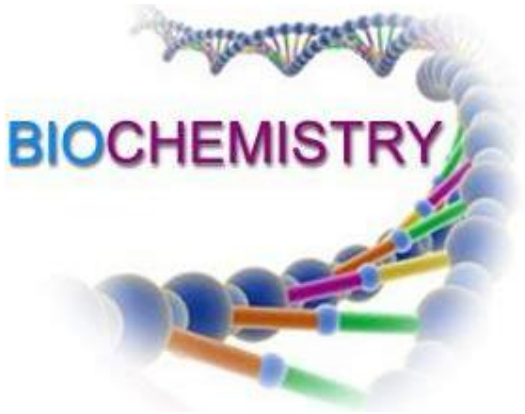
H.S.C (VOCATIONAL)

MEDICAL LABORATORY TECHNICIAN

STD: XII (PAPER-3)

Biochemistry

Theory



INDEX

Chapter 1
Elementary knowledge of Organic Chemistry

Objectives – *At the end of the lesson the student will be able to-*

1. *Understand the basics of organic chemistry*
2. *Know some of the important organic compounds.*

1.1 Introduction

Organic chemistry is a chemistry of carbon compounds. All organic compounds contain carbon in combination with one or more elements. The hydrocarbons contain only carbon and hydrogen. A large number of compounds contain carbon, hydrogen, and oxygen and are considered to be the major elements. Minor elements contain nitrogen, phosphorus, and sulfur, and sometimes halogens and metals.

1.2 Properties of Organic compounds

1. Organic compounds are usually combustible.
2. Organic compounds, in general, have lower melting and boiling points.
3. Organic compounds are usually less soluble in water.
4. Several organic compounds may exist for a given formula. This is known as isomerism.
5. Reactions of organic compounds are usually molecular rather than ionic. As a result, they are often quite slow.
6. The molecular weights of organic compounds may be very high, often well over 1000.
7. Most organic compounds can serve as a source of food for bacteria.

1.3 Sources

Organic compounds are derived from three sources:

1. Natural: Fibers, vegetable oils, animal oils and fats, alkaloids, cellulose, starch, sugars, and so on.
2. Synthetic: A wide variety of compounds and materials prepared by manufacturing processes.
3. Fermentation: Alcohols, acetone, glycerol, antibiotics, acids, and the like are derived by the action of microorganisms upon organic matter.

1.4 The Carbon Atom

Carbon is a tetravalent compound and the four valences are directed towards the four corners of an imaginary tetrahedron. The angle between any two valences is 109.5^0

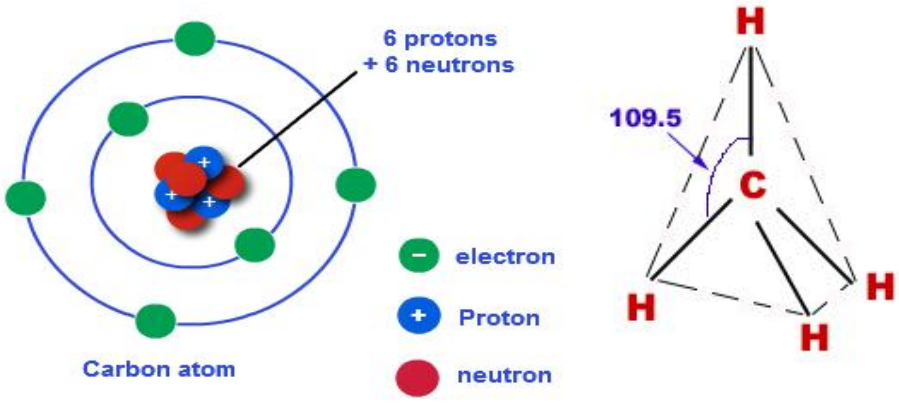


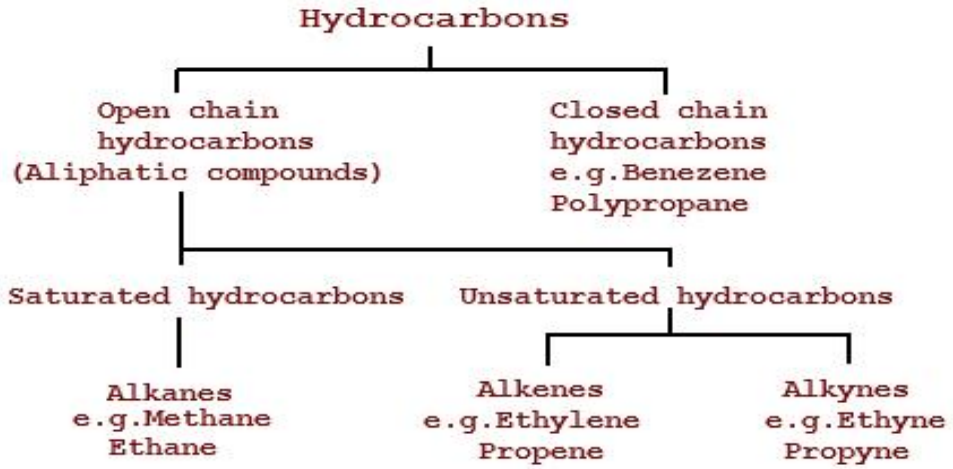
Fig. 1.1 Carbon atom

Carbon atom has four covalent bonds (four electrons to share). This factor alone allows many possibilities, but the most important thing is the ability of carbon atoms to link with one another with covalent bonds which forms a continuous open chain, or a chain with branches or in a ring, or in chains or rings containing other elements.

1.5 Hydrocarbons

The hydrocarbons are compounds of carbon and hydrogen. They are of two types, saturated and unsaturated.

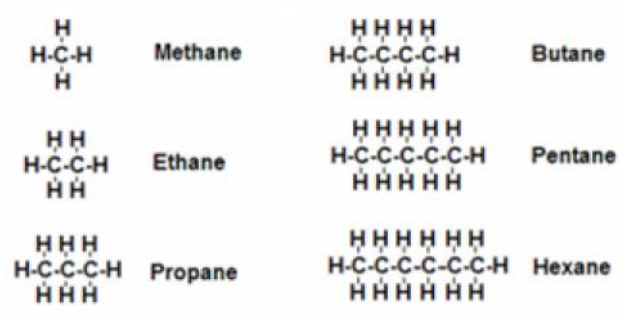
- Saturated hydrocarbons are those in which adjacent carbon atoms are joined with one another by single covalent bond
- Unsaturated hydrocarbons have at least two carbon atoms that are joined by more than one covalent bond. (Double or Triple)



1.5a Saturated Hydrocarbons

Alkanes - Alkanes are the simplest and least reactive, organic molecules, consisting of only carbon and hydrogen and with only single bonds between carbon atoms. Alkanes have the general formula C_nH_{2n+2}. The saturated hydrocarbons form a whole series of compounds starting with one carbon atom and increasing one carbon atom, stepwise. The simplest alkane is Methane.

Following are few examples of alkanes.



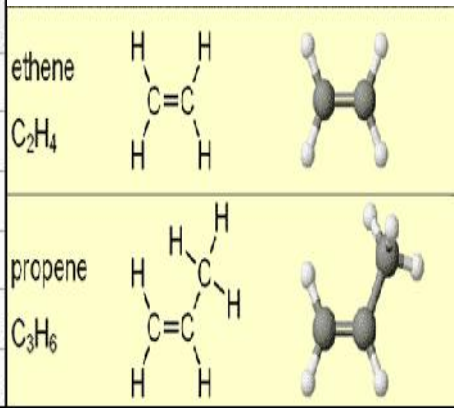
Unsaturated Hydrocarbons

The unsaturated hydrocarbons are usually separated as –

Alkenes - Each member of the alkane group except methane can lose hydrogen to form an unsaturated compound or alkene. Alkenes are aliphatic hydrocarbons containing carbon-carbon double bonds and general formula $\mathbf{C_nH_{2n.}}$The alkenes contain one double bond between two adjacent carbon atoms. Alkenes are named as if they were alkanes, but the "-ane" suffix is changed to "-ene"

Following table shows some Alkenes –

Some example alkenes		
Name	**Formula**	C_nH_{2n}
Ethene	$CH_2=CH_2$	C_2H_4
Propene	$CH_2=CH–CH_3$	C_3H_6
1-Butene	$CH_2=CH–CH_2–CH_3$	C_4H_8
2-Butene	$CH_3–CH=CH–CH_3$	C_4H_8
1-Pentene	$CH_2=CH–(CH_2)_2–CH_3$	C_5H_{10}
1-Hexene	$CH_2=CH–(CH_2)_3–CH_3$	C_6H_{12}



Alkynes - The alkynes have a triple bond between adjacent carbon atoms. They exhibit neither geometric nor optical isomerism. The simplest alkyne is ethyne (HCCH), commonly known as acetylene.

Table showing Alkynes -

No. of C-Atoms	Molecular formula	Structure formula	Common name	IUPAC name
2	C_2H_2	HC≡CH	Acetylene	Ethyne
3	C_3H_4	CH_3— C≡CH	Methyl acetylene (or allylene)	Propyne
4	C_4H_6	CH_3—C≡C—CH_3	Dimethyl acetylene (crotonylene)	Butyne-2

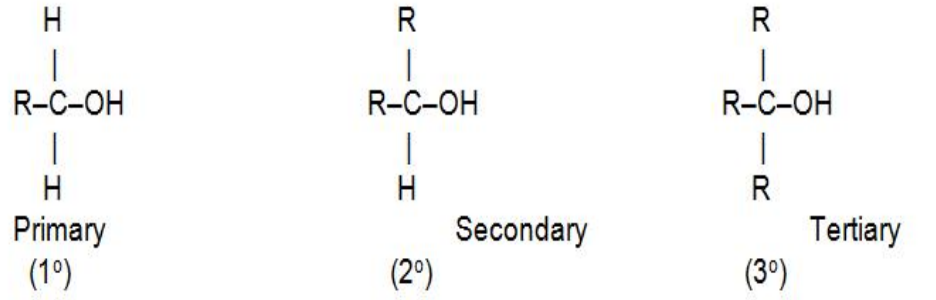
1.6 Some of the commonly used organic compounds -

1. **ALCOHOL**

Alcohols are the family of compounds that contain one or more hydroxyl (-OH) groups attached to a single bonded alkane. Alcohols are represented by the general formula -OH. It is any organic compound in which the hydroxyl functional group (-OH) is bound to a saturated carbon atom. Alcohols are important in organic chemistry because they can be converted to and from many other types of compounds. A common source for producing alcohols is from carbonyl compounds. An alcohol is often called with the name of the corresponding alkyl group followed by the word "alcohol", e.g., methyl alcohol, ethyl alcohol. Propyl alcohol may be *n*-propyl alcohol or isopropyl alcohol, depending on whether the hydroxyl group is bonded to the end or middle carbon on the straight propane chain. Simple alcohols, in particular ethanol and methanol, possess denaturing and inert rendering properties, leading to their use as anti-microbial agents in medicine, pharmacy, and industry.

Alcohols are then classified into different groups based upon the number of carbon atoms connected to the carbon atom that bears the hydroxyl functional group.

a) **Primary alcohols** - have general formulas RCH_2OH; methanol (CH_3OH is the simplest primary alcohol (R=H), and after it, ethanol ($R=CH_3$).
b) **Secondary alcohols** - can be referred to with the shorthand RR'CHOH; 2-propanol is the simplest example ($R=R'=CH_3$).
c) **Tertiary alcohols** - can be referred to with the shorthand RR'R''COH; tert-butanol (2-methylpropan-2-ol) is the simplest example ($R=R'=R''=CH_3$).



Following chart shows different Alcohols with their formulae-

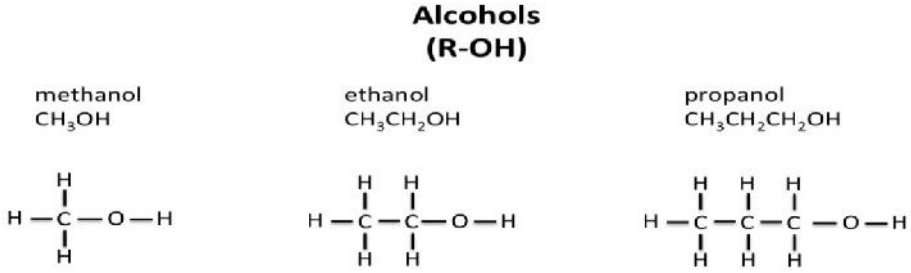
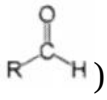


Table 1.1 Showing different types of alcohols –

Sr no.	**Chemical Formula**	**IUPAC Name - Monohydric alcohols**	**Common Name**
1	CH_3OH	Methanol	Wood alcohol
2	C_2H_5OH	Ethanol	Alcohol
3	C_3H_7OH	Isopropyl alcohol	Rubbing alcohol
4	C_4H_9OH	Butyl alcohol	Butanol
		Polyhydric alcohols	
1	$C_2H_4(OH)_2$	Ethane-1,2-diol	Ethylene glycol

2	$C_3H_6(OH)_2$	Propane-1,2-diol	Propylene Glycol
3	$C_3H_5(OH)_3$	Propane-1,2,3-triol	Glycerol
		Unsaturated aliphatic alcohols	
1	C_3H_5OH	Prop-2-ene-1-ol	Allyl alcohol
		Alicyclic alcohols	
1	$C_6H_6(OH)_6$	Cyclohexane-1,2,3,4,5,6-hexol	Inositol
2	$C_{10}H_{19}OH$	2 - (2-propyl)-5-methyl-cyclohexane-1-ol	Menthol

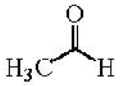
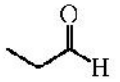
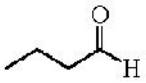
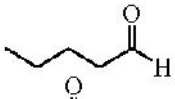
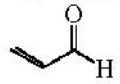
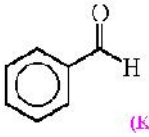
2. **ALDEHYDES**

An aldehyde or alkanal is an organic compound containing a formyl group. The formyl group is a functional group, with the structure R-CHO, consisting of a carbonyl center (a carbon double bonded to oxygen) bonded to hydrogen and an R group,[1] which is any generic alkyl or side chain. The group without R is called the aldehyde group or formyl group Aldehydes (

) and ketones (

) are both carbonyl compounds. They are organic compounds in which the carbonyl carbon is connected to C or H atoms on either side. An aldehyde has one or both vacancies of the carbonyl carbon satisfied by a H atom, while a ketone has both its vacancies satisfied by carbon.

Aldehyde Nomenclature

Structure	Common Name	Systematic Name
	Formaldehyde	Methanal
	Acetaldehyde	Ethanal
	Propionaldehyde	Propanal
	Butyraldehyde	Butanal
	Valeraldehyde	Pentanal
	(Acrolein)	2-Propenal
	Benzaldehyde	Benzenecarbaldehyde

(Know all common names except acrolein)

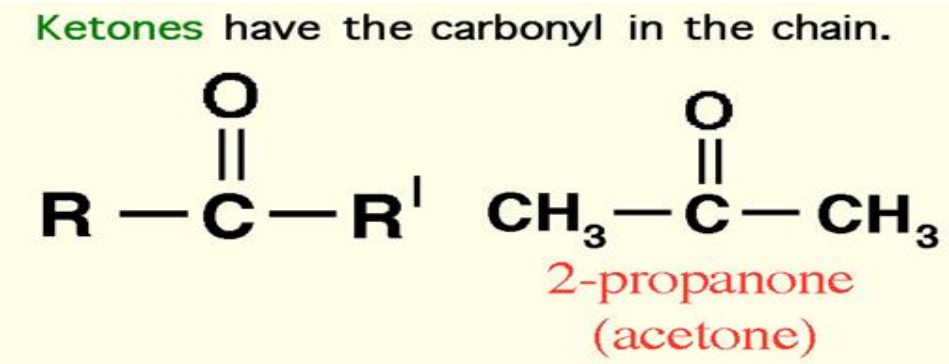
3. **KETONES**

Ketone is an organic compound with the structure RC (=O)R', where R and R' can be a variety of carbon-containing substituents. Ketones and aldehydes are simple compounds that contain a carbonyl group (a carbon-oxygen double bond). They are considered "simple" because they do not have reactive groups like -OH or -Cl attached directly to the carbon atom in the carbonyl group, as in carboxylic acids containing -COOH.Many ketones are known and many are of great importance in industry and in biology. Examples include many sugars (ketoses) and the industrial solvent acetone.

Ketones are classified on the basis of their substituents.

One broad classification subdivides ketones into symmetrical and asymmetrical derivatives, depending on the equivalency of the two organic substituents attached to the carbonyl center. Acetone and

benzophenone (C_6H_5C (O) C6H5) are symmetrical ketones. Acetophenone (C_6H_5C (O) CH3) is an asymmetrical ketone.



4. CARBOXYLIC ACIDS

A carboxylic acid is characterized by the presence of the *carboxyl group* -COOH. The chemical reactivity of carboxylic acids is dominated by the very positive carbon, and the resonance stabilization that is possible should the group lose a proton.

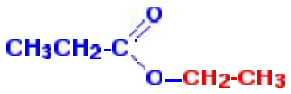
TABLE 1 Common Names of Carboxylic Acids

Name	*Structure*	*Source*	*Etymology*
Formic acid	H—C(=O)—OH	Ant	*Formica* (Latin)
Acetic acid	CH_3—C(=O)—OH	Vinegar	*Acetum* (Latin)
Butyric acid	CH_3—CH_2—CH_2—C(=O)—OH	Butter	*Butyric* (Latin)
Caproic acid	CH_3—CH_2—CH_2—CH_2—CH_2—C(=O)—OH	Goat	*Caper* (Latin)
Steric acid	CH_3—$(CH_2)_{16}$—C(=O)—OH	Tallow	*Steak* (Greek)

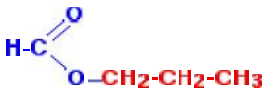
5. **ESTERS**

Esters are derived from carboxylic acids. A carboxylic acid contains the -COOH group, and in an ester the hydrogen in this group is replaced by a hydrocarbon group of some kind. This could be an alkyl group like methyl or ethyl, or one containing a benzene ring like phenyl.

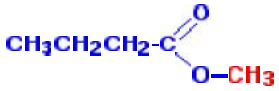
Different types of Esters are shown below-



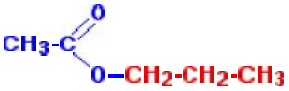
ethyl propanoate



propyl methanoate



methyl butanoate

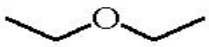


propyl ethanoate

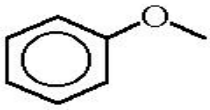
6. **ETHERS**

Ether is a class of organic compounds that contain an ether group — an oxygen atom connected to two alkyl or aryl groups — of general formula R–O–R'. A typical example is the solvent and anesthetic diethyl ether, commonly referred to simply as "ether" (CH_3-CH_2-O-CH_2-CH_3). Ethers are common in organic chemistry and pervasive in biochemistry, as they are common linkages in carbohydrates and lignin.

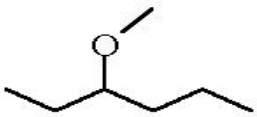
Ether Nomenclature



Diethyl ether, ethyl ether, ether



Methyl phenyl ether, anisole



3-methoxyhexane

Tetrahydrofuran

Depending on the groups at R and R', ethers are classified into two types:

1. Simple ethers or symmetrical ethers; e.g., Diethyl ether, dimethyl ether, etc.
2. Mixed ethers or asymmetrical ethers; e.g., Methyl ethyl ether, Methyl phenyl ether, etc.

Review Questions:

1. What are organic compounds? What are their sources?
2. What are properties of organic compounds?
3. Classify hydrocarbons.
4. Write a note on alcohol/aldehydes/ethers/ketones/esters.
5. Describe structure of a carbon atom.

****

Lesson 2 Glassware used in biochemistry laboratory.

Objective -At the end of this lesson student will able to state about

a.Different glasswares used in pathology laboratory.

b. Uses of different glassware.

d. Care of different glasswares.

e. Cleaning of glasswares.

f. Proper storage of glasswares.

6.1. List of glassware

Glassware are divided into groups –

1. Containers and receivers –
2. Volumetric glassware –
3. Other glassware

1. Containers and receivers –These are most common glassware in the lab. They are available in different volumes. E.g

- Beakers
- Flasks
- Test tubes
- Bottles

2. Volumetric glassware –

These are also known as graduated glassware. E.g.

1) Pipettes
2) Burettes
3) Measuring cylinders
4) Volumetric flask
5) Graduated conical testing glasses

3. Other glassware -

Slides, Cover slip, Petri dishes, syringes, thermometer, etc.

Figure 6.1 Volumetric Glassware

Figure 6.2 Pasteur pipette

Figure6.3 Petri dish

Figure 6.4 Test tubes and Flasks

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6.2. Care and cleaning of glassware –

A. Care of glass ware -

While working in a laboratory the technician must be familiar with the types of glassware handled in the lab. He should use them properly. Improper use of glassware may lead to their breakage.

There are basically two qualities of the glassware's.

- Borosilicate glassware.
- Sodium glassware.

Borosilicate glassware is heat and chemical resistant. It can also stand mechanical stress and will not break due to sudden change of temperature (Thermal shock). This is ideal for beakers and other glassware which are subjected to heating. They are expensive.

Soda lime glassware is less resistant to mechanical shock and thermal shock. It is cheaper than borosilicate glass and is ideal for storage of reagents. It is easy to bend by heat and is used in preparing certain glassware.

Some common tips for the care and maintenance of glassware -

- Never leave the glassware unattended when it is heated, it will crack or explode when dry condition approaches.
- Avoid thermal shock by putting the hot glassware on an asbestos pad.
- Avoid scratching of glass in its daily use. Whenever possible, use a rubber tipped glass rod or plastic rod if the solution is not too hot.
- For cleaning, use plastic brushes instead of metal brushes.
- Use heat resistant glass while preparing solution of acids and alkalis.
- Polish glass tubing before attempting to insert it into rubber tubing.

B.Cleaning of glassware –

Clean glass wares is important requirement of a clinical lab. The glassware should be clean physically, chemically and in certain cases bacteriologically. If glassware are not clean, it will give wrong results.

These glassware can be grouped into four groups –

1) Cleaning of new glassware –

The new glassware that has been never used is slightly alkaline in nature. They should be first neutralized. For that do the following procedure –

i) Prepare a basin full of 3 litres of water and 6 ml of Conc. HCl. (0.2 % HCl)

ii) Leave the new glassware immersed in the above solution for completely 24 hrs.

iii) Rinse twice with ordinary water and once with distilled water.

2) Cleaning of Dirty, stained and infectious glassware – Any glassware should be first immersed in tap water or detergent water immediately after use. Dirty, stained or infected glassware first require different type of special treatment and then, they cleaned in same manner.

- **Preliminary treatment –**
- For dirty glassware – These first boiled in water for 10 minutes.
- Stained glassware – Never rinse blood stained glassware with hot water, as the proteins gets deposited on inner aspect of the glassware. If glassware have stains of colours then remove these with the help of **Potassium dichromate** solution or chromic acid solution.
- Infectious glassware is to be soaked in 5 % Phenol for 24 hrs.

After this preliminary treatment, all glassware have given following treatment –

- **Soaking in detergents –**

Prepare a bowl of water with washing powder or liquid detergents. Put all glassware in this bowl. Brush inside the container with test tube brush. Leave in bowl for 2-3 hours.

- **Rinsing –**

Remove the articles one by one from the bowl. Rinse each one thoroughly under the tap. Then soak them all in a bowl of ordinary water for 30 minutes. Rinse each article in plain water or in the stream water. A small trace of detergent left in the glassware will lead to wrong results. Final rinse always should be with distilled water.

- **Draining –**

Place the glassware on a rack upside down in a wire basket for complete draining.

- **Drying –**

Dry the glassware in oven or keep the basket in sunny spot.

1.5 Micropipette –

Micropipette is one type of pipette which is used in lab commonly. Now a day it has almost totally replaced previously used glass pipettes. Micropipette is a laboratory tool commonly used in lab to transport a measured volume of liquid. This liquid may be chemical or liquid lab sample. These pipettes come in several designs. They may be single volume, adjustable volume. Electronic pipettes are also available.

Advantage of Micropipettes –

- They are more accurate and precise in dispensing the volume of liquid.
- They are easy to use.
- Mouth pipetting is avoided

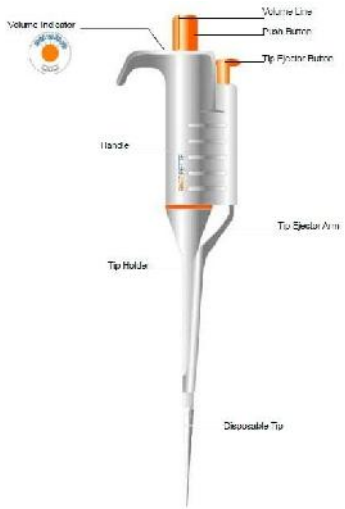


Figure 6.8 Micro pipette

Figure 6.6 Tips of MicropipettesFigure 6.7 Way to Use a Micro pipette

Review Questions:

1. Enlist glasswares used in Pathology lab?
2. How will you clean new glassware?
3. What special treatment you will give while cleaning infected glassware.
4. Write full procedure of cleaning of dirty glassware.

Assignment

Clean new glassware in your lab.

Chapter3

Instrumentation

Objective: -Students shall learn structure and working of various instruments used to carry out different biochemical estimation in serum or plasma.
They will handle instruments and perform tests in proper way.

3.1 Introduction

Biochemical investigations are routinely asked by clinical specialists for screening, diagnosis and monitoring of patients. Quantitative analysis gives an exact amount of substance present in the sample unlike qualitative tests which only give information of presence or absence of compound in the given sample. While semi quantitative tests give an approximate ranges to be present.

