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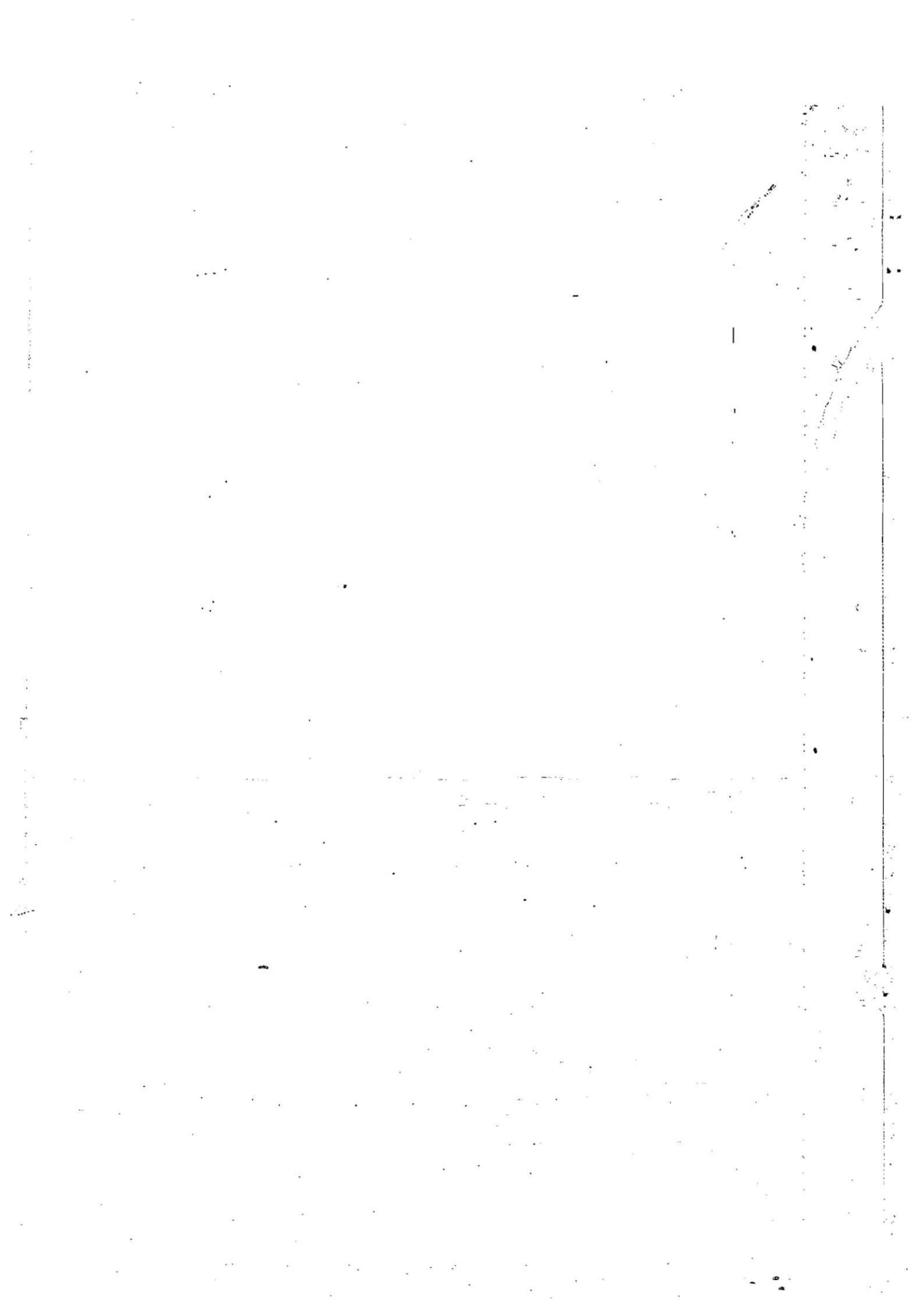
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BIOCHEMISTRY AND CLINICAL PATHOLOGY

IMPORTANT QUESTIONS AND ANSWERS



SYLLABUS

- 1. CARBOHYDRATES**
- 2. PROTEINS**
- 3. LIPIDS**
- 4. VITAMINS**
- 5. ENZYMES**
- 6. ROLE OF MINERALS & WATER IN LIFE PROCESS**
- 7. PATHOLOGY OF BLOOD & URINE**
- 8. METABOLISM**

BIOCHEMISTRY & CLINICAL PATHOLOGY NOTES (340)

CHAPTER-I CARBOHYDATES

Q.1 Define and classify carbohydrates giving suitable examples

Answer- Carbohydrates – Carbohydrate are polyhydroxy aldehyde or ketones or compounds derived from them. The general formula of carbohydrate is $C_x(H_2O)_y$

Classification - Carbohydrates are classified in to four major groups.

1. Monosaccharide's: They contain only one molecule of sugar. Those cannot be broken down into simple substances on hydrolysis.

Example: trioses, pentoses, hexoses

2. Disaccharides: These yield two molecules of monosaccharide's on hydrolysis.

Example: Sucrose, maltose, lactose.

3. Oligosaccharides: These yield two to ten molecules of monosaccharide's on hydrolysis. Disaccharides are come in this group.

Example: Sucrose.

