV irradiation that may cause ***thymine dimer formation.***
- From various chemicals that interact with DNA.

The rapid repair of DNA damages are necessary since they may be lethal to the cell or cause mutations that may result in abnormal cell growth.

Q.73. State the methods available for DNA repair.

At least three methods are available. They are:

- ***Excision repair:*** can be of 2 types:
  - Repair of thymidine dimers.
  - Repair of spontaneous deamination of cytosine to uracil.
- Photo reactivation and
- Recombinational repair.

Q.74. What is xeroderma pigmentosum? What are the clinical features?

- An **inherited disorder** in which DNA repair mechanisms are defective.
- ***Defect:*** DNA damage produced by UV irradiation specially thymine dimers cannot be incised due to inborn deficiency of the enzyme ***nicking endonuclease.***

- ***Clinical manifestations:***
  - Increased cutaneous sensitivity to UV rays of sunlight, produces blisters on skin,
  - Dry keratosis, hyperpigmentation and atrophy of skin,
  - May produce corneal ulcers.

Q.75. What is the progonsis of xeroderma pigmentosum?

- Usually a fatal condition.
- Death occurs due to formation of squamous cell carcinoma of skin.

Q.76. What is ataxia telangiectasia? What are the clinical features?

- A familial disorder which shows increased sensitivity to X-rays and UV Rays.
- ***Clinical manifestations:***
  - Progressive cerebellar ataxia
  - Oculo-cutaneous telangiectasia
  - Frequent sino-pulmonary infections.
- IgE deficiency has been demonstrated in 67% of cases.
- Lymphoreticular neoplasms are common in this condition.

Q.77. What is recombinant DNA?

Combinations of portions of DNA from one molecule to another in the same cell or cell of one species to another yield recombinant DNA.

Q.78. What are the types of DNA recombinant?

Two types:

- Natural
- Artificial.

Q.79. How natural DNA recombinant is produced?

Natural recombination of DNA could occur between:

- Molecules of DNA, and
- Homologous chromosomes.

Q.80. How artificial DNA recombinant is produced?

Artificial recombinant DNA technology is also called ***genetic engineering*** which effect artificial modifications of the genetic constitution of a living cell *by introduction of foreign DNA through experimental techniques.*

Q.81. What are the biological materials required for DNA recombinant technology?

The various biological materials required to bring about genetic manipulation are:

- Various enzymes
- Passenger DNA
- Vehicle DNA.

Q.82. Name at least six enzymes necessary in genetic engineering?

- ***Restriction endonucleases:*** Most important enzyme, which exhibit a two-fold symmetry in cutting a DNA molecule.
- ***Exonucleases:*** To cut at 5' terminus
- ***Endonucleases:*** To cut in the interior to produce "nicks"
- ***Reverse transcriptase***
- ***DNA Polymerases***
- ***DNA ligase.***

Q.83. What is vehicle DNA? Name the types of DNA used.

- DNA which acts as the carrier is called as the vehicle DNA.
- Two types of DNA has been employed as vehicles:
  - Plasmid DNA and
  - Bacteriophage DNA.

Q.84. What is plasmid DNA?

- Plasmids are extrachromosomal DNAs that occur in closed circular forms in bacteria.
- ***Characteristic features:***
  - They can exist and multiply independently of the organism's own genome.
  - Each plasmid carries its own replicator region and retains its characters after recombining with foreign DNA.
  - It may carry additional information such as antibiotic resistance factor.

Q.85. What is passenger DNA? State the types of passenger DNA used?

- Foreign DNA which is passively transferred from one cell to another cell or organ is known as passenger DNA.

- **Types of DNA used as passenger DNA are:**
  - cDNA (complementary DNA)
  - Synthetic DNA, and
  - Random.

Q.86. What is cDNA? How it is produced?

- cDNA is synthesized on RNA template with the help of the enzyme ***reverse transcriptase***. The DNA-RNA hybrid is digested with alkali like NaOH which dissolves RNA. The newly synthesized DNA is realeased as a single-stranded DNA.
- This single-stranded DNA is now used as a template and a complementary DNA strand (cDNA) is synthesized with the enzyme ***DNA polymerase.***
- The semicircular single-stranded loop connecting the two DNA strands is removed by the enzyme ***"S_1 nuclease".***
- The cDNA so produced can be linked to a vehicle DNA.

Q.87. What is DNA cloning?

Foreign DNA may be transcribed in a new host during the replication of vehicles in the bacteria. Alongwith the vehicles the passenger DNA also replicates and forms a single copy from which multiple copies of specific DNA fragments are obtained. This is known as DNA cloning.

Q.88. What is a cosmid?

Cosmid is a hybrid of phages and plasmids with the addition of "Cos" site (cohesive sites of the phages to plasmids). ***Cosmid is also used as a vector in recombination DNA technology.***

Q.89. What is the use of Southern Blot test? How it is carried out? Give the steps.

Southern Blot test is ***used for analysis of chromosmal DNA.***
Steps of Southern Blot test involves:

- ***Cleavage:*** DNA is cleaved with the help of the enzyme ***restriction endonuclease*** at specific sites.
- ***Electrophoresis:*** DNA thus obtained will be in fragments. These DNA fragments are separated by agarose-gel electrophoresis or polyacrylamide gel (slab) electrophoresis.

- ***Blotting:*** The separated DNA fragments are transferred to a sheet of nitrocellulose by a flow of buffer. These fragments bind to the nitrocellulose creating a replica of the pattern of DNA fragments.
- ***Hybridization:*** Next step is the hybridization of fragments with a labelled probe. This probe has a homology with the gene of interest. The probe is hybridized to filter.

The probe can be a
  - Purified RNA,
  - cDNA or
  - A segment of cloned DNA

- ***Autoradiography:*** This is the final step. The pattern of bands that contain the DNA fragments or the fragments that contain the gene are visualized by virtue of radiation from the probe.

Q.90. What is Northern Blot test? What is its use?

- ***It is used for analysis of RNA.***
- ***Steps:***
  - ***Electrophoresis:*** Total cellular RNA or isolated m-RNA is subjected to agarose gel electrophoresis in the presence of a denaturing agent to remove secondary structure contraints.
  - ***Blotting:*** The gel is blotted in a similar manner as given in Southern Blot test with the difference that paper has been treated chemically so that it covalently binds RNA.
  - ***Hybridization:*** A labelled probe is used. This allows visualization of the RNA species that are complementary to the probe.

Q.91. What is Western Blot test. What is its use and how it is carried out?

- ***Used for analysis for proteins.*** Proteins are first isolated from the tissues and identified.
- ***Steps:***
  - ***Electrophoresis and fixation:*** Electrophoresis of the whole protein is done and transferred to a nitrocellulose membrane and fixed.
  - ***Probing:*** After fixing the protein is probed with radio active antibody.

– ***Autoradiography:*** This is the final step. The pattern of bands that contain protein are visualized by virtue of radiation from probe.

Q.92. State the applications of recombinant DNA technology.

- ***In production of proteins/hormones:***
  – Hormones like insulin, growth hormone (GH) and interferon are being synthesized.
  – Recently insulin analogues have been prepared.

  Examples:
  – Short or fast acting insulin analogues, e.g. B_9 ASP B_{27} Glu and Lys (B_{28}) Pro (B_{29}) also called ***lisproinsulin.***
  – Intermediate acting insulin analogues, e.g. diarginyl insulin, DES 64,65 HPI (D-PRO)
  – Long acting insulin analogue, e.g. Novosol basal.
  – ***In diagnosis of hereditary defects:*** of inherited disorders like sickle cell anemia, thalassemia, etc.
  – Cloned genes are being used as hybridization probes for the clinical diagnosis of infectious micro-organisms that contain homologs of such genes.
  – In genetherapy.

CHAPTER

20 Chemistry of Respiration

Q.1. What is respiration?

Respiration is defined as the process in which there is utilization of O_2 from inspired air by the body and elimination of CO_2 produced by the metabolites in the cells in the expired air.

Q.2. State the composition of atmospheric air and expired air.

	Atmospheric air (Inspired air)	*Expired air*
• **Oxygen (O_2)**	20.96%	15%
• **Carbon dioxide (CO_2)**	0.04%	5%
• **Nitrogen**	79%	79%

Q.3. What is the oxygen tension (PO_2) in alveolar air and in venous blood?

- The oxygen tension (PO_2) is the pressure of the dry gas with which the dissolved oxygen in the blood is in equilibrium.
  - Oxygen tension in alveolar air: 107 mm Hg.
  - Oxygen tesnion in venous blood: 40 mm Hg.

Hence, a pressure difference of 67 mm Hg drives oxygen (O_2) from the alveoli of the lung into the blood.

Q.4. What is CO_2 tension in alveolar air and in venous blood?

The CO_2 tension in alveolar air: 36 mm Hg and CO_2 tension in venoun blood: 46 mm Hg. The difference of 10 mm Hg is sufficient to drive CO_2 from the blood into the lung.

Q.5. How oxygen is transported in blood?
Oxygen is transported in the blood as follows:
- ***A small amount*** of O_2 is carried ***dissolved in blood*** in solution.
- Mainly transported in ***combination with Hb*** as oxy-Hb.

Q.6. How much oxygen is carried by 100 ml of blood? What is the total oxygen carrying capacity of blood?
- When fully saturated, ***one gram of Hb can carry 1.34 ml*** of oxygen. A person with normal Hb concentration of 14.5 gm/dl, carries 14.5 × 1.34 = 19.43 ml of O_2.
- The oxygen carried in physical form in dissolved state is around 0.3 ml/dl.
- Hence, the total amount of O_2 carried by 100 ml of blood in a normal individual is about 20 vol%. This ***20 vol% is known as 100% saturation of Hb or total oxygen carrying capacity of blood.***

Q.7. What is meant by oxygen capacity of blood?
It is the number of ml of oxygen in 100 ml of blood when it is fully saturated with air.

Q.8. What is meant by oxygen combining power of blood?
It is the number of ml of oxygen combined with Hb of 100 ml of fully saturated blood.

Q.9. What is meant by oxygen dissociation curve?
Oxygen dissociation curve is the curve which shows graphically the relationship between partial pressure of oxygen (PO_2) in mm Hg against the % (Percentage) saturation of Hb with O_2.

Q.10. What is the shape of oxygen dissociation curve?
- It is S-shaped.

Q.11. Why oxygen dissociation curve is S-shaped?
It happens so due to the combination of one molecule of Hb with 1 to 4 molecules of oxygen under physiologic conditions.

Q.12. What does the lower part of S-shaped curve indicate?
It indicates a fall in partial pressure of O_2 and causes a much greater dissociation of oxygen from oxy-Hb.

Q.13. State the factors that influence oxygen dissociation curve.
- ***Po_2:*** The curve shows that greater the **Po_2,** higher is the saturation.

- ***Pco_2:*** With increasing **Pco_2** the affinity of Hb in the blood for O_2 decreases. Thus oxy-Hb dissociates releasing free O_2.
- ***pH value:*** Increase in pH value increases dissociation of oxy-Hb shifting O_2-dissociation curve to the right.
- ***Temperature:*** Increase in temperature shifts the dissociation curve to the left.
- ***Electrolytes:*** Oxy-Hb dissociates more readily in presence of electrolytes at low **Po_2** levels.
- ***2:3-DPG:*** Presence of 2:3-DPG facilitates the delivery of O_2 shifting the curve to the right.

Q.14. What is hypoxia? And what is anoxia?

- ***Hypoxia:*** When the supply of O_2 to the cells is inadequate leading to insufficiency of tissue oxidation, the condition is called hypoxia.
- ***Anoxia:*** When there is absolutely no supply of O_2 to the tissues it is called as anoxia.

Q.15. State the types of hypoxia.

Four types:

- Hypoxic hypoxia
- Anemic hypoxia
- Stagnant hypoxia
- Histotoxic hypoxia.

Q.16. What is hypoxic hypoxia? Name some conditions where it can occur.

Insufficient O_2 supply to the tissues is called as hypoxic hypoxia. It may be due to:

- Low **Po_2** as occurs in high altitude.
- Thickening of lung alveoli hampering diffusion of O_2. Blood bypasses the arterial alveoli and does not come in contact with O_2 as in congenital heart disease and lobar pneumonia.

Q.17. What is anemic hypoxia?

In anemia the Hb levels of blood is reduced which adversely affect the supply of O_2.

Q.18. What is stagnant hypoxia?

This is principally due to circulatory insufficiency, the rate of blood flow through the tissues is retarded resulting to reduced O_2 supply.

Q.19. What is meant by histotoxic hypoxia?

This type of hypoxia has nothing to do with the mechanism of transport and delivery of O_2. However, when the tissue cells are poisoned they are unable to utilize O_2. These poisons inhibit the respiratory enzymes cytochrome oxidase.

Q.20. How CO_2 is transported in the blood?

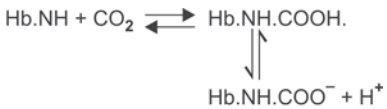
- **Small amount** of CO_2 is carried in **physical solution** (dissolved state) and as **carbamino compound.**
- Major portion is transported as **bicarbonates.**

Q.21. How much CO_2 is carried in physical solution?

- CO_2 is carried in physical solution by plasma as well as RB cells. CO_2 is more soluble in plasma and is directly proportional to the partial pressure.
- In venous blood at **Pco_2** of 46 mm Hg, 3.5 ml of CO_2 is in physical solution per 100 ml of blood.

Q.22. What is a carbamino compound? How much CO_2 is carried as carbamino compound?

- CO_2 combines with -NH_2 group of globin of Hb and forms carbamino compound.



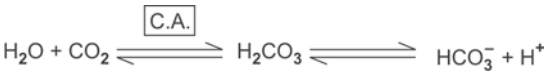
- Hb and certain other plasma proteins carry CO_2 as above. ***In arterial blood,*** it ***is around 3 ml/100 ml of blood and 3.7 ml in venous blood.***
- Reduced Hb and oxy-Hb carry 8 ml and 3 ml of CO_2 as carbamino compound per 100 ml of venous and arterial blood respectively.

Q.23. How much CO_2 is transported as bicarbonates?

- This is the most important means of CO_2 transport in the blood amounting to ***42 ml of CO_2 per 100 ml of arterial blood and 44.8 ml of CO_2 per 100 ml of venous blood.***
- CO_2 produced in the cells finds its way into the RB cells. (CO_2 diffuses through the red cells membrane), where the enzyme ***carbonic anhydrase*** catalyzes the formation of H_2CO_3 (Carbonic acid).

Q.24. What is chloride shift or Hamburger's phenomenon?

- At physiological pH of 7.4, the carbonic acid is dissociated into $H^+ + HCO_3^-$



Some of HCO_3^- combines with intracellular K^+ and rest diffuses out into plasma due to concentration gradient. Since HCO_3^- is an electrolyte, its diffusion out disturbs the electrolyte balance. This is overcome by shifting of equal numbers of chloride (Cl^-) ions from plasms into the red blood cells. This is called as ***chloride shift*** or ***Hamburger's phenomenon.***

Q.25. How much nitrogen is present in plasma? What is its role?

- Nitrogen is found in plasma at a concentration of 2.5 to 3 ml/100 ml of blood.
- The percentage of N_2 is similar in atmospheric, alveolar air and expired air indicating that it has no role in metabolic processes.
- The major function of nitrogen in respiration is probably to act as an inert diluent of O_2.

Q.26. What is Caisson disease or bends?

- When a person goes to high altitude or comes up from deep sea abruptly (as in sea divers) it can cause bad effects.
- In deep seas due to high pressure excessive amount of N_2 gets dissolved in plasma. When a sea-diver comes up abruptly, the N_2 is quickly released by plasma which may collect in the form of bubbles at the joints, adipose tissue and other tissues which cause intense pain. Sometimes the tissues are damaged impairing their functions. Intravascular clotting can also occur.
- This is called as ***bends*** or ***Caisson disease.***

Q.27. "Oxygen is necessary for life." But it can be toxic also. What is the cause of toxicity?

Univalent reduction of molecular oxygen in tissues can give rise to ***superoxide radical*** (O_2^-). It is one of the ***free*** radicals produced in the body. Superoxide radical forms other free radicals. These free radicals are injurious to biomembranes.

Q.28. Why oxygen is more porne to produce superoxide radical (O_2^-)?

- Molecular oxygen is paramagnetic and contains two unpaired electrons with parallel spins. These unpaired electrons reside in separate orbitals unless their spins are opposed.
- Reduction of oxygen by direct insertion of a pair of electrons, e^-, into its partially filled orbitals is not possible without inversion of one electronic spin and such an inversion of spin is a slow change.
- Hence electrons are added to molecular oxygen as single electron successively. When oxygen molecule takes up one electron (e^-), by univalent reduction, it becomes ***superoxide anion (O_2^-).***

$$O_2 + e^- \longrightarrow O_2^-$$

Q.29. State the other free radicals formed in the body?

In addition to superoxide radical (O_2^-), the other free radicals formed in the body are:

- **Hydrogen Peroxide (H_2O_2)**
- **Free hydroxy radical ($OH^\bullet$)**
- **Singlet Oxygen ($^1O_2$)**
- **Hydroperoxy radical ($HOO^\bullet$).**
- Other toxic "free" radicals produced in the body are free radical CCl_3^- and free halogen radical like Cl^- formed from CCl_4.

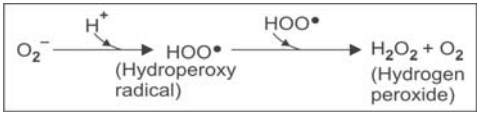
Q.30. How H_2O_2 and hydroperoxy radical can be formed from superoxide anion?