Most of the biochemical tests are performed on blood (serum or plasma) . But other samples like urine, CSF, pleural and peritoneal fluid, etc are also analyzed for different constituents.

All biochemical investigations are performed with the help of various instruments depending on need. Different methods of instrumentation are -

A. Colorimetry
B. Spectrophotometry
C. Flame photometry
D. Paper chromatography
E. Electrophoresis

3.2 Different instruments

A. Colorimeters

Colorimetry is a technique which is frequently used in biochemical investigations. It involves quantitative estimation of color. A substance to be estimated colorimetrically must be either colored or capable of forming chromogen through the addition of reagents. The instrument commonly used is known as **Colorimeter.** It measures the amount of light absorbed.

Visual and Photoelectric colorimetry-

Two methods are used to measure the colour intensities of standard and unknown (Test).

The visual method- Here colours are matched by the eyes using Duboscq type (visual colorimeter).

The photoelectric colorimeter- Here intensity of color is measured by determining the amount of electric current developed by a photoelectric cell when light transmitted by the colored solution falls upon it.

This has replaced the visual colorimeter. It is more sensitive so can determine the small amounts of substances present in the sample.

Principle-The concentration of compounds is determined by measuring the intensity of color. The intensity of color is proportional to the concentration of the compound being measured. But many compounds of clinical significance present in body fluids like sugar, proteins, etc are not colored.These compounds can form coloured complexes by specific chemical reactions and the intensity of colour is directly proportional to the concentration of compound undergoing chemical reaction.

Beer-Lambert law

Photometry or colorimetry is based on two fundamental laws.

Beer's law- When monochromatic light passes through a light absorbing medium, the intensity of the transmitted light decreases exponentially as the concentration of light absorbing material increases.

OR The optical density of a solution is directly proportional to the concentration of the solution.

Lambert's law- when monochromatic light passes through a light absorbing medium the intensity of the light transmitted decreases exponentially as the length of the light path through the light absorbing material increases.

OR The optical density of a coloured solution is directly proportional to the path of a light i.e. diameter of cuvette.

What is Absorbance and transmittance?

When light passes through coloured solution, some amount of light is absorbed by the solution, depending on the concentration of the light absorbing compound, while remaining light is transmitted. The amount of light absorbed is termed as "**Absorbance**" or "**Optical density**", whereas the amount light transmitted is termed as "**Transmittance"**.

Components of colorimeter

The basic elements of colorimeter are as follows-

1. Light source (L) - This provides radiant energy. Tungsten lamp is used for the visible range and hydrogen lamp for the UV range and lower visible range.
2. Entrance slit (ES) – The light emerging from tungsten lamp is allowed to pass through a narrow slit that renders parallel rays to fall on the next component.
3. Filter (F) – Filters provide the desired monochromatic light by excluding other wavelengths. Filters are made up of thin layer of colored glass or dyed gelatin. The color of filter is complementary to the color of the solution. This allows only appropriate wavelength of

light to pass through colored solution. Complementary filters should be used in order to increase the sensitivity of the photometric measurements.

Complementary filters

Color of solution	Filter used	Peak transmission
Bluish green	Red	680
Blue	Yellow	580
Purple	Green	520
Red	Blue-green	490
Yellow	Blue	470
Yellowish-green	Violet	430

Table 3.1

4. Cuvette (C) – It is a special glass tube which the solution to be analyzed in a colorimeter. Cuvettes should have uniform thickness, inner diameter and refractive index. They are either round like a test tube or rectangular (often called cells). Cuvettes usually have 1cm light path.
5. Photocell (P) – This converts light energy in to electrical energy. An electric current is generated when light transmitted by a coloured solution falls on it.
6. Galvanometer (M) – It is readout device that measures electric current generated by the photocell. The electric signal is read directly by a pointer moving over a dial giving direct value of optical density.

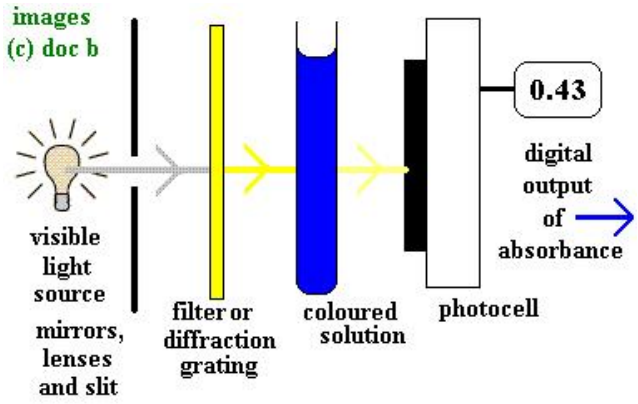


Figure 3.1 Components of photometer

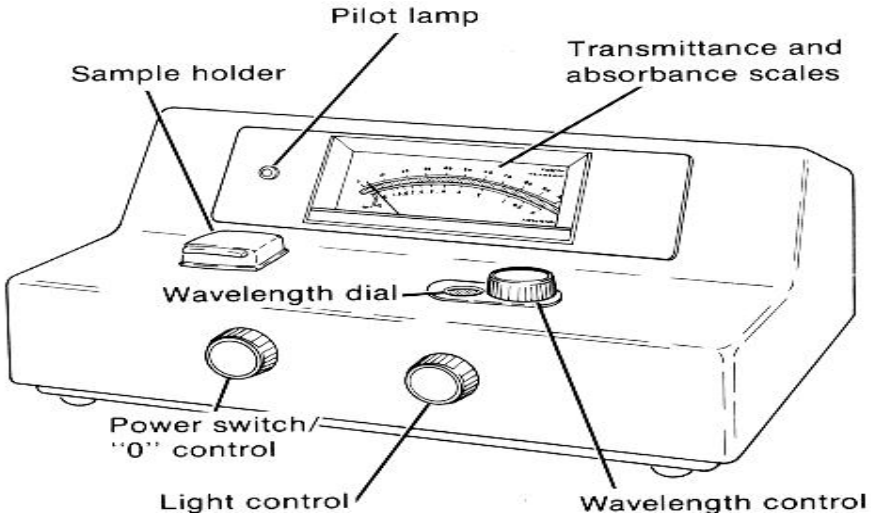


Figure 3.2Photoelectric colorimeter.

Technique-

Colorimetric technique involves three basic steps

1. Chemical reaction-Development of color through a chemical reaction. If the testing compounding is chromogenic no need of chemical reaction.
2. Physical measurement- Intensity of the colour is measured both for test solution and the standard solution (In standard there is known concentration of the test compound is treated identically as the test solution). Use of appropriate filter gives desired quality of light.

3. Calculations- Results are either calculated by the mathematical formula or read from calibration curve prepared from various concentrations of standard and the corresponding absorbance readings.

Use of blank

To eliminate error two types of blanks are used.

a. Water blank- It is used to adjust O.D. to "0" or % Transmittence to 100.
b. Reagent blank- The preparation of blank is essential because some amount of light is absorbed by the colored reagent itself. So the blank is prepared by adding all reagents except the substance to be tested.

Use of standard solution

It is the solution of known concentration of the substance in pure form to be tested. Both the concentration and O.D. of standard is known, so the concentration of unknown can be calculated.

Procedure for taking reading on colorimeter (Operation steps)-

a. Select the proper filter.
b. Fill the cuvette about three fourth with distilled water and place it in a cuvette slot.
c. Switch on the instrument and allow it to warm for 4-5 minutes.
d. Set the instrument to zero optical density.
e. Take reagent blank solution in another tube and keep in slot and read the optical density.
f. Take standard solution and test (unknown) solution in the cuvettes and record optical density. Then calculate concentration of unknown analyte.

Note

- The reading should be taken with lighter solution (lower concentration) first and then darker solution (more concentration) to avoid errors in readings due to carryover effect.

- Satisfactory results are obtained when O.D. values are in the range 0.1- o.7.

Applications

- Colorimeter is used for the estimation of various biochemical compounds in different biological samples like blood, serum, plasma, CSF, urine,etc.
- Colorimetric technique is routinely used for biochemical estimation of glucose, urea, creatinine, cholesterol. SGOT, SGPT, etc.

B. Spectrophotometer

Instrument that is used to measure transmission at various wavelengths continuously is called spectrophotometer. They differ from filter photometer which can be used at only a relatively few wavelengths and the breadth of the wavelength band that must be employed.

The components of spectrophotometer are basically same as photoelectric colorimeter. The differences are

- In spectrophotometer filters are replaced by a continuously adjustable monochromator which is capable of isolating much narrower band of wavelengths.
- A monochromator is an assembly of a dispersing element (a quartz prism or diffraction grating) along with two narrow slits – an entrance and an exit which controls the spectral width.
- The diffraction grating or the prism breaks white light falling on to it into spectrum of its component colours. By adjustment only one particular portion can be allowed to pass through the opening.

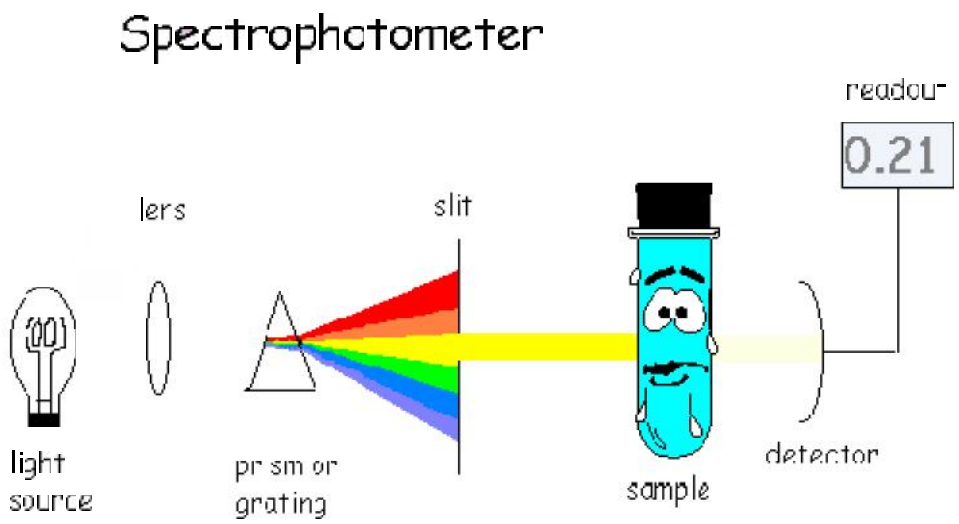


Figure 3.3 Components of Spectrophotometer

Types of spectrophotometer

Depending on spectral region over which they operate;

1. With glass optics, sensitive roughly from 350-800nm. Tungsten lamp is the light source.

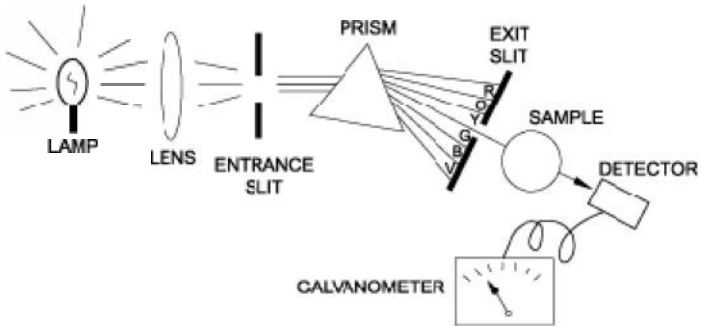


Figure 3.4 Visible light spectrophotometer

2. Those with optical parts of quartz which extends through the uv and visible, roughly 200-1000nm; glass cuvettes cannot be used as glass absorbs at wavelengths less than 400nm. Only quartz or plastic cuvettes that do not absorb UV radiation can be used. Either hydrogen or deuterium lamp is the light source for UV region.

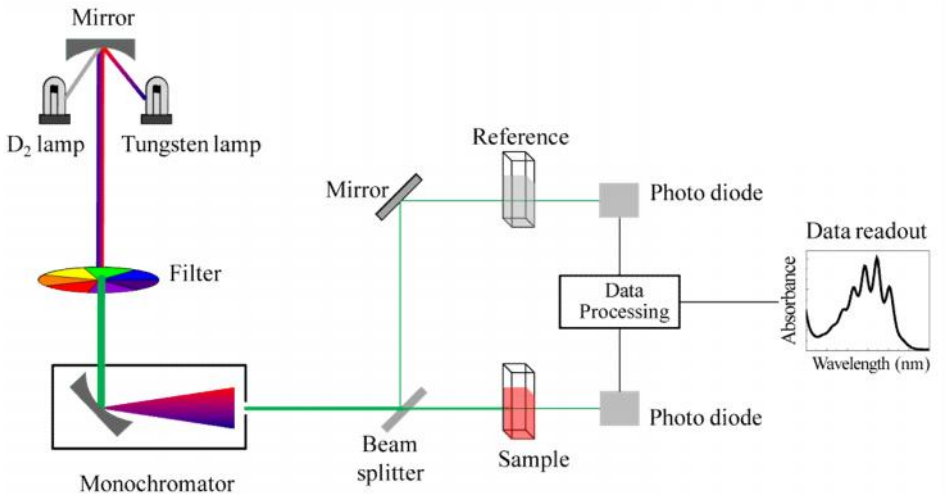


Figure 3.5 UV-VIS Spectrophotometer

3. Infrared spectrophotometer covering the range from 1000nm (1µ) upward.

C. Flame photometer

The special technique of 'emission flame photometry' is widely used in clinical laboratory to determine the concentration of sodium and potassium in biological fluids like serum, urine and sweat. It can be used to measure the serum lithium levels. Lithium is used in therapeutic dose for some psychiatric disorders.

Advantages

- It offers one of the most convenient, accurate and precise measurements of Na^+, K^+.
- The chemical procedures for Na and K are long and tedious.
- The flame photometry is simple, accurate and speedy requiring much less work than chemical procedure.

Principle-

Atoms of alkali metals like Na, K, Li when placed in the heat of a flame, become "excited" and emit light of specific wavelengths while they

return to their “ground stage” which is characteristic of each element. The amount of light emitted is proportional to the number of excited atoms present or the concentration of the elements in the solution. Quantitation of emitted light is done by photodetector system.

Components of Flame photometer-

1. Nebulizer/atomizer/aspirator system- this helps to convert the sample into a steady fine spray of droplets, which is then driven to the flame of gas burner. It is usually of the ‘scent type’ i.e. a forced stream of air passes over a capillary tube which dips in the test solution.
2. Flame- it is used to generate heat that evaporates the elements that becomes luminious when it returns to the ground state. The most common gas mixture that provides optimum temperature for the routine determination of sodium and potassium is propane-butane or natural gas.
3. Filter/ Monochromator- These increase the specificity of the analysis. Simple filters are used. For sodium, filter used is orange (589nm) and for potassium deep red (766nm). These are not complementary filters against the colour of the flame. Heat filters are placed between the flame and monochromator filter to stop the passage of heat. The filters screen out all other wavelengths of light except the specific one emitted by the element analysed.
4. Detector- it converts light energy into electrical energy.
5. Galvanometer- It is output device that can give an output for sodium and potassium concentration simultaneously.

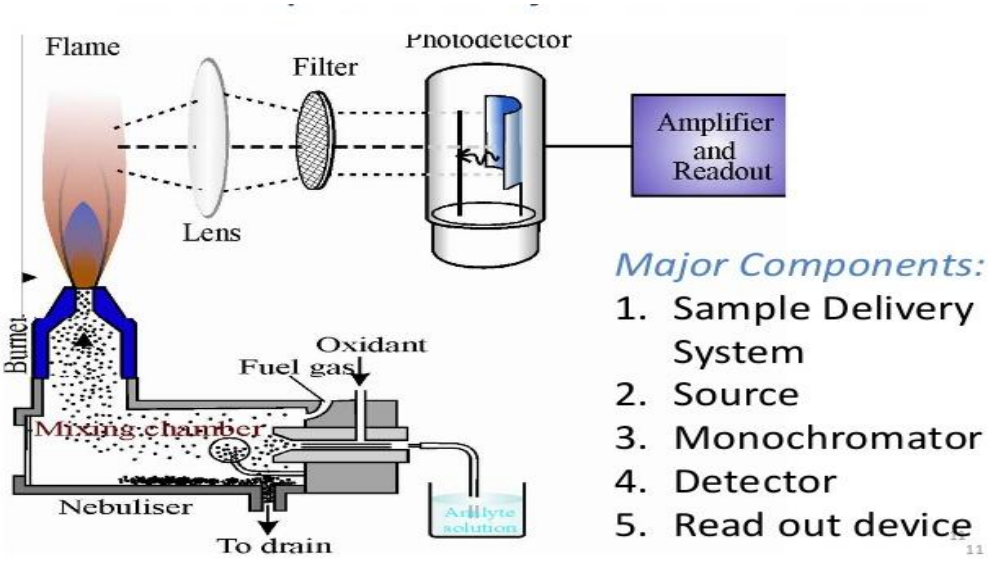


Figure 3.6 Flame photometer

Procedure-

1. Dilute the plasma to an appropriate extent (1 in 100).
2. Insert the proper filter.
3. Ignite the flame.
4. Regulate the pressure to convert sample into steady fine spray of droplets.
5. Place a cup containing distilled water under the aspirator system and adjust the flame with distilled water.
6. Adjust galvanometer reading to zero.
7. Use appropriate standards corresponding to the elements to be assayed and note the galvanometer reading.
8. Then spray the diluted sample and note the reading.

Normal range-

Serum sodium- 135-145 mEq/L

Serum potassium- 3.5-5.1 mEq/L

D. Paper chromatography

Chromatography is a technique used to separate solutes on the basis of their differential distribution between two phase solvent systems where one phase (mobile phase) is caused to move over the other phase (the immobile or stationary phase).

The porous medium through which mobile phase migrates is called support.

Stationary phase- In some chromatographic technique solid support itself forms the stationary phase, while in other it adsorbs liquid phase which in turn acts as a stationary phase. Thus stationary phase may be solid or liquid.

Mobile phase- is one which flows over or through stationary phase and carries sample along with it, when the separation of its components results. It is either liquid or gas.

There are five types of chromatography

1. Paper chromatography
2. Thin layer chromatography
3. Column chromatography
4. Gas liquid chromatography
5. High pressure liquid chromatography

Principles of chromatographic separation:

- Adsorption: Solubility of a compound in mobile phase and adsorption on stationary phase decides separation. The component which is less soluble and strongly adsorbed will be retained on the support. On the other hand more soluble and less adsorbed will flow out faster.
- Partition- Stationary and mobile phases are immiscible with each other. Inert support may be needed for stationary phase. Separation occurs due to differential solubility of components in two phases.

- Gel filtration- As the solute mixture passes through a column of porous beads the solute separates on the basis of molecular size. The smaller particles pass into the pores of beads and are retained in the column and come out later, while bigger particles pass between the beads and come out earlier. This is also known as molecular sieving. It is used for determination of molecular weight. Different gels used are- agarose, polyacrylamide, etc.
- Ion exchange- Here separation of charged solutes is achieved. The solute mixture is allowed to pass through a column of ion exchange resins. There are two types of ion exchange resins-
  - Cation exchange resins which contains negatively charged ions and retain positively charged ions.
  - Anion exchange resins which contains positively charged ions and retain negatively charged ions.

Paper chromatography

Paper chromatography is a partition chromatographic technique in which over the cellulose supporting medium the solvent moves. Water is considered as stationary phase as it is bound to polar cellulose and the organic solvent that moves over the hydrated cellulose fibres is mobile phase.

There are two techniques which are commonly employed for paper chromatography.

1. Ascending- Mobile phase moves up. The sample spot should be in a position just above the surface of the solvent so that as the solvent moves vertically up the paper by capillary action separation of sample is achieved. This is often preferred due its simplicity of set up.

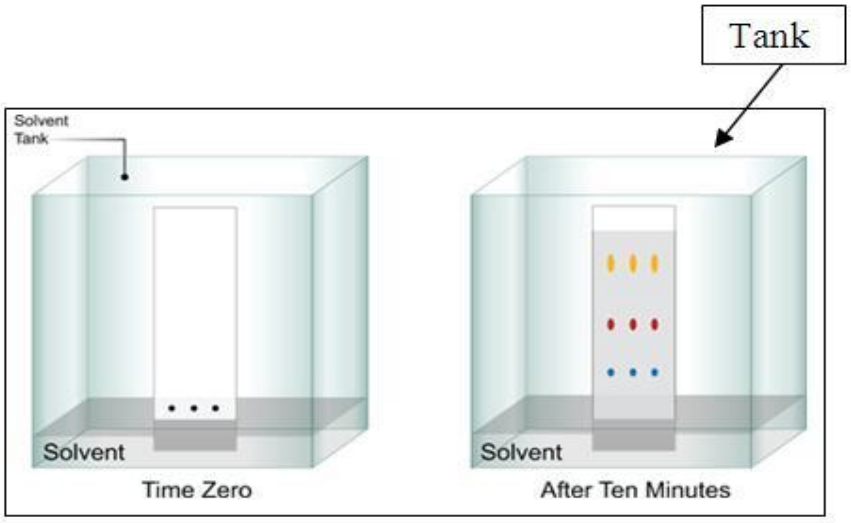


Figure 3.7 Ascending paper chromatography

2. Descending- Mobile phase moves down. The solvent moves downwards under the gravity. Here flow of solvent is faster than ascending.

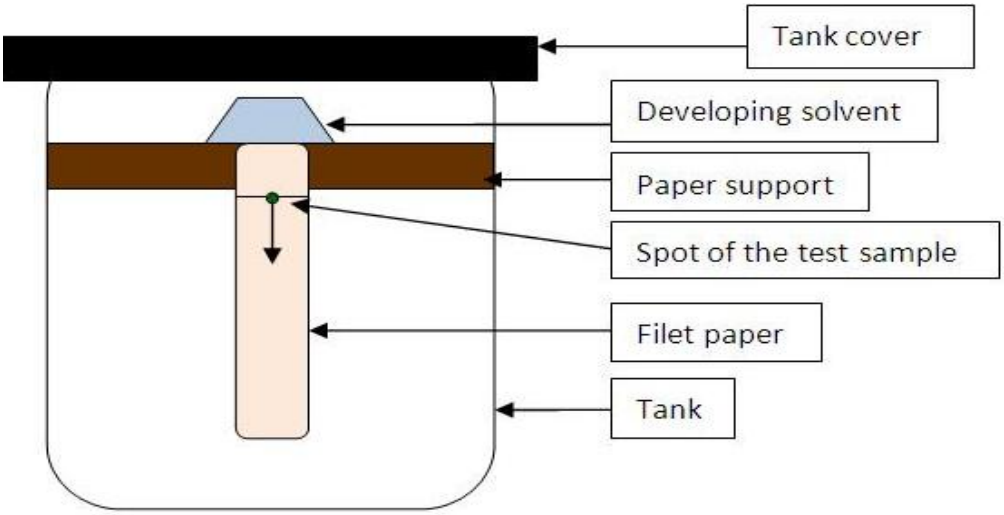


Figure 3.8 Descending paper chromatography

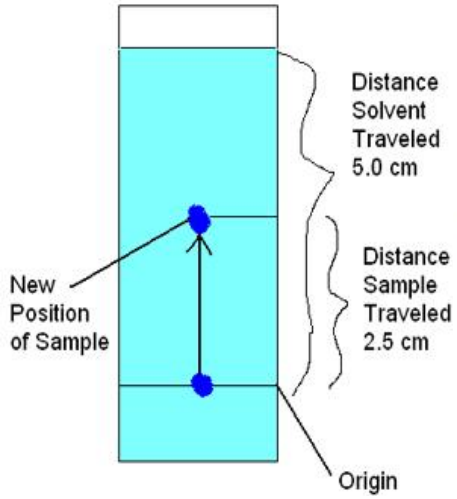
Support medium- A sheet of Whatman filter paper no. 3 (square or circular) soaked in aqueous solvent.

Solvent mixture- Contains non-polar and polar solvent (Butanol:Aceticacid:Water). Polar solvent is absorbed by paper and acts as stationary phase while non-polar acts as the mobile phase.

Procedure-

1. Soak the paper in solvent mixture in a tray which absorbs aqueous part of solvent mixture.
2. The sample (10µl) is then spotted 8cm from the one end of filter paper along with known standards. Make two spots for each sample.
3. The samples are applied repeatedly (allowing drying in between) with the help of fine capillary.
4. The paper is then mounted on stand and kept in solvent tray inside an air tight chromatography chamber.
5. Solvent mixture is then allowed to run almost to the upper edge of paper.
6. Then paper is removed, dried and sprayed with appropriate staining solution.
7. The colored spots are identified by comparing with the spot of known compounds. Give a semiquantative report by measuring the area. For this outline the spot on tracing paper, cut out the tracing paper and weigh. The standard with known concentration of test compounds will be the reference.
8. OR The colored spots are then identified by Rf values, which are specific for different substances.

   $$\text{Rf (Ratio of fronts)} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$



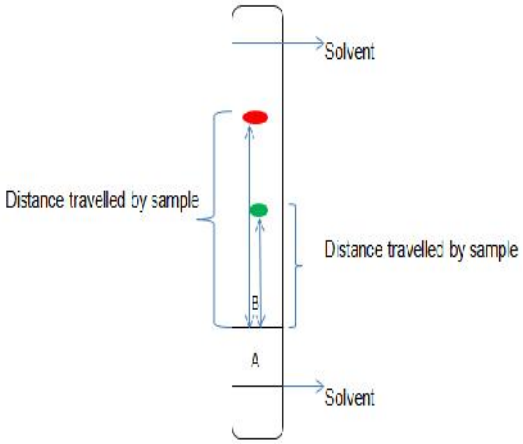


Fig. 3.9 Fig 3.10

Measurement of Rf value

9. For quantitation the spots may be cut out and eluted i.e. dissolved in suitable solvent and intensity of color is measured by photoelectric colorimeter.

Uses

Paper chromatography is for separation of amino acids and sugars.

E. Electrophoresis

Electrophoresis is the technique used to separate a mixture of charged particles by migration under the influence of an electric field.