4. Polysaccharides: These yield more than ten molecules of monosaccharide's on hydrolysis.

Example: Starch, glycogen, dextrin, cellulose

a. Homo Polysaccharides: They yield only one type of monosaccharides on hydrolysis.

Example: starch, glycogen

b. Hetero Polysaccharides: They yield more than one type of monosaccharides on hydrolysis.

Example: Heparin.

Q.2 Write a note on Mutarotation.

Answer - The monosaccharide's explain the phenomenon of mutarotation.

'A change in specific rotation' on standing the aqueous solution of sugar is known as mutarotation. When glucose is dissolved in water its specific rotations gradually change until it reaches a constant value. The phenomenon is shown by almost all reducing sugars except few ketones.

For example, when a glucose prepared by crystallization from water below $50^{\circ}C$, is dissolved in water. Its initial specific rotation of $+112^{\circ}$, falls gradually to a constant value of $+52.5^{\circ}$. Similarly when the glucose crystallized from water above $95^{\circ}-98^{\circ}C$ is dissolved in water its initial specific rotation of 19° gradually rises to $+52^{\circ}$ i.e. when either of the solutions is kept for some time the solutions is kept for some time, the rotation gradually changes to $+52.5^{\circ}$ and remains constant. This phenomenon is known as mutarotation and explained by the existence of two optical isomers of glucose

BIOCHEMISTRY & CLINICAL PATHOLOGY NOTES (340)

Q.3 Discuss Glucose Tolerance test.

Answer - Glucose tolerance test is done to know the ability of an individual to respond in maintain blood sugar homeostasis by giving or loading the glucose and the diabetes can be diagnosed the basis of glucose tolerance test.

In this test the person should be supplemental with carbohydrate rich food for at least 3 days prior to the test and person should be avoid the exercise on the previous day of the test; the person should be fastened for overnight.

- | | | |
|--|---|---|
| | β - Glucose
(Sn-rotation = +19°) | β - Glucose
(Sp-rotation = +19°) |
|--|---|---|
- Procedure:**
1. GTT should be conducted preferably in the morning (9-11 am).
 2. A fasting blood sample is drawn and urine is collected.
 3. Person is given 75gms of glucose dissolved in about 300ml of water, to be drunk in about 5 minutes.
 4. Blood and urine sample are collected at intervals of 30, 60, 90, 120 minutes.
 5. The blood sample is analyzed for glucose concentration and urine sample are qualitatively tested for presence of glucose.
 6. The fasting sample of urine is also tested of acetone.

Q.4 Write a note on Diseases related to carbohydrate metabolism.

Answer - The important diseases related to carbohydrate metabolism are:

- Diabetes mellitus (Hyper glycaemia)
- Hypo glycaemia
- Glycosuria

DIABETES MELLITUS (Hyper glycaemia): Diabetes mellitus is a condition caused due to raised glucose level in the blood due to deficiency of insulin.

Symptoms of Diabetes mellitus:

Hyper glycaemia, Glycosuria, Weakness / tiredness, Loss of body weight, Thirst → polydipsia, Dehydration, Breathlessness, Polyuria - ↑ urine output, Reduced visual activity, Pains in legs, Dry skin, cracked lips, Raised pulse, Polyphagia → increase appetite

Cause of Diabetes mellitus:

Genetic factor, Obesity, Dietary intake of sugar is excess, Increases rate of glucose absorption from intestine, Decreased level of insulin.

Treatment:

- Use of oral antidiabetic agents. E.g. metformin, glipizide
- Use of insulin in the form of injection.
- High protein and low carbohydrate and fat

BIOCHEMISTRY & CLINICAL PATHOLOGY NOTES (340)

2. **GLYCOSURIA:** The condition which abnormal quantities glucose found in urine. It can be classified as follows:

I. Alimentary Glycosuria: Glucose may appear in urine temporarily due to high intake of carbohydrate, without any defect in blood glucose regulatory mechanism.

II. Renal Glycosuria: In this there is a defect in tubular mechanism that leads to glucose excretion in urine.

III. Diabetic Glycosuria: This type of Glycosuria occurs in diabetes mellitus due to hyperglycemia resulting from insulin deficiency.

3. **HYPOGLYCEMIA:** It is condition in which the blood sugar level is below 60mg/dl. The symptoms of hypoglycemia are feeling of hunger, headache, and lack of concentration tremors, sweating convulsion, coma and death. The cause may be:

- Increase production of insulin due to tumors in pancreas.
- Liver damage caused by liver poisons.

Q.5 Describe any four quantitative tests for carbohydrates

1. **Answer - Molisch's test (General test):** To 2-3 ml of aqueous test solution, add few drops of Molisch's reagent, (α -Naphthol in alcohol) Shake, and add few drops of Conc. Sulfuric acid from the side of test tube without shaking. Violet to purple colored ring at the junction of two liquid produced, presence of carbohydrates.

Principle: In this test carbohydrate react with strong mineral acid like conc. concentrated sulphuric acid to produce furfural compound, in 2nd step interact with α -Naphthol to produce violet to purple colour conjugate because of alcoholic α -Naphthol and strong mineral acid are not miscible with each other.

2. **Benedict's test:** Mix equal volume of Benedict's reagent and test solution in test tube. Heat on a boiling water bath for 5min. Solution appears Green, yellow, red or brick red depending upon amount of reducing sugars present in test solution.