- Superoxide anion O_2^-, after its formation can capture further electrons to form **"hydrogen peroxide" (H_2O_2).**

$$O_2^- \xrightarrow{e^-,\ 2H^+} H_2O_2$$

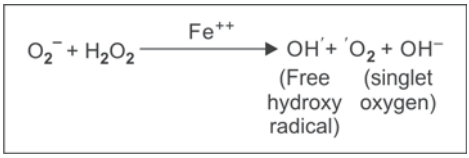
(Hydrogen peroxide)

- Superoxide anion O_2^- can accept an H^+ and form **"hydroperoxy"** radical, which can further form H_2O_2.



Q.31. What is Haber-Weiss-Fenten reaction?

H_2O_2 can react with superoxide anion, O_2^-, in presence of Fe^{++} (Ferrous) to form **"hydroxyl"** radical and **"singlet"** oxygen. This reaction is called as ***Haber's reaction*** (or ***Haber-Weiss-Fenten reaction).***



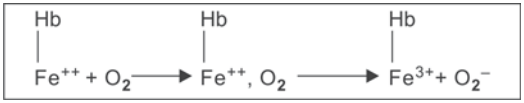
Q.32. How ceruloplasmin is useful in halting Haber's reaction and thus acts as an antioxidant?

Ceruloplasmin acts as ***ferroxidase I*** and can serve as an antioxidant. It can convert $Fe^{++} \rightarrow Fe^{3+}$ (ic) and thus it can halt Haber's reaction preventing formation of highly reactive "free hydroxy radical".

Q.33. Mention some metabolic pathways in the body where superoxide anion O_2^- may form.

- Cytosolic oxidations by auto-oxidizable Fp-dependent enzymes, e.g.:
- Oxidative deamination by L-amino acid oxidase
- Xanthine oxidase
- Aldehyde dehydrogenase.
- During univalent oxidations with molecular oxygen in ETC:

- During methemoglobin formation:



- During cytosolic hydroxylations of drugs, steroids, xenobiotics by cyt. P_{450} system.
- Can be formed when exposed to ionizing radiations.

Q.34. State the effects of free radicals on biomembranes.

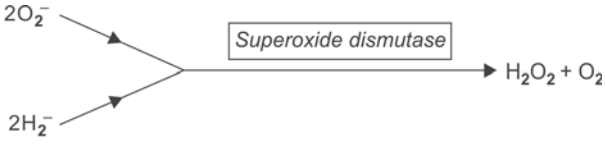
- "Free" radicals are highly reactive. It can initiate chain reaction and brings about lipid peroxidation producing lipid peroxides and lipoxides.
- They can oxidize:
  - -SH groups containing membrane proteins in cells and biomembranes to S-S group.
  - Methionine sulphur is oxidized to its sulphoxide.
  - Membrane lipids, unsaturated FA to lipid peroxides and lipoxides.

Q.35. Name the scavengers of free radicals present in the body.

- ***Superoxide dismutase*** enzyme-cytosolic and mitochondrial
- ***Catalase***
- ***Glutathione peroxidase***
- **Ferricytochrome**
- **Endogenous ceruloplasmin.**

Q.36. What is the role of superoxide dismutase?

- *Superoxide dismutase* is an enzyme present in the cytosol and also in mitochondria, which can destroy the superoxide anion, O_2^-,



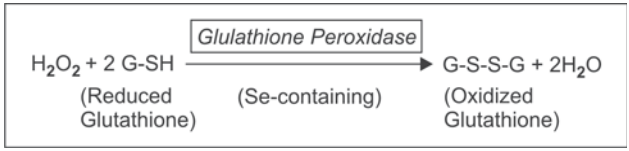
- The enzyme protects the tissues from deleterious effect of O_2^-.

Q.37. What is the role of catalase?

- ***Catalase*** having high Km value can destroy H_2O_2 formed in the tissues to form O_2 again.

Q.38. What is the role of glutathione peroxidase?

- ***Glutathione peroxidase,*** a selenium containing enzyme present in cytosol and mitochondria, having **low Km,** can destroy H_2O_2 with the help of reduced glutathione (G-SH).



Q.39. Name the anti-oxidants which reduce the rate of chain initiation. (Preventive antioxidants).

Antioxidants which reduce the rate of chain initiation are:

- ***Catalases***
- ***Peroxidases***
- ***Endogenous Ceruloplasmin***
- Chelators of metal ions like:
  - **DTPA** (diethylene triamine penta-acetate) and
  - **EDTA** (Ethylene diamine tetra-acetate).

Q.40. Name the anti-oxidants which interfere with the chain propagation (Chain breaking antioxidants).

- ***Superoxide dismutase*** (both cytosolic and mitochondrial)
- **Vitamin E** (Tocopherols)
- Se-containing ***glutathione peroxidase.***

Q.41. What is the clinical importance of free radicals?

- "Free" radicals play an important role in ageing and aggravate certain disease processes like Diabetes mellitus, atherosclerosis, rheumatoid arthritis, cancer, etc.
- "Pre-term" babies are more prone to harmful effects of "free" radicals.

- Diseases like broncho-pulmonary dysplasia, retinopathy of prematurity, necrotizing enterocolitis, periventricular leukomalacia, etc. are now classified as ***neonatal oxygen radical diseases.***

Q.42. What is the role of free radicals in phagocytosis?

- A characteristic feature of phagocytosis by macrophages is increased utilization of glucose by HMP shunt. There is also a great increase in uptake of O_2. NADPH produced by HMP-shunt can react with O_2 to produce superoxide anion, O_2^- which subsequently produce H_2O_2.

- ***H_2O_2 is used in killing micro-organisms by phagocytosis.***

Q.43. Why G-6-PD deficient persons are resistant to infection with plasmodium falciparum?

- Normally, the malarial parasites generate H_2O_2 by oxidizing NADPH, produced by HMP-shunt for their survival.
- In G-6-PD deficient persons, the parasites fail to thrive as they cannot produce H_2O_2 for their survival as NADPH production is lacking.

CHAPTER

21 Biophysics

Q.1. What is pH?

pH is defined as the negative logarithm of hydrogen ion concentration to the base 10. The hydrogen ion concentration $[H^+]$ is expressed as moles/liter.

$$\boxed{pH = -\log_{10}[H^+] = \log 1/[H^+]}$$

Q.2. What does the notation pH means?

pH means ***Puissance hydrogen,*** Potenz hydrogen or hydrogen power. In this, negative powers vanish and positive powers result.

Q.3. What is the pH of water?

The H^+ of water is 10^{-7} g/liter

$H^+ = 10^{-7}$g.

$\log 10\ H^+ = \log_{10} 10^{-7} = -7$

Negative log of $H^+ = 7$

$(-\log 10\ H^+)$.

$\therefore$ pH of water = 7 (neutral pH).

Q.4. What is the pH scale?

The pH scale ranges from 0 to 14 for dilute aqueous solutions like biological fluids. pH 7 is considered the neutral pH. Acidic solution is below 7 (0 to 7) and alkaline solution is above 7 (7 to 14).

Q.5. What is the pH of ECF?

- pH of ECF usually does not vary beyond the range 7.35 to 7.5 and is maintained approximately at pH 7.4 (slightly to alkaline side).
- ***pH of arterial blood is approximately 7.43 and venous blood is 7.4.***

Q.6. What is Henderson-Hasselbalch equation? Show how it is derived.

Henderson-Hasselbalch equation is given as follows:

$$\mathbf{pH = pK + \log \frac{A}{HA}}$$

- It is derived as follows:

Let us consider HA, a weak acid that ionizes as follows:

$$HA = H^+ + A^-.$$

Equilibrium constant K can be written as:

$$K = \frac{H^+ \quad A}{HA}$$

Simplifying the above, we get:

$$K \times [HA] = [H^+][A^-]$$

$$\text{or } [H^+] = \frac{K \times [HA]}{[A^-]}$$

Taking log of both sides,

$$\log [H^+] = \log K + \log \frac{HA}{A}$$

Change sign of both sides,

$$-\log [H^+] = \log K + \log \frac{HA}{A}$$

Now, we know:

$$-\log (H^+) = pH$$
$$-\log K = pK$$

Substituting in above equation, we get,

$$pH = pK + \log \frac{[A^-]}{[HA]} \text{ (Henderson - Hasselbalch equation)}$$

Q.7. What is the significance of pK?

pK of an acid group is that pH at which the protonated and unprotonated species are present at equal concentration.

Q.8. What are the uses of Henderson-Hasselbalch equation?

- Can be used to determine pH of blood, if the concentration of salt i.e. bicarbonate and acid, i.e. carbonic acid is known.

Example:

- Approximately concentration of bicarbonate in normal health = 0.025 M.
- Approximately concentration of carbonic acid is = 0.00125 M.
- pK of carbonic acid is = 6.10.
- By applying Henderson-Hasselbalch equation:

$$\begin{aligned} pH &= 6.10 + \log \frac{0.025}{0.00125} \\ &= 6.10 + \log 20 \\ &= 6.10 + 1.3 \\ &= 7.4 \end{aligned}$$

- Can be used to calculate the pH of any buffer by knowing the dissociation constant of the acid and the concentration of the salt and acid.

Q.9. State some biochemical importance of pH.

- Maintenance of constant blood pH is one of the prime requisites of life, any material variation on either side endangers life.
- Most of the enzymes have optimum pH for its action. In extremes of pH, the enzyme gets destroyed.
- Amino acids and proteins exist as ***zwitterions*** at iso-electric pH (pI).
- Lactam/Lactim forms of nucleic acid bases depend on pH. Specific tautomeric forms exist at pH 7.4.
- Abrupt changes in pH may destroy the ionic and hydrogen bonds and change the 3-D structure of a protein resulting in its inactivation and denaturation.
- MPS, phosphoglycerides, sphingolipids, etc. exist as ionized form either as cations or anions depending on the pH:
- pH effects membrane structure by influencing, the ionization of membrane proteins and lipids.
- pH is responsible for the Gibbs-Donnan membrane equilibrium.

Q.10. What is meant by conjugate acid-base pairs?

- The stronger the acid, the weaker the base, which results from its dissociation and vice versa. Such pairs have been termed conjugate acid-base pairs.
- The conjugate base of the strong acid HCl is the Cl^- ions.

Q.11. Define buffer, what is the compositon?

- A buffer may be defined as a solution which resists the change in pH which might be expected to occur upon the addition of acid or base to the solution.
- Buffer consists of mixture of weak acids and their corresponding salts, alternatively weak bases and their salts.

Q.12. Give two examples of buffer used in the laboratory.

- A weak acid and its salt, e.g. acetic acid and sodium acetate.
- A weak base and its salt, e.g. ammonium hydroxide and ammonium chloride.

Q.13. Name the buffer pairs present in plasma.

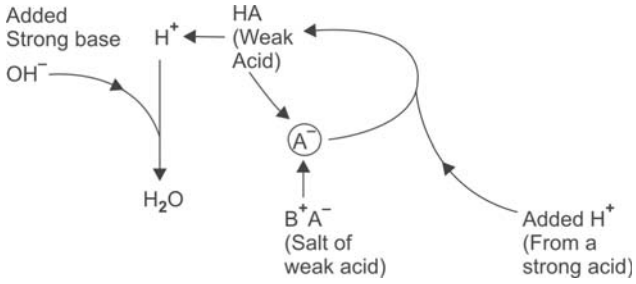
- $\frac{NaHCO_3}{H_2CO_3}$ (Bicarbonate buffer)
- $\frac{Na_2HPO_4}{NaH_2PO_4}$ (Alkaline phosphate) (Acid phosphate) (Phosphate buffer)
- $\frac{Na.Pr.}{H.Pr.}$ (Protein buffer)
- $\frac{Na.\ organic\ acid}{H.organic\ acid.}$

Q.14. Name the buffers pairs present in red blood cells.

- $\frac{K.HCO_3}{H_2CO_3}$ (Bicarbonate buffer)
- $\frac{K_2HPO_4}{KH_2PO_4}$ (Phosphate buffer)
- $\frac{K.Hb}{H.Hb}$ (Haemoglobin buffer)
- $\frac{K\,HbO_2}{H\,HbO_2}$
- K organic acid
  H. organic acid

Q.15. State the mechanism of action of a buffer.

- Action of the buffer against added acid or base may be shown schematically as follows:



- ***Added H^+ ions***, in the form of a strong acid, combine with anions A^- largely from the salt component of the buffer, to form the weakly dissociable HA, so that pH does not become as acid as it would be in absence of the buffer.
- ***Added OH^- ions***, in the form of a strong base, combine with H^+ ions derived from the weak acid HA of the buffer and forms H_2O. Hence pH does not become as alkaline as would happen in absence of buffer.

Q.16. What are Indicators?

- Certain dyes which can determine the hydrogen ions in a solution are called as indicators. The dyes are weak acids (or less commonly weak bases).
- Indicators are used to determine the titratable acidity or alkalinity.

Q.17. Name some indicators commonly used, with their pH range and colour changes.

Indicator	*pH range*	*Colour change*
• **Methyl Red**	4.3 to 6.3	Red → yellow
• **Phenolphthalein**	8.3 to 10.0	Colourless → Red
• **Thymol blue**	8.0 to 9.6	yellow → Blue
• **Methyl orange**	3.1 to 4.4	Red → Yellow
• **Bromphenol blue**	3.0 to 4.6	Yellow → Blue

Q.18. What is diffusion?

Diffusion is defined as a process in which solute particles move from a more concentrated environment to a less concentrated one in order to bring a uniform concentration throughout.

Q.19. What is Fick's law?

Diffusion between two planes "X" and "Y" in a non-homogeneous solution can be expressed quantitatively by Fick's law a follows:

$$\boxed{\frac{ds}{dt} = DA\frac{dc}{dx}}$$

Where,

$\frac{ds}{dt}$ = The rate of movement of solute.

D = Diffusion constant.

A = Area of the planes.

$\frac{dc}{dx}$ = The concentration gradient, i.e. the difference in concentration between X and Y/distance between X and Y.

Q.20. What are the clinical importance of diffusion in biological systems?

- Respiratory exchange of gases O_2 and CO_2 involves diffusion.
- Intestinal absorption of pentoses, some minerals, water soluble vitamins and renal absorption of urea are carried out by diffusion.
- Water, ions and small molecules pass largely by diffusion through plasma membrane.
- Diffusion of ions across cell membranes influences polarization of membranes and membrane potentials.
- Diffusion of respiratory gases across red cells membranes is much faster in small red blood cells of mammals than in larger erythrocytes owing to higher surface: Volume ratios in the smaller cells.
- Increased thickness of inflamed alveolar membrane reduces the diffusion of O_2 in pneumonic consolidation. Similarly, in chronic smokers many small alveoli fuse to

form fewer and larger alveoli, hence total alveolar surface is reduced and the diffusion of gases decreased.
- Keratinized epidermis minimizes loss of water due to reduced diffusion.

Q.21. What is diffusion trapping?
- Weak acids such as salicylic acid and phenobarbital are excreted passively in distal tubule and collecting duct of kidney by the ***diffusion trapping*** mechanism ***so long as the urine is alkaline.*** They diffuse from the tubule cells to tubular lumen down their concentration gradient, as the tubular membrane is permeable to them, though not to their anions.
- In the alkaline urine, they ionize into anions, hence they cannot diffuse back into the tubule cells but lower the concentrations of the respective non-ionized weak acids, enabling their diffusion to continue.

Q.22. Define osmosis.

Osmosis is defined as the spontaneous flow of water into a solution or from a more dilute to a more concentrated solution when the two solutions are separated from each other by semipermeable membrane. Osmosis occurs in the direction opposite to that in which diffusion occurs.

Q.23. Name the artificial semipermeable membrane.

The most selective artificial semipermeable membrane is $Cu_2Fe(CN)_6$.

Q.24. Define osmotic pressure.

Osmotic pressure may be defined as the pressure which must be applied on the concentrated solution to prevent the osmotic inflow into the solution, when they are separated by a semipermeable membrane.

Q.25. State van't Hoff's laws of osmotic pressure.
- The osmotic pressure of a solution varies directly with the concentration of the solute in the solution.
- It also varies directly with the absolute temperature.

Q.26. How do red blood cells behave with isotonic, hypotonic and hypertonic solution?
- ***With isotonic solution,*** the red blood cells remain intact. No damage to red blood cells occur.

- If red blood cells are kept in ***hypotonic solution***, the water will flow through red blood cells membrane, and red blood cells will swell up and rupture ***causing haemolysis***.
- When the red blood cells are kept in ***hypertonic solution***, water comes out of the cells causing shrinkage of the red cells. This is called ***crenation***.

Q.27. State the difference between osmotic pressure and oncotic pressure.

- The osmotic pressure will be greater if the molecular weight of a substance is low.
- The oncotic pressure increases when the molecular weight of the substance is high.
  The plasma albumin having high molecular weight does not increase the osmotic pressure since it is impermeable to the membrane. Hence, it increases the oncotic pressure.

Q.28. What are the biomedical importance of osmosis?