It is a versatile and powerful analytical technique used to separate and analyze a diverse range of ionized analytes.

Principle-

Thin filter paper strip soaked in sample (serum) is applied on to the support medium and voltage is applied. After a definite period of run the supporting medium is removed and the separated fractions are visualized by staining with dye. The intensities of colored bands compared with normal pattern gives an idea about separated fractions.

Support medium- Agar, filter paper, cellulose acetate or agarose are used as support medium. They have large pores.

Components of Electrophoretic apparatus

1. An electrophoresis chamber-
    - It consists of two compartments separated from each other by a dividing wall, on one side is anode and other side contains cathode (platinum wires).
    - Each side is filled with a buffer at the same level.
    - A 'bridge' across the top of dividing wall holds the support medium so that each end is in contact with buffer in one of the compartments. Contact is made with filter paper strips.
2. Power pack (Power supply)-

    This provides an output of of about 120-150 volts with about 2-5 mAmp of current drawn per side. When a voltage is applied current is carried across the porous medium from the cathode (negative pole) to the anode (positive pole) by the buffer ions.

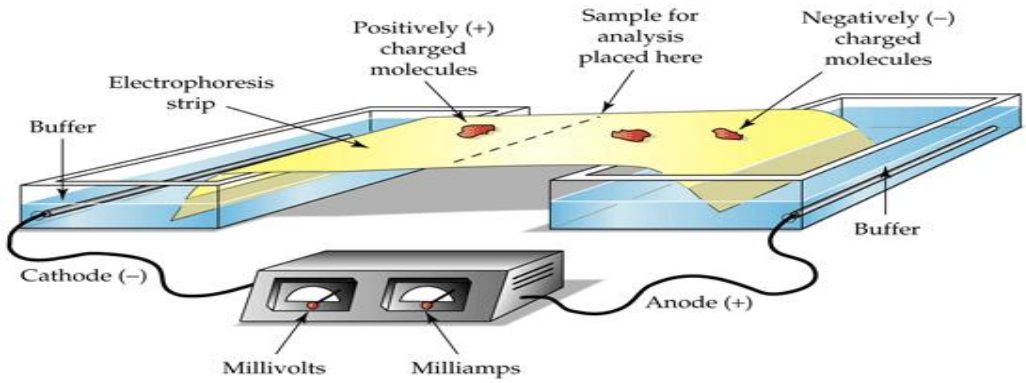


Figure 3.11 Components of Electrophoretic apparatus

Reagents

1. Buffer-

Barbital buffer, pH 8.6

2. Staining solution-

1 % solution of amidoschwartz 10B (amidoblack 10B) in methanol containing 10% glacial acetic acid.

3. Wash solution-

3% acetic acid (v/v).

Procedure-

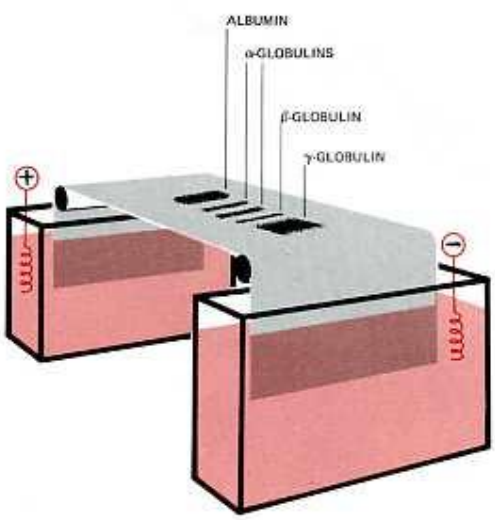
1. Fill the chamber with buffer and level the liquid.
2. Dissolve 10mg of agar in 10ml of warm buffer in a test tube. (The support medium should be made in buffer to conduct the current). Deliver this solution on a clean glass slide carefully without air bubbles. Allow the gel to set uniformly for few minutes.
3. Keep this agar slide on the bridge in the chamber. Place two filter paper strips having the same width as slide on both the ends of slide and connect to the two buffer compartments.
4. Soak a thin strip of filter paper (Whitman 3 1mm×5mm) with the sample (serum) and place it carefully on the agar gel at about $1/3^{rd}$ distance from the cathode end of slide.
5. Close the chamber, switch on the current and adjust the flow to 3mAmp/slide.
6. At the end of the two hours' switch off the current. Remove the agar slide and fix it to prevent diffusion of separated fractions.
7. Visualize the separated fractions by staining with appropriate stain for 2 minutes.
8. Remove the excess stain by gently agitating slide in three rinses of 'wash solution'.
9. Dry the slide and observe the various bands and compare the colour with normal pattern.
10. Quantification can be done by elution and taking reading on colorimeter or spectrophotometer.

<u>Applications-</u>

It is valuable diagnostic tool in clinical biochemistry laboratories.

- Used for separation of proteins in the serum.

- Useful in separation of different forms of haemoglobin, immunoglobulins and isoenzymes.



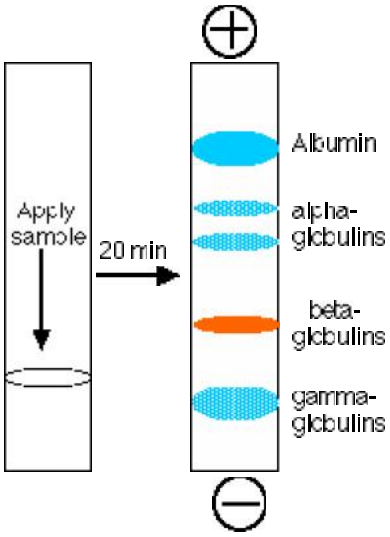


Fig 3.12 Fig 3.13

Serum protein separation by electrophoresis

Review questions-

1. What is role of different instruments in clinical biochemistry?
2. What Beer's and Lambert's law?
3. What are different components of
   a. Photoelectric colorimeter?
   b. Flame photometer?
   c. Electrophoresis?
4. What is principle of chromatography / flame photometer / electrophoresis?
5. What are different types of chromatography?
6. What is ascending and descending paper chromatography?
7. What is Rf value?
8. Difference between colorimeter and spectrophotometer.
9. Name different complementary filters used in colorimeter.

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Chapter 4

Basic laboratory techniques

Objective – Students shall learn basic techniques used in laboratory such as measuring liquid, separation of solids from liquids, centrifugation, filtration, etc.

4.1. Introduction

Biochemical analysis demands great accuracy as the constituents in biological fluids are in minute quantities. The accuracy is attained only when one is well versed with the basic techniques like pipetting, weighing, measuring, etc.

4.2. Methods of measuring liquids

For measuring glassware most frequently used are graduated cylinders, volumetric flasks and pipettes.

4.2.1. Units of measurements - Most commonly measurement is made in litres or its fractions. In metric system deci is $1/10^{th}$, centi is $1/100^{th}$, milli is $1/1000^{th}$ and ul is $1/1000^{th}$ of $1/1000^{th}$ i.e. 1/10,00,000, so that

1Liter = 10 dl = 100 cl = 1000 ml =10,00,000 ul.

1.Weight	1 kg	=	1000 gms
	1gm	=	1000 mgs
	1mg	=	1000ug (microgram)
	1µg	=	1000 ng (nanogram)
	1mg	=	10^{-3}gm
	1µg	=	10^{-6}gms
	1ng	=	10^{-9}gms
2.Volume (unit for measurement of liquids)	1L	=	1000 ml
	1 ml	=	1000µl (microliter)

	1ml =	10^{-3} L
	1µl =	10^{-6} L
3.Length	1 m =	100 cm
	1cm =	10 mm
	1mm =	1000 µm (micrometer)
	1µ(micron)=	1000 mµ (millimicron)
	1mu =	1 nm (nanometer)
	1nm =	10 A (Angstrom units)
4. Strength of solution	1M =	1000mM (milimolar)
	0.1 M =	100mM
	0.01 M =	10 mM
	0.001 M =	1mM
	1mM =	1000 µM (micromoles)
	1Eq/l =	1000mEq/L

Table. 4.2.1. Units of measurement

Note:

1. 1c.c. is equal to 1.004 ml approximately. But for practical purpose 1cc = 1ml.
2. As we go from left to right value of unit increases. For e.g. Nanometer (nm) – micrometer (µm)—millimeter (mm) — centimeter (cm)—meter (m) – kilometer (km)

4.2.2. Graduated cylinders and volumetric flasks –

Volume above 25 ml is often measured using graduated cylinders. While using graduated cylinder lower meniscus of the liquid column in the

cylinder is made to coincide with the definite mark on the cylinder.
Consider the upper meniscus if it is a coloured fluid.

But volumetric flasks are preferred when it is desired to transfer fixed volumes like 25 ml, 50 ml, 100 ml, 250 ml, 500 ml etc.
It is remembered that the solution required to be measured is always allowed to attain room temperature before measuring because liquids expand to heat and contract to cold temperature.
The use of pipettes is very important in clinical analysis; if the pipette has been inaccurate the result is worth nothing. So, correct method of pipetting is very important to get accurate results. There are serological, volumetric or bulb pipette and Ostwald pipettes.
While transferring fluids using pipettes if it is a pipette graduated upto the tip i.e. blow-out type of pipette, the last portion of fluid in the pipette is delivered into the container; otherwise up to the lowest graduation on it, if it is not a blow out type.
While using volumetric or bulb pipette, the portion of the fluid in the tip or nozzle is not collected. In these instances fluid is allowed to drain by itself with the tip of pipette touching the bottom or the wall of the container.
Ostwald pipettes are used to deliver viscous fluids like blood, serum or plasma usually in fixed volume less than an ml. In this case portion of the fluid in the tip is delivered and collected.
It is important to bear in mind that meniscus level is observed holding the pipette vertical and line of vision is parallel to the ground. Otherwise there is error of parallax.

4.3. Separation of solids from liquids

This is done either by centrifugation or by filtration.

Centrifugation – Principle, different types of centrifuge, care & maintenance and application

Filtration using funnel

a. Centrifugation –

4.3.1. Principle – Centrifuges are devices by which suspension of solid material in liquid phase is spun at high speed. As a result the solid phase separates out and forms the sediment at the bottom and the liquid forms the supernatant at the top. At the time of centrifugation, centrifugal force of spinning pushes the solid particles of higher density outwards which pack in the bottom of the centrifuge tube and form a pellet. The packing of the solid particles facilitates the separation of supernatant which is then decanted out.

4.3.2. Different types of centrifuge:

Centrifuge machine is divided in many different ways. Following are the types -

1) Electrically operated and Manually operated (hand drive) centrifuge

2) Table top model and Floor model

3) Free floating type and Angle head type centrifuge

1) Manually operated are used only in spaces where electric supply is not dependable or electricity is not available at all. Speed of this centrifuge is less and this centrifuge can hold only 2 or 4 tubes. It can be used only for preparation of urinary sediment and for concentration of parasite in faecal material.

Electrically operated centrifuges are good and are commonly used. There speed is high and can hold more tubes.

2) Floor model centrifuge are large centrifuge and are used in blood bank. They have arrangement of refrigeration.

Table top models are used in Pathology laboratory.

3) Free floating type also called as horizontal rotor or swing head type is centrifuge where tubes are in vertical position at rest and assume horizontal position when centrifuge revolves. Here sediment surface stays in a straight line at right angle to the wall of the centrifuge tube. Because in this type friction is more speed is comparatively less than angle head type. This centrifuge cannot hold more than 4 – 8 tubes.

Angle head type centrifuge has fixed angle rotors and here tubs are held at fixed angle of 45^{O}. In this type sediment is laid at an angle and there is also less chance that sediment will be disturbed when the centrifuge stops. This centrifuge can hold more centrifuge tubes Speed is high and also attains this high speed in short time.This type is used in blood bank.

Diagram-4.3.1

4.3.3. Care & maintenance:

Maintenance of the centrifuge should be according to the manufacturer's directions.

1. Balance the tubes properly before starting centrifugation. Observe for usual normal vibration noise during operation. The centrifuge may vibrate excessively if the tubes inside are not balanced properly. This should be checked immediately.

2. Do oiling of moving parts on regular basis.

3. Keep it on sturdy or firm platform.

4. Put lid during operation.

5. Never open the (centrifuge) chamber until the rotator has come to a complete stop.

6. Always clean the centrifuge with soft cloth.

7. The chamber or bowl of centrifuge should be kept clean. If there is breakage of tubes with infective material, clean the bowl with disinfectant like 5 % phenol or Lysol.

8. Never take the cushions out of tube unless for cleaning. Cushions prevent breakage of glass tubes.

9. Centrifuge and work area around it should be kept clean at all times. Place a cover on the centrifuge when not in use.

4.3.4. Use or application of centrifuge tube –

General application of centrifuge include

a. Separation of two immiscible liquids

b. Separation of solid particles in a suspension.

In Pathology laboratory centrifuge is mainly used for –

1. Separation of serum or plasma from red blood cells

2. Separation of sediment in urine.

3. Separation of protein free filtrate

4. Washing red blood cells in normal saline.

4.3. b. Filtration using funnel:

4.3.5 Filtration is commonly the mechanical or physical operation which is used for the separation of solids from fluids (liquids or gases) by interposing a medium through which only the fluid can pass. In Pathology laboratory medium commonly used for filtration is filter paper. The fluid

that passes through is called the filtrate. Oversize solids in the fluid are retained, but the separation is not complete; solids will be contaminated with some fluid and filtrate will contain fine particles (depending on the pore size and filter thickness).

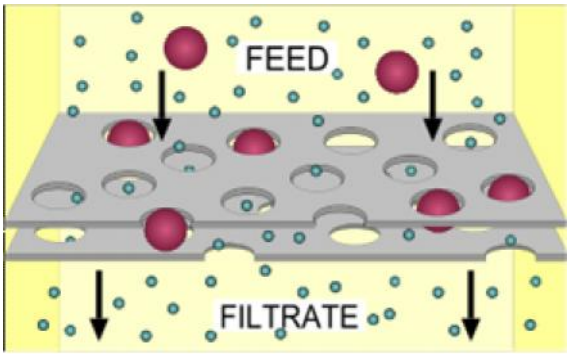


Diagram of simple filtration 4.3.2.: oversize particles in the **feed** cannot pass through the lattice structure of the filter, while fluid and small particles pass through, becoming **filtrate**.

Ordinary filter papers are available but filter papers commonly used are Whatman filter paper. They are available in different sizes and hence using which the rates of filtration are different.

Sr. No.	**Whatman No.**	**Filter speed**	**Separate**
1	4,54	Fast	Coarse and gelatinous precipitates
2	1,43	Medium fast	Medium crystalline suspension
3	2,40,52	Medium	Crystalline suspension
4	5,6,42,44,50	Slow	Fine crystalline suspension

Table - 4.3.1.Grades of Filter papers

Procedure of filtration – Select the circular filter paper or cut into circle. Fold it twice to obtain a cone shape. Place it into funnel. Pour the fluid into funnel. Collect the clear filtrate in dry vessels.

Ordinary filter papers serve the purpose in most cases with thick precipitates. If precipitate is in suspension or gelatinous; then better to use Whatman filter paper of required grade.

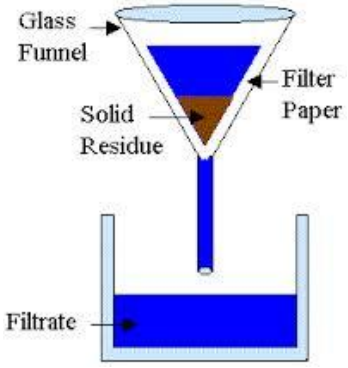
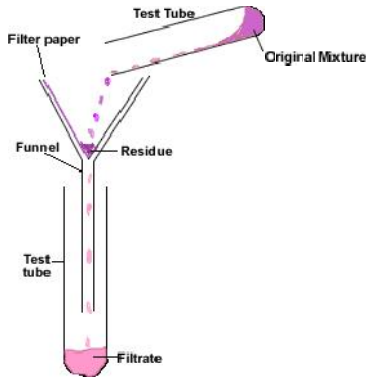


Fig. 4.3.3.a) Funnel



b)Filter paper

4.3. Weighing: Different types of balances used, care and maintenance

4.4.1. Introduction -

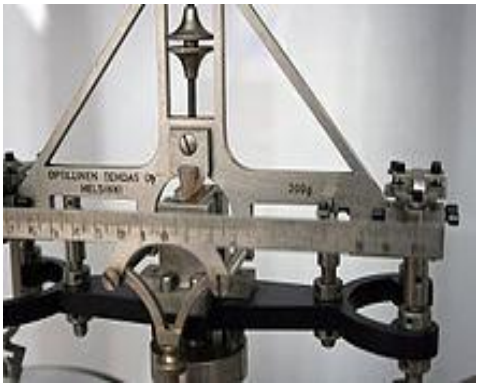
Balances are essential laboratory instruments that are widely used for weighing of various substances (powders, crystals and others) in the laboratory. For instance, to prepare reagents, stains and culture media, balances are required to weigh accurately and precisely within the needed range.

Balances are of different sensitivity depending on their use. The physical balance is less sensitive than the analytical balance. For routine laboratory purposes, sensitivity of balance can be considered to be the smallest mass that can be weighed accurately. For example, physical

balance may require more weight to move the pointer than the analytical balance.

If the sensitivity of balance is 1 mg, this means that a weight of at least 1.0 mg is needed to move the pointer over one scale. For routine laboratory purposes the sensitivity of a balance can be considered to be the smallest weight that it will measure accurately. Usually the larger the amount of substance to go into a reagent, the least accuracy is required.

4. 4.1.a.Mechanical analytical balance



4. 4.1.b Mechanical analytical balance (detail)

4. 4.1.c.Digital analytical balance

4.4.1.d.Image showing the way of operation of balance.

4.4.2. Types of balances –

Balances in medical laboratory may be:

A. physical balance (mechanical balances or Rough balance)

B. Analytical balances

Both types can be single pan or double pan.

A) Physical balance:

Physical balances are of several types. Some of them use sliding scale, some have a single or double pan (s) and others utilize dial - operated fractions. They are used for weighing substances, which do not call for extreme accuracy. While operating, they do not require electricity or battery power and are currently less expensive than analytical balances of the similar sensitivity. Some rough balances weigh accurately to 0.01 to 0.1 gm (10 - 100 mg) of a substance.

It is used:

1. To weigh large amounts (up to several kilo grams)
2. When a high degree of accuracy is not required.

Example: 20.5 gm, 36. 5gm etc. The sensitivity of a two pan physical balance is less i.e. 0.5 gm.

B) An **analytical balance** is a class of balance designed to measure small mass in the sub-milligram range. The measuring pan of an analytical balance (0.1 mg or better) is inside a transparent enclosure with doors so that dust does not collect and so any air currents in the room do not affect the balance's operation. This enclosure is often called a draft shield. The use of a mechanically vented balance safety enclosure, which has uniquely designed acrylic airfoils, allows a smooth turbulence-free airflow that prevents balance fluctuation and the measure of mass down to 1 μg without fluctuations or loss of product. Also, the sample must be at room temperature to prevent natural convection from forming air currents inside the enclosure from causing an error in reading.

Electronic analytical scales measure the force needed to counter the mass being measured rather than using actual masses. Electronic analytical balance requires electricity. They are also called as digital type analytical balance and they require electricity.

Analytical balances are costly but more sensitive. Now-a-days they are most popularly used balances in medical laboratories. This is to provide a precision and accuracy for reagent and standard preparation.

It is used:

1. To weigh small quantities usually in milligram (mg) range.
2. When great accuracy is required.

Example, 2.750mg, 0.330 mg, 5.860mg, etc

Its sensitivity is 0.5 mg to 1 mg depending on the model.

Note: The accuracy of a balance should be checked regularly.

To ensure proper operation of an analytical balance, study the instruction manual provided by the manufacturer o the instrument.

4.4.4. Care and maintenance of analytical balance –

Balance is a delicate instrument that requires practical instruction in its correct use.

The following should be applied when using a balance:

1. Read carefully the manufacturer's instructions.
2. Always handle a balance with care.
3. This is sensitive balance. Position the balance on a firm bench away from vibration, draughts and direct sunlight, corroding fumes, dirt. Better to keep it in air-conditioned room.
4. Before starting to weigh, zero the balance as directed by the manufacturer. The pointer should show equal oscillations on either side of '0'on the scale; otherwise work on terminal adjustment screws and make the weights of empty pans balance exactly. If using a beam balance, check the position of the beam.
5. Weigh the chemicals in a weighing scoop or small beaker. Never put the chemicals directly on the balance pan.
6. Do not weigh the substances unless they come to room temperature.
7. When adding or removing a chemical, remove the container to avoid spilling any chemical on the balance.
8. When using an analytical double pan balance, bring the pans to rest before adding or removing a chemical. This protects the sharpness of agate knife edges and hence sensitivity of balance.
9. Do not give jerky movement to knob.
10. Before weighing see that the platform is horizontal indicated by index. Work on leveling screws to make horizontal.
11. Always use forceps to add or remove weights. The weights are places in right sided pan. Protect the weights from dust, moisture and fungal growth. When not in use keep them in their box.
12. Use small brush to remove any chemical, which may have been spilt on the balance.

13. A container of self - indicating silica gel should be kept inside the analytical balance. This is to remove any moisture present in the atmosphere.
14. Keep the balance clean, being particularly careful not to let dirt accumulate near the pivots and bearings.

Remember, all determinations in biological fluids are worthless if weighing and pipetting are not done accurately.

Review questions -

1. Write different units of measuring the liquids.
2. What precautions should be taken white measuring liquids with graduated cylinders?
3. What is Ostwald pipette?
4. Name different methods used for separation of solids from liquids?
5. Write principle of centrifuge machine.
6. Write on care and maintenance of centrifuge.
7. Write application of centrifuge.
8. Write principle of working of filtration.
9. Write types of balances? Which type is commonly used and why?
10. Write on care and maintenance of balances.
11. What is sensitivity, precision, accuracy?

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Chapter 5

Carbohydrates

Objectives- At the end of this chapter student shall understand the sources, classification, properties and significance of carbohydrates.

5.1 Introduction-

Definition – A carbohydrate is generally defined as a neutral compound made of Carbon, Hydrogen and Oxygen the last two elements remaining in the same proportion as in water.

The general Formula is $\mathbf{C_n (H_2O)_n}$

There are many exceptions for example Rhamnose is carbohydrate having formula $C_6H_{12}O_5$ in which H&O are in different proportions.

There are also certain other compounds such as formaldehyde (HCHO), acetic acid CH_3COOH etc. which have got same empirical formula of carbohydrate but they are not carbohydrates.

Therefore carbohydrate are chemically defined as the aldehyde and ketone derivatives of higher polyhydric alcohol having more than one OH group.

Sources- Carbohydrates are found in sugar, jam, cereals, bread, potatoes, fruits, vegetables and milk

5.2 Classification of carbohydrate

Carbohydrates are classified according to the number of simple sugar units present in the molecule

1) Simple Carbohydrates.
2) Compound Carbohydrates.

Simple Carbohydrates

Simple Carbohydrate is also known as monosaccharide or simple sugar .It contains only one unit of simple sugar example Glucose, Fructose, Galactose.

It is further sub divided into two sub groups

A. According to the number of carbon atoms present in the molecule example monose, diose, triose, tetrose, pentose, hexose, heptose etc. containing 1,2,3,4,5,6,7 Carbon atoms respectively.
B. According to the nature of the reducing group they contain example
   a. Aldoses - example Glucose and reducing group is aldehyde (-CHO) radicle
   b. Ketoses – Example Fructoses reducing group is Ketone (C=O)
      i. General Formula of simple carbohydrate is $C_n(H_2O)_n$

5.3- General Properties of simple carbohydrates

1) They are colourless, crystalline compounds having a sweet taste
2) Formation of esters – By virtue of the alcohol group they easily form esters with acids example acetates, benzoates.
3) Reducing power - By virtue of the aldehyde or the ketone group the simple sugar are powerful reducing agent. They easily reduce alkaline copper, bismuth or silver solution.
4) Optical Rotation – Simple sugar rotates the plane of polarized light and therefore may exist in either dextro or laevo forms
5) Condensation – Simple sugar condense and form higher carbohydrate molecules example polysaccharide
6) Osazone Formation – All reducing Sugar condense with phenylhydrazine and produce osazone compound
7) Fermentation – Sugar in general undergo fermentation by yeast and other micro organism

<u>Compound Carbohydrates</u>

These are made up of from two to one thousand or more than thousand monosaccharide units either with or without non carbohydrate units. Compound carbohydrate are principally of two types

a. Simpler Compound Carbohydrate.
b. More Complex Compound Carbohydrate.

<u>Simpler Compound Carbohydrate</u>.

These are also known as oligosaccharides. These compounds contain two to ten monosaccharide units. It includes

a) Disaccharide.
b) Trisaccharide.
c) Tetrasaccharide.

a) Disaccharides – These can be regarded as condensation products of two monosaccharide units with the elimination of one molecule of water. There general formula is $C_n(H_2O)_{n-1}$

   Example – Lactose, Maltose and sucrose.

   Lactose – It is composed of one molecule of glucose and one molecule of galactose. Found in the milk of mammals.

   Maltose (Malt Sugar) – It is composed of two glucose units it is an intermediate product in the digestion of starch.

   Sucrose (Cane Sugar) – On Hydrolysis it gives rise to one molecule of glucose and one molecule of fructose. It is one of the chief forms of sugar taken in diet. It is widely distributed in many plant juices such as sugar cane, sugar maple, pineapple and also in sugar beets

b) Trisaccharide – on hydrolysis it gives rise to three monosaccharide example mannotriose, rabinose, rhaminose etc

   These oligosaccharide are crystalline, water soluble and gives sweet taste.