Principle: This test is positive for all reducing sugars, benedict's reagent contain cupric ions (cupric sulphate) in alkaline medium and different chelating agents to keep the cupric ions in solution. When sugar is heated with Benedict's reagent the cupric ions reduced to cuprous ions. Which may be green, yellow, orange or red in colour produced, depending upon the concentration of sugar in the solution.

3. **Fehling's test:** Mix each 1ml of Fehling's A and B solutions, and add equal volume of test solution. Heat it on a boiling water bath for 5 min. First yellow then brick red precipitate observed Indicates the presence of reducing sugars.

Principle: The Fehling's solution is composed of copper sulphate, sodium-potassium tartarate, and sodium hydroxide. Carbohydrates with free aldehyde and ketone group having ability to reduce copper sulphate to cuprous oxide forming a yellow or brick red colored precipitate.

BIOCHEMISTRY & CLINICAL PATHOLOGY NOTES (340)

4. **Barfoed's test:** Mix equal volume of Barfoed's reagent and test solution in test tube. Heat it for 1-2 min on boiling water bath. Brick red precipitate. At the bottom of test tube if red cupric oxide is formed, monosaccharide is present. Disaccharide on prolong heating (about 10min) may also reduction, owing to partial hydrolysis to monosaccharide's.

Principle: Barfoed's reagent, cupric acetate in acetic acid is slightly acidic and is balanced so that it can only be reduced by monosaccharides but not less powerful reducing sugars. Disaccharides may also react with this reagent, but the reaction is much slower when compared to monosaccharides. Perform this test with glucose, maltose and sucrose.

5. **Tollen's test/silver mirror test:** Add few drops of freshly prepared Tollen's reagent to 2 ml of aqueous solution of drug and 2-3 drops of sodium hydroxide solution in clean test tube and heat gently. Formation of black mirror on the side wall of test tube indicates the presence of aldehydic group.

Principle: The sugar is heated with Tollen's reagent (Ammonical silver nitrate). If the sugar has a free aldehyde or ketone group, it changes to enediol which then reduces AgNO_3 to metallic silver precipitating it in the form of a shining mirror.

6. **Seliwanoff's test (resorcinol test for ketones.):**

To dilute aqueous solution add crystals of resorcinol and equal volume of concentrated hydrochloric acid and heat on a water bath. Formation of cherry red colour or reddish brown colour indicates presence of ketoses like fructose or sucrose.

Principle: Seliwanoff's test distinguishes between aldose and ketose sugars. Ketoses are distinguished from aldoses via their ketone/aldehyde functionality. If the sugar contains a ketone group, it is a ketose and if it contains an aldehyde group, it is an aldose. This test is based on the fact that, when heated, ketoses are more rapidly dehydrated than aldoses.

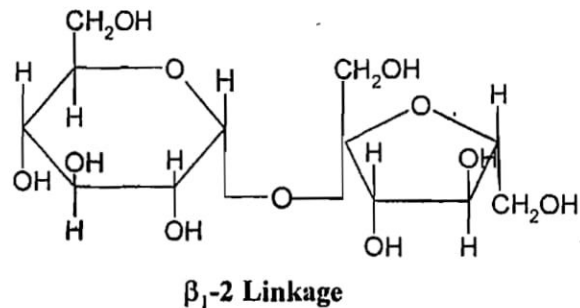
7. **Osazone formation test:**

To 0.5 g of phenyl hydrazine hydrochloride add 0.1 g of sodium acetate and 10 drops of glacial acetic acid. To this mixture add 5 ml of test solution and heat on a boiling water bath for about 30 min. Allow the tube to cool slowly and examine the crystals under a microscope. Glucose, fructose and mannose produce needle-shaped yellow osazone crystals, whereas lactosazones are balls of prickles shaped. Different osazones show crystals of different shapes. Maltose produces sunflower-shaped crystals. The ketoses and aldoses react with phenyl hydrazine to produce a phenyl hydrazine which in turn reacts with another two molecules of phenyl hydrazine to form the osazone.

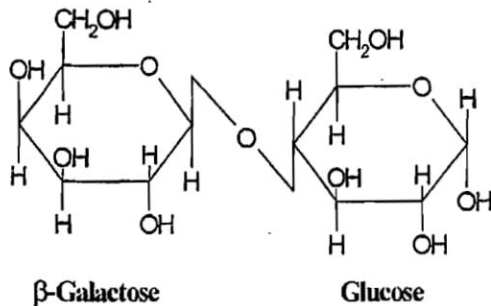
Principle: For osazone test, the reaction mixture should be between pH 5 and 6. Fructose takes 2 min to form the osazone whereas for glucose it is 5 min. The disaccharides take a longer time to form osazones. Disaccharides form crystals only on cooling

BIOCHEMISTRY & CLINICAL PATHOLOGY NOTES (340)

Structure of Lactose



Structure of Sucrose



Q.6 Write a note on the role of carbohydrates.

Answer - Functions or biological role or Importance of carbohydrates:

1. Carbohydrates work as a main source of energy.
2. Glycogen is a reservoir of energy, where glycogen is stored in the liver and skeletal muscle.
3. Heparin is a naturally occurring anticoagulant that prevents blood coagulation.
4. Gluconic acid acts as a detoxifying agent by forming a complex with toxic substances.
5. Hyaluronic acid acts as a lubricant.
6. Ribose is useful for nucleic acid synthesis.
7. Galactose is necessary for forming lactose in the milk of gluco-proteins.
8. Starch is an important food source for humans.