- Practice of giving 5% glucose solution and 0.9% saline for IV infusions as these are iso-osmotic with plasma.
- ***Cause of oedema in cases of albuminuria:*** In albuminuria plasma proteins, mainly albumin are excreted, reducing concentration of plasma proteins leading to lowering of oncotic pressure of plasma. This increases hydrostatic pressure causing accumulation of excess of fluid in tissue spaces and edema.
- Another clinical application of osmotic pressure is the injection of hypertonic solutions of salts such as NaCl or $MgSO_4$ to reduce cerebral edema, water is withdrawn from the brain osmotically.
- ***In pure water depletion*** (primary dehydration), the ECF becomes hypertonic, this leads to withdrawal of cellular fluid producing intracellular dehydration.
- ***Osmotic fragility test:*** The extent of hemolysis of Red blood cells is observed in a series of tubes with hypotonic NaCl solutions, adding a drop of blood and observing after about two hours. In normal health, compete hemolysis occurs only below 0.35% NaCl, the cells resist hemolysis above 0.45%. ***Diminished resistance (increased fragility) is seen in hemolytic anemia (upto 0.7 to 0.8%).***

- ***Purgative action of epsom salt ($MgSO_4$):*** The strong solution of this salt prevents the absorption of water in the intestine causing dilution of intestinal contents and purgative action.
- Urine excretion is assisted by osmosis to keep the blood isotonic with the cells.

Q.29. What is meant by electroendosmosis?

The movement of a liquid through a capillary under the influence of an electric potential is termed as electroendosmosis.

Q.30. What is dialysis?

- Dialysis is the process of separating crystalloids from colloids by diffusion through a membrane adopting osmotic force.
- Membranes are made of cellophane, parchment, collodion or inert cellulose esters like cellulose nitrate or acetate.

Q.31. What is electrodialysis?

In this form of dialysis, the semipermeability and migration of electrolytic ions in an electric field are utilized in separating colloid particles from electrolyte ions.

Q.32. What is the biomedical importance of dialysis?

- Dialysis is essential to separate proteins from a mixture of salts and to separate out macromolecules of cell extract.
- It is also required for preventing enzymatic/or metabolic reactions by removing small cofactor molecules from the cell extract.
- ***Biological ultrafiltrates:*** Many extracellular fluids like interstitial fluids, cerebrospinal fluid, glomerular filtrate of kidney are formed by ultrafiltration. ***Proteins do not appear in ultrafiltrate.***
- ***Dialysis by artificial kidney:*** Patients with uremia and acute renal failure, dialysis of blood is performed in artificial kidneys, to eliminate non-electrolyte waste products and the excess of electrolytes. The blood cells are proteins which are retained in plasma.

Q.33. What is surface tension?

- The force with which the surface molecules are held is called the surface tension of the liquid.
- It is ***defined*** as the force in dynes acting at right angles to any imaginary line of 1 cm. length on the surface.

Q.34. Name the substances that increase and decrease the surface tension.

- Some of the substances that increase surface tension are: Alkalies, sodium chloride and most of inorganic salts.
- Substances that decrease surface tension are: Bile salts, soaps, oils, proteins, fatty acids, ammonia, potassium permanganate, DPL (Dipalmityl lecithin), strong mineral acids.

Q.35. Name the instrument used to determine surface tension.

Traubes stalagmometer is used for determination of surface tension of liquids.

Q.36. State the Gibbs-Thompson principle in relation to surface tension.

- The substances that lower the surface tension become concentrated at the interface.
- The substances that increase the surface tension tend to move away from the interface.
- Lipids and proteins that are effective in lowering surface tension are found concentrated in the cell wall.

Q.37. State the biomedical importance of surface tension.

- ***Emulsification of fats:*** Bile salts lower surface tension of fats and brings about emulsification of fats, which is a prerequisite and important for digestion and absorption of fats.
- ***Alveolar exudation:*** The inward force generated by lung alveolar surface tension increases diffusion of fluids into alveoli from alveolar capillaries. Lung surfactant reduces such fluid exudation and ***prevents pulmonary edema by lowering surface tension.***
- In postnatal life, type 2 alveolar epithelial cells in lungs secrete a surfactant called ***dipalmityl lecithin (DPL)*** which prevents collapse of lung alveoli during expiration. ***Absence of DPL in newborns produces respiratory distress syndrome (RDS).***

Q.38. Mention one practical application of surface tension.

- ***Hay's test*** for bile salts: The presence of bile salts in urine can be detected by Hay's sulfur test.

If the urine contains bile salts, the fine sulphur particles sprinkled on its surface settles down due to lowering of surface tension by bile salts.

If the urine does not contain bile salts, the fine sulphur powder continue to float on the surface due to surface tension.

Q.39. What is viscosity?

Viscosity is the internal resistance against the free flow of a liquid to the frictional forces between the fluid layers moving over each other at different velocities.

Q.40. What is meant by co-efficient of viscosity?

The coefficient of viscosity (n) is the force (dynes) required to maintain the streamline flow of one fluid layer of 1 cm^2 area over another layer of equal area, separated from one another by 1 cm, at a rate of 1 cm/sec.

Q.41. What is the instrument used for determination of viscosity of a fluid?

Viscosity is generally determined by **Oswald's viscosimeter.**

Q.42. What is the unit of viscosity?

The unit of viscosity is ***Poise,*** named after ***Poiseuille,*** a french man who first devised methods for measuring viscosity.

Q.43. Define Poise. What is the absolute viscosity of water?

- Poise is ***defined*** as the force in dynes, necessary to be applied to an area of 1 sq cm between two parallel planes 1 sq cm in area and 1 cm apart, to produce a difference in streaming velocity between the liquid planes 1 cm/sec.
- Absolute viscosity of water at 25°C is 0.00395 poise and is generally used in plotting the viscosity of liquid systems.

Q.44. What are the effects of temperature and colloids on viscosity?

Viscosity decreases with the rise in temperature and increases with the increase in colloids.

Q.45. What is the viscosity of plasma and whole blood?

- Plasma has a normal viscosity of 15 to 20 m poises at 20°C.
- Whole blood viscosity ranges from 30 to 40 m poises at 20°C.

Q.46. Which exerts more viscosity—albumin or fibrinogen?

Fibrinogen is large and it has an irregular shape and exerts more viscosity in plasma than albumin.

Q.47. What is Fahraeus-Lindguist effect?

Increase in blood viscosity decreases blood viscosity particularly in capillaries. However, such rises in blood viscosity are partly compensated by a fall in viscosity during blood flow through narrow vessels of diameter less than 150 mm. This is called ***Fahraeus-Lindguist effect.***

Q.48. State the biomedical importance of viscosity.

- Blood viscosity helps in stream-lining blood flow.
- The viscosity of blood offers resistance to the heart during circulation. The heart muscle functions best while working against a certain resistance.
- The viscosity of whole blood depends on the number of red blood cells. Hence it is high in polycythaemia and low in chronic anemias.

Q.49. What is hydrotropy?

Hydrotropy is the process by which water-insoluble substances are made water soluble by hydrotropic substances.

Q.50. Name some water insoluble biomolecules.

Lipids, sterols, uric acid, phospholipids, calcium and magnesium phosphates.

Q.51. Name some hydrotropic substances.

Benzoic acid, cholic acid, hippuric acid, phenyl acetic acid and soaps of higher fatty acids.

Q.52. State the biomedical importance of hydrotropy.

- The hydrotropic substances have the capacity to reduce surface tension.
- Substances dissolved by hydrotropy are diffusible through membranes.
- Hydrotropic substances are useful in helping absorption and transport of insoluble substances.

Q.53. What is adsorption?

- Adsorption is the process of taking up substances from solution on surface.
- It is a surface phenomenon, a reversible process and decreases with rise in temperature.

Q.54. State the biomedical importance of adsorption.

- Surface adsorption help to combine enzymes with substrates to give reaction product.
- Adsorption on the cell membranes promote many vital chemical reactions.
- Adsorption is used in purification of enzymes.
- Drugs and poisons which are adsorbed on cell surfaces exert their effects from that location. Selective adsorption may be related to specific action.
- Many chemical reactions are speeded up by the presence of adsorptive surface.

Q.55. What are colloids?

Certain substances such as proteins, polysaccharides which do not diffuse through parchment or animal membrane, although they form homogeneous or heterogeneous solution are called as colloids.

Q.56. What is meant by colloidal state?

- When a solution has two phases it is called a colloidal state.
- Colloidal state is a heterogeneous state and consists of ***disperse phase*** and the ***continuous phase*** with distinct boundaries between them.

Q.57. What is the size of colloid particles?

Any substance is said to be in a colloidal state if the diameter of its particles ranges from 1 mm to 200 mm.

Q.58. What is meant by Helmholtz-Gouy electrical double layer?

- There is statically charged electrical system around each colloid particle.
- The colloid particle has a definite electrical charge. This charge in the case of proteins, is due to primary ionization of groups-COOH groups and $-NH_2$ groups. In addition, there is adsorption of ions on the surface. When there is no primary ionization, the electrical

charge is only due to the adsorption of ions. Adsorption results in the formation of two layers: one ***immobile layer*** and the other ***mobile***. This envelope of ions on the colloid is termed the ***Helmholtz-Gouy*** electrical double layer.

Q.59. What are the different types of potentials present in colloid? Explain their nature.

There are **three types** of potentials formed as a result of the double layer:

- ***Epsilon potential,***
- ***Zeta potential and***
- ***Stern potential.***

- The potential drop across all ionic layers from the surface into the solution is the ***epsilon*** potential.
- The potential between the immobile layer of ions and the mobile layer is termed the ***zeta*** protential.
- The potential between immobile layer and the particle surface is the ***stern*** potential.

Q.60. What is Tyndall effect?

The path of light through a true solution is invisible. But colloid solution, transmits only a part of the incident light. The remaining light is scattered by the particles of the dispersed phase. This makes these particles appear like continously changing, moving tiny specks or flashes in the path of light. This scattering of light is called ***Tyndall effect*** and is ***characteristic of colloidal solution.***

Q.61. What are the types of colloidal solution?

Two types of colloidal solutions are:

- ***Suspensoids*** and
- ***Emulsoids***

Q.62. What are the essential differences between the suspensoids and emulsoids?

Suspensoids	*Emulsoids*
• Particles carry a definite electric charge which determines the stability of the suspensoids • Easily precipitated if charges are neutralized.	• Particles carry electric charges: some carry +ve and -ve charges simultaneously, e.g. protein molecules • They are very stable and not easily precipitated by salts.

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Contd...

Suspensoids	*Emulsoids*
• Once precipitated they cannot be brought back to original colloidal solution again ***(irreversible change)***	• When precipitated, they are easily redissolved to from a colloidal solution again ***(reversible change)***
• Suspensoids are not hydrated, hence they are ***hydrophobic*** or ***Lyophobic*** colloids	• Emulsoids have great affinity for water, hence they are ***hydrophilic*** (water-loving colloids). Emulsoids can be changed to suspensoids by removal of water (by dehydration). Most of the colloids in living cells exist as emulsoids
• Surface tension and viscosity are nearly the same as those of solvent	• Have lower surface tension and much higher viscosity than the solvent

Q.63. What are the types of emulsoids?

An emulsoid can exist in two forms:

- Sol or
- Gel.

Q.64. What are the characteristics of "Sol"?

- The continuous phase in "Sol" is water (or a dilute solution) and the disperse phase is a concentrated solution.
- "Sol" can be converted to "Gel" by changes of temperature, pH and salt concentration.

Q.65. What are the characteristics of "Gel"?

- Gel is a semi-rigid, jelly-like mass obtained by coagulating a "Sol", particularly a lyophilic Sol under certain conditions and it contains the whole of the liquid present in the Sol. A Gel can be considered a colloidal system of a liquid dispersed in a solid.
- Gels are of two types:
  - Elastic and
  - Non-elastic.
- Formaldehyde can remove water from the gel and hence it is used for fixing the tissues for histopathology.

Q.66. What is imbibition?

Elastic gels when placed in water imbibe large quantities of water and swell up in size. This process is called ***imbibition.***

Q.67. What is meant by syneresis?
The imbibed gel, on keeping, exudes the water and shrinks in size. This process is called ***syneresis*** or ***weeping*** of the gel.

Q.68. State an example of imbibition and syneresis *in vivo*.
The glandular secretions are due to imbibition of water by the gel-like colloids in the gland which give out the water of imbibition as glandular secretion by syneresis.

Q.69. Give a common example of emulsion used in our diet daily.
Mild is a common example of an emulsion and contains droplets of liquid fats dispersed in an aqueous medium. The emulsifier is the protein casein.

Q.70. What is meant by protective colloids? Give suitable examples.
- Protective colloids are those ***which prevent precipitation.*** They play important role in the body.
- ***Examples of protective colloids:*** 1% Gum-ghatti solution used in blood urea estimation by Nesslerization method acts as protective colloid and prevents turbidity to develop.
- When a gelatin solution (emulsoid) is added to gold solution (suspensoids), the particles of gelatin are adsorbed by the particles of gold and the gold particles become more resistant to precipitation.

Q.71. State some biomedical importance of protective colloids.
- Protective colloids present in urine prevent formation of urinary stones.
- ***Bile salts act as protective colloids,*** and prevents precipitation of cholesterol and bilirubin salts (Ca-salts of bilirubin). In absence of bile salts, the solubility of cholesterol and Ca-bilirubinate may suffer and they get precipitated to form gall stones.

Q.72. What is meant by Gibbs-Donnan membrane equilibrium?
When two solutions containing diffusible and non-diffusible ions are separated by a semipermeable membrane, the non-diffusible ions enhance the diffusion of the oppositely charged diffusible ions. The diffusion takes

place towards non-diffusible ions containing side. This also reduces the diffusion of like charged ions to that side. As a result, on the side which contains non-diffusible ions, diffusible counter-ions are more concentrated while the like charged diffusible ions concentrate more on the opposite side. This is called as ***Gibbs-Donnan effect.*** However, the total number of cations and anions are equal on both sides at equilibrium.

Q.73. State some biomedical importance of Gibbs-Donnan effect.

- **Proteins in plasma and interstitial fluid (ISF):** Proteins have much higher concentration in the plasma than ISF. This is due to impermeability of capillary walls to proteins. As per Gibbs-Donnan effect non-diffusible ions like proteins in plasma enhance the outward diffusion of anions like Cl^- from blood vessel to ISF.
- **Concentration of RB cells chlorides:** Due to Gibbs-Donnan effect it is observed that the Cl^- concentration in erythrocytes is only 1/4th of its plasma concentration.
- **Chloride shift or Hamburger phenomenon:** Is due to Gibbs-Donnan effect.
- **Concentration of Na^+/K^+ in renal glomerular filtrate:** Slightly higher Cl^- concentration and slightly lower concentration of Na^+ or K^+ are found in renal glomerular filtrate than in plasma.
- **pH of RB cells:** Due to retention of H^+ as a result of Gibbs-Donnan effect, pH of red blood cells is slightly lower than that of plasma.

CHAPTER

22 Radioactivity: Radio-isotopes in Medicine

Q.1. What are Isotopes?

Isotopes are the elements having the same atomic number but differing in mass number. In isotopes the number of neutrons is different. Identity of atomic number places the isotopes in the same position in periodic table.

Q.2. What are the types of isotopes?

Two types:

- ***Stable isotopes and***
- ***Radioisotopes.***

Q.3. What are stable isotopes?

- Stable isotopes do not emit radiations, their atomic number and atomic mass are constant and they are found abundantly in nature.
- The important stable isotopes of biological interest are ^{2}H (deuterium, heavy hydrogen), ^{15}N, ^{18}O and ^{13}C.

Q.4. What are radioactive isotopes?

- Radio isotopes possess radioactivity and give out radioactive emanations like X-rays, β-rays, and γ-rays. In radioisotopes, the atomic number and/or atomic mass are constantly changing.
- The important radioisotopes clinically used are ^{131}I, ^{132}I, ^{32}P etc.

Q.5. What are natural radioactive elements?

Natural radioactive elements belong to one of three different series:

- Uranium series
- Thorium series
- Actinium series.

Q.6. What is radio-activity?

The process by which unstable nucleus of some atoms of same elements spontaneously emits photons of radiation is termed radio-activity.

Q.7. What are artificial radioisotopes?

- Radioisotopes can be produced artificially by bombarding the stable nucleus of some natural radioactive elements with **α**-particles, neutrons or **γ**-rays or accelerated particles like protons, electrons, tritons or deuterons.
- Nuclear chain reactors may be used for generating neutrons for this purpose by nuclear fission.

Q.8. What are the types of radioactive emissions?

- They are mainly **3 types:**
  - **α**-rays
  - **β**-rays
  - **γ**-rays.
- Damaging effects depend on the penetrating power. They interact with matter to produce ion-pairs (ionization) and hence, called as ionizing radiations.

Q.9. What are the properties of α-rays?

- They are streams of positively (+vely) charged particles.
- Each particle is a tight cluster of two neutrons and two protons corresponding to a mass number of 4 and carries two positive charges and is identical with helium nucleus (He^{2+}).
- Penetrating power is much less but they ***produce dense ionization in small area and causes greater damage.***

Q.10. What are β-rays?

- **β**-rays are streams of very light, negatively (-vely) charged accelerated particles. They resemble electrons but they originate from the atomic nucleus and not electron shells.
- They have ***greater penetrating capabilities*** than α-particles and ***produces less ionization in a greater area.***

Q.11. What are γ-rays?

- **γ**-rays are electromagnetic radiations which originate from nuclear part.

- They can penetrate through and can produce ionization in the path of penetration. Thus, they can affect multiple organs and can cause damage to greater area.

Q.12. List the essential differences between radioactive isotope and stable isotope.

Radioactive isotope	*Stable isotope*
• Atomic number and atomic mass are continually changing	• Atomic number and atomic mass are constant
• Spontaneous emissions of radiations (α, β, or γ rays) or neutrons	• No emission of any kind of radiations
• New elements are produced upon disintegration or decay.	• No new elements are produced
• Detected by external detectors, e.g. G-M counter or scintillation counter	• Cannot be detected by external counters
• Emits radiations and causes harmful effects on biological tissues	• Are not hazardous
• Are used for generation of power (energy) in nuclear reactors	• Not so
• Has special applications in medical diagnosis and therapy	• No such use
• Can be used for sterilization of medical equipments.	• Cannot be used
• Used in research to induce mutation and in special techniques like autoradiography	• No such research applications
• Can be used for nuclear weapons	• No such use

Q.13. What is meant by radioactive decay?