More Complex Compound Carbohydrate

These are also known as polysaccharide and they are made of a large number of monosaccharide units during condensation all the active radicles become engaged so that they do not show any reducing power and do not produce any osazone and are not sweet to taste. They are soluble in water except cellulose the empeical formula of polysaccharide is $(C_6H_{10}O_5)_n$ Examples are starch, glycogen, dextrins, cellulose and inulin.

a) Starch – This is the chief form of carbohydrate taken in diet it is manufactured by the plants and plays the same role in them as glycogen does in animals that is easily available sugar store. It is the main constituent of food grain example rice, wheat, jowar etc. It is insoluble in cold water. Due to the presence of an outer cellulose layer around the granule, on boiling it ruptures and starch enters into a colloidal solution. It gives blue colour with iodine, it has no reducing power and is tasteless.

b) Glycogen – It is called animal starch because it is this form that glucose remains stored in the liver and muscles of animal body. Glycogen is also found in those plants which do not posses any chlorophyll such as yeast, fungi etc. Glycogen is soluble in water, makes an opalescent solution and gives reddish colour with iodine.

c) Dextrin– They do not occur naturally but they are the split products of starch digestion therefore it is also known as derived carbohydrates.

d) Cellulose – It is a stable, insoluble compound found in the plant and never present in the animal body. Cellulose is taken with vegetable food. It cannot be digested by the human beings; herbivorous animals can digest cellulose with the help of bacteria. Although indigestible yet cellulose is of considerable importance in human dietetics because it acts bulk to the intestinal contents to stimulates peristalsis and thereby help in the evacuation of faeces.

e) Inulin – It is polysaccharide compound of d-fructose units. It is white crystalline powder and is readily soluble in hot water. It does not give any colour with iodine.

Other Polysaccharide containing carbohydrate an non carbohydrate units are heteropentosans, heterohexosans and mucopolysaccharide

Functional Importance of carbohydrate

1) It is readily available fuel of the body it provides most of the energy needed for life
2) It also constitutes the structural material of the organism
3) It also acts as important storage of food material of the organism
4) Carbohydrate plays as a key role in the metabolism of amino acids and fatty acids.

Review questions:

1. What are carbohydrates?
2. Classify carbohydrates.
3. What are properties of monosaccharides?
4. Note on monosaccharides, disaccharides, polysaccharides.

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Chapter6

Proteins

Objectives- At the end of this chapter student shall understand the sources, classification, properties and significance of proteins.

6.1 Introduction

A third principal group of organic compounds is protein. They are complex compounds containing carbon, hydrogen, oxygen, nitrogen, sulphur and phosphorus. They differ from carbohydrate and lipids by always containing nitrogen and sometimes phosphorus and sulphur.

The elementary composition of protein is as follows-

Carbon 54%, Hydrogen 7%, nitrogen 16%, oxygen 22% some may contain sulphur 1% while others phosphorus 0.6%.

The complex protein molecule is built up by the union of larger number of amino acids. The amino acids should be considered as the units with which the protein molecule is composed

Sources-

Proteins are the chief nitrogenous constituents tissues of the body and of the food we eat.

They are obtained chiefly from meat, eggs, milk, cheese, fish, cereals and certain vegetables such as peas and beans.

6.2 Classification of Protein

Proteins are classified in various ways. The following classification is advocated by the English school of physiologist

1. Simple Proteins
2. Conjugated Proteins
3. Derived Proteins

1) **Simple Protein** – These are pure Protein not combined with anything else. The important members of this group are as follows.

a) **Protamines**– Found in the sperm of certain fishes. Strongly basic in character and it is not coagulated by heat
b) **Histones** – Occur in thymus Gland and also in the haemoglobin (globin part). Coagulates on boiling in presence of salts, soluble in water.
c) **Albumins** – Soluble in distilled water as well as in salt solution. Coagulated by heat, acids and alkalis**.** It includes
    - Egg albumin – Forms 10 to 12 % of egg white
    - Serum albumin – Occurs in blood serum (4 to 5%). Lymph and tissue fluid etc.
    - Lacto albumin – In Milk.
    - Mayo albumin – In muscle.
    - Leucosin – Is a vegetable protein found in wheat.
d) **Globulins** – Insoluble in distilled water but soluble in dilute salt solution. Coagulated by heat an acid. It includes
    - Serum Globulins – Occurs in blood serum.
    - Ovo Globulins – In Egg yolk.
    - Crystallin – Found in the crystalline lens of the eye..
    - Fibrinogen – Present in blood plasma and is converted into fibrin during clotting.
e) Prolamines – Found in maize, barley, wheat etc
f) Glutelins– Obtained from cereals.
g) Scleroproteins– They are least soluble of all the proteins.
    - Keratin – Found in horn, nails, hairs etc.
    - Elastin – From elastic tissues, cartilage, ligaments etc
    - Collagen – From white fibrous tissue, bone and cartilage etc.
    - Ossein – Bone and teeth.

2) **Conjugated Protein** – In these compounds the protein molecules are conjugated with another non protein prosthetic group. It Includes
    a) **Chromo protein**– In these group non protein prosthetic group is a colouring matter. Examples
    1. **Haemoglobin**– Simple protein Globin remains united with iron containing pigment haem
    2. **Visual purple (Rhodopsin)** – In the retina of the eye prosthetic group is a carotenoid pigment.
    3. **Cytochrome** – Conjugated protein containing haem.

b) **Phosphoprotein** – Protein molecules remain combine with phosphoric acid example caseinogen of milk, vitellin of egg yolk
c) **Nucleoproteins** – This protein contains phosphoric acid and also other prosthetic group hence it is placed in a special group. (Discussed in detail in next chapter)
d) **Glycoprotein and Mucoprotein**– In these compounds protein molecule remains combine with a carbohydrate radical example Glucosamine
e) **Lipoprotein** – In this compound protein remains united with lipids example phospholipid
f) **Metalloprotein**– Protein containing metallic elements Fe, Cu, Mg, etc Found in large variety of enzymes

3) **Derived Protein** – This protein are not present in nature as such. They are found as products of hydrolysis of the native protein molecule. The Gradual stages are follows

   Protein → Protean →Metaprotein→Proteose→ Peptone → Peptides →Aminoacids.

6.3 Amino Acids

Amino acids are the building blocks of proteins. In Protein formation amino acids combine to form more complex molecules while water molecules are lost. The bonds formed between amino acids are called peptide bonds.

Amino acid is an organic acid in which one or more hydrogen atom is replaced by – NH_2 and a carboxyl group (-COOH). The empirical formula is R-$CHNH_2$COOH. At least 20 different amino acids are found in protein. A great variety of proteins is possible because each variation in the number of or the sequence can produce a different protein.

The amino acids which cannot be synthesized from the body cells are called Essential amino acids example methionine, threonine, tryptophan, valine, isoleucine, phenylalanine, lysine, leucine and histidine. Those amino acids which can be synthesized by body cells are called non essential amino acids example are Glycine, alanine, serine, aspartic acid, glutamic acid, cystine, prolene, hydroxyproline, etc. Long chains of amino acids linked by peptide bonds are called polypeptides.

The amino acids are colorless, crystalline substances, soluble in water, easily diffusible and except glycine all are optically active.

6.4 Properties of Proteins

Proteins are colloidal in nature but many of them can be crystallized. They are generally soluble in water, weak salt solution, dilute acids and alkalis.

Each Protein has got a particular isoelectric point at which it is precipitated. During precipitation proteins do not undergo any intramolecular change. They simply separate out because the medium is not favorable for solution.

Coagulation – Most of the protein undergo heat or acid coagulation involves intermolecular change.

Denaturation – It is said to occur when a protein undergoes changes structure or composition, Chemical and physical agents which cause these changes are called denaturing agents such as shaking, change of temperature, addition of neutral salt, radiation etc.

Denaturation results in an unfolding of the protein molecule or intermolecular arrangement due to the destruction of hydrogen bonds mainly these changes often affect the viscosity, particle size solubility and even loss of certain amino acids or peptides of low molecular weight.

In the same species each type of tissue contains proteins which are distinct from those found in the other tissues. No two proteins are found to be exactly the same in there physiological properties.

Vegetable protein differ from animal protein in the fat that the former is generally poorer in essential amino acid.

6.5 Functional Importance of proteins

1. Protein acts as a growth material for the organism.
2. Structures of living materials are composed of different types of protein molecule.
3. It also acts as a part of fuel of the organism.

4. All the Hormones example pituitary, hypothalamic, placental, pancreatic are protein in nature. These hormones regulate various physiological processes in the body.
5. All enzymes are protein in nature. They regulates biochemical reactions.
6. Protein serves as contractile elements in muscle tissue.
7. Proteins serve as antibodies to protect the body against invading micro organisms.
8. Protein transports vital substances throughout the body example haemoglobin transport CO_2 and O_2.

Review Questions-

1. What are proteins?
2. Classify proteins with examples.
3. What are properties of proteins?
4. Note on amino acids.

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Chapter 7

Lipids

Objectives- At the end of this chapter student shall understand the sources, classification, properties and significance of lipids.

1.1 Introduction-

Lipids (Greek: lipos-fat) is a source of high energy to the body. It may be regarded as organic substances relatively insoluble in water, soluble in organic solvents like alcohol, ether, chloroform, etc. and are utilized by living cells.

7.2 Classification of Lipids: - Broadly classified as

1. Saponifiable –

   A. Simple lipids

   B. Compound or complex lipids

1. Unsaponifiable –

   A. Derived lipids

   B. Miscellaneous lipids

Simple Lipids-

These are esters of fatty acids with alcohol. They are mainly of 2 types:

a) Fats and Oils- Also called as triacylglycerols. These are esters fatty acids with glycerols.

b) Waxes- Esters of fatty acids with alcohol other than glycerol.
   E.g. Cetyl alcohol

Compound Lipids or complex Lipids-

They are esters of fatty acids with alcohols containing additional group such as phosphate, nitrogenous base, carbohydrate, protein, etc. They are further classified as:-

a) Phospholipids- It contains phosphoric acid with a nitrogenous base and alcohol and fatty acids.

   They are of 2 types

   i. Glycerophospholipids e.g Lecithin, cephalin.

   ii. Sphingophospholipids e.g. Sphingomyelin.

b) Glycolipids- These are lipids containing a fatty acid with carbohydrate and nitrogenous base. The alcohol is sphingosine hence also called glycosphingolipids. Glycerol and phosphate is absent. E.g. Cerebrosides, gangliosides.

c) Lipoproteins- it contains proteins and hence very large molecules of lipids. they are of five types –

   a. Chylomicrons
   b. HDL– High density lipoproteins.
   c. LDL- Low density lipoproteins.
   d. VLDL – Very low density lipoproteins.

d) Steroids – Steroids contains cyclic steroid nucleus. If it contains 1 or more hydroxyl group it is called sterol. The different steroids are cholesterol, bile acids, vitamin D, sex & adrenocortical hormones, etc.

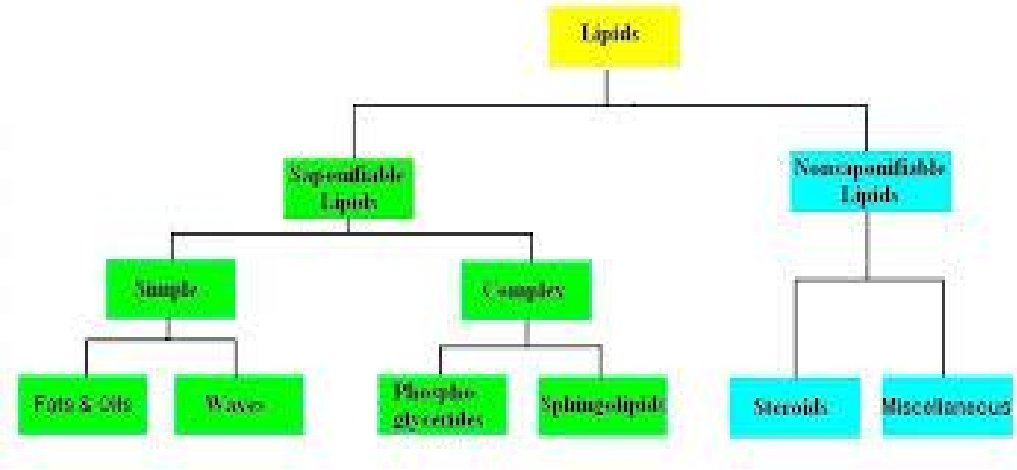
e) Others- e.g. Sulpholipids, amino lipids, lipopolysaccharides.

Derived Lipids-

They are obtained on hydrolysis of group 1 and group 2 lipids. They possess the characteristics of lipids. E.g Glycerol, Fatty acids, mono and diacylglycerols, fat soluble vitamins, steroid hormones, hydrocarbons and ketone bodies.

Miscellaneous Lipids-

They possesses characteristics of lipids e.g carotenoids, squalene and hydrocarbon (pentacosane) terpenes.



7.3 **Functions of lipids:-**

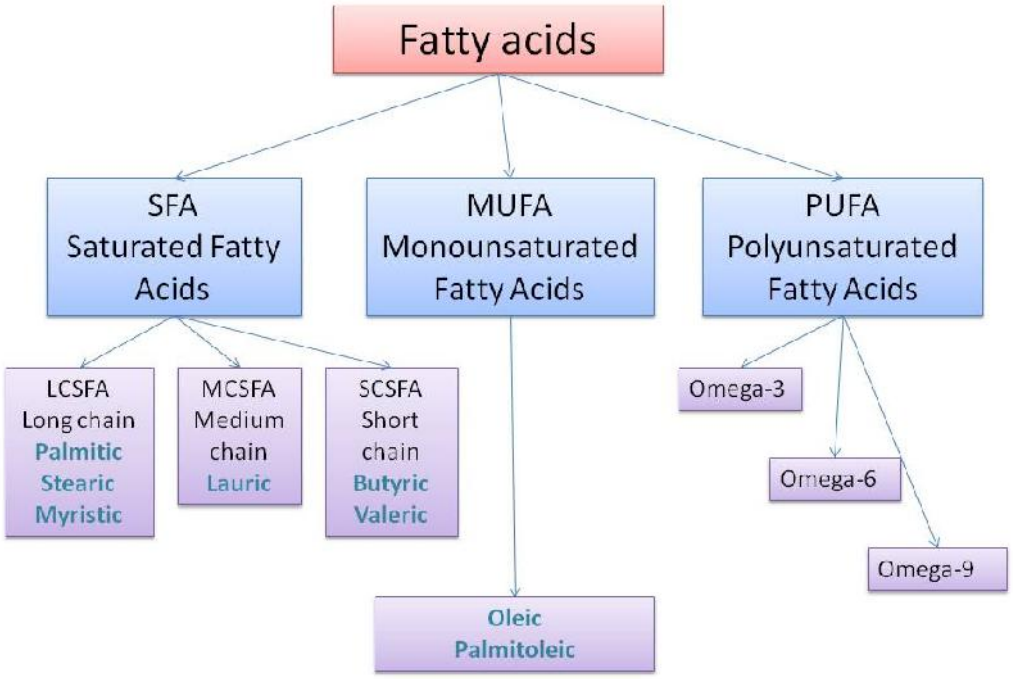
1. They are the concentrated fuel reserve of the body (triacylglycerols)
2. Lipids are the constituents of membrane structure and regulate the membrane permeability (phospholipids and cholesterol)
3. They serve as a source of fat soluble vitamins (A,D,E and K)
4. Lipids are important as cellular metabolic regulators (steroid hormones and prostaglandins)
5. Lipids protect the internal organs, serve as insulating materials and give shape and smooth appearance to the body.

Note: Neutral lipids – These are uncharged lipids e.g. mono, di and triacylglycerols, cholesterol, cholesteryl esters.

7.4 Fatty acids –

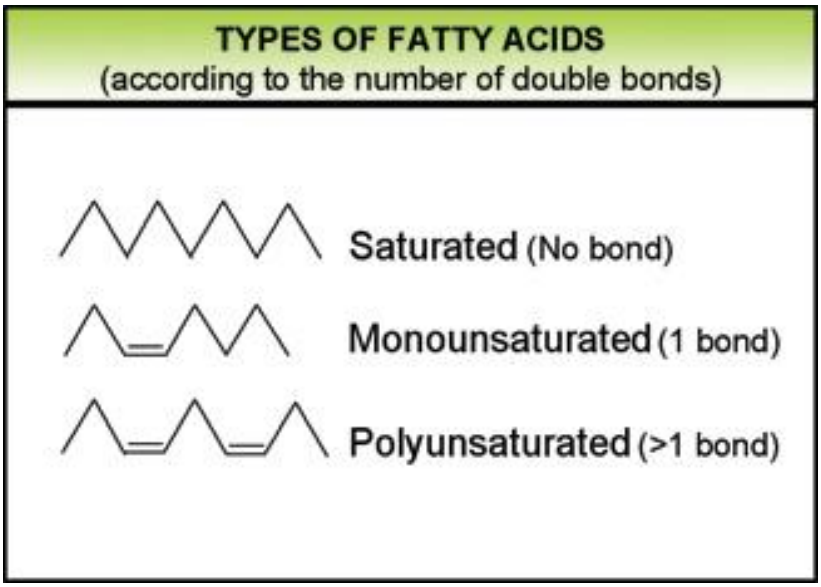
Fatty acids are simplest form of lipids. They are carboxylic acid with hydrocarbons. They occur in the esterified form as major constituents of various lipids or unesterified form as free fatty acids. Even carbon fatty acids are Pamitic acid, Stearic acid etc. and odd carbon fatty acids are propionic acid ,valeric acid etc. Saturated fatty acids do not contain

double bond where as unsaturated fatty acids contains. Fatty acid with 1 double bond is called monounsaturated and those with 2 or more are called polyunsaturated fatty acids (PUFA).



7.5 Essential fatty acids

Essential fatty acids (EFA) are not formed by body, hence has to be obtained from food. Chemically they are polyunsaturated fatty acid e.g. linoleic acid and linolenic acid.



7.6 Properties of lipids-

1. Hydrolysis – Lipids undergo hydrolysis to liberate free fatty acid and glycerol in the presence of lipases.
2. Saponification – The hydrolysis of lipids by alkalies to form glycerol and soap is called saponification.
3. Rancidity – Deterioration of fats and oils leading to unpleasant taste is called rancidity.

Review Questions-

1. What are lipids?
2. Classify lipids.
3. What are properties of lipids?
4. What are fatty acids? Classify them.
5. What are functions of lipids?

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Chapter 8-Nucleic Acids

Objectives- Students shall learn about different types of nucleic acids, difference between DNA and RNA etc.

8.1 Introduction-

Nucleic Acids are compounds first discovered in the nuclei of cells are exceedingly large organic molecule containing carbon, hydrogen, oxygen, nitrogen and phosphorus. They are divided into two principal kinds.

Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA).

The basic structural units of proteins are amino acids and the basic units of nucleic acids are nucleotides.

8.2 DNA – A molecule of DNA is a chain composed of repeating nucleotide units, each nucleotide of DNA consist of three basic parts

1. It contains four possible nitrogenous bases which are adenine, thymine, cytosine and guanine. Adenine and guanine are double ring shaped structures collectively referred to as purine, thymine, and cytosine are smaller single ring structures called pyrimidines.
2. It contains a pentose sugar called deoxyribose.
3. It also contains phosphate group.
4. The nucleotides are named according to the nitrogenous base that is present e.g. a nucleotide containing thymine is called thymine nucleotide. One containing adenine is called adenine nucleotide and so on.

The chemical components of the DNA molecule were known before 1900 but it was not until 1953 that a model of the organization of the chemical was constructed by **J.D .Watson and F.H. Crick.**

a. The molecule of DNA consists of two strands with cross bars. The Strands twist about each other in the form of double helix so that shape resembles a twisted ladder.

b. The upright DNA ladder consists of alternating Phosphate group and the deoxyribose portion of the nucleotide.

c. The rungs of the ladder contain paired nitrogenous bases. Adenine always pairs with thymine and cytosine always pairs with guanine.

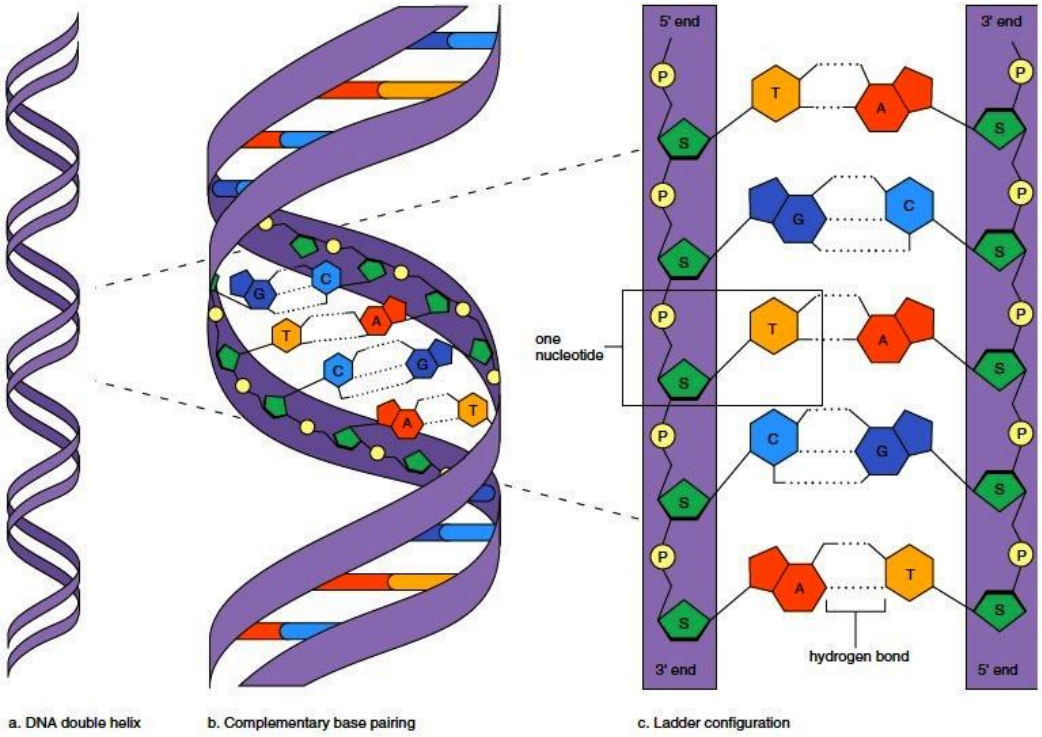

Figure 8.1 DNA structure

8.3 Importance-

Cells contain hereditary material called **genes** each of which is a segment of DNA molecule,genes determine traits (Characters) we inherit. Generally half character from the father and half from the mother according to the Mendelian law of inheritance. Genes control all the activities that take place in our cells throughout a life time. When a cell divides its hereditary information is passed on to the next generation of cells and this is possible because of the unique structure of DNA.

8.4RNA –

The second principal kind of nucleic acid differ from DNA in several respects.

1. RNA is single stranded where as DNA is double stranded.
2. Sugar in the RNA is pentose ribose where as dexoiribose in DNA.
3. RNA does not contain the nitrogen base thymine, instead of thymine, RNA contains uracil.

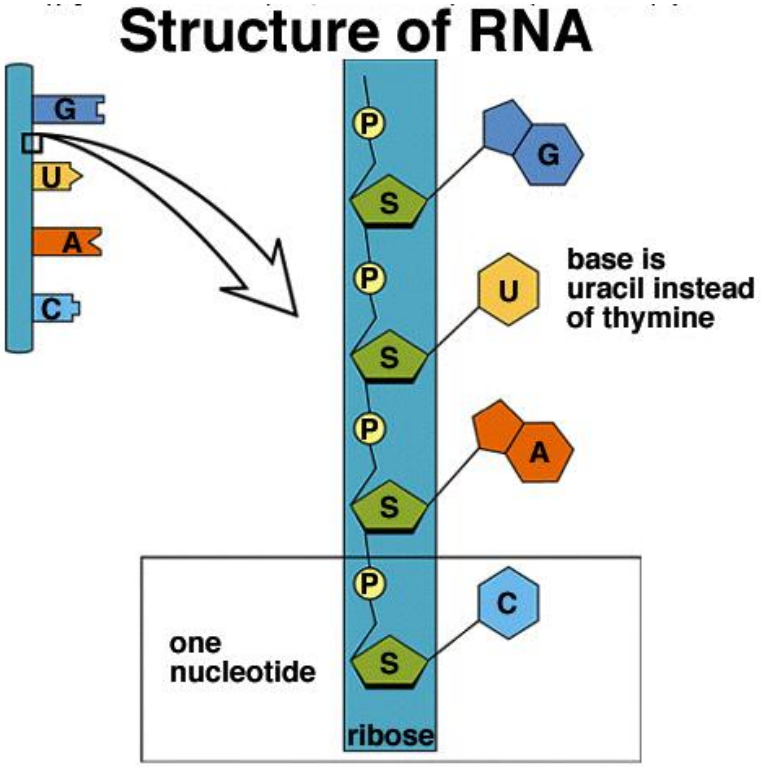

Fig. 8.2 RNA

There are at least three different kinds of RNA have been identified in cells. Each type has a specific role to perform with DNA in protein synthesis reaction. Three types of RNA's are mRNA, tRNA, rRNA.

a. m RNA (Messenger RNA) – It is formed on the strands of DNA and is responsible for carrying information from the nucleus of the cell to the area where protein is synthesized. It directs the synthesis of protein in the cell.

b. tRNA (Transfer RNA) – It is responsible for transferring specific amino acids to the size of protein synthesis where mRNA has already stretched over the ribosomal surface and thus expresses the genetic information. The amino acid sequences are determined by the tRNA.

c. rRNA (RibomalRNA) – It is found as minute granules in the ribosome, it is a protein synthesis centre in the cells.