BIOCHEMISTRY & CLINICAL PATHOLOGY NOTES (340)

CHAPTER-2 PROTEINS

Q.1 Define and classify proteins with examples.

Answer - Proteins are high molecular weight polyhydroxy peptides containing alpha amino acids joined together by peptide linkage (bond) C—CO—Na. They contain —C, H, N and S sometimes phosphor also.

The molecular weight may range from 6000 to many millions. Proteins are made up of amino acids which from the fundamental units so the properties and behavior of proteins depend on the amino acids present.

Classification: Proteins are classified into the following groups:

1. **Simple Proteins:** Examples: Albumins, Globulins, Prolamines, Histamines
2. **Conjugated Proteins:** Examples: Nucleo proteins, Phospho proteins, Glycoproteins, Lipoproteins, Flavoproteins, Metalloproteins
3. **Derived Proteins:**
 - (I) Primary derived proteins: e.g. Fibrin, from fibrinogen, Meta proteins.
 - (II) Secondary proteins: e.g. Proteoses, peptones, Peptides.

1. SIMPLE PROTEINS:

Simple proteins are those proteins which yield only one amino acid on hydrolysis.

(a) Albumin:

1. Soluble in water
2. Coagulated by heat
3. Precipitate at high salt concentration.

Example: Serum albumin, Egg albumin, Plasma albumin

(b) Globulin:

1. Insoluble in water
2. Coagulated by heat
3. Precipitate by half saturated salt solutions.

Example: Plasma globulin Serum globulin

(c) Glutelins:

1. Insoluble in water
2. Soluble in acids and acid base
3. Coagulated by heat

Example: Glutelin of wheat, oryzenin of rice

(d) Prolamines:

1. Insoluble in water,
2. Soluble in ethanol
3. Not Coagulated

Example: Gliadin of wheat, Zenin of maize

(e) Prolamines:

1. Soluble in water,
2. Not coagulated by heat

Example: Salmine and cryptinine in the sperm of certain fish

BIOCHEMISTRY & CLINICAL PATHOLOGY NOTES (340)

2. CONJUGATED PROTEINS: These contain simple proteins molecules united with non-protein group called prosthetic group and on hydrolysis they yield other non-proteinous substance in addition to amino acid on the basis of prosthetic group the conjugated proteins are classified as follows.

(a) **Nucleo proteins:** Proteins with nucleic acid as prosthetic group.
Examples: Nucleo protamines and Nucleo histamines.

(b) **Lipoproteins:** Proteins with lipids (like fatty acids phospho lipids, cholesterol).
E.g. Lipoprotein of blood

(c) **Glycoproteins:** Proteins with carbohydrates as prosthetic group.
E.g. Mucin of saliva, Ovomuroid of egg yolk

(d) **Phospho proteins:** Proteins with phosphorous radical as prosthetic group.
E.g. Casein of milk, Vitellin of egg

(e) **Metalloproteins:** Proteins with metal ions as the prosthetic group (i.e. Zn^{++} , Co^{++} , Fe^{++} , Cu^{++} , Mg^{++} , Mn^{++} etc) E.g. Ferritin which contains iron (Fe^{++}), Ceruloplasmin which contains (Cu^{++})

(f) **Chromo proteins:** Proteins with colored substance like porphyrin as the prosthetic group.
E.g. Haemoglobin, Cytochrome

3. DERIVED PROTEINS: Then derived proteins are *intermediate hydrolytic* products which are formed by the action of physical chain, *chemical or enzymatic agents* on natural proteins.

(I) **Primary derived proteins:** They are formed by the action of heat, acid, and alkalis on proteins denaturation occurs without hydrolytic cleavage of the protein molecule.

(a) **Pröteans:** e.g. Fibrin from fibrinogen

(b) **Meta proteins:** e.g. Acid alkalis from meta protein

(c) **Coagulated proteins:** e.g. Cooked proteins

(II) **Secondary proteins:** They are formed by hydrolytic cleavage of proteins at their peptide linkage.

(a) **Proteoses:** e.g. Albumose, globumose

(b) **Peptides:** e.g. Glycyl-alanine, Leucyl-glutamic acid

Q.2 Write a note on structure of proteins.

Answer - Structure of Proteins: - The structure of proteins can be studied as follows.

Primary structure

Secondary structure

Tertiary structure

Quaternary structure

1. **Primary structure:** Primary structure of protein means simply a polypeptide chain (Peptide bond). The peptide bond occurs between the carboxyl ($COOH$). Group of one amino acid amino group (NH_2) another.