The property of spontaneous nuclear changes and conversion of an unstable radioactive nucleus to a more stable one with simultaneous emission of ionizing radiations is called radioactive decay.

Q.14. Name the different units of measurement of radio activity.

Different units used are:

- Curie (ci)
- (γ) Roentgen,

- Rad (Radiation absorbed dose)
- RBE (Relative biological equivalence)
- REM (Roentgen equivalent mammals).

Q.15. What is a Curie (Ci)?

Curie (Ci) is the international unit of radioactivity based on the radioactivity of 1 gm of radium. One Ci equals 3.7×10^{10} nuclear disintegrations per second. 1 mCi = 10^{-3} Ci and 1 μCi = 10^{-6} Ci.

Q.16. Define Roentgen (r) and radioactive absorbed dose (rad).

- ***Roentgen (r):*** is the amount of **γ** or X-rays, which deposits 8.33×10^{-6} J of energy per gm of air to produce 1.61×10^{12} ion pairs/gm,
- ***Radioactive absorbed dose (rad):*** is the quantity of **γ** or X-rays that can deposit 100 ergs or 1×10^{-5} J/gm in matter including biological tissues.

Q.17. Differentiate RBE and REM.

- **RBE *(Relative biological equivalence):*** It is the ratio between the doses (rad) of the radiation under investigation and of **γ**-rays producing identical biological effects. RBE is 1 for **γ**-rays.
- **REM *(Roentgen equivalent mammals):*** It measures a radiation in terms of its effects on mammalian tissues. REM = rad × RBE.

Q.18. What is meant by the term "radiation hazards"?

Radiations cause harmful effects on biological system. Thus, radioactivity is hazardous to living organs. The understanding regarding the biological effects of radiations has come mainly from somatic and hereditary damages sustained by the atomic explosions.

Q.19. What are the effects of radiations?

The effects of radiation can be divided into ***three categories:***

- ***Immediate effects,***
- ***Delayed effects and***
- ***Genetic effects.***

Q.20. What are the immediate effects of radiation?

Immediate effects are seen only at very high doses of exposure such as nuclear accidents, nuclear explosions or

high doses of radiation therapy. **Three types of syndromes** are associated with such high doses:

- ***Bone marrow syndrome:*** Whole body exposure of 200 to 1000 rads results in severe damage to hematopoietic system due to higher sensitivity of proliferating cells. Depletion of leucocytes and precursors leads to gross immuno suppression and increased susceptibility to infection. Death occurs within 10 to 20 days.
- ***Gastrointestinal syndrome:*** The radiation exposure of 1000 to 5000 rads causes severe damage to mucosal epithelia, fluid loss, electrolyte imbalance and hemorrhages in GI tract, resulting to nausea, vomiting and acute diarrhea. Death occurs within 3 to 5 days.
- ***Central nervous system syndrome:*** Higher radiation exposure to 5000 to 10,000 rads are lethal for even non-dividing cells like neurons. The blood-brain barrier is lost leading to cerebral vasculitis, meningitis, and choroid plexitis. Death occurs within 8 to 48 hours.

Q.21. What are the delayed effects of radiation?

The effects of radiation seen after a lapse of time are called delayed effects which are mainly due to somatic mutations.

- ***Carcinogenesis:*** Ionizing radiations can cause cancers. The different tissues have different susceptibility. Bone marrow and other rapidly dividing cells are particularly prone to produce leukaemia. There may be breast cancer, bone cancer, polycythemia, etc.
- ***In utero radiation exposure:*** It can lead to three types of damages:
  - Growth retardation
  - Congenital malformations and
  - Foetal or neonatal death.
- ***Miscellaneous effects:*** Endocrine imbalance, decreased fertility/or sterility, cataract, nephrosclerosis, etc.

Q.22. What are the genetic effects of radiation?

- Each interaction of radiation with a target molecule can lead to molecular damage of the target molecule. When the target molecule is DNA, it will undergo a damage-mutagenesis. The manifestation of mutation will depend upon the efficiency of DNA repair mechanism.

Even small doses of radiation are capable of inducing mutation.

- The magnitude of genetic damage depends on:
  - The stage of germ cell development
  - The dose rate
  - Dose fractionation
  - Interval between exposure and conception.

Q.23. Name the equipment used for detection of radiations from radioactive isotope.

- Geiger-Müller counter (G-M counter)
- Scintillation counter
- Semiconductor detectors.

Q.24. What is meant by half-life?

- Radioactive elements continuously lose radio activity. This loss of radioactivity takes place in a well-defined manner in a logarithmic fashion.
- **Two types of half-life** recognized:
  - ***Physical half-life:*** The time required for a given isotope to disappear to half its original value is called the ***"physical half life"*** of that isotope.
  - ***Biological half life:*** The time required by an isotope to reduce its body concentration to half of that administered is known as the ***"biological half life"***.

Q.25. What is the recommended level of maximum individual radiation exposure?

- The maximum individual radiation exposure for occupational workers is 5.0 rem per year.
- For general public who is not involved in occupational exposure the maximum is 0.5 rem per year.

Q.26. What measures you will take to protect from radiation?

- The most popular **"triad"** of radiation protection is:
  - Time
  - Distance
  - Shield
- ***Time:*** Minimum possible time should be spent near the radiation zone.
- ***Distance:*** Handling of radio active materials should be done from a maximum possible distance.
- ***Shield:*** Individual should be shielded by lead.

Continuous monitoring of the dose received by the occupational worker must be done. Film badge should be used.

Q.27. State some of the radioisotopes used for studies of volume and space.

- Following tests utilize dilution principle for estimation of volume and space:
  - ***Plasma volume:*** ^{131}I labelled human serum albumin given IV (RIHSA method).
  - ***Red cell volume:*** Injection of Cr^{51}-labelled RB cells from the patient itself is given IV.
  - ***Total body water:*** By H^3-tritiated water given orally.
  - ***ECF volume:*** Br^{82} given orally or IV.
  - ***Total exchangeable Na*** and ***Sodium space:*** Na^{24} given orally or IV.

Q.28. What is the principle of dilution studies for measuring volume and space?

When a known amount of radioactive "tracer" is introduced into an unknown volume and if after thorough mixing for a known time, the concentration of the radioactive "tracer" is estimated. Then the total volume in which the "tracer" has been diluted is given by the formula:

$$V = \frac{N}{n}$$ Where: V = Volume to be measured

N = Total number of counts injected.

n = Number of counts per ml.

Q.29. Mention the uses of Fe^{59} in the body.

With the help of radioisotope Fe^{59}, the following can be determined:

- The rate of absorption of Fe from the GI tract.
- The rate of disappearance of Fe^{59} from plasma.
- The rate "turnover" of plasma Fe indicates the extent of activity of the erythropoietic bone marrow.
- The rate of incorporation of Fe^{59} into the red blood cells indicates the effective erythropoiesis.

Q.30. How will you estimate GI protein loss in protein losing enteropathy?

- Gastrointestinal (GI) protein loss can be estimated by giving IV injection of ^{131}I-labelled human serum albumin. "Tracer" normally remains in the vascular compartment and will not appear in faeces unless there is GI protein loss.
- ***In normal healthy persons*** less than 0.1 to 1% of injected radioactivity appear in 24 hours stool collection. ***More than 2% of injected radioactivity in faeces will indicate excessive protein loss.***

Q.31. How will you measure GI blood loss?

Cr^{51}-labelled RB cells when given IV will not appear in the faeces except a loss through GI tract. By measuring the faecal radioactivity over a 72-hour period and by comparing it with blood radioactivity, the faecal blood loss can be quantitated. By this method as little as 5 ml of blood loss in GI tract can be determined.

Q.32. What is Schilling test?

- Schilling test is used to study the absorption of vitamin B_{12} from the gut.
- In this measurement of the urinary excretion of labelled B_{12} following a saturating dose of nonlabelled stable B_{12} ***(flushing out dose)*** is done.
- The following radioisotopes for labelling B_{12} can be used:

  Co^{60} or Co^{58} (1/2 life = 71 days)
  Co^{57} (1/2 life = 27.0 days)

- Normal urinary excretion is greater than 15% of the test dose. In patients with pernicious anemia or with B_{12} deficiency associated with intestinal malabsorption or other causes, the excretion is less than 5%.

Q.33. What is scanning? What information can be obtained from scanning an organ?

An organ can selectively concentrates a **γ**-emitting radioisotope when administered. An image of the distribution of the **γ**-emitter can be obtained by using a scanner

over the organ, which can provide the following informations:

- Position of the organ, any *"ectopic"* site like thyroids
- Size and boundaries of the organ
- Shape of the organ
- Presence or absence of any lesion: A localized impairment of function like cyst, abscess etc. may be noticed as a ***void*** (called as ***cold area***). On the other hand, an abnormal accumulation of radioactivity much in excess of the surrounding tissues appears as ***dense*** (called as ***hot area***) indicating presence of tumors.

Q.34. Name the radioisotopes used for scanning of different organs.

- ***For thyroid Scan:*** ^{131}I is used. Recently, 99^{m} technetium pertechnate (Tc^{99m}) has been used.
- ***For Renal Scan:*** Hg^{203} or Hg^{197}-labelled chlormerodin is used.
- ***For Brain Scan:*** Technetium (Tc^{99m}) and indium (In^{133m}) are used.
- ***For Lung Scan:*** I^{131}-human serum albumin macro-aggregates are used.

Q.35. State the uses of radioisotope P^{32} in diseases.

P^{32} is used in the treatment of following diseases:

- Polycythemia vera
- Multiple myeloma
- Carcinoma of the breast
- Chronic myeloid leukaemia
- Primary hemorrhagic thrombocytosis
- Carcinoma of prostate.

Q.36. State the therapeutic uses of ^{131}I in the body.

^{131}I has been used for the therapeutic uses of following:

- For treatment of thyroid cancers.
- Has been used for treatment of primary thyrotoxicosis: should not be used in young patients below the age group of 25 and in pregnancy.
- Has been used in cardiac disease in euthyroid patients.
- For control of ectopic arrhythmias.

Q.37. What is extracorporeal blood irradiation?

Has been used in chronic leukemic patients. The blood is taken out from the patient via forearm artery, then it is

circulated around a cesium-137 source which emits powerful γ-rays and then the irradiated blood is returned to the same patient via a forearm vein.

Q.38. How $Boron^{10}$ neutron irradiation is useful in brain tumors?

$Boron^{10}$ has been used recently in the treatment of the inoperable rapidly fatal brain tumor like glioblastoma multiforme. When $Boron^{10}$ is injected IV in the patient, it is readily and selectively taken up by the tumor tissue in brain. After ten minutes, the head of the patient is placed in a beam of slow neutrons. $Boron^{10}$ in tumor tissue absorbs neutrons rapidly and becomes transformed to $Boron^{11}$ which disintegrates almost immediately to lithium isotope giving out α-particles. Ionizing property of α-particles destroy the tumor tissue.

Q.39. What is thyroid uptake study? What information is obtained?

- Thyroid uptake of ^{131}I is routinely measured 24-hours after the administration of oral dose. A 4-hour uptake or 48-hour uptake can also be measured when rapid "turnover" or "delayed uptake" situation is expected.
- Dose of ^{131}I = 10 μCi given orally.
- Thyroid accumulation of radio-iodine is measured externally over the gland.
- Normal range of uptake is 20 to 40%.
- An abnormally high RAI uptake is usually consistent with hyperthyroid states.
- Abnormally, "low" uptake is characteristic of hypothyroidism.

Q.40. State some uses of radioimmuno assay (RIA)?

- RIA is an elegant technique used in the determination of biological compounds in microgram to picogram quantities.
- RAI is used for:
  - Estimation of hormones like Insulin. T_4, PTH, T_3, cortisol, testosterone, estrogen, etc.
  - Estimation of "tropins" like ACTH, FSH, LH, etc.
  - Estimation of vitamins like B_{12} and folic acid.

- Drugs like digoxin, and digitoxin.
- Oncogenic "markers" like carcinoembryonic antigen (CEA), α-feto-protein, etc.

Q.41. What is ^{131}I-labelled Rose Bengal test? What for it is used?

- The test is used for assessment of hepatic function.
- ^{131}I-labelled Rose Bengal is administered IV then count is taken over the neck and abdomen. Initially count is more in the neck and practically nil over the abdomen.
- If Liver function is alright, as the dye is excreted through liver, neck count goes down, and count over abdomen increases.
- In parenchymal liver diseases: High count in the neck persists and there is practically no rise in count over the abdomen, as the dye is retained.

CHAPTER

23 *Clinical Enzymology*

Q.1. What is meant by clinical enzymology?
Clinical enzymology is a branch of biochemistry dealing with the diagnostic value of enzyme estimation in serum and tissues in diseases.

Q.2. What are the types of enzymes present in plasma?
The enzymes present in plasma are **mainly of 2 types:**
- Plasma-derived enzymes and
- Cell-derived enzymes.

Q.3. What are plasma-derived enzymes?
These are enzymes which act on substrates in plasma and their activity is higher in plasma than in cells, e.g. the coagulation enzymes.

Q.4. What are cell-derived enzymes?
These enzymes are normally present in plasma in small quantities. They have a high activity in cells and they overflow into the plasma.

The cell-derived enzymes enter the plasma in small amounts as a result of:
- Continuous normal ageing of the cells, and
- Owing to diffusion through undamaged cell membranes.

Q.5. How the serum enzyme assay is useful in clinical practice?
Single or serial assay of the serum activity of a selected enzyme or enzymes may provide information on the nature and extent of disease process.
They are useful:
- In diagnosis of a disease
- In differential diagnosis

- Serial assay is helpful in ascertaining prognosis
- Useful in early detection of a disease.

Q.6. What is international unit of serum activity?

One **"international unit"** [IU] is defined as the activity of the enzyme which transforms one **μ**-mole of substrate per minute under optimal conditions and at defined temperature and is expressed as IU/ml.

- When milli-micromole of the substrate is transformed per minute, it is IU/L or m-IU/ml.

Q.7. Name three enzyme assays which are useful in acute myocardial infarction?

- ***Creatine phosphokinase*** /or creatine kinase [CPK or CK]
- ***Aspartate transaminase*** [AST], also called S-GOT.
- ***Lactate dehydrogenase*** [LDH].

Q.8. What is creatine kinase?

Creatine kinase is an enzyme which catalyzes the following reaction:

$$\text{Creatine} \sim (P) + ADP \longrightarrow \text{Creatine} + ATP$$

Q.9. What is the normal serum level of CK in a normal healthy adult?

Normal serum activity of CK varies from 4 to 60 IU/L (at 37°C).

Q.10. What is the behavior of serum CK activity in acute myocardial infarction?

After myocardial infarction, serum value is found to increase after about 4 to 6 hours, reaches a peak level in 24 to 30 hours and returns to normal level in 2 to 4 days (Usually 72 hours).

Q.11. What is the effect on serum activity of CK on storage?

There is 50% loss of serum CK activity after 6 hours storage at room temperature and 24 hours of storage at refrigerated temperature.

- ***All determinations of serum CK activity should be done on fresh blood samples.***

Q.12. Which transminase is present in heart muscle?

Concentration of ***aspartate transaminase*** (AST) is very high in myocardium. ***Alanine transaminase*** (ALT) is practically absent in myocardium.

Q.13. What is the normal value of serum AST in a healthy adult?
Normal serum activity of AST (S-GOT) varies 4 to 17 IU/L (10 to 35 Karmen spectrophotometric units/ml).

Q.14. What is the behavior of serum AST activity in acute myocardial infarction?
In acute myocardial infarction, serum activity rises sharply within the first 12 hours, with a peak level at 24 hours or over and returns to normal within 3 to 5 days.

Q.15. What is LDH?
Lactate dehydrogenase (LDH) is an enzyme which catalyzes the reversible conversion of pyruvic acid (PA) to lactic acid (LA).

Q.16. What is the normal serum LDH activity in a healthy adult?
Normal serum LDH activity ranges from 60 to 250 IU/L (120 to 500 Karmen spectrophotometric units/ml).

Q.17. What is the behavior of serum LDH activity in acute myocardial infarction?
In acute myocardial infarction, serum activity of LDH rises within 12 to 24 hours, attains peak level at 48 hours (2 to 4 days) reaching about 1000 IU/L or more and then return gradually to normal from 8th to 14th day.

Q.18. What precaution one should take in estimating serum LDH activity?
- Red blood cells are rich in LDH, hence **avoid hemolysis.**
- ***Hemolyzed serum samples should not be assayed for LDH activity.***

Q.19. Is serum LDH enzyme estimation specific for myocardial infarction?
The LDH enzyme is rather non-specific for myocardium. The enzyme is wide spread in body cells of many organs/tissues, hence co-existent disease processes in other organs may cause increased serum LDH.

Q.20. If a suspected case of myocardial infarction is seen after two weeks, which serum enzyme assay may be useful?
The estimation of ***γ-glutamyl transpeptidase*** (G-GTP) also called γ-glutamyl transferase (γ-GT) may be useful.

Q.21. What is the enzyme γ-glutamyl transpeptidase?
γ-Glutamyl transpeptidase (G-GTP) catalyzes the transfer of the γ-glutamyl group from one peptide to another peptide or to an amino acid.