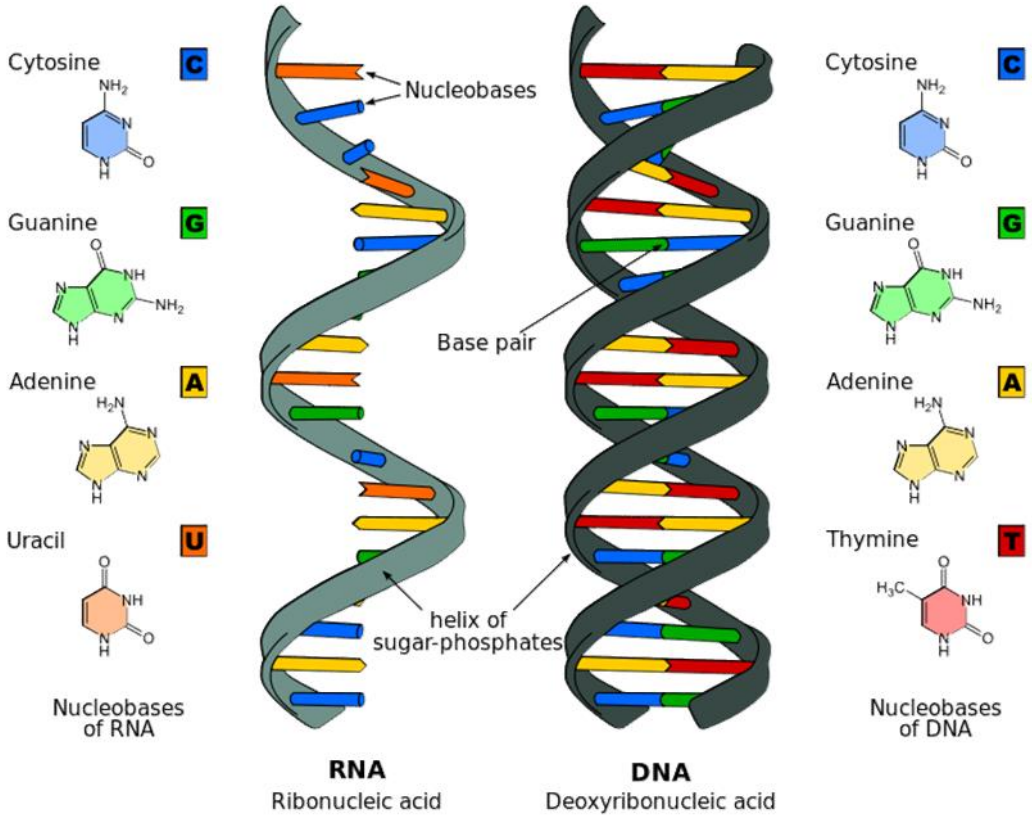

Figure 8.3 Differences between DNA & RNA

Review Questions-

1. What are nucleic acids?
2. Name different nitrogenous bases.
3. Differentiate between DNA and RNA.
4. What are types of RNA?
5. What are functions of DNA and RNA?
6. Describe structure of DNA.

Chapter 9 Enzymes

Objectives:

To obtain knowledge about some of the basic facts about enzymes.

To understand diagnostic importance of enzymes.

9.1 - Introduction:

- Enzymes are soluble organic catalysts formed by living cells but their activity does not depend upon lividity of cell.
- They are protein in nature.
- Colloidal in character, destroyed by heat and specific in their action.
- Enzymes increase the rate of chemical reactions within the body without itself undergoing any change.
- In the laboratory, hydrolysis of proteins by a strong acid at 100°C takes at least a couple of days. The same protein is fully digested by the enzymes in digestive organs at 37°C within a couple of hours. This easily explains the importance of enzymes as biocatalyst in various chemical reactions taking place in side our body.
- The substance on which the enzyme acts is known as “substrate”. The substance obtained after the enzymatic reaction is called as “product”.

$$\text{Substrate} \xrightarrow{\text{Enzyme}} \text{Product}$$

- Some of the enzyme levels in serum are often studied in the pathological laboratory.
- The enzyme levels carry definite diagnostic importance.

9.2- Classification of enzymes:

I. Classification I

Enzymes are classified into two broad groups.

A. Intra cellular enzymes: - Such enzymes act within the cell where they are synthesized.

B. Extracellular enzymes: - These enzymes are active outside the cells. All the digestive enzymes belong to this group.

II. Classification II

The International Union of Biochemistry classified enzymes according to their type of reaction. In this way enzymes are classified into six types. They are as follows.

1. Oxidoreductases: Enzymes involved in oxidation – reduction reactions e.g. alcohol dehydrogenase.
2. Transferases: Enzymes that catalyze the transfer of functional groups. E.g Hexokinase, transaminases.
3. Hydrolases: Enzymes that bring about hydrolysis of various compounds. E.g .Lipase, pepsin.
4. Lyases: Enzymes specialized in the addition or removal of water, ammonia etc. e.g. Aldolase.
5. Isomerases: Enzymes involved in all the isomerization reactions. e.g. phosphohexose isomerase.
6. Ligases: Enzymes catalyzing the synthetic reactions where two molecules are joined together and ATP is used. E.g. succinate thiokinase.

9.3- Properties of enzymes:

Enzymes being protein in nature, exhibit all the general properties of proteins.

1. Solubility: Enzymes form colloidal solutions in water instead of true solutions.
2. Molecular weight: Depending on no. of amino acids present their molecular weight is variable from enzyme to enzyme.
3. Shape: Most of the enzymes are globular or oval in shape.
4. Precipitation: Enzymes can be precipitated by dehydration or neutralization of polar groups.
5. Coagulation: Irreversible denaturation results in coagulation.
6. Denaturation: Denaturation means disorganization of original structure. This can be caused by agents like heat, U-V rays, acids, alkalis etc.

9.4Mechanism of enzyme action

Enzyme – substrate complex formation:

In the process of enzyme catalysis substrate combines with the enzyme (E) to form enzyme substrate complex (ES) which result in product formation (P).

$$E + S \longleftrightarrow E + P$$

Some theories are there to explain mechanism of enzyme – substrate complex formation.

Lock & key model or Fischer's template theory:

According to this model, the structure of enzyme is rigid. The substrate fits into the active site just as a key fits into the proper lock.

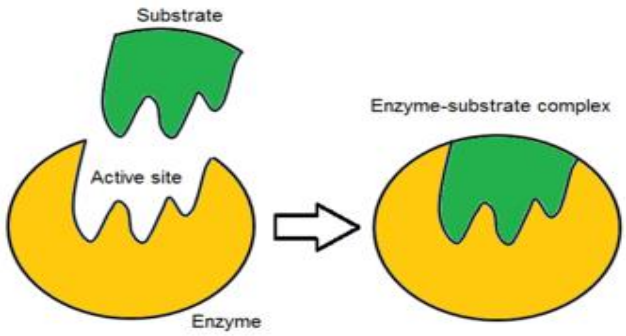

Figure 9.4.1 Lock & key model

Induced fit theory or koshland's model:

As per this model active site is not rigid and preshaped. The interaction of the substrate with the enzyme induces a fit or a conformation change in the enzyme, resulting in the formation of strong substrate binding site.

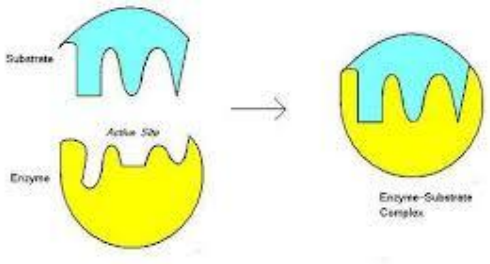

Figure 9.4.2 Koshland's Model

Substrate strain theory:

In this model, when a substrate binds to the preformed active site, the enzyme induces a strain to the substrate. The strained substrate leads to the formation of product.

A combination of induced fit model with substrate strain is considered as operative in the enzymatic action.

9.4- Diagnostic importance of serum enzymes:

Estimation of enzyme activities in plasma / serum is of great diagnostic importance. The normal serum level of an enzyme indicates the balance between its synthesis and release in the routine cell turnover. The raised enzyme levels can be due to reasons as follows:

- Cellular damage
- Increased rate of cell turnover
- Proliferation of cells
- Increased synthesis of enzymes

Thus the serum enzymes are conveniently used as markers to diagnose some diseases. In some diseases plasma activities of the enzymes may be lower than normal.

Table 1: Increased enzyme levels and diseases:

Sr. No.	Important enzymes in the diagnosis of disease	
	Serum enzyme (elevated)	**Disease**
1.	Amylase	Acute pancreatitis
2.	Serum glutamate pyruvate transaminase (SGPT)	Liver diseases (hepatitis)
3.	Serum glutamate oxaloacetate transaminase (SGOT)	Heart attacks (myocardial infarction)
4.	Alkaline phosphatase	Rickets, obstructive jaundice
5.	Acid phosphatase	Cancer of prostate gland
6.	Lactate dehydrogenase (LDH)	Heart attacks, liver diseases
7.	Creatinine phosphokinase (CPK)	Myocardial infarction (early marker)
8.	Aldolase	Muscular dystrophy

Table 2:

Sr. No.	Increase in plasma (serum) enzymes in the diagnosis of diseases		
	Enzymes	**Normal Value**	**Diseases in which increased**
1.	Digestive enzymes Amylase Lipase	80-180 Somogyi units / dl 0.2-1.5 IU/l	Acute pancreatitis, mumps (acute parotitis), obstruction in pancreatic duct, severe diabetic ketoacidosis.

			Acute pancreatitis, moderate elevation in carcinoma of pancreas.
2.	Transaminases Alanine transaminase (ALT) or serum glutamate pyruvate transaminase (SGPT) Aspartate transaminase (AST) or serum glutamate oxaloacetate transaminase (SGOT)	3-40 IU/I 4-45 IU/I	Acute hepatitis (viral or toxic), jaundice, cirrhosis of liver. Myocardial infarction, liver diseases, liver cancer, cirrhosis of liver.
3.	Phosphatases Alkaline phosphatase (ALP) (pH optimum 9-10) Acid phosphatase (ACP) (pH optimum 4-6)	In adults 3-13 king Armstrong (KA) units/dl or 25-90 IU/I .In children 15-30 KA/dl 0.5-4 KA units/dl or 2.5 – 12 IU/l	Bone diseases (related to higher osteoblastic activity) rickets, Pagets' disease, hyperparathyroidism, carcinoma of bone. Liver diseases-obstructive jaundice (cholestasis), infective hepatitis, cirrhosis of liver. Prostatic carcinoma i.e. cancer of prostate gland (tartarateliablile ACP

			serves as a marker for diagnosis and follow up), pagets's disease, Gauchers' disease.
4.	Enzymes of carbohydrate metabolism Aldolase Isocitrate dehydrogenase (ICD) Lactate dehydrogenase (LDH)	2-6 IU/I 1-4 IU/I 50-200 IU/l	Muscular dystrophy, liver diseases, myocardial infarction, myasthenia gravis, leukemias Liver diseases Iinflammatory toxic or malignant) Myocardial infarction, acute infective hepatitis, muscular dystrophy, leukemia, permiciousanaemia.

9.5- Diagnostic importance of some frequently used enzymes:-

1. **SGOT - (serum Glutamate oxaloacetate transaminase)**

   Normal values**: -** 4-45 IU /l SGOT level is increased in myocardial infarction, liver diseases, liver cancer, cirrhosis of liver etc.

2. **SGPT (Serum Glutamate pyruvate transaminase)**

   Normal value**: -** 3 to 40 IU/l SGPT level is increased in acute hepatitis, jaundice and cirrhosis of liver.

3. **Acid phosphatase:-**

Normal values:- 0.5 to 4 KA units / dl or 2.5 to 12 IU/l Acid phosphatase level is useful in diagnosis & follow up of cancer of prostate.

4. **Alkaline phosphatase:-**

Normal value:- Adults – 3-13 KA units / dl or 25 to 90 IU / l
Children – 15-30 KA units / dl

Alkaline phosphatase level is increased in obstructive jaundice, infective hepatitis, cirrhosis of liver. It is also increased in bone diseases like rickets, hyperparathyroidisim, carcinoma of bone, paget's disease.

5. **Lactate dehydrogenase:-**

Normal value:- 50-200 IU/l

6. LDH level is increased in myocardial infarction, acute infective hepatitis, muscular dystrophy leukemia.

Different isoenzymes of LDH (LDH1, LDH2, LDH3, LDH4 & LDH5) can be separated electrophoretically which has its more specific diagnostic applications. In myocardial infarction LDH1, is greater than LDH2, in liver diseases LDH5 is greater.

7. **Creatinine phosphokinase:-**

Normal value - 10-50 IU/l CPK is increased in myocardial infarction, muscular dystrophy, hypothyroidism & alcoholism.

8. **Amylase:-**

Normal value:-80 to 180 somogyi units / dl Increased serum amylase level is observed in acute pancreatitis, mumps, severe diabetic ketoacidosis.

9. **Lipase:-**

Normal Value: - 0.2 to 1.5 IU/l

Serum lipase level is increased in acute pancreatitis.

Review Questions:

1. Define Enzyme. What is their physiological importance?
2. Describe classification of enzymes.
3. Enlist any five enzymes with their normal values.
4. Enzymes have diagnostic importance. Explain with any two examples.
5. Write names of various enzymes useful in diagnosis of myocardial infarction with their normal values.

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Chapter 10 Vitamins

Objective: At the end of this topic students will learn about different types of vitamins required to maintain normal health.

10.1 Introduction

Definition: Vitamins may be defined as organic compounds which are found in natural food and are essential for the normal growth and nutrition of human body.

- They are required by the body in small amounts for metabolism, to protect health,and for proper growth in children.
- Vitamins also assist in the formation of hormones, blood cells,nervous-system chemicals, and genetic material.
- They generally act as catalysts, combining with proteinsto create metabolically active enzymes that in turn produce hundreds of important chemical reactionsthroughout the body.

10.2 Classification of vitamins:-

On basis of solubility vitamins are classified as:-

1. Fat soluble vitamins: - A, D, E, K

2. Water soluble vitamins: - C and B complex (B1 to B12).

Characteristics of fat / water soluble vitamins.

Fat soluble vitamins	**Water soluble vitamins**
Soluble in fat	Soluble in water
Require a lipid carrier	Not so

Stored in the body	Excess excreted except B_{12}
Biologically active (not always a coenzyme)	Act as coenzyme
Not excreted in urine	Excreted in urine
Excess causes hypervitaminosis	Non toxic
Deficiency symptoms develop slowly	Develop rapidly
Require bile salts for absorption	Not so
e.g. :- Vitamin A, D, E, K	e.g.:- Vitamin. B complex and Vitamin C

FAT SOLUBLE VITAMINS

10.3 Vitamin A:

Vitamin A is a pale yellow primary alcohol derived from carotene. It includes Retinol (alcoholic form),Retinal (Aldehyde form) and Retinoic acid (acidic form).

Sources:

Carotenoids are the chief source of vitamin A in diet. They are found in leafy vegetables, carrots, pumpkin, sweet potato, turnip, tomato, corn and apricot and orange & yellow fruits like papaya, mango, peach etc.

Animal sources of vitamin A are egg yolk, butter, liver and fish liver oils and milk.

Fig 10.3 Vitamin A sources

Functions:

1. Vitamin A accelerates normal formation of bone and teeth.
2. Vitamin A helps in maintaining the normal structure and functions of epithelial tissue such as epithelial layer of skin.
3. Vitamin A plays an important role in maintaining the normal chemical changes taking place in photoreceptor cells of retina, i.e. cones and rods. Retinal is a component of visual pigment, rhodopsin.
4. Spermatogenesis in male.

Daily requirement:

- Infants: 1500 I.U. Children: 2000 – 3500 I.U
- Adult: 5000 I.U. equivalent to 1000 retinol
- Pregnancy and lactation: 6000 – 8000 I.U

Clinical significance:

1. **Deficiency**: This will result in

- Night blindness (Nyctalopia): it is the inability to adapt when suddenlyproceeding from bright light to dim light.
- Xerophthalmia: Absence of tears and drying of eyes with irritation and conjunctivitis with Bitot spots (white spots).

- Keratinization of skin and mucous membranes, they will become dry,scaly and rough.

2**. Toxicity**:

- Excessive intake of vitamin A produces a toxic syndrome calledHypervitaminosis A. It leads to hyperirritability, tender swelling of long bone, dry scaly lips and enlarged liver, loss of hair, vomiting.

10.4 Vitamin D

The D vitamins are a group of sterols that have a hormone-like function.

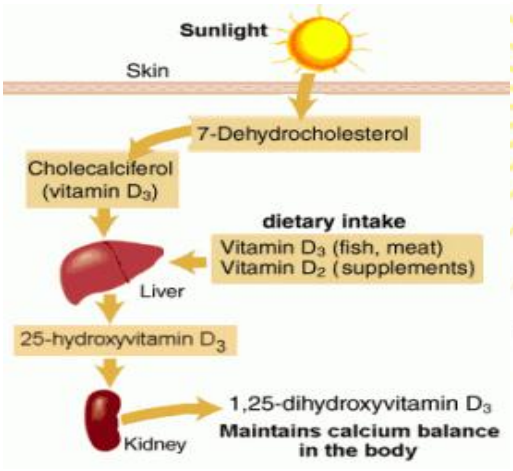
Two main forms are discovered-

1. Vitamin D2 (calciferol) which is the activated form of ergosterol
2. Vitamin D3 the activated 7- dehydrocholesterol.

The activation occurs by their exposure to ultraviolet rays present in sunlight.

Sources

1. Diet: D2 in plants (mushrooms) and D3 in animals (egg yolk, fortified milk, butter, liver and fish like sardines, tuna, and salmon).
2. Endogenous precursor: 7-dehydrocholesterol is converted to D3 in the dermis and epidermis of humans exposed to sunlight.



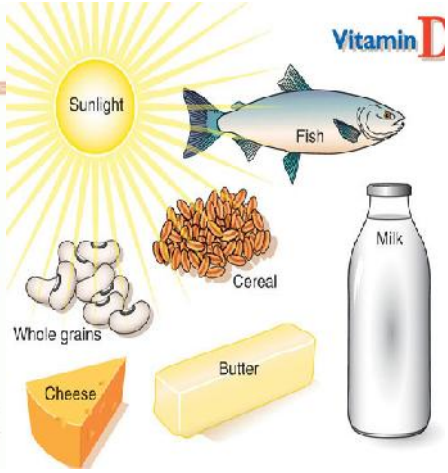


Fig. 10.4.1 **Fig. 10.4.2**

Functions:

1. It is essential for the development of bones and normal growth of the body.
2. Vitamin D increases the excretion of phosphate by kidney and lowers the serum phosphate concentration.
3. Vitamin D increases the absorption of calcium and phosphorus from the intestine and maintains their regulated supply to body fluids.
4. It protects teeth and bones against the effects of low calcium intake by making more effective use of calcium and phosphorus.
5. It increases the citrate level of blood, bone, kidney and heart tissues as well as the excretion of citric acid.
6. It decreases the pH in the lower intestinal tract which helps in increasing the absorption of calcium and phosphorus.

Daily requirement:

Infants and children – 400 I.U. Adults: 400 – 800 I.U

Clinical significance

1. **Deficiency-** Deficiency of vitamin D causes

- Rickets in children and Osteomalacia in adults.
- In Rickets abnormalities of skull and rib cage (Bead like structure on ribs (rachitic rosary), contracted pelvis and bow legs due to failure of the body to absorb calcium and phosphorus.
- In osteomalacia softness of pelvic girdle, ribs and femoral bones. Bones become softer with increased susceptibility to fractures.

2. Toxicity-

- Excessive consumption of vitamin D for long periods produce Hypervitaminosis as vitamin is fat soluble and it is stored in body. Symptoms like weakness, vomiting, diarrhea, polyuria and thirst. Serum calcium and phosphorous levels are elevated and urinary calcium excretion is increased .Deposition of calcium in a soft tissue and kidney (renal calculi).

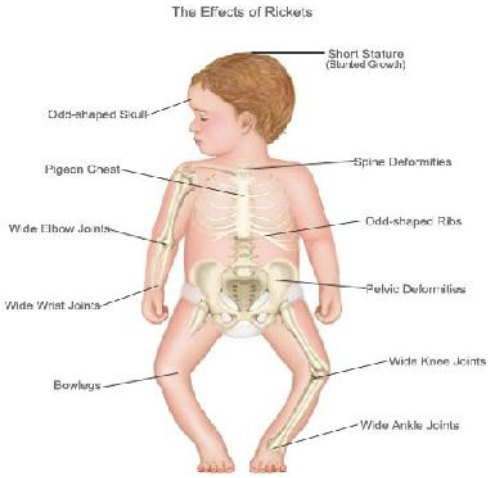


Fig. 10.4.3

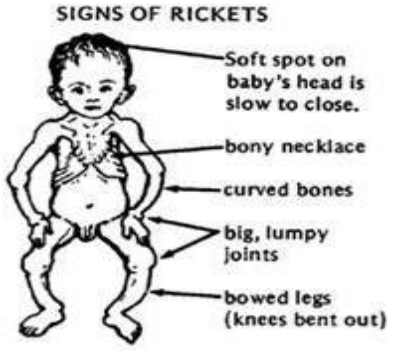


Fig. 10.4.4

10.5 Vitamin E

Compounds having vitamin E activity are called Tocopherols.

- Four such compounds have been found to occur in natural sources, α, β, γ, and δ – tocopherol.
- α-tocopherol is the predominant isomer in plasma and the mostactive form of vitamin E.

- Vitamin E is stored in adipose tissue, liver & muscle

Sources:

1. Vegetable oil, fresh leafy vegetables, brown rice, oils of wheat, soya and corn, sunflower and cotton seed and egg yolk.
2. It is also present in almonds, olives, apricots.
3. Eggs, meat, liver and fish are also good sources of vitamin E.

Vitamin E type	Dietary source
Alpha-tocopherol	Sunflower oil, safflower oil, wheat germ, canola oil
Gamma-tocopherol	Corn oil, soybean oil, margarine, dressings, canola oil
Delta-tocopherol	Soybean oil
Tocotrienols	Palm oil

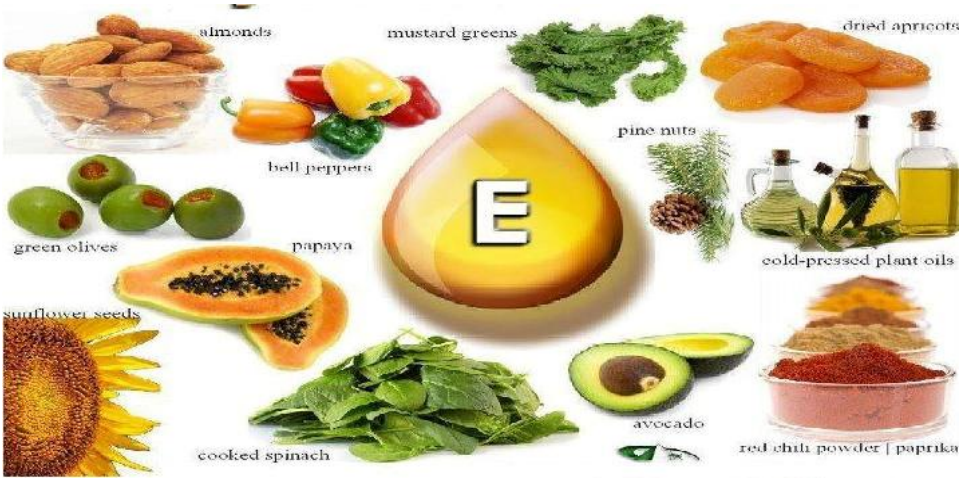


Figure 10.5

Functions:

1. Vitamin E is essential for the membrane structure and integrity of the cell; hence it is regarded as a membrane antioxidant.
2. It protects RBCs from hemolysis by oxidizing agents.
3. It is closely associated with reproductive functions and prevents sterility.
4. It increases the synthesis of haem by enhancing the activity of enzymes required for it.

5. Vitamin E protects liver from being damaged by toxic compounds such as carbon tetrachloride.
6. It works in association with vitamins A, C and β-carotene, to delay the onset of cataract.

Daily requirement:

The average daily diet should contain about 25 – 30 mgs of vitamin E.

Clinicalsignificance

1. Deficiency:

- Rare but occurs in two groups; premature or very low birth weight babies, and children with defective lipid absorption.
- Increased sensitivity of erythrocytes to peroxidation resulting in Hemolytic anemia.

2. Toxicity

- Overdose : Unknown

10.6 Vitamin K (Phylloquinone or Antihemorragic Vitamin or Coagulation Vitamin)

- Vitamin K is a complex unsaturated hydrocarbon found in two forms
    1. Vitamin K1 (Phylloquinone)
    2. Vitamin K 2 (Menaquinone).

Sources: -

1. Green vegetables like spinach, alfalfa and cabbage contain vitamin K1.
2. Vitamin K2 is produced by most bacteria present in human intestine.
3. Animal source include milk, egg, cheese & meat.

Figure 10.6

Functions

1. Vitamin K is required for synthesis of coagulation factors VII, IX, X and prothrombin (II). It allows chelation of calcium during coagulation.
2. It is required for prothrombin synthesis which is required to produce fibrin.
3. Concerned with mineralization of bones.
4. It is associated with electron transport chain and oxidative phosphorylation.

Daily requirement:

- Since the total requirement of Vitamin K is met by its production by the intestinal bacteria in man, there is no dietary requirement of VitaminK.
- Requirement of Vitamin K is 70 – 140 μg / day.

Clinical significance-

1. **Deficiency:** due to its defective absorption from the intestine or the inhibition of intestinal bacteria. It leads to
   - Hypoprothrombinemia in which the blood clotting time is prolonged.
   - Uncontrolled hemorrhages.

2. **Toxicity**-
    - Not seen in adults but large doses in infants can lead to hemolytic anemia and jaundice due to breakdown of RBCs.