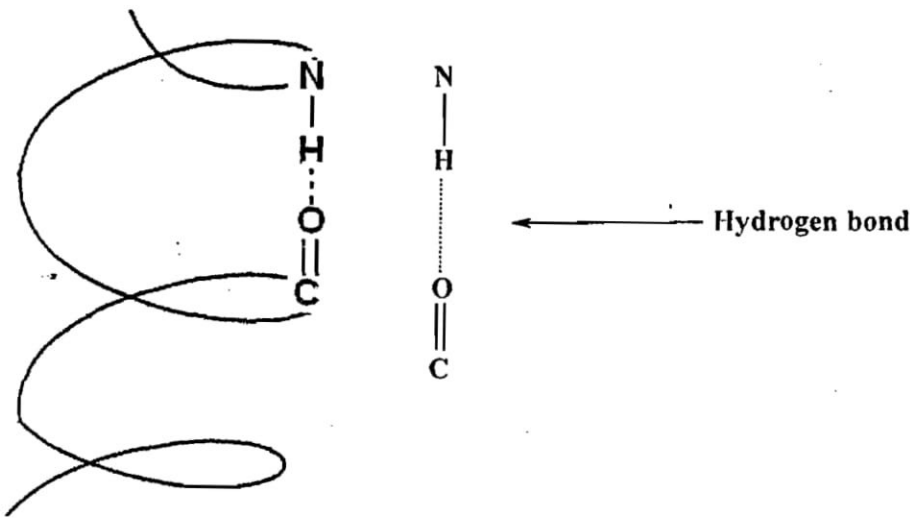


BIOCHEMISTRY & CLINICAL PATHOLOGY NOTES (340)

2. Secondary structure: Secondary structure refers to the coiling of the polypeptide chain into a helical amino structure. In this structure there is hydrogen bonding between amino group and carbonyl oxygen of peptide bond i.e. O of CO is linked by H to N of NH hydrogen bond makes helix. This is called as secondary structure.

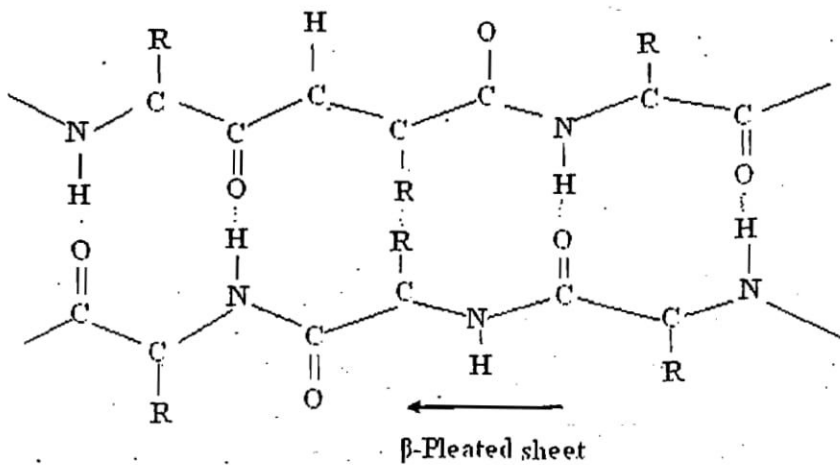
There are two types of Secondary structure are possible.

1. α -helix structure: In this case the polypeptide chain twists into a right hand side, so that hydrogen bond is formed between C = O and NH.



2. β - Pleated sheet: In this structure a number of polypeptide chains occurs side by side (in an extended state instead of coiled state). The polypeptide chains are linked by hydrogen bond which occurs between

C = O and N—H group of adjacent polypeptide chains.



BIOCHEMISTRY & CLINICAL PATHOLOGY NOTES (340)

3. Tertiary structure: Tertiary structure refers to the folding of protein chain to form a tight and compact three dimensional structure. It is stable final structural form which decides biological and functional properties of that particular protein. Different types of bonds involved in the formation of stable tertiary structure of proteins are as follows.

Vander waals, Electrostatic forces, Hydrogen bond, Hydrophobic bond, Disulphide bonds

3. **Quaternary structure:** Two or more polypeptide chains bind to give raises the quaternary structure. This binding may be brought by hydrophobic interaction, hydrogen bonds and electrostatic bonds. E.g. Proteins such as hemoglobin this consist of more than one polypeptide chain.

Q.3 Explain Denaturation of proteins

Answer - Denaturation of proteins: The breach down or disruption of secondary, tertiary and quaternary structure of protein resulting in the alterations of the physical, chemical and biological characteristic of protein by a variety of agents such as.

1. Physical agents: e.g. Heat, U.V. light, X-rays
2. Chemical agents: e.g. Acids, alkalis, Heavy metals, Salt

Changes occur during denaturation of proteins are.

1. Physical changes:

- (a) Solubility decreases
- (b) Denaturation of proteins cannot be crystallized.

2. **Chemical change:** Denaturation by chemical agents leads to splitting of linkages hydrogen bond and disulfide bond in the protein molecule. These leads to unfolding and uncoiling of peptide chain.

3. **Biological change:** Denaturation decreases the activity of enzymatic and hormonal.

Significance:

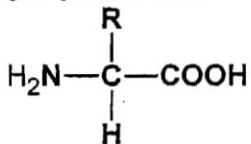
Precipitation of proteins by denaturation helps in clinical laboratory.

Blood and serum samples are easily analyzed by removing protein fraction.

Q.4 Write a note on Isoelectric point

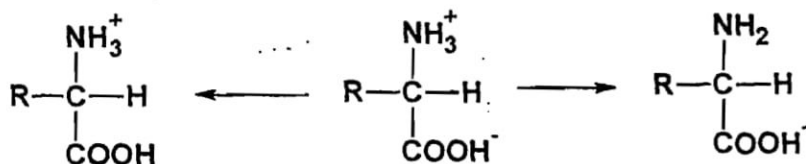
Answer - Isoelectric point (pH) or zwitterion: The properties of proteins are electrolytes are determined by the ionizable groups in the molecule.

As each protein chain one free —NH_2 group (Amine group) i.e. basic and one free (—COOH) group i.e. acidic.