Q.22. What is the normal serum activity of G-GTP (γ-GT)?
Normal serum activity of G-GTP is as follows:
- **Men:** 10 to 47 IU/L.
- **Women:** 7 to 30 IU/L.

Q.23. What is the behavior of serum γ-GT activity in acute myocardial infarction?
Increase in serum γ-GT activity is found late in the disease, peak activity seen between 7th and 11th day and significantly elevated level of the enzyme lasts for a month.

Q.24. Does myocardium have γ-GT enzyme? Which are the organs rich in γ-GT?
Normal heart contains practically no γ-GT activity.
- The organs which are rich in γ-GT are kidney and liver. Also high activity seen in lungs, pancreas and prostate.

Q.25. What is the source of increased γ-GT in myocardial infarction?
The enzyme γ-GT does not come from heart muscle. It is postulated that increased serum and tissue levels of enzyme activity develop with the repair process. ***Source of enzyme is from vascular endothelium from angioblastic proliferation in repair process.***

Q.26. Name three enzymes which are commonly estimated in liver diseases?
- ***Serum transaminases:*** Both ***alanine transaminase*** [ALT] and ***aspartate transaminase*** [AST]
- ***Serum alkaline phosphatase*** [ALP]
- ***Serum γ-GT***.

Q.27. What is normal serum activity of alanine transaminase?
Normal serum activity of alanine transaminase [ALT], also called S-GPT: 3 to 15 IU/L [6 to 32 Karmen spectrophotometric units/ml].

Q.28. What is the behaviour of serum transaminases in hepatic diseases?

Increase in ***both*** transaminases are found in liver diseases, with alanine transaminase [S-GPT] much higher than aspartate transaminase [S-GOT].

- Their determinations are of limited value in differential diagnosis of jaundice because of considerable over-lapping.
- But their determination is of ***extreme use in assessing the severity and prognosis [serial assays]*** of parenchymal liver diseases specially acute infectious hepatitis and serum hepatitis. In these two conditions highest values in thousand units are seen.

Q.29. How S-GPT assay be useful as a screening test in viral hepatitis out break?

S-GPT assay has been found to be extremely useful as a ***screening test*** in outbreak of infectious hepatitis [viral hepatitis]

- The increase in S-GPT can be detected in prodromal stage when jaundice has not appeared clinically. Such cases can be isolated and segregated from others so that spread of the disease can be prevented.

Q.30. Name the organs which are rich in the enzyme alkaline phosphatase?

The enzyme alkaline phosphatase [ALP] is found in a number of organs, most plentiful in bones and liver, then in small intestine, kidney and placenta.

Q.31. What is the normal value of serum ALP in a healthy adult?

Normal serum ALP activity is 3 to 13 KA units/100 ml [23 to 92 I.U/L]. KA units stand for King Armstrong units.

Q.32. What is the source of serum ALP in normal adult?

- Hepatic soure of ALP

Q.33. What is the principal source of serum ALP in growing child?

- Bone ALP [Osteoblasts].

Q.34. How serum ALP measurement is useful in differential diagnosis of jaundice?

For many years, serum ALP has been used as a reliable parameter for differential diagnosis of jaundice.

- Serum ALP is increased in both viral hepatitis and posthepatic jaundice [extra hepatic obstruction], but ***rise is usually much greater in cases of obstructive jaundice.***

Q.35. What is the dividing line for differentiating parenchymatous jaundice and extra hepatic obstructive jaundice?

Dividing line suggested is 35 KA units per 100 ml. Values higher than 35 KA units per 100 ml are strongly suggestive of diagnosis of extra hepatic obstructive jaundice in which very high figures even 200 units or more may be found.

- There is certain amount of over-lapping mostly in the range of 30 to 45 KA units/100 ml.

Q.36. What is the usefulness of serum ALP in space occupying lesions of liver?

Higher values of serum ALP are obtained in ***space occupying lesions*** of liver, e.g. abscess, primary carcinoma [Hepatoma], metastatic carcinoma, infiltrative lesions viz., lymphoma, granuloma, and amyloidosis.

- ***A diagnostic "triad"*** suggested for these conditions:
  - ***High serum ALP activity,***
  - ***Impaired BSP-retention,***
  - ***Normal or almost normal serum bilirubin.***

Q.37. What is the mechanism of increase in serum ALP in liver diseases?

Increase in the activity of serum ALP in liver diseases is not due to hepatic cell disruption, nor to a failure of clearance, but it is ***due to increased synthesis of hepatic ALP.*** The stimulus for this increased synthesis in patients with liver disease has been attributed to bile duct obstruction either extra hepatically by stones, tumors or strictures or intrahepatically by infiltrative disorders or "space occupying lesions".

Q.38. Serum ALP activity is increased in both hepatic as well as bone diseases, which enzyme estimation will be useful in hepatic diseases and not affected in bone diseases?

- **Serum 5'-nucleotidase is useful;** it is more specific for liver diseases. It is not affected in bone diseases.

- Similarly, **serum LAP activity** increases in liver diseases but not affected in bone diseases.

Q.39. What is the normal serum activity of 5'-nucleotidase in healthy adult?
Normal serum activity of 5'-nucleotidase is 2 to 17 IU/L.

Q.40. What is serum HBD (hydroxy butyrate dehydrogenase)?
It is an enzyme which acts on **α**-OH-butyrate. It has been found to be useful aid in liver diseases and myocardial infarction.

Q.41. What is the normal serum activity of HBD?
Normal serum activity of HBD is 56 to 125 IU/L.

Q.42. How serum HBD and LDH be useful in differentiation of liver diseases from myocardial infarction?
- For differentiation, ***ratio of LDH/SHBD*** has been found to be useful.
- **Normal ratio** of LDH/SHBD=1.18 to 1.60.
- Less than 1.80 is observed in most cases of myocardial infarction and a value greater than 1.60 is observed in liver diseases.

Q.43. Name the enzyme which has been used for screening alcohol abuse.
- **Serum γ-gultamyl transferase (γ-GT)**
- Sudden increase in **γ**-GT in chronic alcoholics suggests recent bout of drinking alcohols.

Q.44. State the enzymes used in diagnosis of pancreatic disorders.
- *Serum amylase*
- *Serum lipase.*

Q.45. What is the normal value of serum amylase?
Normal serum Amylase is 80 to 180 Somogyi units/100 ml.

Q.46. What is the behavior of serum amylase in pancreatic diseases?
- Serum amylase assay is the investigation of choice in the diagnosis of acute pancreatitis. Serum enzyme activity greater than 1000 units seen within 24 hours and returns to normal within 3 days.
- Uninary amylase also increases and persists a little longer than serum activity.

Q.47. What is macro amylasemia?

- In some individuals, a form of amylase with a high molecular weight occurs in circulation. It cannot pass through the glomerular filter and hence accumulates in blood.
- ***Macroamylase*** may be formed by ***combination of serum amylase with an antibody.*** Probably results from the polymerization of the enzyme molecule.
- Macroamylasemia should be suspected:
  - When there is an increase in serum amylase, but no increase in urinary amylase.

Q.48. State one condition other than acute pancreatitis where very high serum amylase may be obtained.

In mumps: Very high serum amylase values but less than 1000 units are seen.

Q.49. What is the normal serum lipase?

- Normal serum lipase activity is 0.06 to 1.02 Cherry-Crandall units.
- By colorimetric assay: 9 to 20 m-I.U.

Q.50. What is the behaviour of serum lipase in pancreatic disorders?

- Serum lipase assay is more specific in pancreatic disorders and remains raised for longer periods.
- In acute pancreatitis, serum lipase activity increases promptly, very high values are obtained. Elevated levels persist for 10 to 14 days or longer.

Q.51. State the enzyme assays used in muscle diseases.

- ***Transaminases: S-GOT/S-GPT***
- ***Aldolase***
- ***Creatine Kinase.***

Q.52. Which enzyme estimation is more specific for muscle diseases?

Serum creatine kinase estimation is more specific. Raised values of serum CK are seen in most cases of muscular dystrophies and dermatomyositis, Usually 1000 IU/L. or more. Highest values are found in Duchenne type of muscular dystrophies.

Q.53. What is the normal serum aldolase?

Normal serum aldolase activity is 2 to 6 m-I.U.

Q.54. What is the behavior of serum aldolase in Muscle diseases?

- Moderate increase seen in dermatomyositis and muscular dystrophies. Highest values are seen in Duchenne type of muscular dystrophy.
- Normal values are seen in neurogenic muscle diseases, e.g. in peripheral neuritis, poliomyelitis.

Q.55. State at least five enzymes which are useful in malignancies.

Enzyme Assay	*Used in Malignancies*
• **Serum acid phosphatase (AP)**	• Cancer of prostate with or without metastasis.
• **Serum alkaline phosphatase (ALP)**	• Osteoblastic metastasis in bones. • Metastasis in liver (secondaries) • Primary bone tumors. • Jaundice due to carcinoma of head of pancreas.
• **β-Glucuronidase in urine**	• Cancer of urinary bladder.
• **Serum LDH**	• In widespread malignancies. • Advanced leukemias.
• **LDH in effusion fluids**	• Local malignancies.

Q.56. What is the normal value of serum acid phosphatase (AP)?
Normal serum acid phosphatase activity is 0.6 to 3.1 KA units/100 ml.

Q.57. What precautions should be taken in estimation of serum acid phosphatase?

- Serum acid phosphatase is an extremely ***labile*** enzyme. Enzyme assays should be done on ***fresh*** samples immediately.
- RB cells are rich in the enzyme, hence hemolysis should be avoided. ***Hemolyzed samples should not be used for assay.***

Q.58. What is the clinical usefulness of serum acid phosphatase assay?
Main value is in diagnosing metastasizing prostatic cancer. Enzyme is formed from mature prostatic epithelial

cells, not formed by immature prostatic epithelial cells. Highly anaplastic carcinoma may not produce the enzyme.

Q.59. What is tartrate-labile serum acid phosphatase? What is the normal value? State its clinical use.

- Acid phosphatase of prostate is inhibited by L-Tartrate.
- Tartrate-labile acid phosphatase is more specific for metastasizing prostatic cancer.
- Normal value of "tartrate-labile" AP is 0.0 to 0.5 KA/dl.

Q.60. Name one lipid storage disease in which increased serum acid phosphatase activity is seen.

Marked rise of serum acid phosphatase occurs in ***Gaucher's disease*** and it is characteristic of this disorder.

Q.61. What is an isoenzyme (or isozyme)?

Isoenzymes (or Isozymes) are the physically distinct forms of the same enzyme but catalyze the same chemical reaction or reactions and differ from each other structurally, electrophoretically and immunologically.

Q.62. State the isoenzymes of LDH. How do they differ from each other structurally and electrophoretically?

- In human serum, there are at least ***five*** isoenzymes of LDH. They are: LDH-1, LDH-2, LDH-3, LDH-4 and LDH-5.
- Each isoenzyme is a ***tetramer.*** Each monomeric unit may be one of two types: "H" (stands for heart muscle) and `M' (stands for skeletal muscle). The different isoenzymes contain 'H' and 'M' in different proportions.
- Thus:

Type of LDH	*Polypeptide chains*	*Electrophoretic mobility*	*Tissue rich in isoenzyme type*
• **LDH-1**	H_4	Fast-moving (Fastest)	Heart muscle
• **LDH-2**	H_3M	-	-
• **LDH-3**	H_2M_2	-	-
• **LDH-4**	HM_3	-	-
• **LDH-5**	M_4	Slowest moving	Liver

Q.63. Which LDH isoenzyme is found more in normal serum?

- LDH-2 (H_3M)

Q.64. Which LDH isoenzymes are increased in serum in acute myocardial infarction?

In myocardial infarction, the faster LDH isoenzymes LDH-1 and LDH-2 predomiante.

Q.65. Which LDH isoenzymes are increased in serum in viral hepatitis?

In acute viral hepatitis, the slowest isoenzymes LDH-5 and LDH-4 predominate.

Q.66. What is the pattern of LDH and LDH isoenzymes in malignancies?

- Total serum LDH is usually elevated in all types of neoplastic diseases (malignancies) but the LDH isoenzyme patterns may be different.
- In malignancies of lung, colon, uterus there is an increase in slow moving enzymes LDH-5, LDH-4 and LDH-3 usually increases.
- An increase in LDH-5 seen in liver carcinoma, breast cancer, prostatic cancer and malignancies of CNS.
- In leukemias, increase is more of LDH-2 and LDH-3.
- Malignant tumors of testes and ovary show rise in LDH-2 and LDH-3 (called ***"seminoma" pattern***). Also there may be increase in LDH-4.

Q.67. Mention the chemical methods by which LDH isoenzymes can be estimated.

- **Three chemical methods** available which are based on:
  - Heat stability
  - Inhibition with urea
  - Reaction with change substrate oxo-butyrate.
- LDH-1 (myocardial LDH) is more heat-stable than that of hepatic LDH (LDH-5).
- Hepatic LDH (LDH-5) is inhibited by urea.
- Cardiac LDH (LDH-1 and 2) utilizes oxo-butyrate as substrate preferentially to pyruvate; whereas liver LDH (LDH-5 and 4) has relatively low activity with oxo-butyrate.

Q.68. What are the isoenzymes of CPK (or CK)? How do they differ structurally and electrophoretically?

- In human tissues and serum, CPK exists as three different isoenzymes called CPK-1 (BB), CPK-2 (MB) and CPK-3 (MM).

- Each isoenzyme is a ***dimer*** and composed of two monomeric units "M" (Muscle) and "B" (Brain).
- They differ structurally and electrophoretically as follows:

Type of CPK Isoenzyme	*Polypeptide chains*	*Electrophoretic mobility*	*Tissue distribution*
• ***CPK-1***	BB	Fast moving (More -ve charge)	Brain
• ***CPK-2***	MB	Intermediate in mobility	Myocardium
• ***CPK-3***	MM	Slow moving	Skeletal muscle.

Q.69. Which isoenzyme of CPK is useful in myocardial infarction?

- CPK-2 (MB) is useful in clinical practice for early diagnosis of acute myocardial infarction.
- Normally CPK-2 (MB) is very small, accounts for about 2% of total CPK activity of plasma.

Q.70. What is the behavior of CPK-MB in acute myocardial infarction?

In myocardial infarction, increase of CK-2 (MB) occurs within 4 hours, maximum reaches in 24 hours and then falls rapidly. CK-MB accounts for 4.5 to 20% of total CPK activity in plasma in patients with a recent acute myocardial infarction and remains elevated up to 20-fold above normal.

Q.71. State the clinical significance of CK-1 (BB).

CK-1 (BB) is increased in retinal diseases, in cerebrovascular accidents, in brain tumors and in Reye's syndrome. Recently it has been shown that CK-1 (BB) increases significantly in prostatic cancer (adenocarcinoma).

Q.72. State the clinical significance of CPK-3 (MM).

CPK-3 (MM) is elevated in muscular dystrophies. Highest values seen in Duchenne type of muscular dystrophy. Moderate rise is seen in myositis.

Q.73. What are the two atypical isoenzymes of CPK?

Two atypical isoenzymes reported are:

- ***Macro-CK (CK-macro)***
- ***Mitochondrial type (CK-Mi).***

Q.74. What is atypical Macro-CK (CK-macro) isoenzyme? State its significance.

- Macro-CK isoenzyme is formed by aggregation of CK-BB with Igs, usually with IgG but sometimes with IgA. May also form by complexing CK-MM with lipoproteins. Electrophoretically, migrates between CK-MB and CK-MM.
- Ranges from 0.8 to 1.6%, occurs most frequently in women over 50 years of age.
- No specific disorder has been associated with this isoenzyme. It is not of any clinical importance.

Q.75. What is atypical CK-mi (mitochondrial CK isoenzyme)? State its clinical significance?

- ***CK-mi is not present in normal serum.*** It is present bound to the exterior surface of inner mitochondrial membrane of muscle, liver and brain. Electrophoretically, migrates towards cathode and is behind CK-MM band. Incidence is from 0.8 to 1.7%.

Clinical significance:

- It is only present in serum ***when there is extensive tissue damage*** causing breakdown of mitochondria and cell wall. Its presence in serum indicates severe illness.
- It has been detected in cases of malignant tumors and cardiac abnormalities.

Q.76. What are the isoenzymes of alkaline phosphatase (ALP)?

By starch gel electrophoresis at pH-8.6, at least ***Six*** isoenzymes of ALP have been detected. They are:

- Hepatic isoenzyme (α-2)
- Bone isoenzyme (Pre-β and β)
- Skeletal muscle isoenzyme
- Renal isoenzyme
- Small intestinal isoenzyme (γ)
- Placental isoenzyme ***(heat-stable).***

Q.77. State the clinical significance of hepatic isoenzyme of ALP.

- Major liver band (α_2) increased in liver cells damage.
- A subsidiary smaller fraction called ***fast*** liver band (α_1) is increased also in:
  - Viral hepatitis
  - Metastatic carcinoma of liver
  - Cholangitis

- Two subsidiary bands form a ***doublet,*** which is of diagnostic significance in extra hepatic obstructive jaundice.

Q.78. State the clinical significance of bone isoenzyme of ALP.
Bone isoenzyme of ALP (pre-**β** and **β**) increases due to osteoblastic activity and is normally elevated in children during periods of growth, and in adults over age of 50. In these cases elevated ALP level may cause difficulty in interpretation.