WATER SOLUBLE VITAMINS

10.7 Vitamins B complex:

- The B vitamins in the past were thought to be a single vitamin, referred to as vitamin B.
- Later on research on these vitamins showed that they are chemically distinct vitamins that often coexist in the same foods.
- In general, supplements containing all eight are referred to as a vitamin B complex.
- These are fragile, water-soluble substances, several of which are particularly important to carbohydrate metabolism.
- The members of this group are:
    1. Thiamine (B1)
    2. Riboflavin (B2)
    3. Niacin (B3)
    4. Pantothenic acid (B5)
    5. Pyridoxine (B6)
    6. Biotin (B7)
    7. Folic acid (B9)
    8. Cyanocobalamin (B12)- Extrinsic factor of castle

Thiamine (B1)

It is soluble in water and heat labile.

Sources: Cereal grains, pulses, beans, peas, nuts, green leafy vegetables, organ meats (liver, heart and kidney), egg and milk. It is concentrated in the outer layer of cereals.

Figure 10.7.1 Vitamin B1 sources

Functions:

1. Acts as a coenzyme in citric acid cycle.
2. Acts as a catalyst in carbohydrate metabolism.

Daily requirement:

Infant- 0.4mg Children - 2.8 mg

Adults - 1.5 mg (for diet of 2000 – 3000 Calories).

Significance- Deficiency of vitamin B1 causes

Beriberi: Palpitation, cardiac hypertrophy, pulmonary congestion, Peripheral polyneuritis, muscular weakness.

<u>Riboflavin (B2)</u>

- It is water soluble and heat stable compound which is sensitive to light.

- Riboflavin imparts yellow orange color to Vitamin B complex tablets.
- Urine of a person taking riboflavin appears florescent yellow due to excretion of excess riboflavin in urine.

Sources:

1. Green leafy vegetables, tomatoes, cheese, almonds.
2. Fermenting bacteria, milk, kidney, liver and heart & fish are the best source of riboflavin.

Figure 10.7.2 Vitamin B2 sources

Functions:

1. It is essential for carbohydrate metabolism. Enzymes containing riboflavin are called flavoproteins.
2. It acts as a coenzyme for a enzyme catalyzing oxidation-reduction reaction.

Daily requirement:

Infants- 0.6 mg	Children - 1.0 –1.8 mg
Adults-1.5 – 1.8 mg.	During pregnancy - 2.0 mg

Deficiency:

1. Causes glossitis (Inflammation of tongue).
2. Skin lesions particularly around the nose lips. Red and shiny lips and dermatitis.

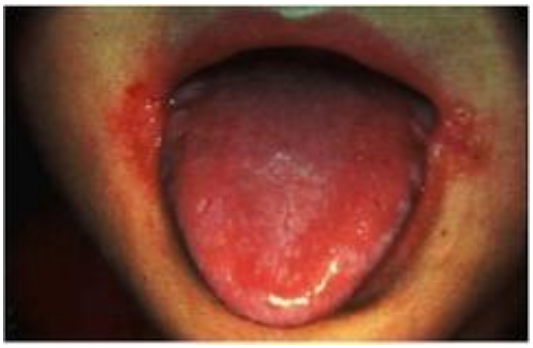


Figure 10.7.3Glossitis

Niacin (Nicotinic acid) B3

Niacin (Nicotinic acid) is pyridine 3 – carboxylic acid

Sources:

Cereal grains, yeast, legumes, liver, kidney, certain nuts, coffee and tea.

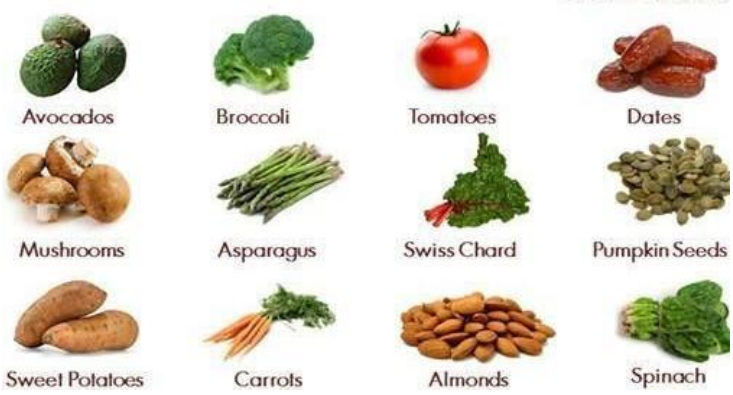


Fig 10.7.4 Vitamin B3 sources

Functions:

1. Required for normal functioning of skin, intestinal tract and nervous system.
2. Acts as Coenzyme in large number of dehydrogenase enzymes.

Daily requirement:

Infants – 5 - 8 mg Children – 8 -15 mgAdults- 17 -21 mg

Deficiency:

Causes Pellagra. The clinical features of this disease include (3Ds): Dermatitis, Diarrhoea, and Dementia.

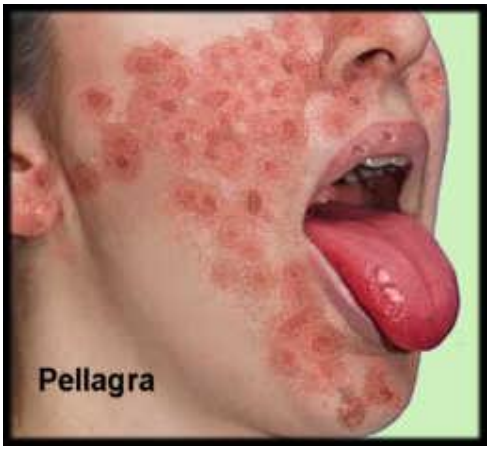


Fig. 10.7.5 Pellagra

Choline (B4)

- Some label choline as an essential dietary nutrient & not under vitamin B complex group. Because it can be synthesized in humans, from serine.
- Choline is a component of phospholipids lecithin.

Sources:

Available from many dietary sources like milk, eggs, liver, cereals etc.

Functions:

1. It is involved in membrane structure and lipid transport.
2. Choline prevents the accumulation of fat in liver (as lipotropic factor)
3. Involved in one carbon metabolism.
4. Choline is a precursor for the synthesis of acetylcholine which is required for transmission of nerve impulse.

Daily requirement:

Adults: 425-550 mgs.

Deficiency:

1. Fatty liver.
2. Atherosclerosis.
3. Neurological disorders.
4. It is important for pregnant women to get sufficient choline, since low choline level may raise the risk of neural tube defects in infants & may affect memory of child.
5. Choline supplements may reduce homocysteine & reduce chances of myocardial infarction.

Pantothenic acid (B5)

Panthothenic acid present in tissues in the form of acetyl- CoA.

Sources:

One of the most widely distributed vitamin found in plants & animals. Rich sources are yeast, kidney, liver, eggs, milk and peanuts.

Fig. 10.7.6 Vitamin B5 sources

Functions:

1. Synthesis of adrenocortical hormones and cholesterol from acetyl - CoA.
2. Plays a major role in metabolism of carbohydrates, fats and proteins

Daily requirement:

No specific requirement .Synthesized by the intestinal flora in sufficient amount to meet the requirement.

Deficiency:

Deficiency of this vitamin in man leads to inadequate growth, failure in gaining weight and fatty liver.

Pyridoxine (B6)

Pyridoxine occurs in nature in the form of pyridoxal phosphate and pyridoxyamine phosphate which act as coenzymes for several enzymes.

Sources:

Yeast, rice polishing, cereals grains and egg yolk, liver, muscle, kidney and fish etc.

Fig 10.7.7 Vitamin B6 sources

Functions:

1. Necessary for absorption and metabolism of amino acids. Pyridoxal phosphate acts as coenzyme in transamination and decarboxylation of amino acids.

2. Maintains healthy brain function.
3. Helps in formation of RBCs & antibodies.

Daily requirement:

Infants- 0.3mg Adults - 2.0mg

Deficiency:

- Retarded growth, failure in gaining weight,
- Anaemia,
- Skin lesions and
- Epileptiform convulsion in infants.

Biotin B7

Biotin is known "Anti egg white injury factor or H-factor".

Sources:

Egg yolk, kidney, liver, yeast, milk and tomato are good sources of biotin.

Functions:

1. Biotin functions as coenzyme for several enzymes e.g. CO_2 fixation reactions, acetyl CoA carboxylase etc.
2. Helps in synthesis of fatty acids.

Daily requirement:

- Since large amounts of biotin are produced by intestinal bacteria, there is no dietary requirement.

Deficiency:

- Deficiency of biotin caused by the destruction of intestinal bacteria or by feeding high amounts of raw egg white develops dermatitis of extremities, muscular pain, anorexia, nausea and anemia.

Folic acid group B 9

Sources:

- Yeast, kidney, liver and green leafy vegetables are good sources of folic acid.
- Avoid over cooking of food since it destroys folates.

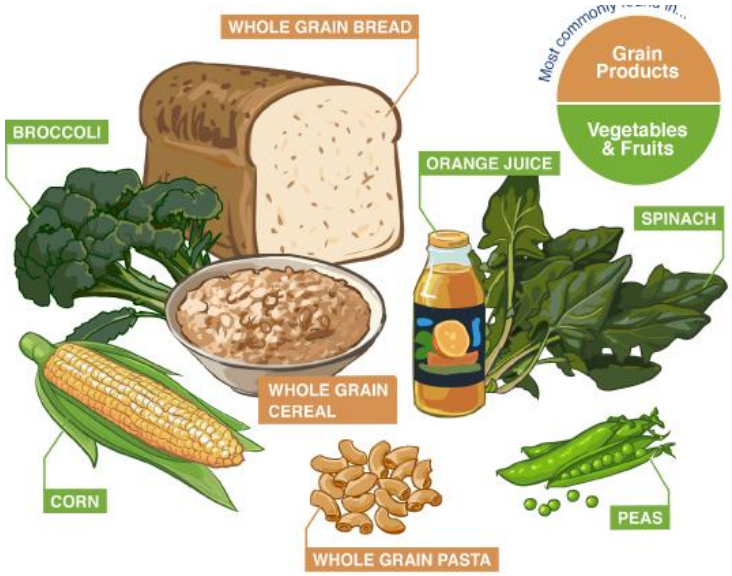


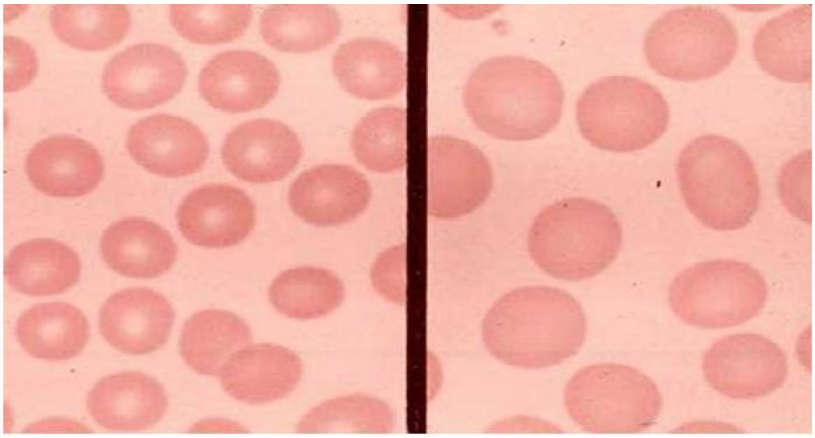
Fig 10.7.8 Folic acid sources

Functions:

1. Formation and maturation of RBCs.
2. Synthesis of DNA,
3. Helps in digestion & utilization of protein.

Deficiency:

1. Macrocytic and Megaloblasticanaemia.
2. Weakness.
3. Retardation of growth.



normal red cells macrocytic red cells Fig 10.7.9

Cyanocobalamin (B12) –

- Anti pernicious anemic factor.
- The absorptions of vitamin B_{12} (Extrinsic factor) which take place from ileum, requires prior combination with a mucoprotein called 'intrinsic factor' present in the normal gastric juice. The failure of the formation of this complex leads to vitamin B_{12} deficiency.

Sources:

Liver, eggs, milk, meat and fish are the best source of vitamin B_{12}.

Functions:

1. Vitamin B_{12} along with folic acid is essential for the development of red blood cells.
2. It is necessary for functioning of nervous system.
3. Vitamin B_{12}acts as coenzyme in the conversion of methyl - malonyl – CoA to succinyl - CoA.
4. Required in minute amounts for the formation of nucleoproteins and proteins.

Deficiency:

1. Megaloblastic anemia (pernicious anemia)- Ineffective production of RBCs.

2. Mucosal atrophy and inflammation of tongue (glossitis) and mouth.

3. Peripheral sensory disturbances.

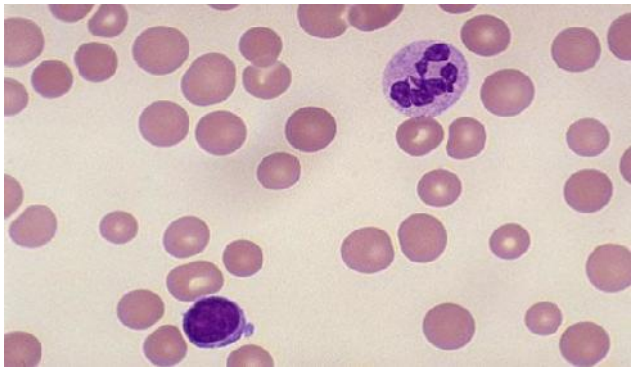


Fig 10.7.10 Pernicious anaemia

10.8 Vitamin C (Ascorbic acid)

- Ascorbic acid is white crystalline water – soluble substance with sour taste.
- It is hexose derivative & a strong reducing substance.
- It is distributed throughout the body and not stored in any particular organ.

Sources:

Citrus fruits like lemon oranges, amla(gooseberry) ,guavas, Tomatoes, berries and green vegetables.

Fig 10.7.11 Vit c sources

Functions:

1. Formation and maintenance of collagen that supports many body structures.
2. Plays major role in the formation of bones and teeth.
3. Participate in the synthesis of steroid hormones both in adrenal cortex and corpus luteum.
4. Enhances the absorption of iron from foods of vegetable origin.
5. Role in wound repair.
6. Protects body against stress.
7. In bile acid formation.
8. Ascorbic acid may act as water soluble antioxidant.

Daily requirement:

Infants- 30 mg	Children- 80 mg
Adults- 75 mg	Pregnancy & lactation- 100 -150 mg

Deficiency:

Causes **scurvy**. It is related to defective collagen synthesis. Features are -

- Internal haemorrhages, subcutaneous haemorrhages(perifollicular or petechial or ecchymosis).

- Muscle weakness.
- Swelling, sponginess, tenderness and bleeding of gums and loosening of tooth.
- Delayed wound healing.
- Susceptibility to infections.

Fig. 10.8.1 Bleeding gums

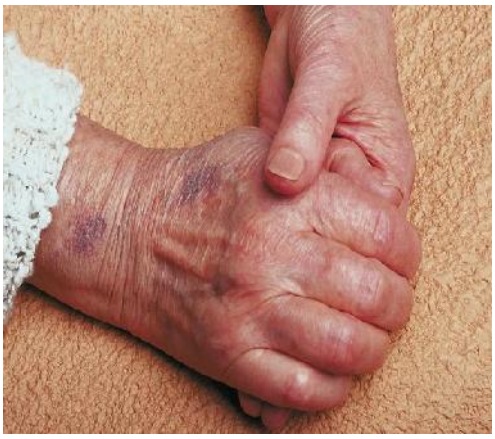


Fig. 10.8.2 Discoloration of skin

10.9 Biomedical/ Clinical Concepts

1. Distinct deficiency conditions of certain B – complex vitamins are known

Thiamine - Beriberi	Riboflavin – Cheilosis, glossitis
Niacin - Pellagra	Pyridoxine – Peripheral neuropathy
Folic acid - Macrocytic anaemia	Cobalamin - Pernicious anaemia

2. B -complex vitamin deficiencies are usually multiple rather than individual with overlapping symptoms.
3. A combined therapy of vitamin B12 and folic acid is commonly employed to treat the patients of megaloblastic anaemias.
4. Megadoses of niacin are useful in the treatment of hyperlipidemia.
5. Long term use of isoniazid for the treatment of tuberculosis causes B6 deficiency.
6. Folic acid supplementation reduces elevated plasma homocysteine level which is associated with althrosclerosis and thrombosis.

7. Sulfonamides serve as antibacterial drugs by inhibiting the incorporation of PABA to produce folic acid.
8. Aminopterin and amethopterin, the structural analogues of folic acid are employed in the treatment of cancers.
9. Lipoic acid is therapeutically useful as an antioxidant to prevent stroke, myocardial infarction, etc.

	Vitamin	Sources	Functions (essential for)	Deficiency diseases
1	Vitamin A	Oil, fish, liver egg, milk, butter and carrots	Eye and lungs	Night blindness
2	Vitamin D	Animal fat, milk ghee, butter	Bones and teeth formation	Rickets
3	Vitamin E	Vegetable, milk, egg yolk and vegetable oils	Sex glands	Hemolysis & sterility
4	Vitamin K	Liver, spinach cauliflower green tomatoes	Blood clotting	Haemorrhage
5	Vitamin B_1	Cereals, wheat, carrot, milk	Nervous system	Beri-beri
6	Vitamin B_2	Cereals, milk, egg, liver	Eyes, skin, blood	Slow growth, sore eyes
7	Vitamin B_4	Meat, fish, cereals, peanuts	Gum and tongue	Inflammation of the tongue and lateral margins of tongue and gums become swollen and red
8	Vitamin C	Lemon, grapes, tomatoes, oranges, apples and vegetables	Gums and wound healing	Scurvy

Review Questions:

1. Define Vitamin. Write classification of Vitamins. What are differences between fat soluble & water soluble vitamins?
2. Write a note on Vitamin A, with reference to functions, sources, deficiency manifestations.
3. Write a note on Folic acid &Vitamin B 12 .
4. Write a note on Vitamin C.
5. Write a note on Vitamin D.
6. Write a note on Vitamin E.

Chapter 11

Carbohydrate Metabolism

Objective- Students shall understand the basic processes occurring in carbohydrate metabolism and commonly used different terms.

11.1- Introduction

1. **Metabolism** – It refers to all the chemical reactions of the body that either require or release energy. It is a energy balancing act between anabolic (Synthesis – require energy) and catabolic (Degradative – release energy) reactions.

a. **Anabolic reactions (Anabolism)** – In Living cells those chemical reactions that combine simple substances into more complex molecules are collectively known as anabolic reactions, e.g. monosaccharides condense together to form polysaccharide. These reactions require energy to form new chemical molecule.

b. **Catabolic reactions (Catabolism)** –The chemical reactions that break down complex organic compounds into simple ones are collectively known as catabolism. This is generally hydrolysis reactions that release the available chemical energy in organic molecule which is stored in ATP (Adenosine triphosphate)

E.g. – Glucose is broken down to form energy + water + CO_2

2. <u>Oxidation – Reduction Reaction</u>

a. **Oxidation** – It is removal of electron or hydrogen (H^+) ions from a molecule and results in a decrease in the energy content of the molecule, most of the biological oxidation involve loss of hydrogen atoms they are called dehydrogenation reaction

Example – conversion of lactic acid to pyruvic acid

b. **Reduction** – It is the addition of electron or Hydrogen ions to a molecule and results in an increase in the energy content of the molecule. It is opposite of oxidation.

Example – Conversation of pyruvic acid to lactic acid.

Within a cell Oxidation and reduction reactions are always coupled that is whenever a substance is oxidized, another is almost simultaneously reduced. This coupling of reaction is referred to as oxidation reduction reaction.

11.2- Physiology of Carbohydrate metabolism

Carbohydrate metabolism begins in the digestive system during the process of digestion polysaccharides and disaccharides are digested or hydrolyzed into monosaccharides.

Example – Glucose, Fructose and Galactose

Glucose represents about 80% of the monosaccharides. The free monosaccharides are absorbed into the capillaries of the villi of the small intestine. These absorb monosaccharides carried through the hepatic portal vein to the liver. Fructose and Galactose ultimately converted into Glucose in the Liver, so the carbohydrate metabolism is really the glucose metabolism.

11.3- Fate of Glucose in the human body

Since Glucose is the body's preferred source of energy, the fate of absorbed glucose depends on the body cells energy needs. If the cells require immediate energy, the glucose is oxidized by the cells. Each gram of carbohydrate produces about 4 kilocalories.

The glucose not needed for immediate use is handled in several ways.

a. First the liver can convert excess glucose to glycogen by the process known as glycogensis and then store it in the liver and skeleton muscle.

b. Second if the glycogen storage areas are filled up liver cells and fat cell can transform the glucose to fat that can be stored in adipose tissue.
c. Third excess glucose can be excreted in the urine normally this happens only when meal containing mostly carbohydrate and no fats is eaten. When the cells need more energy the glycogen can be converted back to glucose by the processes called glycogenolysis. When body requires more energy e.g. during exercise or heavy work or there is decrease in the supply of carbohydrates e.g. fasting, starving or lack of appetite due to tuberculosis, AIDS etc. then stored fat gets converted to glucose. When stored fat is exhausted proteins of the body get converted to glucose. The conversion of fat and protein into glucose is known as Gluconeogenesis.

Glucose Catabolism

The oxidation of glucose is also known as cellular respiration. It occurs in every cell in the body except blood cells which lack mitochondria and the provides the cells chief source of energy. The complete oxidation of glucose to carbon dioxide and water produces large amounts of energy. It occurs in three stages glycolysis, the kreb's cycle and the electron transport chain.

Glycolysis – The term glycolysis refers to a series of chemical reactions in the cytoplasm of the cell that converts a six carbon molecule of glucose into two -three carbon molecule of pyruvic acid. Most of the energy produce during glycolysis is used to generate ATP; the remainder is expended as heat energy some of which helps to maintain body temperature.

The fate of pyurvic acid depends on the availability of oxygen if anaerobic (without oxygen) conditions exist in certain cells e.g. during strenuous exercise pyruvic acid is reduced by the addition of two

hydrogen atoms to form lactic acid. The lactic acid may be transported to the liver where it is converted back to pyruvic acid or it may remain in the cells until aerobic (with oxygen) conditions are restored. Then it is converted to pyurvic acid in the cells. Under aerobic conditions the process of the complete oxidation of glucose continues in mitochondria and pyruvic acid is oxidized to form Carbon dioxide and water in two sets of reaction, the kreb's cycle and the electron transport chain, this is known as aerobic respiration.

Kreb's Cycle – It is also known as citric acid cycle or tricarboxylic acid cycle (TCA cycle). It is a series of biochemical reactions that occur in the matrix of mitochondria in which the large amount of potential energy stored in intermediate substances ultimately derived from pyruvic acid is released step by step by oxidation reduction reactions and transferred through electron transport chain for the generation of ATP (Adenosine Tri Phosphate).

Glucose Anabolism – Most of the glucose in the body is catabolised to supply energy; however some of the glucose participate in number of anabolic reactions, one is the synthesis of glycogen from many glucose molecule by the process called **glycogenesis** another is the manufacture of glucose from the break down product a protein and lipids called gluconeogenesis.

11.4- Different processes occurring in glucose metabolism-

Glycogenesis – If Glucose is not needed immediately for energy it is combined with many other molecules of glucose to form a long chain molecule called glycogen this process is called **Glycogenesis.** The body can store about 500gm of glycogen in the liver and skeletal muscles (80%).

Glycogenolysis – When the body needs energy the glycogen stored in the liver is broken down into glucose and released into the blood stream to be

transported to cells where it will be catabolised to produce energy. The process of converting glycogen back to glucose is called glycogenolysis.

Gluconeogenesis – Formation of glucose from proteins and fats. When glycogen storage in the liver is decreased, body starts catabolising fats and proteins. The process of convertion of fat and protein or non carbohydrate molecules into glucose is called Gluconeogenesis. In this process moderate quantities of glucose can be formed from certain amino acids and glycerol portion of fat molecules. About 60% of amino acids in the body can undergo this conversion.

Glucosuria – The presence of chemically detectable amount of glucose in urine is called glycosuria or more specific glucosuria. A small amount of glucose (2 to 20 mg/dl) maybe present in fasting urine which is not detectable by chemical method. The quantity of glucose that appears in the urine is dependent upon

a. Blood sugar level.
b. The rate of Glomerular filtration.
c. The degree tubular absorption.

The normal renal threshold for glucose is 160 to 180 mg/dl when the glucose exceeds the renal threshold the tubules cannot reabsorbs all of the filtered glucose and then glucose is excreted in the urine called glycosuria. The main reason for is hyperglycemia is elevated level of blood glucose. It is found in

- Diabetes Mellitus is the most common cause of hyperglycemias.
- Patients with endocrine hyper activity e.g. hyperthyroidism, hyperpituitarism, hyperadrenalism etc.
- There are various other non pathological causes of transitory hyperglycemia which results into glycosuria. E.g.
    a. Pregnancy – Glucosuria is due to lower renal threshold.

b. Stress and anxiety – Hyperglycemia is due to an increased output of epinephrine and glucocorticoid hormones.
c. Alimentary Glucosuria – Is due to intake of large amount of carbohydrate.

Renal glycosuria – The presence of chemically detectable amount of glucose in urine due to the defect in the kidney, reabsorptive ability of the renal tubules and due to the subsequent lowered renal threshold. Glucose appears in the urine following ingestion of food. Blood sugar level however is normal (<180 mg%).

Ketosis- The metabolism of glucose normally provides the body with its energy requirements. If however intake of glucose is insufficient as in starvation or glucose metabolism is defective due to lack of insulin as occurs in untreated or uncontrolled diabetes the body obtains its energy by breaking down fats. It is this increase in fat metabolism which leads to increase in ketone body formation. These ketone bodies are

1. Acetone
2. Acetoacetic acid
3. Beta hydroxyl butyric acid

Accumulation of these ketone bodies in the tissues is called ketosis.