The amine group NH_2 can accept proton (H^+) and form cation (NH_3^+). The carboxyl group (—COOH) can donate H^+ and form anion (COO^-) so at acidic pH. The amino acids are positively charged at intermediate pH the change is zero since it carries both +ve and -ve changes. This pH is called isoelectric pH. At the isoelectric pH the proteins (amino acids) exists as zwitterion which carries equal number of +ve and -ve charges.

BIOCHEMISTRY & CLINICAL PATHOLOGY NOTES (340)



Q.5 Explain protein deficiency diseases

Answer - Deficiency of proteins in diet produces two important diseases in children, they are.

1. Kwashiorkor, (2) Marasmus

1. Kwashiorkor: It is a protein deficiency disease occurring mainly in the children when they change from breast feeding to a diet low in protein.

Symptoms:

Retarded growth and generalized edema.

Skin changes like pigmentation, ticking cracks and laceration.

Enlargement of liver.

Vomiting and diarrhea

2. Marasmus: It is the protein deficiency disease commonly found in infants below 1 year of age. It occurs due to a diet very low in protein and calories it occurs due to early termination of breast feeding.

Symptoms:

Retard growth, child is very thin

Complete loss of body fat.

Head is large and limbs are thin

Diarrhoea and dehydration

Alteration in pigmentation of skin and hair.

Q.6 Give any four colour reactions of proteins.

Answer -

Biuret test:(General test): - To 3ml of test solution, add few drops of 4% NaOH and few drops of 1% copper sulphate solution. Violet or deep violet colour appears, Presence of proteins.

Principle: This test is characteristic of peptide linkage present in protein molecule. All proteins contain peptide linkages so this is test positive for all proteins it serves as the generalized test for all identification of proteins. This test is also positive for substance contain (-CO-NH₂) group. (Proteins, proteoses, peptones and polypeptides) The colour of the biuret reaction varies from blue to violet depending upon the no of peptide groups present in the protein molecule.

Million's test: Mix 3ml of test solution with few ml of Million's reagent. White precipitate, warm precipitate turns to brick red or the precipitate dissolves giving red colored solution.

Principle: Million's test is given by protein containing amino acid (tyrosine) with acidified mercuric sulphate forms red phenolic complex of the mercury to produce specific red colour or red colored precipitate.

BIOCHEMISTRY & CLINICAL PATHOLOGY NOTES (340)

Ninhydrine test: To the test solution, add 0.5ml of ninhydrine solution on boiling water bath for 2min and cool. Violet colour indicates presence of amino acid.

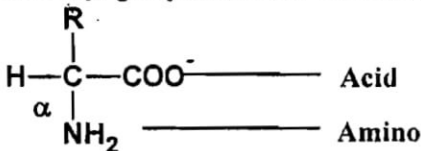
Principle: This test is characteristic of free amino group present in protein molecule and so it is also given by all proteins. In this test ninhydrine is reduced by free amino group of protein form a complex blue colored compound.

Xanthoproteic test: (Test for protein containing tyrosine or tryptophan); Mix 5ml of test solution with 2-3ml of conc. nitric acid and boil. Yellow precipitate is formed. After cooling it, add 40% sodium hydroxide solution. Deep yellow or orange colour is produced.

Principle: The proteins composed of amino acids containing aromatic benzene ring like tyrosine, phenyl alanine; tryptophan gives this colored reaction positive. In this test aromatic group is converted into yellow colored aromatic nitro derivative on treatment with conc. nitric acid further addition of NaOH solution till alkaline changes yellow colour to orange coloration due to ionisation.

Q.7 Define & classify Amino acids with suitable examples.

Answer - Amino acids are the compounds containing one or more amine group and one or more carboxyl group in the same molecule generally amino acids can be represented by the structure.



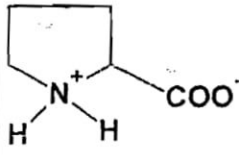
Classification of amino acids:

Amino acids are classified into three groups. (I) Natural, (II) Acidic, (III) Basic

I. Natural amino acids:

A. Aliphatic amino acids:			
Sl. no	Name	Abbreviation	R-group
1	Glycin	Gly	—H
2	Alanine	Ala	—CH ₃
3	Serine	Ser	—CH ₂ —OH
4	Threonine	Thr	$ \begin{array}{c} \text{HO} \\ \\ \text{---CH---CH}_3 \end{array} $
5	Valine	Val	$ \begin{array}{c} \text{CH}_3 \\ \\ \text{---C---H} \\ \\ \text{CH}_3 \end{array} $

BIOCHEMISTRY & CLINICAL PATHOLOGY NOTES (340)

6	Leucine	Leu	$\begin{array}{c} \text{CH}_3 \\ \\ -\text{CH}_2-\text{C}-\text{H} \\ \\ \text{CH}_3 \end{array}$
7	Isoleucine	Ile	$\begin{array}{c} \text{CH}_2 \\ \\ -\text{C}-\text{H} \\ \\ \text{CH}_3 \end{array}$
B. Aromatic Amino Acids:			
1	Phenyl alanine	Phe	$-\text{CH}_2-\text{C}_6\text{H}_5$
2	Tyrosine	Tyr	$\text{CH}_2-\text{C}_6\text{H}_4-\text{OH}$
3	Tryptophan	trp	$\text{CH}_2-\text{C}_8\text{H}_6\text{N}$
C. Heterocyclic amino acids (Imino acids)			
1	Proline	Pro	
D. Sulphur containing amino acids			
1	Cysteine	Cys	$-\text{CH}_2-\text{SH}$
2	Methionine	Met	$-\text{CH}_2-\text{CH}_2-\text{S}-\text{CH}_3$
3	Cystine		$\begin{array}{c} -\text{CH}_2-\text{S} \\ \\ -\text{H}_2-\text{S} \end{array}$