Q.79. What is the characteristic of placental isoenzyme of ALP? State its clinical significance.
- Placental isoenzyme of ALP is ***heat-stable*** and resists heat denaturation at 65°C for 1/2 hour. It is ***inhibited by L-phenylalanine.***
- Placental isoenzyme of ALP increases in pregnancy (During last six weeks of pregnancy).
- ***A sudden decrease of placental isoenzyme in pregnancy suggest placental insufficiency.***

Q.80. State the clinical significance of intestinal isoenzyme of ALP? What are its characteristics?
Intestinal isoenzyme of ALP **(γ)** may increase in:
  - In severe disorders of GI tract
  - In cirrhosis liver
  - In patients undergoing chronic hemodialysis
- Characteristics of intestinal isoenzyme of ALP are:
  - Slow moving in electrophoresis **(γ)**
  - Inhibited by L-phenylalanine
  - Resistant to neuraminidase enzyme.

Q.81. Name two atypical isoenzymes of ALP which have been used as tumour markers (Oncogenic markers).
- ***Regan isoenzyme***
- ***Nagao isoenzyme.***

These two atypical isoenzymes of ALP have been called as ***carcino-placental ALP isoenzymes*** as they resemble placental isoenzyme and found in malignancies. Frequency of occurrence in cancer patients is 3 to 15%.

Q.82. State the characteristics of Regan isoenzyme of ALP and its clinical significance.
- Regan isoenzyme electrophoretically migrates to same position as bone fraction. It is extremely heat-stable and

resists denaturation at 65°C for 1/2 hour. It is inhibited by L-phenylalanine.

- ***Clinical significance:***
  - Regan isoenzyme is produced by malignant tissues. It has been detected in various carcinomas of breast, lungs, colon and ovary. Highest incidence of positivity found in cancers of ovary and uterus.

Q.83. State the characteristics of Nagao isoenzyme of ALP and its clinical significance.

- Nagao isoenzyme may be considered as a variant of Regan isoenzyme. Other properties and electrophoretic mobility is similar to Regan isoenzyme. It is inhibited by L-Leucine.
- ***Clinical significance:***
  Nagao isoenzyme has been detected in metastatic carcinoma of pleural surfaces and adenocarcinoma of pancreas and bile duct.

CHAPTER

24 *Biochemistry of Cancer*

Q.1. What is cancer?

Cancer is a cellular tumor that unlike benign tumor cells can metastasize and can invade the surrounding and distant tissues.

Q.2. What are the characteristics of cancer cells?

Cancer cells are characterized by **three important properties.** They are:

- Diminished or unrestricted control of growth.
- Capability of invasion of local tissues.
- Capable of spreading to distant parts of body by metastasis.

Q.3. State the morphological changes shown by cancer cells.

- Cells have usually ***rounded shape,*** larger than normal cells.
- Cells show ***nuclear and cellular pleomorphism,*** hyperchromatism, altered nuclear: cytoplasmic ratio, abundant mitosis, and sometimes tumor giant cells.
- Cells often grow over one another and form ***multilayers.***
- Can grow without attachment to the surface *in vitro* in culture medium, thus shows ***diminished adhesion.***

Q.4. State the agents that cause cancer?

Agents that can cause cancer can be divided into **three main groups.** They are:

- ***Physical:*** Radiant energy
- ***Chemicals:*** Variety of chemical compounds can cause cancer. Some of the chemicals can act directly and others can act as ***procarcinogens***.
- ***Biological:*** Oncogenic viruses.

Q.5. Name some cancers which are found commonly in children.

- Retinoblastoma
- Neuroblastoma
- Wilms' tumor
- Certain tumors of hemopoietic tissues like lymphomas, and leukaemias
- Sarcomas of bones and skeletal muscles.

Q.6. State four precancerous conditions, which can produce cancer.

- ***Leukoplakia of oral mucosa and genital mucosa:*** It can develop into squamous cell carcinoma
- ***Ulcerative colitis:*** It can produce adenocarcinoma of colon
- ***Cirrhosis liver:*** A few can form hepatoma (hepatocellular carcinoma)
- ***Carcinoma "in situ" of cervix:*** It can produce squamous cell carcinoma of cervix.

Q.7. How does radiant energy cause cancer?

- ***Direct effect:*** Produces damages to DNA which may be as follows:
  - Single or double strand "breaks"
  - Eliminition of purine/pyrimidine bases
  - Cross-linking of strands
  - Formation of pyrimidine dimers.
- ***Indirect effects:*** X-rays and **γ**-rays produce **"free" radicals** *viz* superoxide radical O_2^-, and other free radicals like H_2O_2, $HOO^\bullet$, OH^- etc. which may interact subsequently with DNA and other macromolecules leading to molecular damage.

Q.8. State some chemicals with examples which are carcinogenic?

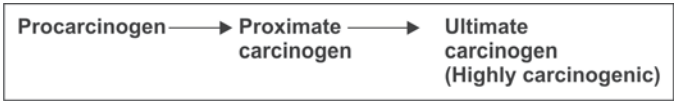
- ***Polycyclic aromatic hydrocarbons:*** Benzpyrene, Dimethyl Benzanthracene
- ***Azo dyes:*** **β**-Naphthylamine, N-methyl-4-amino azo benzene
- ***Nitrosamines and amides:*** Dimethyl nitrosamine and diethyl nitrosamine.
- ***Some drugs:*** Alkylating and acylating agents, e.g. cyclophosphamide, busulfan, nitrogen mustard, **β**-propiolactone.

- ***Naturally occurring compounds:*** Aflatoxin B_1-produced by the fungus aspergillus flavus which may contaminate ground nuts/pea nuts etc.
- ***Miscellaneous agents:*** Asbestos, beryllium, chromium, vinyl chloride, saccharin and cyclamates.

Q.9. What are the mechanisms of chemical carcinogenesis?
Chemical carcinogens can be:
- Direct acting
- Procarcinogens.

Q.10. How does direct acting chemicals act?
A few chemical carcinogens like alkylating agents, e.g. cyclophosphamide, busulfan, etc. can interact directly with the target molecules, DNA, RNA and proteins.

Q.11. How does procarcinogens act?
Most of the chemicals act as procarcinogens. They are not chemically reactive. In the body after metabolism they are converted to ***ultimate carcinogens,*** which are highly carcinogenic. Thus:



Q.12. How does ultimate carcinogen act?
Most of the "ultimate carcinogens" are ***electrophiles,*** i.e. the molecules are deficient in electrons and thus they can readily react with ***nucleophilic electrons rich*** groups in DNA, RNA and various proteins.

Q.13. What is meant by the term metabolic activation?
The process by which a procarcinogen is converted in the body to highly active "ultimate carcinogen" by one or more enzyme-catalyzed reactions, is called as ***metabolic activation.***

Q.14. State enzyme systems involved in metabolic activation.
The enzyme systems involved in metabolic activation are:
- Cytochrome P_{450} species present in the endoplasmic reticulum of cells
- Recently, cytochrome P_{498}, a mono-oxygenase species (also called AHH-aromatic hydrocarbon hydroxylase), has been incriminated in metabolic activation of polycyclic aromatic hydrocarbons.

Q.15. What are proto-oncogenes?

- Proto-oncogenes are normal cellular genes that affect growth and differentiation **(V-onc proto-oncogene)**
- Proto-oncogenes are converted to oncogenes before they can be carcinogenic.

Q.16. What are oncogenes?

Oncogenes are genes whose products are associated with neoplastic transformation.

Q.17. State the different mechanisms by which proto-oncogenes are activated to oncogenes.

At least ***five*** mechanisms are known that alter the structure/expression of proto-oncogenes and convert them to "oncogenes" which produce cancer. They are:

- Single point mutation
- Gene amplification
- Promoter insertion
- Enhancer insertion
- Translocations.

Q.18. What are the types of oncogenic viruses?

Oncogenic viruses are of ***two*** types:

- DNA viruses
- RNA viruses

Q.19. State some DNA viruses which produce cancers in humans.

Many of the DNA viruses cause tumors in animals. But at least ***three*** DNA viruses have been established as causing human cancers. They are:

Class	*Member*	*Associated tumors in humans*
• ***Papova virus***	Human Papilloma virus (HPV)—several types identified	• Warts leading to squamous cell carcinoma of skin (HPV viruses type 1,2,4 and 7 important) • Carcinoma-*in-situ* of cervix leading to squamous cell carcinoma of cervix (HPV type 16 and 18 important)

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Class	*Member*	*Associated tumours in humans*
• ***Hepadna virus***	Hepatitis B virus	• Liver cell carcinoma
• ***Herpes virus***	• Epstein-Barr virus (EBV)	• Burkitt's lymphoma • Immunoblastic lymphoma
	• Herpes simplex (Type-2)	• Nasopharyngeal carcinoma • Cancer cervix

Q.20. What are oncogenic RNA viruses? What are the types?

- ***All oncogenic RNA viruses are retroviruses.***
- They are mainly of ***two types:***
  - ***Acute transforming retroviruses:*** These include type C viruses and cause rapid induction of tumors in animals. Transforming sequences of these viruses are viral oncogenes (V-oncs).
  - ***Slow transforming retroviruses:*** These do not contain V-oncs and are replication competent and cause transformation of the cells slowly. Mechanism of transformation is insertional mutagenesis.

Q.21. Name four retroviruses, their oncogenes and oncogenic products.

Retrovirus	*Corresponding oncogenes*	*Oncogen-Product*
• ***Rous sarcoma virus***	src	*Protein tyrosine kinase*
• ***Simian sarcoma virus***	sis	Truncated PDGF (B chain)
• ***Feline sarcoma virus***	fms	CSF-1 receptor protein tyrosine kinase
• ***Avian erythroblastosis***	erb-B	Truncated EGF-receptor

Q.22. How does oncogene Src act?

- The Product of "Src" gene is a protein *tyrosine kinase* which is responsible for the cell transformation. The protein is produced in cytoplasm by inner cell membrane and called $PP_{60\,Src.}$
- The specific biochemical mechanism involved is abnormal phosphorylations of a number of proteins.

The proteins that are phosphorylated by *protein tyrosine kinase* are:

- Certain glycolytic enzyme proteins
- A critical protein called ***vinculin.***

Abnormal phosphorylation of this critical protein leads to transformation of the cells.

Q.23. State two RNA retroviruses with their oncogenes and products which have been implicated in human cancers.

RNA retro Virus	*Oncogenes*	*Oncogenic Products*	*Human Cancers*
• ***Murine Sarcoma Virus***	"ras"	GTP binding proteins with GTP-ase activity	• Large varieties of human cancer *viz.* lung, bladder, colon • leukemias, • Neuroblastoma
• ***Avian myelocytoma virus***	"myc"	DNA-binding Protein	• Burkitt's lymphoma • Small-cell lung carcinoma • Neuroblastoma

Q.24. What is HTLV-1 virus? What does it produce?

- HTLV-1 virus has been found to be associated with human leukemias and lymphomas
- HTLV-1 contains a segment in its genome called ***tat.*** The proteins coded by "tat" gene are believed to be responsible for transformation. They affect the transcription of certain growth factors and receptors like IL-2 and IL-2R.

Q.25. What is the chromosomal translocation found in Burkitt's lymphoma?

Burkitt's lymphoma, a fast growing cancer of human B lymphocytes. In this, there is ***reciprocal translocation*** between chromosomes 8 and 14 **(t 8:14)** in 90% cases. In 10% cases, there is exchange between chromosomes 8 or 2, or 8 or 22 **(t 8:2 or t 8:22).**

Q.26. What is Philadelphia chromosome?

- In chronic granulocytic leukemia, the "Philadelphia chromosome" involves chromosomes 9 and 22. It is

direct translocation **(t 9:22)** in which "C-abl" gene from chromosome 9 relocates to "bcr" locus on chromosome 22

- The "C-abl-bcr" hybrid gene codes for a chimeric protein that exhibits the *tyrosine kinase* activity.

Q.27. What are cancer-suppressor genes (or anti-oncogenes).

- Recently, it has been shown that genes other than oncogenes can play a role in etiology of certain types of cancers. These are called ***"cancer-suppressor genes" or "growth supperssor genes" or "anti-oncogenes"***
- A loss or inactivation of such genes removes certain mechanisms of growth control and produces cancer.

Q.28. Name some cancers which are produced by loss of anti-oncogenes?

- Retinoblastoma
- Wilms' tumors of kidney
- Oat cell carcinoma of lungs
- One type of breast cancer.

Q.29. What is Rb gene?

Rb gene is an antioncogene located on chromosome 13 q 14. Normally, it exerts an inhibitory effect. Both normal alleles of the Rb locus must be inactivated for the development of retinoblastoma.

Q.30. What are growth factors?

- Growth factors are usually, polypeptides in nature
- They can initiate cell migration, differentiation and tissue modelling
- They can affect many different types of cells, *viz.*, hemopoietic, epithelial tissues, nervous system and mesenchymal tissues
- Play a major role in regulating differentiation of stem cells to form various types of mature cells
- Are mitogenic to target cells
- Products of several oncogenes are either growth factors or parts of receptors for growth factors.

Q.31. State the mechanisms of action of growth factors?

Growth factors may act in **3 different ways:**

- ***Endocrine action***
- ***Paracrine action***
- ***Autocrine action***

Q.32. State five important growth factors, their sources and functions?

Name of growth factors	*Sources*	*Functions*
• ***Epidermal growth factor (EGF)***	• Mouse salivary gland	• Stimulates growth of many epidermal and epithelial cells • Mitogenic for epithelial cells and fibloblasts
• ***Fibroblast growth factors (FGFs)***	• Basic FGF is present in many organs and secreted by activated macrophages • Acidic FGF is found in nerve tissues.	• Binds to heparin and cause fibroblast proliferation and neovasularization
• ***Platelet derived growth factor (PDGF)***	• α-granules of platelets • Also produced by activated macrophages, endothelium and smooth muscles	• Stimulates growth of mesenchymal and glial cells • Causes migration and proliferation of fibroblasts, smooth muscle cells and monocytes
• ***Nerve growth factor (NGF)***	• Mouse salivary gland	• Tropic effect on sympathetic and certain sensory neurons
• ***Transforming growth factor β (TGF-β)***	• Kidneys • Platelets, T-cells, endothelium and macrophages	• Inhibits growth of most cells except fibroblasts • Inhibits collagen degradation • Deactivates macrophages

Q.33. State some biochemical changes that are found in malignant cells.

- Appearance of new antigens and loss of certain antigens
- Show alterations of permeability and surface charge
- Show increased rate of glycolysis both aerobic and anaerobic
- Increased synthesis of DNA and RNA
- Changes in composition of glycoproteins, and glycosphingolipids on cell surfaces.
- Alterations of the oligosaccharide chains
- Alterations of isoenzyme patterns often to a fetal pattern and synthesis of fetal proteins, e.g. α-fetoprotein (AFP), carcinoembryonic antigen (CEA)

- Increased synthesis of growth factors
- Secretion of certain ***proteinkinases and proteases***.

Q.34. What is metastasis?

Metastasis is the spread of cancer cells from the primary site of origin to other tissues, both neighboring and distant, where they grow as the secondary tumors. The spread of cancer cells may be blood-borne/or through lymphatics.

Q.35. What are oncogenic markers (or tumor markers)?

Oncogenic markers (or tumor markers) are defined as a biochemical substance (protein, hormone or enzyme) synthesized and released by cancer cells or produced by the host in response to cancerous substance and are used to monitor or identify the presence of a cancerous growth.

Q.36. What are the uses of tumor markers in cancer patient care?

Ideally tumor makers have following **six** potential uses in cancer-patient care. They are:

- For "screening" specially in asymptomatic population
- For diagnosis in asymptomatic patients
- As a prognostic predictor
- As an adjunct in clinical staging of the cancerous condition
- For monitoring during treatment of the patients, and
- For early detection of relapse/recurrence of the cancerous process.

Q.37. What are the characteristics of an ideal tumor marker?

a. **Clinical criteria:**

- Should be disease-sensitive
- Should have high disease-specificity
- Should be stable and should not show wide fluctuations
- Should correlate well with cancerous process, i.e. its extent and volume of the tumor
- Should correlate well with cure rate
- Should prognosticate the "high risk" cancer patients from "lower risk"
- Should be able to detect relapse/recurrence of the cancer.

b. **Analytical criteria**
- Should have high sensitivity, specificity, accuracy and precision
- Should be simple and easy to measure and should not be costly.

Q.38. Mention some clinically useful tumor markers and their nature.

Tumor maker	*Nature*	*Usefulness in cancer*
• ***Carcinoembryonic antigen (CEA)***	• An oncofetal antigen • Glycoprotein in nature	• Most valuable in ***colonorectal cancer*** • Also useful in small-cell carcinoma of lungs, breast cancer pancreatic cancer
• ***Alpha-fetoprotein (AFP)***	• An oncofetal antigen • Glycoprotein in nature	• Most valuable in ***liver cell carcinoma*** • Also useful in germ cell tumors of testes and ovary
• **β-*HCG (Human chorionic gonadotropin)***	• Produced by syncytiotrophoblastic cells of placental villi • A glycoprotein, a dimer, consisting of α and β subunits	• Most useful in gestational trophoblastic tumors and germ cell tumors viz. seminomas, embryonal, carcinoma, teratocarcinoma and chriocarcinoma

Q.39. What are interferoms?
- Interferoms are cellular glycoproteins produced as soon as virus infects the cells
- It has a molecular weight approximately 20,000 to 25,000
- Other inducers for production of interferons are: inactivated viruses, fungal extracts, synthetic polynucleotides, etc.

Q.40. What are the types of interferons?
There are **three types** of interferons:
- **IFN-α**
- **IFN-β, and**
- **IFN-γ**

- IFN-**α** has been produced by utilizing recombinant DNA technology and being used thereputically.

Q.41. What is the mechanism of action of interferons?