Increased levels in blood are known as Ketonemia. Ketones are toxic to the brain and if present in sufficiently high concentration in the blood they can contribute to the coma found in diabetic ketoacidosis. They are strong acids and may overcome the buffer systems in the blood and so cause ketoacidosis

Ketonuria- The excretion of more than a trace of ketone bodies in the urine is called ketonuria. Untreated and uncontrolled diabetes is the most important disorder in which ketonuria occurs. When this ketonuria occurs in diabetic patient insulin doses or other medical management is indicated

Ketonuria also accompanies the other conditions such as anorexia, fasting, starvation, fever and prolonged vomiting.

Review Questions:

1. Define metabolism.
2. What is oxidation-reduction?
3. Explain glucose anabolism and catabolism.
4. Differentiate between glycosuria and renal glycosuria.
5. Define following terms
    a. Glycogenesis
    b. Glycolysis
    c. Glycogenolysis
    d. Gluconeogenesis
    e. Ketosis
    f. Ketonemia
    g. Ketonuria
6. What is TCA cycle?

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Chapter 12

Lipid metabolism

Objectives- Students shall understand basic processes occurring in lipid metabolism ,metabolism of phospholipids, ketone bodies and oxidation of fatty acids.

12.1- Introduction

Lipids are indispensable for cell structure and function. Due to their hydrophobic and nonpolar nature, lipids differ from rest of the body compounds and are unique in their action. The lipids of metabolic significance include triacylglycerol (triglycerides, neutral fat), phospholipids and steroids together with long chain fatty acids (free fatty acids), glycerol and ketone bodies.

12.2- General lipid metabolism

The chylomicrons are finally deposited either in the liver, or in the storage depots (adipose tissue) which in health form about 15 percent of the body weight. Lipoprotein lipase in the adipose tissue is responsible for the clearance of chylomicrons. Plasma lipoprotein lipase is also responsible for the clearance of a small amount of chylomicrons in plasma. The triacylglycerides undergo hydrolysis by an intra cellular lipase to form free fatty acids (FFA) and glycerol. The released free fatty acids are carried in the plasma as liver, heart, kidney, muscle, lung, testis; brain and adipose tissue which have the ability to oxidize long chain fatty acids by beta oxidation. To oxidize long chain fatty acids are degraded completely to acetyl-CoA, which can be oxidized to CO_2 and water through the citric acid cycle.

The glycerol which has been released by hydrolysis of fat enters general carbohydrate metabolism via-glyceraldehyde and is either converted to glycogen or oxidized. The triglycerides stores in adipose tissue continually undergo lipolysis (hydrolysis) and re-esterification. This is influenced by nutritional, metabolic and hormonal factors.

When the availability of glucose in body tissues is less (as in starvation or diabetes mellitus), the rate of lipolysis exceeds the rate of esterification with subsequent accumulation of FFA and their release into plasma.

Oxidation of triacylglycerol –

Triacylglycerols is first hydrolyzed to fatty acids and glycerol mostly in adipose tissue. The free fatty acids are released into the plasma where they combine with serum albumin. Long chain fatty acids are oxidized in liver, heart, kidney, muscle, lung, testis, brain and adipose tissue.

Glycerol is utilized by liver, kidney, intestine and lactating mammary glands where the activating enzyme **glycerokinase** is present abundantly.

12.3- Oxidation of fatty acids-

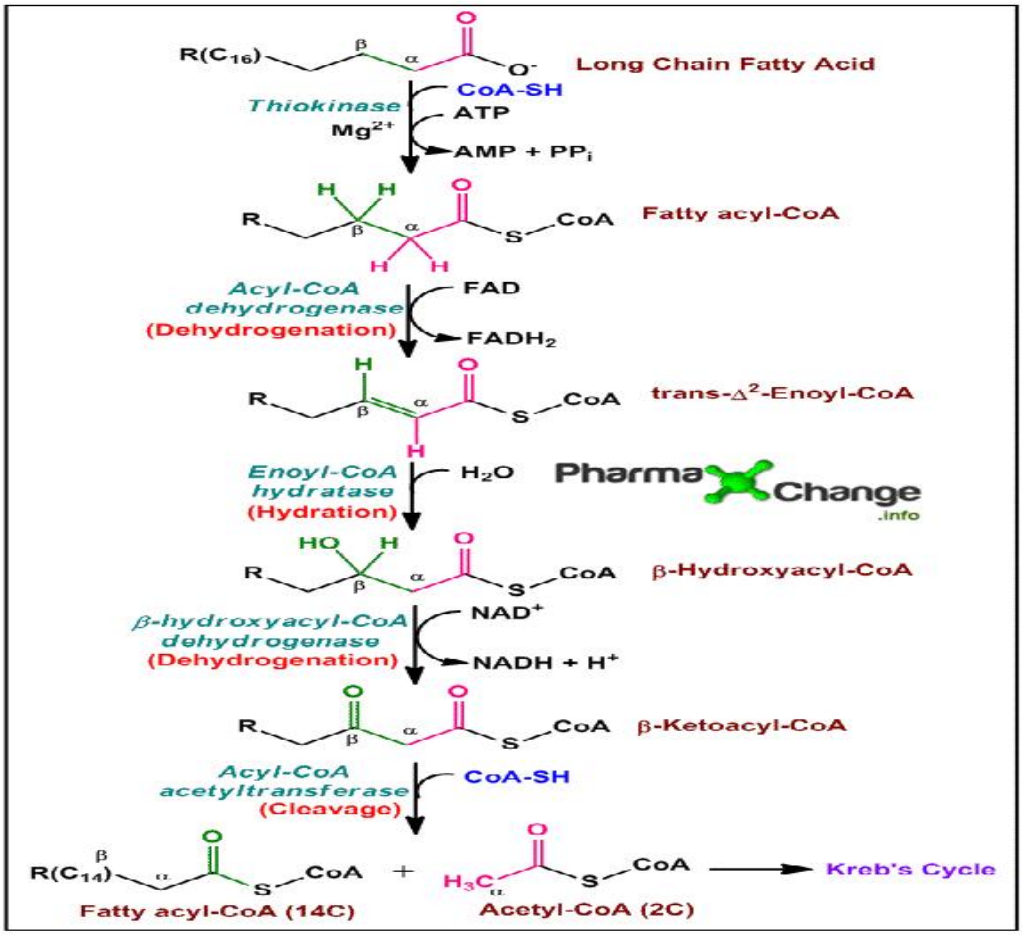
Fatty acids are oxidized by β-, α- and ω- oxidation. Quantitatively, β-oxidation is the most important pathway.The fatty acids containing even and odd number of carbon atoms as well as the unsaturated fatty acids are oxidized by β-oxidation.

β- Oxidation of fatty acids-

1. Several enzymes called fatty acid oxidase are found in mitochondria.
2. Long chain fatty acids are first activated to active fatty acid or acyl-CoA by the enzyme acyl-CoA synthetase in the presence of ATP, coenzyme A and $Mg^{++.}$
3. Long chain acyl-CoA does not penetrate mitochondria without the presence of carnitine. The enzyme carnitine palmitoyl transferase l associated with the mitochondrial membrane allows long chain acyl

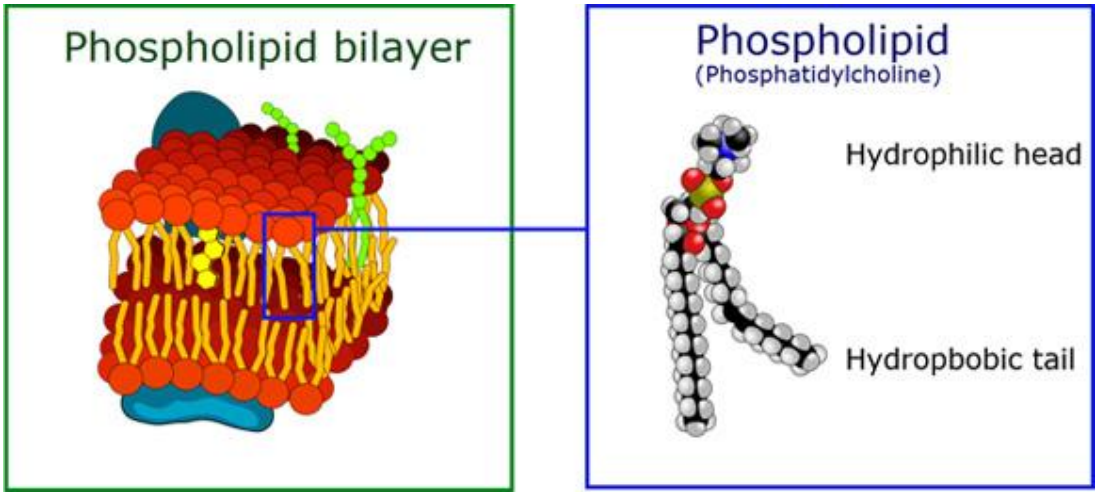
groups to penetrate the mitochondria. The mechanism is shown another enzyme, carnitine palmitoyl transferase 2, present in mitochondria, catalyzes the transfer of short chain acyl groups between CoA and carnitine.

4. Acyl-CoA is then converted to α, β- unsaturated acyl-CoA by the enzyme acyl-CoA dehydrogenase in presence of the coenzyme flavoprotein which contains FAD as prosthetic groups.
5. Water is added to saturate the double bond and form β-hydroxy acyl-CoA, catalyzed by the enzyme enoyl- CoA hydratase (crotonase).
6. The β- hydroxyl acyl-CoA in the presences of NAD and catalyst β-hydroxyl acyl-CoA dehydrogenase. Undergoes dehydrogenation to form β- keto acyl-CoA.
7. Finally β- keto acyl-CoA is split by β- ketothiolase to from acetyl CoA and acyl CoA. In this way a long chain fatty acid is degraded completely.



12.4- Metabolism of phospholipids-

Phospholipids are specialized group of lipids performing a variety of functions. These include membrane structure and function, involvement in blood clotting, and supply of prostaglandins.



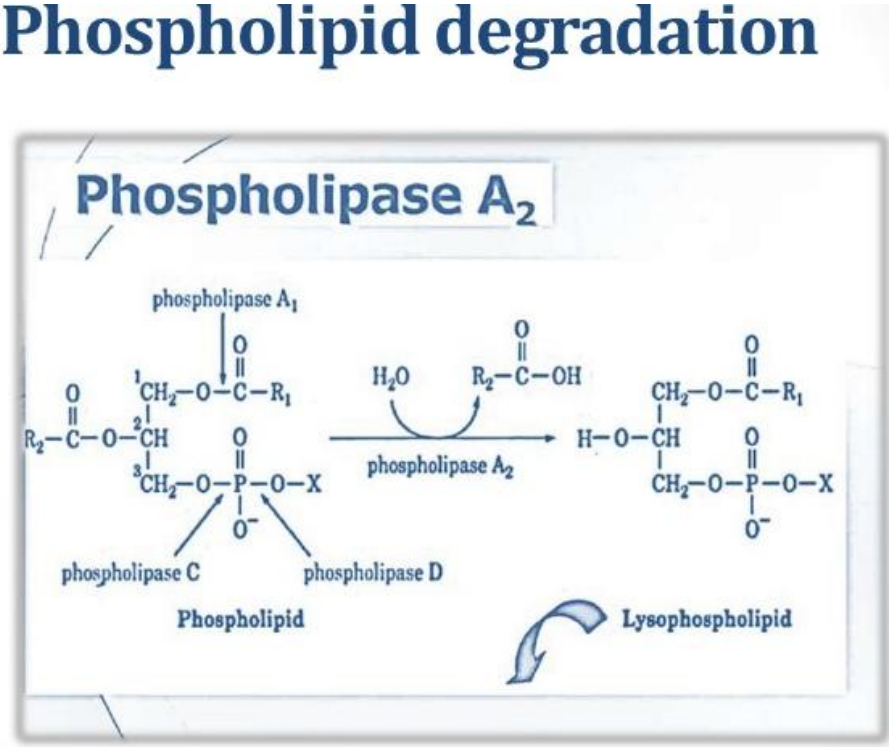
Synthesis of Phospholipids-

Phospholipids are synthesized from phosphatidic acid and 1, 2-diacylglycerol, intermediates in the production of triacylglycrols. Phospholipid synthesis occurs in the smooth endoplasmic reticulum as follows.

1. Formation of lecithin and cephalin.
2. Synthesis of phosphatidylserine.
3. Formation of phosphatidylinositol.
4. Synthesis of phosphatidyl glycerol and cardiolipin.
5. Formation of plasmalogens.
6. Synthesis of sphingomyelins.

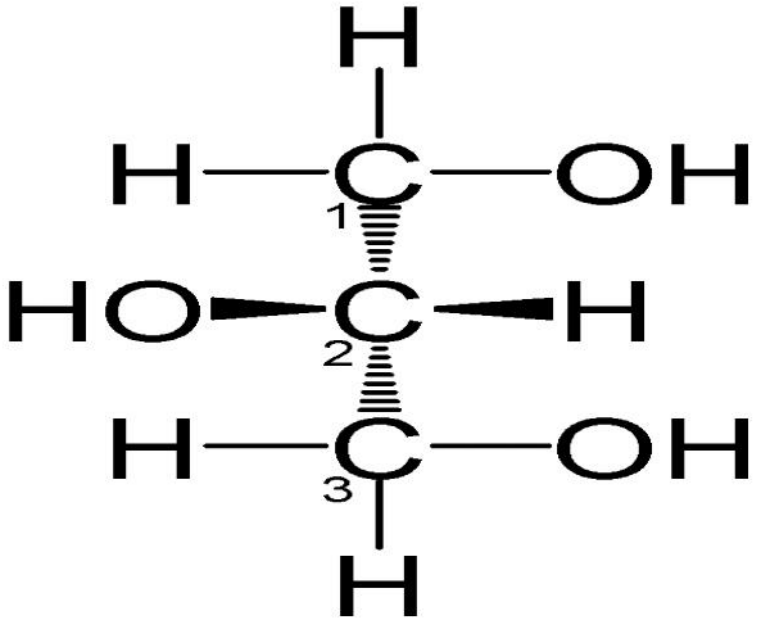
Degradation of phospholipids-

Phospholipids are degraded by Phospholipases which cleave the phosphodiester bonds. Phospholipase A_1 specifically cleaves the fatty acid at C1 position of Phospholipids resulting in lysophospholipids. The latter can be further acted by lysophospholipase, Phospholipase B to remove the second acyl group at C2 position. Phospholipase A_2 hydrolyses the fatty acid at C_2 position of phospholipids. Phospholipase C specifically cleaves the bond between phosphate and glycerol of phospholipids. Phospholipase D hydrolyses and removes the nitrogenous base from phospholipids.



12.5- Metabolism of Glycerols

Glycerols are transported to liver and activated to glycerol-3-phosphate in the presence of glycerol kinase. The glycerol-3-phosphate is then used for Synthesis of triacylglycerols.



12.6- Metabolism ketone bodies

The compounds namely acetone, acetoactate and β-hydroxybutyrate are known as ketone bodies. Acetoacetic acid and β-hydroxybutyrate acid are carried from liver to extra hepatic tissues mainly kidney and muscle where they are oxidized for energy production after conversion to acetyl – CoA. The enzyme responsible for the activation of acetoacetate to acetoacetyl- CoA is absent from liver for which liver can cotunilize these acids. Two reactions take place in extra hepatic tissue for the activation of acetoacetate to acetoacetyl-CoA.

12.7- Synthesis of ketone bodies-

The synthesis of ketone bodies occurs in the **liver.** The enzymes for ketone body synthesis are located in the mitochondrial matrix. Acetyl CoA, formed by oxidation of fatty acids, pyruvate or some amino acids, is the precursor for ketone bodies. Ketogenesis occurs though the following reactions. Two moles of acety CoA condenses to form acetoacetyl CoA. Acetoacety CoA combines with another molecule of acety CoA to produce β-hydroxy β-methyl glutarylCoA(HMG CoA). HMG CoA lyase cleaves HMG CoA to produce acetoacetate and acetyl CoA. Acetoacetate can undergo spontaneous decarboxylation to from acetone. Acetoacetate can be reduced by a dehydrogenease to β-hydroxybutyrate.

Chapter 13

Protein Metabolism

Objectives- Students shall understand protein anabolism and catabolism, deamination, transamination, etc.

13.1- Introduction

Proteins are essential constituents of all living cells. Metabolism of proteins has no. of special features. Some are

a. The power of protein synthesis of the human body is very limited.
b. Storage of proteins can occur only under certain special conditions.
c. Distribution of proteins is also characteristic.
d. Peculiar method for utilization of amino acids.
e. Maintenance of nitrogen equilibrium.
f. Each animal species and type of tissue is made up of characteristic protein and variation in species and tissues is due to variation of protein types in them.

The metabolism of proteins means metabolism of amino acids. Amino acids are continuously returned from the body fluids for the synthesis of tissue proteins, oxidation and other uses and added into tissue fluids by absorption from intestine, break down of tissue proteins and synthesis. There is constant exchange of amino acids from tissue to blood and other body fluids and from fluid to tissue. The amino acid pool remains constant.

13.2- Fate of proteins

Amino acids enter body cells by active transport. This process is stimulated by human growth hormone and insulin. Almost immediately

after entrance they are synthesized into proteins, other amino acids are stored as fat or glycogen or used for energy. Each gram of protein produces about 4 kcal.

Protein Catabolism

- A certain amount of protein catabolism occurs in the body each day although much of this is only partial catabolism. Proteins are extracted from worn out cells such as red blood cells and broken down into amino acids. Some amino acids are converted into other amino acids, peptide bonds are reformed and new proteins are made as part of the constant state of turnover in all cells.
- If other energy sources are used up (glucose,glycogen and fats) or if other sources are inadequate and protein intake is high then liver can convert protein to fat or glucose or oxidize it to produce energy, carbon dioxide and water. However before amino acid can be catabolised they must first be converted to various substances that can enter the kreb's cycle.
- One such conversion consists of removing the amino group (NH_2) from the amino acids a process called **deamination.** The liver cells then convert the NH_2 to ammonia (NH_3) and finally to urea which is excreted in the urine.
- Other conversions are decarboxylation and dehydrogenation.
- Amino acids enter the kreb's cycle for catabolism at different points. They may be converted to pyruvic acid, acetyl coA, alphaketoglutaric acid, succinyl coA, fumaric acid, oxaloacetic acid or acetoacetyl coA. The point is that amino acids can be altered in various ways to enter the kreb's cycle at various locations.

Protein anabolism

- It involves the formation of peptide bonds between amino acids to produce new proteins.
- Protein anabolism or synthesis is carried out on the ribosomes of almost every cell in the body directed by the cell's DNA and RNA.
- Protein synthesis is stimulated by human growth hormone, thyroxine and insulin.
- The synthesis proteins are the primary constituents of enzymes, antibodies, clotting chemicals, hormones, structural components of cells and so forth.
- Because proteins are primary ingredients of most cell structures, high protein diets are essential during the growth years, during pregnancy and when tissue has been damaged by diseases or injury.

13.3- Deamination

It is the process by which the amino radical (---NH_2) is taken away from amino acids. This occurs in liver under the action of enzyme deaminase. The amino acid is broken down in to two parts-

a. Nitrogenous part (Ammonia) - This takes part in formation of urea.
b. Non nitrogenous part (an α-ketonic acid or aldehyde)

13.4- Transamination–

It is reversible and combined process of deamination and amination.Nitrogen is transferred as amino group. It catalysed enzymes transaminases. They are classified as transferases which catalyses a transfer of a group between a pair for two substrates.

Example- Alanine transaminase (ALT or GPT) and Aspartate transaminase (AST or GOT)

1. Glutamic acid + Pyruvic acid $\xleftrightarrow{\text{Transaminase}}$ α- ketoglutaric acid+ Alanine

(SGPT)

2. Glutamic acid + oxaloacetic acid $\xleftrightarrow{\text{Transaminase}}$ α- ketoglutaric acid+ Aspartic acid

(SGOT)

Review questions

1. What are features of protein metabolism?
2. What is deamination and transamination?
3. Explain protein anabolism.
4. Explain protein catabolism.

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Chapter 14

Water & Mineral Metabolism

Objective : After reading this topic **s**tudents will learn about importance of water for living, functions of water, it's distribution in the body & how body balances it's level. They will also learn about serum electrolytes, minerals which are required for normal health & about diseases produced in case of their increase or decrease level.

14.1. Biological importance & functions of water:

1. As a body constituent : Water is an essential constituent of cell structures and provides the media in which the chemical reactions of the body takes place and substance are transported. The fluidity of blood is because of water.

2. Regulation of body temperature : It has a very high specific heat for which, it can absorb or gives off heat without any appreciable change in temperature. It is regulated by the evaporation of water from skin and lungs and it's removal in urine.

3. **Solvent power** – One of the most important properties of water is its capacity to dissolve different kinds of substances. It is therefore the most suitable solvent for cellular components.

4. Catalytic action – Water accelerates a large number of chemical reactions in the body due to it's ionizing power.

5**. Lubricating action** – Water acts as a lubricant in the body and prevents friction in joints, pleura, conjunctiva and peritoneum.

14.2. Distribution of water in body: The average body water content is 60 – 70% of the body weight. In adult male of 70kg the total body water is about 40 – 42 liters. The amount of water in females is little less than males. The distribution of water in the body is given below.

1. Intracellular – The fluid within the body cells is called the intracellular fluid. It constitutes about 50% of the body weight.

2. Extracellular – All the fluid outside the body cells is collectively termed as extracellular fluid. It constitutes about 20% of the body weight. It can be further divided in the following compartments :

a. Plasma – 5.5%.

b. Intestinal and lymph fluids – 80%.

c. Dense connective tissue, cartilage and bone – 6%.

d. Transcellular fluids (aqueous and vitreous humour, CSF, endolymph, etc.) – 5%.

14.3. **Water balance** : An equilibrium is maintained between the intake and the loss of water from the body.

Balance sheet of water (per day)

Water intake		**Water loss**	
Drinks	1350ml.	Lungs	500ml.
Solid	900ml.	Skin	700ml.
		Urine	1400ml.
Oxidation of food	450ml.	Feces	100ml.
	----------		----------
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	2700ml.		2700ml.

Water intake: Thirst is a good guide for adequate fluid intake. The normal intake of water and that formed in the body, together averages 2500 ml /day. It can be approximately divided as follows for a day:

a) Intake of water and other fluids: 1200 – 1500 ml.

b) Foods: 770 – 1000ml.

c) Metabolic water: 200 – 300ml.

Water output : Water is lost from the body by following four routes:

a) The skin: as sensible and insensible perspiration.

b) The lungs: as water vapor in the expired air.

c) The kidneys: as urine

d) The intestine: in the feces.

Water lost through skin both as sensible and insensible perspiration (600 ml). Insensible perspiration is means ` one is not aware of it , it evaporates as it is formed'.

On the other hand, with vigorous activity especially in hot weather, we lose much additional water through visible perspiration.

Sensible perspiration is called active sweating. It depends upon :

i. Habits & ii. Types of activity.

If metabolic rate is high, then water loss will be high. Greater the respiration rate, the higher will be metabolic rate, therefore more loss.

Kidney is the most important guardian of the water content of the body. The water loss through skin, lungs and feces are not controllable but there is an automatic feedback mechanism by the kidney.

Certain volume of urine has to be lost by the kidney and it is called minimum urine volume or obligatory excretion. Kidney controls the excretion of waste products and to dissolve them minimum urine volume of 500 ml is needed. The 500ml constitutes the 2% of body weight which has to be lost even when the body does not take any water. Minimum water excreted by the kidney depends upon concentrating power of the kidney & water intake. Healthier the kidney, the greater will be concentrating power of the kidney.

14. 4 Dehydration :

Defination: "A condition characterized by water depletion in the body".

Causes:

1. When the water intake is less than the body needs.

2. When the fluid loss from the body is abnormally high, e.g. excessive perspiration in hot weather, severe diarrhoea, vomiting, fever, with increased loss from the skin, severe burns with accompanying water losses and in uncontrolled diabetes with frequent urination.

Dehydration is corrected by electrolytes and water.

Edema :

Edema is the accumulation of water or excessive retention of water in the body. It occurs in diseases of heart (impaired circulation), diseases of kidney (unable to excrete sodium in sufficient amount), prolonged protein deficiency (tissues are unable to maintain normal water balance).

MINERAL METABOLISM

Minerals are inorganic substances. Minerals are present in all body tissues and fluids. Unlike carbohydrates, fats and protein, mineral elements do not furnish energy.

Unlike vitamins, the minerals are not destroyed in food preparation. However, they are soluble in water so that some loss will occur if cooking liquid is discarded.

In contrast to the organic substances, which can be considered as energy sources, the inorganic substances do not supply any energy. Their presence is necessary for the maintenance of certain physiochemical conditions which are essential for life.

14.5 Classification : The minerals are classified as principal elements and trace elements.

Principle elements: These are calcium, phosphorous, magnesium, sodium, potassium, chloride and sulfur.

Trace elements : They are subdivided into three categories.

1) Essential trace elements: Iron, copper, iodine, manganese, zinc, and molybdenum, cobalt, fluorine, selenium, and chromium.

2) Possibly essential trace elements: Nickel, vanadium, cadmium and barium.

3) Non-essential trace elements: Aluminium, lead, mercury, boron, silver, bismuth etc.

Basic functions performed by the minerals are:-

1) As structural component of body tissues.

2) In the maintenance of acid- base balance.

3) In the regulation of body fluids.

4) In transport of gases.

5) In muscle contractions.

14.6Electrolytes

Electrolyte Balance: Electrolytes are the compounds which readily dissociate in solution and exist as ions i.e. positively and negatively charged particles. For instance, NaCl does not exist as such, but it exists as cation (Na^+) and anion (Cl^-).The concentration of electrolytes are expressed as milliequivalents (mEq/L). Four major electrolytes are sodium, potassium, chlorides & bicarbonates

Electrolyte composition of body fluids: Electrolytes are well distributed in the body fluids in order to maintain the osmotic equilibrium and water balance. Na^+ is the principal extracellular cation , while K^+ is the intracellular cation. This difference in the concentration is essential for the cell survival which is maintained by Na^+- K^+ pump. Chloride (Cl^-) and bicarbonates (HCO_3^-) are predominantly present in extracellular fluids.