BIOCHEMISTRY & CLINICAL PATHOLOGY NOTES (340)

II. Acidic amino acids				
1	Aspartic acid	Asp	$-\text{CH}_2-\text{COOH}-$	Carboxyl
2	Asparagine	Asn	$-\text{CH}_2-\text{CO}-\text{NH}_2$	Amide
3	Glutamic acid		$\text{CH}_2-\text{CH}_2-\text{COOH}$	Carboxyl
4	Glutamine	Gln	$-\text{CH}_2-\text{CH}_2-\text{CONH}_2$	Amide
III. Basic amino acids				
1	Arginine	Arg	$ \begin{array}{c} -\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{NH} \\ \\ \text{C}=\text{NH} \\ \\ \text{NH}_2 \end{array} $	Guanidino
2	Lysine	Lys	$\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{NH}_2$	Amino
3	Histidine	His	$ \begin{array}{c} -\text{H}_2\text{C} \\ \\ \text{HN} \quad \text{N} \\ \diagdown \quad / \\ \text{C} \\ \\ \text{C} \\ / \quad \backslash \\ \text{N} \quad \text{NH} \\ \\ \text{H} \end{array} $	Imidazole
			$ \begin{array}{c} -\text{CH}_2-\text{C}=\text{CH}_2 \\ \quad \\ \text{N} \quad \text{NH} \\ \\ \text{C} \\ \\ \text{H} \end{array} $	

Q.8 Write the biological importance of Amino Acids and proteins

Answer - Biological importance of amino acids:

- (1) Amino acids are joined with each other by peptide bonds form protein and peptide.
- (2) Some amino acids like glycine and alanine are converted to carbohydrates in the body.
- (3) Specific amino acids can give rise to specialized biological products in body.
 - e.g. Tyrosine gives rise to adrenaline, Histidine gives rise to histamine
 - Certain amino acids are necessary for detoxification of toxic substances.
 - E.g. glycine and cysteine
 - Amino acids are required for synthesis of various enzyme hormones, plasma proteins and immunoglobulins.

Biological value of proteins: The quality of a protein is measured by its ability to supply essential amino acids. This is expressed as biological value (BV).
 Biological value is defined as the percentage of absorbed nitrogen which is retained in the body. it can be estimated as follows.

BIOCHEMISTRY & CLINICAL PATHOLOGY NOTES (340)

1. First the animals are kept on a protein free diet for two days. Then the following estimation is done.

- (a) Fecal N_2 is estimated to obtain the amount of metabolic fecal N_2 .
- (b) Urinary N_2 is estimate to obtain the amount of endogenous urinary N_2

2. Secondary the animal is feeded with a known or measured amount of test protein the fecal and urinary N_2 is determined.

B.V calculated as follows:

$$BV = \frac{(N_2 \text{ intake} - N_2 \text{ in faces}) - N_2 \text{ in urine}}{N_2 \text{ intake} - N_2 \text{ in face}}$$

Q.9 Write a note on essential amino acids & non essential amino acids

Answer - Essential amino acids (EAA): Essential amino acids are not supplied through diet. There are eight essential amino acids. Adequate amount of EAA are required to maintain nitrogen balance otherwise the deficiency may cause nervous breakdown, inhibition of full mental growth and even death in young with complete deficiency.

e.g. casein of milk contains all these essential amino acids, so it is a complete protein. Gliadin of maize lacks in tryptophan so it is not complete protein.

2. Non-essential amino acids (NEAA): NEAA are synthesizing in the body and hence it is not important component of diet. These are synthesizing from lipids, carbohydrate metabolism. They are twelve NEAA

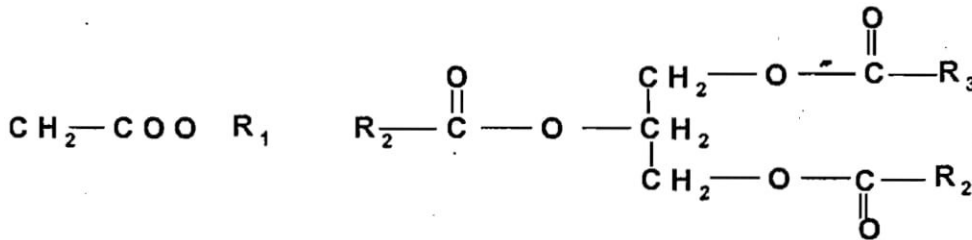
CHAPTER – 3

LIPIDS

Q.1 Define & classify lipids with examples

Answer - Lipids are heterogeneous group of organic compounds related to fatty acids or are substances capable of forming such ester and are utilized by the living organism. Lipids are insoluble in water. Soluble in inorganic solvents, (like alcohol, ether etc)

General structure:



Classification of lipids:

Lipids are classified as follows:

1. **Simple lipids:**
 - (a) Oils and fat
 - (b) Waxes
2. **Compound lipids:**
 - (a) Phospho lipids: e.g. Lecithins, cephalins, plasmalogens
 - (b) Glycolipids: e.g. Cerebrosides, Gangliosides
 - (c) Other compound lipids: Lipoproteins, Sulpholipids, Aminolipids
3. **Derived lipids:**

E.g. Fatty acids, Glycerols, Sterols, Prostaglandins, Sphingolipids

Q.2 Define following: (a) Iodine number, (b) Saponification number, (c) Acid number

Answer -

- **Saponification number (Saponification value):** It is defined as the number of milligrams of KOH or NaOH is required to saponify 1gm of oil or fat. Saponification number is determined by Saponification and titration of excess of alkali with fat or oil.