Interferons act in **two ways:**

- ***Direct inhibition of translation of viral mRNA:*** IFN induces an enzyme, called ***oligo-2′5′-adenylate synthetase*, which activates a latent endonuclease which degrades the viral mRNA**
- ***Inhibition of protein synthesis:*** IFN can inactivate "IF-2" required for protein synthesis.

Q.42. State the functions of interferons.

- ***Antiviral action*** inhibits multiplication of viruses
- ***Anticancerous action:*** antiproliferative

Q.43. State the therapeutic uses of interferons.

IFN-α has recently been used in the following conditions:

- Used in hematological malignancies *viz* multiple myeloma, low-grade non-Hodgkin's lymphoma, Hairy-cell leukemia (HCL)
  - In Myeloproliferative disorders (MPD): found to be highly effective
  - In regressing hemangiomas of infants and young adults: highly effective by its anti-angiogenic effect
- Used in solid tumors:
  - Kaposi's sarcoma
  - Renal cell carcinoma
  - Breast cancer
  - Also used in connective tissue disorders and chronic neurological diseases.

Q.44. What is P^{53}?

- P^{53} is a tumour suppressor gene having a molecular weight of approximately 53,000 and is located on short arm of chromosome 17.
- ***Mechanism of action:***
  It binds various viral proteins forming inactive oligomeric complexes. Probably acts as transcriptional regulator.

Q.45. State the tumors produced by mutations in P^{53} gene.

- Mutations in the P^{53} gene are the most common genetic alterations in human cancer and are frequently associated with cancers of lungs, liver, breast and colon
- Transversions are frequent in lung cancer and liver cancer.

Q.46. List some drugs that are used in cancer chemotherapy.

- ***Poly functional alkylating agents:***
  - Nitrogen mustard, busulfan, chlorambucil, triethylene melamine
- ***Hormones:***
  - Sex hormones—estrogens
  - Corticosteroids—prednisone
- ***Antimetabolites:***
  - Folate antagonist—methotrexate
  - Purine antagonist—mercaptopurine
  - Pyrimidine antagonist—S-fluoro uracil
- ***Antibiotics:***
  - Actinomycin D
  - Doxorubicin
- ***Miscellaneous agents:***
  - Vinblastin,
  - Vincristin,
  - Cisplatin
  - Retine—a non-toxic anticancer agent, a natural constituent of body cells.
  - Herception—for breast cancer. It is antibody that attaches to HERz gene on tumor.

Q.47. What is meant by resistance to drugs?

The drugs are effective initially to cancer chemotherapy but after several months of treatment, the drugs become ineffective by the mechanisms developed by the tumor cells producing acquired resistance.

Q.48. What is meant by multidrug resistance (MDR)?

In multidrug resistance (MDR), the resistance is not only to a particular drug but also to other structurally unrelated anti-cancer agents.

Q.49. What is the role of P-glycoprotein in MDR?

- Phosphorylated glycoprotein (P-glycoprotein) present in the plasma membrane. It contains 1280 amino acids and has a molecular weight of 1,70,000
- It acts as energy-dependent efflux pump expelling a variety of drugs and thus mediating MDR.

Q.50. Name some natural anticancer substances.

- Vitamin A and β-carotene
- Vitamin E (Tocopherols)
- Lycopene—a carotenoid present in ripe tomatoes (also a powerful anti-oxidant)
- Ascorbic acid (vitamin C)
- Selenium—a trace element
- Zinc—a trace element
- Glucosinolates: present in bitter "Brussel sprouts". It contains:
  - ***Sinigrin:*** suppresses development of precancerous cells
  - ***Glucoraphanin:*** It breaks down into a compound called ***sulphoraphane*** which neutralizes carcinogens.

CHAPTER

25 Acid Base Balance and Imbalance

Q.1. What is an acid?
An acid is defined as a substance, ion, molecule or particle that yields H^+ ions (Protons) in solution.

Q.2. What is a base?
A base is defined as a substance, ion, molecule or particle that combines with H^+ ions (Protons).

Q.3. What is a strong acid? Give an example of a strong acid.
A strong acid dissociates extensively to produce large numbers of protons (H^+).
Example: Hydrochloric acid

Q.4. What is a weak acid? Give an example of a weak acid.
A weak acid dissociates less to produce smaller numbers of H^+ (Protons).
Example: Acetic acid

Q.5. What is meant by conjugate acid base pairs? Explain.

- The stronger the acid, the weaker is the base which results from its dissociation. Such pairs have been termed conjugate base pairs.
- HCl is a strong acid by virtue of its extensive dissociation into H^+ and Cl^-. Cl^- is an extremely weak base because it has very little capacity for combining with H^+ ions.

$$\underset{\text{(Strong acid)}}{HCl} \rightleftharpoons H^+ + \underset{\text{(Weak base)}}{Cl^-}$$

Q.6. What is a buffer?
For answer refer to answer of Q 11, Chapter 21, p-407.

Q.7. Describe the mechanism of action of a buffer.
For answer, refer to answer of Q15, Chapter 21), p-408.

Q.8. Name some acids produced in the body.
- Carbonic acid (H_2CO_3): Chief acid produced due to oxidation in the cells.
- Sulphuric acid (H_2SO_4): A strong dissociable acid produced during oxidation of S-containing amino acids.
- Phosphoric acid: Products of metabolism of dietary phosphoproteins, nucleoproteins and phosphatides, etc.
- Organic acids: Pyruvic acid, Lactic acid, β-OH butyric acid, etc.

Q.9. What is the normal pH of ECF?
- Under normal conditions, the pH of ECF usually does not vary beyond the range of 7.35 to 7.5 and is maintained approximately at 7.4.
- pH of arterial blood is approximately 7.43 and venous blood is 7.4.

Q.10. What are the different mechanisms of regulation of blood pH.
The mechanisms of regulation of blood pH involves the following:
- "Front line" defence: They are mainly
  - Blood buffers in the blood which restricts pH change in blood.
  - Respiratory mechanisms regulates excretion of CO_2, thus concentration of H_2CO_3 in ECF.
  - "Second Line" defence: This is achieved by kidneys (Renal mechanisms).
  - Dilution factor: Entrance of a given amount of acid into a smaller volume of fluid as in conditions of relatively greater rise in H^+ concentration.

Q.11. Name the buffer pairs present in plasma.
For answer, refer to Chapter 21, Q 13, p-407.

Q.12. Name the buffer pairs present in red blood cells.
For answer, refer to Chapter 21, Q 14, p-407.

Q.13. Describe the Bicarbonate buffer system present in ECF. What is the normal ratio in blood?
Bicarbonate buffer system consists of weak acid carbonic acid (H_2CO_3) and its corresponding salt with strong base (HCO_3^-), Sodium bicarbonate, NaH CO_3.

- Normal ratio in blood:

$$\frac{NaHCO_3}{H_2CO_3} = \frac{20}{1}$$

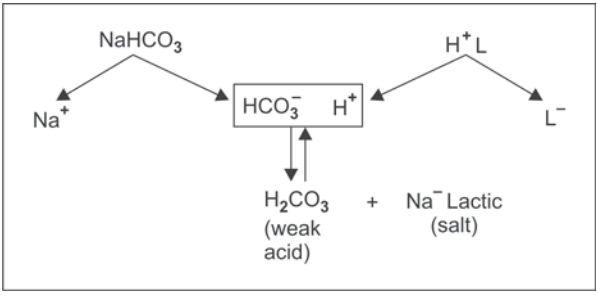
Q.14. What is meant by the term "alkali reserve"?

Alkali reserve is represented by the $NaHCO_3$ concentration in the blood that has not yet combined with strong and non-volatile acid.

Q.15. Explain how bicarbonate buffer system acts in the body.

- Neutralization of strong and non-volatile acids entering the ECF is achieved by the bicarbonate buffers.
- Such acids as lactic acid, HCl, etc. which are strong and non-volatile react immediately with $NaHCO_3$ component.

Example: A strong and non-volatile acid, lactic acid (LA) will be buffered as follows:



Thus a strong and non-volatile acid like LA is converted into weak (less dissociable) and volatile acid H_2CO_3 at the expense of $NaHCO_3^-$ salt component of the buffer.

Q.16. Why bicarbonate buffer system is linked up directly with respiration?

H_2CO_3 (carbonic acid) formed after buffering a strong and non-volatile acid is a volatile weak acid and is eliminated by diffusion of CO_2 through alveoli of lungs. Proper lung functioning is important and essential.

$$H_2CO_3 \xrightarrow[\text{Low } CO_2 \text{ tension}]{\text{Carbonic anhydrase}} H_2O + CO_2 \text{ (expired air)}$$

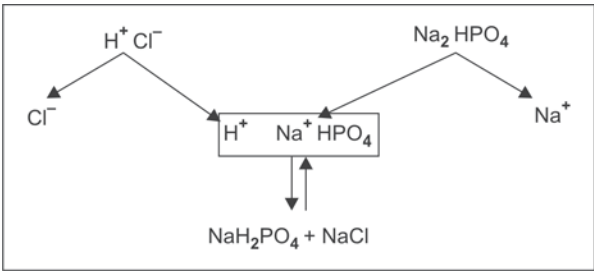
Thus bicarbonate buffer system is directly linked up with respiration.

Q.17. What are the advantages of bicarbonate buffer system?
Bicarbonate buffer system is efficient as compared to other buffer systems because:
- It is present in very high concentration 26 to 28 millimoles per litre.
- Produces H_2CO_3 which is a weak and volatile acid. It breaks up in lung alveoli and CO_2 is exhaled out.
  Thus, it is a very good physiological buffer and acts as a frontline defence.

Q.18. Is bicarbonate buffer efficient chemically?
As a chemical buffer, it is weak, pKa is further away from the physiologcal pH.

Q.19. What is the phosphate buffer system? What is the normal ratio in plasma?
Phosphate buffer system consists of Na_2HPO_4 (alk. PO_4)/ NaH_2PO_4 (acid PO_4).
- Normal ratio in plasma is 4:1.

Q.20. Explain how phosphate buffer system acts in the body.
When a strong acid enters the blood, it is fixed up by Na_2HPO_4 (alk PO_4) which is converted to NaH_2PO_4 (acid PO_4)



NaH_2PO_4 (acid PO_4) thus formed are excreted by the kidneys, hence urine becomes more acidic.

Hence phosphate buffer system is linked up with the kidneys.

Q.21. What are the drawbacks of phosphate buffer system physiologically?
- Concentration of phosphate buffer system is low, 1.0 millimole per litre.
- As a physiological buffer it is less effective.

Q.22. Is phosphate buffer system efficient chemically?
As a chemical buffer, it is very effective and better as pKa approaches physiological pH.

Q.23. What is protein buffer system?
Protein buffer system consists of Na^+ Pr^-/H^+ Pr^- = [salt/acid]. Buffering capacity of plasma proteins is much less than Hb, which operates only in erythrocytes.

Q.24. How does protein buffer act? Explain.
- In acidic medium, proteins act as a **base,** NH_2 group takes up H^+ions from the medium forming NH_3^+, and proteins behave positively charged.
- In alkaline medium, proteins act as an **acid.** Acidic COOH group dissociates and gives H^+, forming COO^-, H^+ combines with OH^- in medium to produce H_2O. Proteins thus become negatively charged.
- Salt component of protein buffer, Na^+ proteinate can combine with strong acids and thus produces weak acid H^+Pr^-.

Q.25. How does Hb act as a buffering agent?
The buffering capacity of Hb depends on the number of dissociable buffering groups, most important being imidazole group.
- Imidazole contains two groups:
  - Fe^{2+} containing group which is concerned with carriage of O_2.
  - Imidazole N_2 group, which can give up H^+ (protons) and accept H^+ depending on pH of the medium.

    Thus buffering capacity of Hb is due to the presence of "imidazole" N_2 group which remains dissociated in acidic medium and conjugate base forms.

Q.26. Oxygenated Hb is a stronger acid than deoxygenated Hb. Explain.
- On oxygenation, the imidazole N_2 group acts as **acid** and donate H^+ (Proton) in the medium.

- Deoxygenated Hb is less acidic, less dissociable and imidazole N_2 group acts as **base** and takes up H^+ (Protons) from the medium.
- Acidity of the medium favours delivery of O_2 and alkalimity of the medium favours oxygenation of Hb.

Q.27. Explain the term "Isohydric transport of CO_2".

At a pH of 7.25, one mole of oxy-Hb donates 1.88 mEq of H^+; on the other hand, one mole of reduced Hb, as it is less ionized, donates only, 1.28 mEq H^+.

- It is to be noted that at the tissues, a change of the mole of oxy-Hb to reduced Hb allows 6 meq H^+ to be bound (buffered), so that these newly formed H^+ ions do not bring about a change in pH.

This circumstance as it relates to the role of Hb buffers is referred to as "isohydric transport of CO_2."

Q.28. What are the factors on which the respiratory mechanism depend upon?

Participation of respiratory mechanism in the regulator of acid-base balance depend on:

- The sensitivity of the respiratory center (RC) to very slight changes in pH and PCO_2.
- Ready diffusibility of CO_2 from the blood across the pulmonary alveolar membrane into the alveolar air.

Lungs should be healthy so that diffusion of CO_2 take place properly.

Q.29. What will be the nature of respiration in $\uparrow pCO_2$ and $\uparrow H^+$ concentration (acidosis) ?

- An increase in blood pCO_2 (0.2%) results in 100% increase in pulmonary ventilation by stimulation of RC. The same occurs with increase in H^+ ion concentration (acidosis). The excess CO_2 is promptly removed from the ECF in the expired air.

Q.30. What will be the nature of respiration in $\downarrow pCO_2$ and $\downarrow H^+$ concentration (alkalosis)?

A decrease in blood $pCO_2 \downarrow$ or $\downarrow H^+$ ion concentration (alkalosis) cause depression of RC with consequent slow and shallow respiration (hypoventilation) resulting to retention of CO_2 in the blood until the normal pCO_2 and pH are restored.

Q.31. State the mechanisms that operate in kidney for regulation of acid-base balance.

Three mechanisms operate in the kidneys for regulation of acid-base balance. They are:

- Bicarbonate mechanism
- Phosphate mechanism
- Ammonia mechanism

Q.32. State the sequence of events that occur in proximal tubular epithelial cells in bicarbonate mechanism.

- H_2O and CO_2 combines to form H_2CO_3 under the influence of the enzyme "carbonic anhydrase".
- H_2CO_3 dissociates to form H^+ and HCO_3^-
- H^+ is secreted in tubular lumen where it reacts with $NaHCO_3$ H^+ replaces Na^+ and forms H_2CO_3 which breaks up to H_2CO and CO_2.
- Na^+ passively enters into the renal epithelial cell and actively excreted in ECF.
- HCO_3^- formed in tubular epithelial cell is absorbed passively in ECF where HCO_3^- combines absorbed Na^+ and reforms $NaHCO_3$.

Q.33. Mention the site where bicarbonate mechanism operates in kidneys.

Bicarbonate mechanisms operate in proximal tubular epithelial cells where the exchange of H^+ proceeds against $NaHCO_3$.

Q.34. Which HCO_3^- is reabsorbed to reform $NaHCO_3$ in ECF?

HCO_3^- formed by dissociation of H_2CO_3 in tubular epithelial cells is absorbed to produce $NaHCO_3$ in ECF.

Q.35. What is Paradoxic aciduria? Explain.

- In K^+ deficiency, K^+ ions leave the cells, H^+ions enter the cells producing intracellular acidosis and ECF becomes alkaline.
- More H^+ secretion from tubular epithelial cells and there is increased excretion of H^+, NaH_2PO_4 and NH_4Cl increasing the titratable acidity.

Hence, in this condition, though the ECF is alkaline, highly acidic urine is excreted. This condition is called as "paradoxic aciduria".

Q.36. Name at least three clinical conditions where paradoxic aciduria can occur due to K deficiency.

- Patients treated for prolonged periods with cortico-steroids.
- In persons with hypercorticism. (Cushing's syndrome).
- In post-operative patients with K-free fluids, in whom depletion may occur due to continued loss in urine and G.I. fluids.

Q.37. What is carbonic anhydrase? What is its function?

- Carbonic anhydrase is a Zn-containing metalloenzyme.
- It catalyzes the formation of H_2CO_3 (carbonic acid) from H_2O and CO_2. It is a reversible reaction.

$$H_2O + CO_2 \xleftrightarrow{\text{Carbonic anhydrase}} H_2CO_3$$

Q.38. State some sites in the body where carbonic anhydrase is found.

In most of the tissues where it catalyzes formation of H_2CO_3 from H_2O and metabolic CO_2. Specially the enzyme is found in:

- Red blood cells (Not in plasma).
- In parietal cells of stomach, involved in secretion of HCl.
- In renal tubular epithelial cells, involved in $H^+ \rightarrow Na^+$ exchange. Recently, also demonstrated in small quantities in:
  - Muscle tissue; pancreas and
  - spermatozoa.

Q.39. Name one drug of clinical importance which inhibits the enzyme carbonic anhydrase.

Acetazolamide (Diamox), a common diuretic used in edema due to congestive heart failure and in hypertensive heart disease.

Q.40. Why urine becomes alkaline in diamox therapy?

- Diamox inhibits the enzyme carbonic anhydrase.
- Increased amount of $NaHCO_3$ excreted in urine due to less

  $H^+ \rightarrow Na^+$ exchange.
- There is also reduction in titratable acidity $\downarrow$ NH_3 formation $\downarrow$ and an increase in K^+ excretion $\uparrow$ from distal tubular epithelial cells.

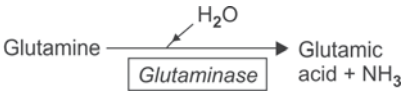
Q.41. What is the ratio of phosphate buffer in urine?
In urine, concentration of NaH_2PO_4 increases and exceeds $Na_2 HPO_4$.
Ratio of $NaH_2 PO_4/Na_2HPO_4$ becomes 9:1.

Q.42. State the site where phosphate buffer operates in kidney.
Phosphate buffer operates in the distal tubules of kidneys.

Q.43. State the sequence of events that occur in distal tubular epithelium by phosphate buffer.
- H_2O and CO_2 combines to form H_2CO_3 under the influence of CA.
- H_2CO_3 dissociates to form H^+ and HCO_3^-.
- H^+ secreted in tubular lumen, where it reacts with Na_2HPO_4. The exchange of Na^+ for secreted H^+ takes place and it changes Na_2HPO_4 to NaH_2PO_4 which is excreted in urine.
- Na^+ from tubular lumen is reabsorbed into tubular epithelial cell passively and then it is secreted in plasma actively by sodium pump.
- Na^+ and reabsorbed HCO_3^- moiety of H_2CO_3 reforms $NaHCO_3$ again.

Q.44. What is ammonia mechanism? Where does it operate?
It operates in the distal renal tubular cells, for the elimination of H^+ ions and the conservation of Na^+, by production of NH_3 by distal renal tubular epithelial cells.

Q.45. What is the main source of NH_3 in distal tubular epithelial cells?
- Main source of NH_3 is from glutamine, amide of glutamic acid.
- NH_3 is produced by the hydrolysis of glutamine by the enzyme "glutaminase" present in distal renal tubular epithelial cells.



Q.46. State other sources of NH_3 in distal tubular epithelial cells of kidney.

If the cells require more NH_3, the additional sources of NH_3 are:

- NH_3 can be formed from other amino acids by oxidative, deamination by L-amino acid oxidase.
- NH_3 can also be formed from glycine by glycine oxidase.

Q.47. State sequence of events that occur in NH_3 mechanism.

- H_2CO_3 is formed from H_2O and CO_2 by carbonic anhydrase.
- H_2CO_3 dissociates into H^+ and HCO_3^-
- H^+ combines with NH_3 to form NH_4^+ ion in tubular lumen, where it acts with Na^+Cl^- to form Na^+ and NH_4Cl.
- Na^+ ion is reabsorbed alongwith HCO_3^- and combines to form back $NaHCO_3$ in ECF. NH_4Cl is excreted in urine.
- Reabsorption of Na^+ from tubular lumen to epithelial cell is passive but absorption from cell to ECF is active.

Q.48. What are the acid-base abnormalities occur clinically?

Acid-base abnormalities can manifest or occur clinically:

- Acidosis
- Alkalosis.

Q.49. What are the types of acidosis?

Acidosis can be of two types: $\frac{BHCO_3}{H_2CO_3}$

- Metabolic acidosis
- Respiratory acidosis.

Q.50. What is metabolic acidosis? What is the other name?

- Metabolic acidosis occurs when there is a reduction or decrease in plasma $HCO_3^- \downarrow$ ($BHCO_3$). In this ratio of $[HCO_3^-]/[H_2CO_3]$ = 20/1 is decreased i.e. pH is decreased resulting to acidosis.

$$= \frac{20\downarrow}{1} = pH\downarrow$$

- The other name for metabolic acidosis is "Primary bicarbonate deficit".

Q.51. How does body tries to compensate?

The compensatory mechanisms are of two types:

- Primary compensatory mechanism
- Secondary compensatory mechanism.

Q.52. Explain primary compensatory mechanism?

In metabolic acidosis, primary compensatory mechanism is of prime importance as body tries to bring the ratio 20:1.

- The RC is stimulated by acidosis causing deep and rapid (kausmal) breathing. This increased ventilation results in CO_2 loss and reduction in $[H_2CO_3] \downarrow$ (carbonic acid).
- As a result, the ratio of $[HCO_3^-]/[H_2CO_3]$ is restored towards normal 20 : 1 ratio, as levels of both in blood are reduced.

This is an immediate reaction and cannot last long.

Q.53. Explain why primary compensatory mechanism cannot last for long?

- Primary compensatory mechanism cannot last long as increased ventilation causes reduction in $pCO_2 \downarrow$ which in turn depresses the RC.
- Two opposing forces start working:
  - Acidosis stimulating RC
  - Low $pCO_2 \downarrow$ depresses RC

Thus respiratory compensation is only partial and temporary.

Q.54. What is uncompensated acidosis?

During the early stages of primary alkali deficit (metabolic acidosis), the organism is in a state of compensated acidosis temporarily and partially.

But as the condition progresses and if the treatment is not instituted, the alkali deficit becomes more pronounced, the primary compensation mechanism fails and the condition becomes one of uncompensated acidosis with an increase in H^+ ion concentration in blood.

Q.55. What is the secondary compensatory mechanism?

Secondary compensatory mechanisms are the renal mechanisms which tries to correct the disturbances as follows:

- By conserving cations i.e., Na^+ by increasing $H^+ \rightarrow Na^+$ exchange.
- By increasing:
  - HCO_3^- reabsorption so that $NaHCO_3$ is reformed.
  - H^+ excretion compared to K^+ excretion in distal tubules, and
  - increased $NH_3 \uparrow$ formation.

Q.56. State the biochemical changes in uncompensated phase and fully compensated phase in metabolic acidosis.

- In uncompensated phase:
  - Disproportionate decrease in $[HCO_3^-]\downarrow$
  - $[H_2CO_3^-]\downarrow$
  - $PCO_2\downarrow$
  - Total CO_2 is decreased $\downarrow$
  - Ratio is decreased $\downarrow$
- In fully compensated phase:
  Total CO_2 is low $\downarrow$ but the decrease in $[HCO_3^-]$ and $[H_2CO_3]$ is proportionate, and the ratio 20:1 and pH is maintained.

Q.57. What are the urinary findings in acidosis?

- pH is acidic.
- Increased excretion of NH_4Cl and NaH_2PO_4.
- Increase in titratable acidity $\uparrow$.

Q.58. Enumerate a few important causes of metabolic acidosis.

- Abnormal increase in anions other than HCO_3^- (acid gain acidosis)
- Endogenous production of acid ions when excessive, e.g.
  - Diabetic acidosis
  - Starvation
  - Violent exercise (LA)
  - High fever
  - Lactic acidosis due to other causes like shock and Hemorrhage.
- Ingestion of acidifying salts.
- Renal insufficiency: retention of acids normally produced.
- Abnormal loss of HCO_3^- e.g. in severe diarrheas, GI fluid loss in fistulas, etc.

Q.59. What is respiratory acidosis? What is its other name?

- If excretion of CO_2 through lungs is impaired, more CO_2 accumulates in blood resulting in increase in $[H_2CO_3]\uparrow$. This results in lowering of the ratio 20:1, pH $\downarrow$ resulting to acidosis.

$$\frac{BHCO_3}{H_2CO_3} = \frac{20}{1} = pH\downarrow$$

- The other name is "primary carbonic acid excess".

Q.60. Which compensatory mechanism is more important in this condition (respiratory acidosis)?
In respiratory acidosis, renal mechanisms are of prime importance. The respiratory mechanism is secondary and less important.

Q.61. How does kidneys help in this condition?
Renal mechanism becomes of prime importance which increases the absorption of more HCO_3^- from tubules in response to raised pCO_2 in blood and ratio of $[HCO_3^-]/[H_2CO_3]$ is restored towards 20:1 as levels of both in blood increase.

Q.62. Explain why respiratory mechanism is not of importance.
Acidosis and increased CO_2 tension ($pCO_2 \uparrow$) stimulate the RC resulting in increased depth and rate of respiration resulting to increase in ventilation.

But this mechanism becomes secondary in importance as the primary defect may be with RC depression or some pathology in the lungs itself. Thus this compensatory mechanism becomes less effective.

Q.63. State the biochemical changes that occur in compensated and in fully compensated phase in this condition.
- In uncompensated phase disproportionate increase in $[H_2CO_3] \downarrow (pCO_2) \uparrow$
- Increase in $[HCO_3^-] \uparrow$
- Increase in total CO_2 content $\uparrow$
- Decrease in $[HCO_3^-]/[H_2CO_3]$ ratio $\downarrow$
- Decrease in pH $\downarrow$
- If fully compensated, the CO_2 content is high but the increase in $[H_2CO_3]$ and $[HCO_3^-]$ are proportionate; the $[HCO_3^-]$; $[H_2CO_3]$ ratio and pH remaining within normal limits.

Q.64. Enumerate a few conditions that can cause respiratory acidosis.
- Conditions in which there is depression/or suppression of respiration:
  - Damage to CNS: brain damage, drug poisoning like morphine and barbiturates.
  - Loss of ventilatory function, e.g. emphysema, pulmonary and mediastinal tumors, etc.
  - Effects of pain, e.g. pleurisy.

- Conditions causing impaired of diffusion of CO_2 across alveolar membrane—reduced alveolar-respiratory function, e.g. Emphysema, pulmonary oedema, pneumonia, etc.
- Conditions in which there is obstruction to escape of CO_2 from alveoli, e.g. obstruction to respiratory tract, rebreathing from a closed space.
- Conditions in which pulmonary blood flow is insufficient, e.g. certain congenital heart diseases

Q.65. State one biochemical test which will differentiate metabolic and respiratory acidosis.

- Total CO_2 content of blood:
  - In metabolic acidosis—total CO_2 content is decreased.
  - In respiratory acidosis—total CO_2 contents is increased.

Q.66. What are the types of alkalosis?

Alkalosis can be 2 types :

- Metabolic alkalosis
- Respiratory alkalosis

Q.67. What is metabolic alkalosis? What is the other name?

- Metabolic alkalosis results from an absolute or relative increase in $[HCO_3^-]$

$$\frac{HCO_3}{H_2CO_3} = \frac{20}{1} = pH\uparrow$$

- The other name is "Primary alkali excess".

Q.68. Explain the mechanism by which body tries to compensate.

The RC is inhibited by alkalosis causing shallow, irregular breathing.

- The reduced ventilation results in CO_2 retention and increases the carbonic acid level $[H_2CO_3]\uparrow$.
- The ratio of $[HCO_3^-]/[H_2CO_3]$ is restored towards 20:1, as levels of both in blood are increased pH is maintained.

Thus respiratory mechanism is the primary mechanism that tries to compensate.

Q.69 Explain why primary compensatory mechanism cannot last for long.

Decreased ventilation raises $pCO_2 \uparrow$ which tends to stimulate respiratory center (RC). Thus two opposing forces start operating.

- Alkalosis depressing RC ↓ and
- Raised pCO_2 ↑ stimulating the RC start working simultaneously and the respiratory compensation is temporary and incomplete.

Q.70. How does kidneys help in compensation?

Renal mechanisms increases the excretion of:

- Cations ↑ (Na^+)
- $HCO_3^- \uparrow$ (replacing Cl^- in urine). Both due to decrease $H^+ \rightarrow Na^+$ exchange.
- K^+ excretion increases in the distal tubules instead of H^+
- Reduced NH_3 formation ↓ and excretion of non-volatile acids viz lactic acid and ketoacids.

Q.71. State the biochemical changes that occur in uncompensated and fully phase of metabolic acidosis.

- Uncompensated phase is characterized biochemically (plasma/or blood) as follows:
  - Disproportionate increase in $[HCO_3^-] \uparrow$.
  - Increase in $[H_2CO_3] \uparrow$, $pCO_2 \uparrow$
- Increase in total CO_2 content ↑
- Increase in $[HCO_3^-]/[H_2CO_3]$ ratio ↑
  - Increase in pH ↑
- If fully compensated, the CO_2 content is high, but the increase in $[HCO_3^-]$ and $[H_2CO_3]$ are proportionate and the $[HCO_3^-]/[H_2CO_3]$ ratio and pH is maintained within normal limits.

Q.72. Mention some of the can that can produce metabolic alkalosis.

Some important causes that can produce metabolic alkalosis are:

- Excessive loss of HCl
  - Prolonged gastric lavage
  - Pyloric obstruction,
  - High intestinal obstruction
- Alkali ingestion and alkali administration.

- Excessive loss of K^+ leading to K^-deficiency.
- X-ray therapy, uv radiation and radiation therapy.

Q.73. State additional clinical features that may be associated with alkalosis.

In both types of alkalosis, the following additional clinical features may be associated:

- Tetany due to low ionic Ca^{++},
- K^+ depletion-hypokalemia
- Ketosis and ketonuria may develop.
- Kidney damage—degenerative changes in tubules leading to N_2 retention and oliguria may occur.

Q.74. What is respiratory alkalosis? What is the other name?

- Respiratory alkalosis occurs when there is a decrease in $[H_2CO_3]\downarrow$ fraction with no corresponding change in $[HCO_3^-]$ in plasma .

$$= \frac{20}{1}\uparrow = \text{pH}\uparrow \text{ (alkalosis)}$$

- The other name is "Primary carbonic acid deficit."

$$\frac{B.HCO_3}{H_2CO_3}$$

Q.75. How does body compensate in respiratory alkalosis?

Increased loss of CO_2 due to hyperventilation, results in dimination of $[H_2CO_3]\downarrow$. The ratio of $[HCO_3^-]/[H_2CO_3]$ is increased $\uparrow$ i.e. pH is increased and is termed respiratory alkalosis (carbonic acid excess).

- Due to increased CO_2 loss, pCO_2 is low, which leads to less $H^+ \rightarrow$ Na exchange and less bicarbonate is reabsorbed, i.e. more HCO_3^- is excreted by renal tubules and the ratio of $[HCO_3^-]/[H_2CO_3]$ returns towards normal, i.e. 20:1, as levels of both in blood are decreased and pH is maintained.

Q.76. Explain the compensatory mechanisms that operate in respiratory alkalosis.

In respiratory alkalosis, principal compensatory mechanism is renal:

- Excretion of alkali in the form of HCO_3^-.
- Decreased excretion of acid.
- Decreased excretion of NH_3 in the urine.
- Retention of Cl^- in the blood .

Q.77. State the biochemical changes that occur in uncompensated and in fully compensated phase in respiratory alkalosis.

- If uncompensated, it is characterized biochemically (plasma or blood) as follows:
  - Disproportionate decrease in $[H_2CO_3] \downarrow$ and $pCO_2 \downarrow$
  - Decrease in $[HCO_3^-] \downarrow$
  - Decrease in total CO_2 content
  - Increase in $[HCO_3^-]$: $[H_2CO^3]$ ratio $\uparrow$
  - Increase in pH $\uparrow$
- If compensated, the CO_2 content is low, but the decrease in $[HCO_3^-]$ and $[H_2CO_3]$ is proportionate the $[HCO_3^-]$: $[H_2CO_3]$ ratio and pH remaining within normal limits.

Q.78. State the urinary findings in alkalosis

- pH of urine alkaline
- The urinary $NH_3\downarrow$ and titratable acidity $\downarrow$ are both decreased (if kidneys are functioning normally)

Q.79. State one biochemical test which will differentiate metabolic and respiratory alkalosis.

- Measurement of total CO_2 content of blood:
  - In metabolic alkalosis: total CO_2 will be high $\uparrow$
  - In respiratory alkalosis: Total CO_2 content of blood will be decreased $\downarrow$.

Q.80. Enumerate some causes that can produce respiratory alkalosis.

- Stimulation of respiratory center (RC):
  - CNS diseases like meningitis, encephalitis, etc.
  - Salicylate poisoning
  - Hyperpyrexia
- Other causes:
  - Hysteria
  - Apprehensive blood donor
  - High attitude ascending
  - Injudicious use of respirators.
  - Some cases of hepatic coma

Q.81. What is meant by the term "anion gap"?

The "anion gap" is a mathematical approximation of the difference between anions and cations that are routinely measured in serum.

Q.82. What is the normal value of anion gap?

- If the Cl^- and the total CO_2 concentrations are summed up and subtracted from the total of Na^+ and K^+ concentrations, the difference should be less than <17 mEq/L (mmol/L)

Q.83. If the anion gap exceeds 17 mmol/L, what does it indicate?

This usually, indicates significantly increased concentrations of unmeasured anions viz $PO_4^=$, $SO_4^=$, proteins and organic acids.

Q.84. Mention some conditions where anion gap can exceed 17 mmol/L.

- Uremia with retention of fixed acids.
- Ketotic states, e.g. diabetes mellitus, alcoholism, starvation
- Lactic acidosis, e.g. shock and hemorrhage.
- Toxins ingestion, e.g. methanol, salicylates, ethylene glycol.
- Increased plasma proteins, e.g. in dehydration

Q.85. If the anion gap is less than <10 mmol/L, what does it signify?

Decreased anion gap less than 10 mmol/L can result from either:

- An increased in the unmeasured cation viz Ca^{++}, Mg^{++}.
- A decrease in unmeasured anions viz. $PO_4^=$, $SO_4^=$, proteins and organic acids.

Q.86. Mention some conditions in which an increase in unmeasured cations can occur.

- Hyper magnesemia
- Lithium intoxication
- Multiple myeloma
- Polyclonal gammopathy
- Polymyxin B therapy-juice the drug is polycationic.

Q.87. State some conditions in which a decrease in unmeasured anions can occurs.

- Hypoalbuminemia
- Hyponatremia with normal or increased ECF, e.g. SIADH which is supposed to result from the selective renal excretion of unmeasured anions.

Q.88. Mention the clinical use of anion gap.

The anion gap is useful for quality control of laboratory results for Na^+, K^+, Cl^- and total CO_2.

If an increased or decreased anion gap is calculated for a set of electrolytes from a healthy individual, this would indicate that one or more of the laboratory results are erroneous.