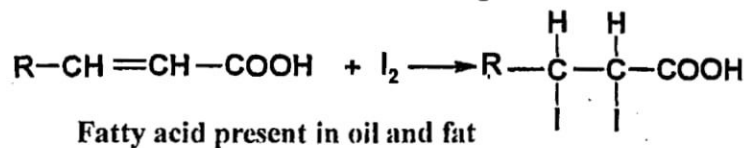
Sl. No	Fat or oil	Saponification value
1	Butter fat	210-230 milligrams
2	Human fat	195-200 milligrams

BIOCHEMISTRY & CLINICAL PATHOLOGY NOTES (340)

Significance:

- High Saponification value indicates low molecular weight fatty acid and vice versa.
- It tells about the content of free or bound fatty acids in a given oil or fat

2. **Iodine number (Iodine value):** It is defined as number of grams of iodine absorbed by 100gm of fat or oil. In actual reaction iodine is added to C=C of unsaturated fatty acids present in oils or fats. So unsaturation is removed i.e. C=C the halogenation reaction occurs as below.



Iodine value of some oil or fats:

1. Butter fat: 26 to 28gm
2. Human fat: 65 to 70 gm

Significance:

- It shows degree of unsaturation in oil and fat.
- Lower the iodine number less is the degree of unsaturation.

3. **Acid number (Acid value):** It is defined as number of milligrams of KOH required to neutralize 1gm of fat or oil.

Significance:

1. It gives an idea about rancidity of oil or fat.
2. It represents amount of free fatty acid.
3. It helps to judge the quantity of given fat or oil. i.e. higher the acid value more is the rancidity.

Q.3 Explain rancidity

Answer - Rancidity of fat and oils: When oils and fats are exposed to light, air, heat and moisture for a longer period of time resulting in an unpleasant odour and taste thus oil or fat is said to be rancid this phenomenon is called as rancidification.

1. The bad and objectionable odor is because of liberation of volatile oil, fatty acids like butyric acids caproic acid capric acid.
2. Rancid oils show high acid types.

Rancidity is of two types.

(a) **Hydrolytic rancidity:** Some oils and fats undergo hydrolysis and produce bad odour is called as hydrolytic rancidity.

(b) **Oxidative rancidity:** If the rancidity caused due to oxidation of double bonds in fats and oils.

Note - Rancidity can be prevented by addition of antioxidants like Vitamin-E, ascorbic acid

BIOCHEMISTRY & CLINICAL PATHOLOGY NOTES (340)

Q.4 Write a note on essential fatty acids.

Answer - Essential fatty acid: They are poly unsaturated fatty acids which not synthesized in the body but can be supplied through diet hence they are called as essential fatty acids. They are represented by the general formula $-C_n H_{2n-1} COOH$

These are characterized by the presence of one or more double bond in the molecule. Because of the presence of double bonds the unsaturated fatty acids are much more reactive than the saturated fatty acid the reactivity increases with increasing number of double bond.

The examples of essential fatty acids (unsaturated fatty acids):

- (1) Linoleic acid: Two double bond
- (2) Linoliric acid: Three double bond
- (3) Oleic acid: Two double bond
- 4) Palmitoleic acid:
- (5) Arachidonic acid: Four double bond

Q.5 Write a note on qualitative test for lipids

Answer - QUALITATIVE TESTS FOR LIPIDS:

1. **Grease spot test:** A drop of oil is applied over a piece of ordinary filter paper. It forms a translucent spot on paper indicates the presence of fixed oils.
2. **Specific gravity:** Take 5ml of water in a beaker and add a drop of oil. The oil will float on water because of lesser specific gravity.
3. **Solubility:** To oil or fat add benzene, chloroform, ether alcohol it is soluble. Fat or oil with water it is insoluble.
4. **Emulsification test:** 2ml of alcoholic solution of fat is mixed with 2ml of dilute bile salt solution in a test tube. It forms a stable emulsion indicates the presence of lipids.
5. **Saponification test:** 10 drops of given oil, 20 drops of 40% KOH and 2ml of glycerin are mixed in test tube. The mixture is boiled for 3 minute till saponification occurs. The saponified solution is divided into two parts.
 - To the one test tube add saturated solution of nail. This leads to separation of soaps which floats on the surface.
 - To other test tube a few drops of $CaCl_2$ solution. A white precipitate of calcium salt of fatty acid is formed.

DISEASES RELATED TO LIPIDS METABOLISM:

- | | |
|-----------------|---------------------------|
| (1) Obesity | (4) Arthrosclerosis |
| (2) Fatty liver | (5) Hyper lipoproteinemia |
| (3) Ketosis | (6) Hyper proteinemia |