14.7 Sodium

Sources: Salt added to prepared to food and seasoning, salted food like cheese, eggs, nuts etc.

Functions: (a) Principal cation in extracellular fluid.

(b) Regulates plasma volume, acid base balance, nerve and muscle function.

Metabolism: Regulated by aldosterone (progesterone,ACH).

Deficiency:Hyponatremia

(a) Unknown on normal diet.

(b) Always secondary to injury or illness e.g. diarrhoea , vomiting, Addison's disease, excessive sweating, overuse of diuretics, salt losing nephritis, diabetes mellitus. It is characterized by headache, nausea, cramps, fall in B.P., oliguria, increased pulse rate.

Toxicity :**(Hypernatremia**)

Excess is met with in cardiac failure, hepatic cirrhosis, acute glomerulonephritis , premenstrual phase of cycle, excess of ACTH, ACH, testosterone, In hypersensitive individual it causes elevation of blood pressure and oedema.

Plasma level: 135 – 145 MEq / lit.

Daily Requirement : 5 – 15 gm.

14.8 Potassium

Sources : Vegetables, fruits like banana, oranges, peaches, pineapples Etc, nuts, potatoes & chicken.

Functions :

a. Principal cation in intracellular fluid. Within a cell , it plays important role in maintenance of acid – base balance, osmotic pressure& water retention.

b. Regulates nerve and muscle function as well as cell membrane function.

Regulation : By aldosterone.

Deficiency:**Hypokalemia**

a) Characterised by muscular weakness , paralysis and mental confusion , ileus, thirst (polyurea) apathy , paraesthesia, small T wave and ST segment depression in ECG.

b) It is seen in acute severe diarrhoea ,vomiting , malabsorption syndrome, diabetes mellitus (after treatment) use of ACTH , corticosteroids, diuretics, Cushings syndrome, aldosteronism, postoperative period.

Plasma level: 3 – 5 MEq / L**.**

Daily Requirment : 4 gm.

Toxicity :**Hyperkalemia**

An excess causes cardiac arrest, small bowel ulcers. It is a feature of diabetic ketoacidosis (before treatment), severe burn ,blood loss, acute renal failure ,Addison's disease, chronic renal disease.

14.9 Chloride

Chloride is the major extracellular anion of the body. Blood serum contains 99 to 108 mEq /L, principally as sodium chloride. Its primary role in the body is to maintain proper water distribution, osmotic pressure, and normal anion-cation balance in the external fluid (plasma).

Clinical Significance:

Low chloride values are observed in the condition such as:

(1) Prolonged vomiting

(2) Burns

(3) Salt losing renal disease and in

(4)Over hydration.

High chloride values are observed in the conditions such as (1) Dehydration (2) Renal tubular disease and in condition causing decreased renal blood flow, i.e. congestive heart failure.

14.10 Phosphorus

Functions :-

1. Phosphorus along with calcium is essential for bones and teeth.
2. Buffering action, i.e. phosphate buffers.
3. In the formation of high energy compound, i.e. ATP.
4. In the synthesis of RNA & DNA.
5. In the synthesis of phospholipids.
6. In the synthesis of phosphoproteins.

Absorption :

The absorption of phosphorus is related to that of calcium. Normally 1/3 rd of the ingested phosphorus is passed in the feces and 2/3rds in the urine. A high calcium diet, diminishes the phosphorus absorption by forming insoluble calcium phosphates.

Phosphorus is present in the blood as :

a. Inorganic phosphorus

b. Organic Phosphorus

c. Lipid phosphorus

The normal serum inorganic phosphorus level is 2.5 to 4.00 mg%.

It is higher in children, the value being 4 – 6 mg%

Increase in serum phosphorus is found in :

1. Chronic nephritis.
2. Hypoparathyroidism.

Decrease in serum phosphorus is found in :

1. Rickets.
2. Hyperparathyroidism

3. Fanconi's syndrome

14.11Calcium

Calcium is present in the body in the largest amount of all the minerals present in the body. Calcium comprises 2% of the body weight.RBC is devoid of calcium. The normal serum level is 9-11 mgs %.

Calcium is present in three forms:

1. Ionized form: This form is physiologically active form. It constitutes about 50% of total calcium.

2. Nonionized: This form is physiologically inert. It is combined with plasma proteins.

3. In combination with citrates: Protein bound fraction is nondiffusible whereas other two fractions are diffusible. (about 10%)

Absorption of Calcium

1. Calcium salts are more soluble in acidic media than the alkaline media. Greater the acidity, the more will be the absorption.

2. Certain foodstuffs contain phytic acid (present in cereals) and oxalates (present in spinach) which inhibit calcium absorption by forming insoluble calcium salts.

3. When fat absorption is not proper, the free fatty acids present react with calcium to form soaps (calcium salts of fatty acid) will hinders absorption.

4. On a high protein diet the calcium absorption will be more.

5. Vitamin D is necessary in the diet to promote the absorption of calcium.

6. The optimum calcium phosphorus ratio in the diet should be 1:1.

Functions

1. Calcium along with phosphorus is essential for bones and teeth formation.

2. In blood coagulation: Calcium activates the conversion of prothrombin to thrombin.

3. In enzyme activation: Calcium activates large number of enzymes such as adenosine triphosphatase (ATPase), succinic dehydrogenase, lipase, etc.

4. In muscle contraction.

5. In normal transmission of nerve impulses.

6. In neuromuscular excitability.

Regulation of Blood Calcium Level

1. Indirect Factors :Dietary factors which have an effect on calcium absorption, which have been discussed in the absorption of calcium.

2. Direct factors: Those which have direct effect on blood calcium. These are:

a. Hormones

i. Parathyroid hormone regulates the concentration of ionized serum calcium.

ii. Calcitonin lowers calcium level by inhibiting bone absorption and thus decreases the loss of calcium from bones.

b. Serum proteins

Decrease in serum proteins will result in decrease in total calcium level as most of the calcium bound to protein will be less.

c. A reciprocal relationship exists between calcium and phosphorus in the blood. Increase in serum phosphorus causes decrease in serum calcium and vice versa.

Sources

Dairy products such as milk, cheese are the best sources. It is also present in lentils, nuts, egg yolk, cauliflower, green leafy vegetables.

Daily Requirement

Adults	800 mg
In females during pregnancy and lactation	1200 mg
Infancy	350-540 mg
Children from 1 to 8 years	0.8-1.2 gm.

Rickets:

Deficiency of vitamin D gives rise to rickets in children. The main symptom of rickets is, sufficient calcification by calcium phosphate of the bone in growing children. The bones, therefore, remain soft and deformed by the body weight.

Osteoporosis:

Osteoporosis is a disease of demineralization or decalcification of the bones.

It is a condition when calcium is withdrawn from the bones. The bone become week and porous and hence breaks. It is more prevalent in older woman than in men.

Toxicity: Occurs in hypervitaminosis D and hyperparathyroidism or idiopathic hypercalcimia. It is characterized by vomiting,abdominalcramps,nephrocalcinosis.

Importance of Ca: P ratio: The ratio of plasma Ca: P is important for calcification of bones. The product of Ca x P (in mg/dl) in children is around 50 and in adults around 40. This product is less than 30 in rickets.

14.12 Iodine

The total body contains about 20 mg iodine, most of it (80%) being present in the thyroid gland. Muscle, salivary glands and ovaries also contain some amount of iodine.

Biochemical functions:

The only known function of iodine is its requirement for the synthesis of thyroid hormones namely, thyroxine (T4) and triiodothyronine (T3). These hormones are involved in several biochemical functions. Functionally, T3 is more active than T4.

Dietary requirement:

Adults	-	100 – 150 µg/day
Pregnant women	-	200 µg/day

Sources :

Seafoods, drinking water, vegetables, fruits (grown on seaboard . High altitudes are deficient in iodine content in water as well as soil. Plant and animal foods of these areas, therefore, contain lesser amount of iodine. In these regions, iodine is added to drinking water or to table salt.

Absorption, storage and excretion:

Iodine as iodide is mainly absorbed from the small intestine. Normally, about 30% of dietary iodine is taken up by the intestinal cells. Iodine absorption also occurs through skin and lungs.

About 80% of body's iodine is stored in the organic form as iodothyroglobuline (a glycoprotein) in the thyroid gland. This protein contains thyroxine, diiodotyosine and triiodotyrosine in different proportions.

Excretion of iodine mostly occurs through kidney. It is also excreted through saliva, bile, skin, and milk (in lactating women).

Plasma iodine :

The normal concentration of plasma iodine is 4 – 10 mg/dl. Most of this is present as protein bound iodine (PBI) and represents the iodine contained in the circulating thyroid hormones. PBI level decreases in hypothyroidism & increases in hyperthyroidism. RBC do not contain iodine.The conversion of iodide(I^-) to active iodine(I^+) is an essential step

for it's incorporation into thyroid hormones.TSH promotes the oxidation of iodide to active iodine. The ratio of T3 to T4 in thyroglobulin is usually around 1:10.

Biochemical functions of thyroid hormones:

1. Influence on the metabolic rate: Thyroid hormones stimulate the metabolic activities and increases the oxygen consumption in most of the tissues of the body. Obesity in some individuals is attributed to a decreased energy utilization.

2. Effect on protein synthesis: Thyroid hormones act like steroid hormones in promoting protein synthesis. They function as anabolic hormones and promote growth and development.

3. Influence on carbohydrate metabolism: Thyroid hormones promote intestinal absorption of glucose and it's utilization with overall effect of enhancing blood glucose level.

4. Effect on lipid metabolism:Lipid turnover and utilization are stimulated by thyroid hormones. Hypothyroidism is associated with elevated plasma cholesterol levels which can be reversed by thyroid hormone administration.

Regulation of T3 and T4 synthesis

The increased synthesis of TSH and TRH occurs in response to decreased circulatory levels of T3 and T4 (Feedback metabolism).

Abnormalities of thyroid function

Among the endocrine glands, thyroid is the most susceptible for hypo or hyperfunction.

(1)**Goiter**: Any abnormal increase in the size of the thyroid gland is known as goiter. Enlargement of thyroid gland is mostly to compensate the decreased synthesis of thyroid hormones and is associated with elevated TSH.

Simple endemic goiter: This is due to iodine deficiency in the diet. It is mostly found in the geographical regions away from sea coast where the water and soil are low in iodine content. Consumption of iodized salt is advocated to overcome the problem of endemic goiter.

Hyperthyroidism: This is also known as **thyrotoxicosis** and is associated with overproduction of thyroid hormones. Hyperthyroidism is characterized by increased metabolic rate (higher BMR) nervousness, irritability, anxiety, rapid heart rate, loss of weight despite increased appetite, weakness, diarrhea, sweating , sensitivity to heat and often protrusion of eyeballs (exopthalmos).

Hypothyroidism: This is due to an impairment in the function of thyroid gland that often causes decreased circulatory levels of T3and T4.Disorders of pituitary or hypothalamus also contribute to hypothyroidism. Women are more susceptible than men. Hypothyroidism is characterized by reduced BMR, slow heart rate, weight gain, sluggish behavior , constipation, sensitivity to cold, dry skin etc.

Hypothyroidism in children is associated with physical and mental retardation, collectively known as **cretinism**. Early diagnosis and proper treatment are essential. Hypothyroidism in adult causes **myxoedema,** characterized by bagginess under the eyes, puffiness of face, slowness in physical and mental activities.

Interpretation

TSH	T4	T3	Interpretation
High	Normal	Normal	Mild(subclinical) hypothyroidism
High	Low	Low or normal	Hypothyroidism
Low	Normal	Normal	Mild(subclinical) hyperthyroidism
Low	High Or normal	High or normal	hyperthyroidism
Low	Low or normal	Low or normal	Non-thyroidal illness; Rare pituitary (secondary) hypothyroidism

Other trace elements:

Minerals	Uses	Sources
Chromium	Increases effectiveness of insulin and promote healthy weight.	Broccoli, Green Beans, Garlic, Potatoes, Turkey, Apples, Bananas, Whole Grains
Copper	Aids in formation of red blood cells and elastin, a skin protein. Supports healthy nerves and joints.	Almonds and Nuts, Sunflower Seeds, Avocados, Broccoli, Garlic, Oranges, Raisins, Salmon
Iodine	Important for physical and mental development. Helps regulate metabolism.	Cod, Tuna, Turkey, Navy Beans, Milk, Eggs
Iron	Production of hemoglobin and myoglobin and the oxygenation of red blood cells.	Squash and Pumpkin Seeds, Nuts, Lamb, Dark Leafy Greens, Whole Grains
Magnesium	Acts as a catalyst in utilization of carbohydrates, fat, protein & other minerals.	Dark Leafy Greens, Nuts, Fatty Fish, Beans, Whole Grains, Avacados, Bananas
Manganese	For skeletal development, healthy nervous and immune system, and sex hormone production.	Avocados, Nuts, Flax Seed, Soybeans, Wheat Bran
Molybdenum	Helps iron transport from liver, promotes normal cell function. Extremely small amounts needed.	Dark green leafy vegetables, Legumes, Peas
Phosphorus	Necessary for blood clotting, bone and tooth formation, cell growth, contraction of the heart muscle, normal heart rhythm and kidney function.	Seeds, Salmon, Asparagus, Wheat Bran, Nuts, Yogurt
Potassium	Necessary for heart muscle function, kidneys & nervous system.	Dark Leafy Greens, Fatty Fish, Beans, Mushrooms, Avacados, Bananas, Yogurt
Selenium	Works with E to promote antibodies. Maintains elasticity of tissue and artery.	Tuna, Mackerel, Whole Grain, Seeds, Lamb, Poultry, Mushrooms
Zinc	Aids in healing process, used by prostate gland & immune system.	Lamb, Spinach, Squash and Pumpkin Seeds, Nuts, Beans, Mushrooms

Questions :

4) Define dehydration and edema.

5) Write about functions of water. How it is balanced in the body?

6) Name the principal elements or minerals and trace elements.

7) Write about functions and metabolism of calcium.

8) Write a note on phosphorus metabolism.

9) Write a note on sodium, potassium and chloride metabolism.

10) Write a note on Iodine metabolism.

Chapter15

Renal Function test

The diagnosis of kidney disease, to a great extent is made in clinical chemistry laboratory. The renal or kidney function test when properly conducted can give valuable information about the status of kidney function and frequently about the location of the defect.

However kidney has a considerable functional reserve and this test may be normal even in the presence of relatively severe renal pathology. Kidney function test in general can affected by

1) Prerenal cause – Due to decrease plasma volume (dehydration) or due to decrease blood flow (excessive bleeding, shock cardiac failure etc) will cause decrease in kidney function.

2) Renal cause – The condition affecting

(a) Glomerular filtration rate.

(b) Tubular function.

(c) Any changes in the renal vascular system that decreases the blood flow.

3) Postrenal cause – Decrease in renal function is due to the obstruction of urine flow and it may cause by enlargement of the prostrate, stones in the urinary track or tumour of the bladder etc.

Kidney or renal function test

These are separated into the following group

Group 1 test

i) Blood urea nitrogen.

ii) Serum creatinine.

iii) Routine urine examination.

These test can be helpful to differentiate prerenal condition from post and renal conditions, by estimating blood urea nitrogen. Examination of urine for protein cells and cast give idea of an active lesion in the kidney.

Group 2 test

These test are performed in the case of renal diseases to asses the severity of the diseases.

i) Serum total protein, albumin, globulins and A/G ratio.

ii) Quantitative determination of urinal proteins.

iii) Electrophoretic fractionation of serum protein

iv) Serum cholesterol.

In nephritis type-I (Glomerulonephritis) moderate loss of protein in the urine is observed and in nephritis type-II (nephritic syndrome) massive loss of protein in the urine is observed

Group 3 tests

Tests measuring glomerular filtration

i) Creatinine clearance test

ii) Urea clearance test

These tests measure the actual excretory capacity of the kidney clearance test are considerably more sensitive and clinically more useful then the test measuring retention of substances such as urea and creatinine

Group 4 tests

Tests measuring tubular function.

i) Determination of specific gravity, Concentrating and diluting ability of kidneys.

ii) Determination of serum and urine osmolarity.

iii) Determination urine ammonia.

iv) Phenosluphonphthalein (PSP) test.

The specific gravity test designed to determine concentration power and dilution power of kidneys. Osmolarity is a measure of the total concentration of dissolved particle in the specimen.

Determination of ammonia in urine gives measure of the ability of the tubules to produce ammonia in state of acidosis.

PSP test gives measure of the secretory capacity of the kidney tubules the rate at which injected dye appears in the urine depends upon the action of tubular epithelial as well as the renal blood flow.

Group 5 tests

Test determining acid base status.

i) Serum Electrolytes (Sodium, potassium and chlorides)

ii) Serum in organic phosphorus and calcium.

iii) Serum bicarbonates.

iv) Blood gases (PO_2PCO_2) and blood PH.

Metabolic acidosis is a characteristic complication of renal diseases. It is caused by accumulation of phosphates, sulphates and non protein nitrogenous substances in blood.

Reabsorption of sodium is defective which causes hypokalaemia and severe dehydration may occur secondary to the electrolyte depletion.

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Chapter 16

LIVER FUNCTION TESTS

Objectives:

1. To learn laboratory evaluation of liver function which is one of the vital organs of the body.
2. To understand liver function tests from the view of patient's diagnosis, prognosis as well as management.

Introduction:

1. Liver function tests means various biochemical investigations performed in the laboratory to measure the functioning of liver.
2. Liver is the master organ of body's metabolism. Apart from its role in metabolism, liver performs many other important functions.
3. Liver Function Tests will help to detect the abnormalities and the extent of liver damage.

Functions of liver:

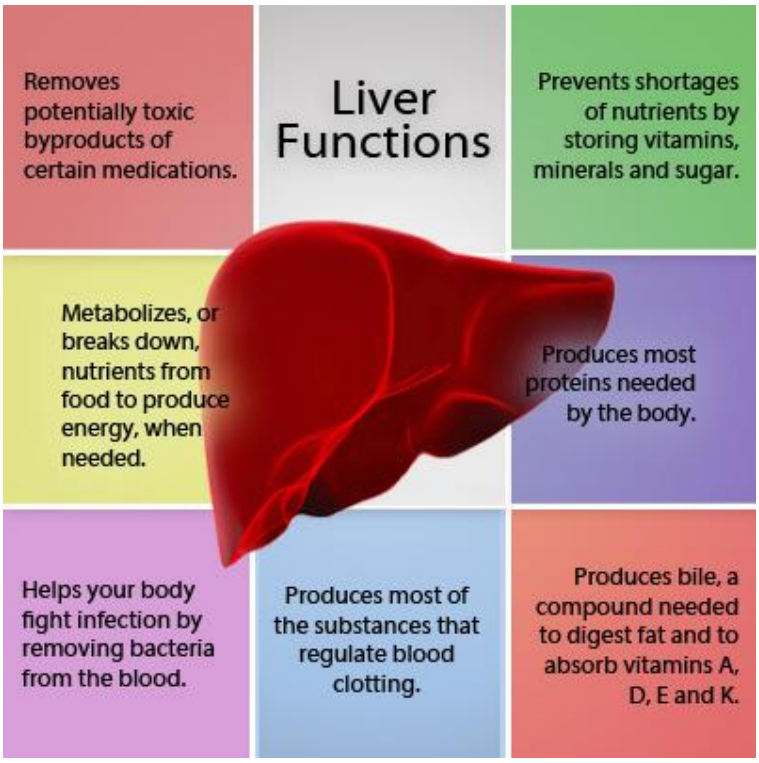
1. **Metabolic Functions:** Liver actively participates in carbohydrate, lipid, protein, mineral and vitamin metabolisms.

2. **Excretory Functions:** Bile pigments, bile salts and cholesterol are excreted in the bile into intestine.

3. **Protective Functions and detoxification:** Kupffer cells of liver perform phagocytosis to eliminate foreign compounds. Ammonia is detoxified to urea. Liver is responsible for detoxification.

4. **Hematological Functions:** Liver participates in the formation of blood (particularly in the embryo), synthesis of plasma proteins (including blood clotting factors) and destruction of erythrocytes.

5. **Storage Functions:** Glycogen, vitamins A, D and B12 and trace element iron are stored in liver.



Liver function tests:

The liver function tests (LFT) are the biochemical investigations to assess the capacity of the liver to carry out any of the functions it performs. LFT will help to detect the abnormalities and the extent of liver damage. Commonly used liver function tests are as follows.

I. Serum Bilirubin:

Bilirubin is the excretory end product of haem degradation. It is conjugated in liver and excreted in bile. When serum bilirubin level increases up to 3 mg/dl patient can develop icterus. Icterus means yellow discoloration of skin mucous membrane, which is easily seen in white of eyes. The normal concentration of serum bilirubin is in the range of 0.2-1.0 mg/dl. Of this, the conjugated bilirubin is about 0.2-0.4 mg/dl, while the unconjugated bilirubin is 0.2-0.6 mg/dl.

Van den Bergh reaction:

This is a specific reaction to identify the increase in serum bilirubin (above the reference level). Normal serum gives a negative van den bergh reaction.

Mechanism of the reaction: van den Bergh reagent is a mixture of equal volumes of sulfanilic acid (in dilute HCl) and sodium nitrite. The principle of the reaction is that diazotized sulfanilic acid (in the above mixture) reacts with bilirubin to form a purple colouredazobilirubin.

Direct and indirect reactions: Van den Bergh reagent reacts with conjugated bilirubin and gives a purple colour immediately (normally within 30 seconds). This is referred to as a direct positive van den Bergh reaction. Addition of methanol (or alcohol) dissolves the unconjugated bilirubin which then gives the van den Bergh reaction (normally within 30 minutes) positive and this is referred to as indirect positive. If the serum contain both unconjugated and conjugated bilirubin in high concentration, the purple colour is produced immediately (direct positive) which is further intensified by the addition of alcohol (indirect positive). This type of reaction is known as biphasic.

Van den Bergh reaction and jaundice: This reaction is highly useful in understanding the nature of jaundice.

Indirect positive – Hemolytic jaundice

Direct positive – Obstructive jaundice

Biphasic – Hepatic jaundice.

II. Bilirubin in urine:

The conjugated bilirubin, being water soluble, is excreted in urine. This is in contrast to unconjugated bilirubin which is not excreted. Bilirubin in urine can be detected by Fouchet's test or Gmelin's test.

Jaundice:

Jaundice is yellow discoloration of skin ,mucus membrane .It is observed in sclera of eyes & skin. When serum bilirubin increases more than 3 mg %, jaundice (icterus) can be seen. Various reasons for developing jaundice are as follows:

1) Prehepatic (Haemolytic jaundice):

It occurs due to excessive destruction of red cells resulting in increased bilirubin formation. It is caused by diseases like Thalassaemiamajour, Sickle cell anaemia, Congenital spherocytosis, G6PD deficiency.

2) Hepatic jaundice:

Hepatic jaundice means liver cells' functioning is affected due to different reasons resulting in jaundice. Inective hepatitis caused by virus comes in this category.

3) Posthepatic (obstructive) jaundice:

This occurs because of interference with normal passage of bile into the duodenum. It can be because of tumour of bile duct, bile duct stricture or cancer of head of pancreas.

Table showing variation in bilirubin level & type of jaundice:

Test	**PrehepaticHaemolytic**	**Hepatic**		**Posthepatic Obstructive**
		Congenital	**Infective toxic**	
Serum total	++++	+	+++	++++

bilirubin				
Direct’	N	N	++	++
Indirect”	+++	+	++	++
Urine bile Pigmets	N	N	++	++
Bile salts	N	N	++	++
Urobilinogen	++++	+	+	Nor
‘N’: Normal ‘+’: increased ‘++’: Moderately increased ‘+++’: High ‘++++’: Very High ‘-’: absent				

III. Serum Enzymes derived from liver:

Liver cells contain several enzymes which may be released into the circulation in liver damage. Measurement of selected enzymes in serum is often used to assess the liver function. It must, however, be noted that there is no single enzyme that is absolutely specific to liver alone. Despite this fact, serum enzymes provide valuable information for LFT. Some of these enzymes are discussed as follows.

IV. Transaminases or aminotransferases:

The activities of two enzymes – namely serum glutamate pyruvate transaminase (SGPT; recently called as alanine transaminase – ALT) and serum glutamate oxaloacetate tansaminase (SGOT; recently known as aspartate transaminase – AST) – are widely used to assess the liver function.

- **SGOT:** Serum glutamate oxaloacetate transaminase or AST .The activity of this enzyme is used to assess liver function .Normal value of SGOT or AST is 5 to 45 IU/l. This level is increased in liver damage. It has limitations as far as prognosis of patient is concerned.

- **SGPT:** Serum glutamate pyruvate transaminase or ALT as recently called. (Alanine transaminase). Normal SGPT level is 5 to 40 IU/l. SGPT level is increased in liver damage. SGPT level is more sensitive as compared to SGOT level for assessment of liver function. Estimation of serum transaminases cannot identify the causes (etiology) of hepatic damage.

- **Alkaline Phosphatase:**

Alkaline phosphatase (ALP) is mainly derived from bone and liver. Normal alkaline phosphatase level is 3 to 13 KA units /dl. A rise in serum alkaline phosphatase level associated with elevated serum bilirubin is an indicator of billiary obstruction (obstructive jaundice or cholestasis). ALP is also elevated in cirrhosis of liver and hepatic tumors.

- **Glutamyltranspeptidase:**

This is a microsomal enzyme widely distributed in body tissues, including liver. Measurement of y-glutamyltranspeptidase (GGT) activity provides a sensitive index to asses liver abnormality.

- **Total proteins:**

Estimation of proteins assesses synthetic function of liver.

Normal values-

Total proteins-6 to 8.5gm/dl

Albumin- 3.5 to 5.5gm/dl

Globulin- 2.3 to 3.5 gm/dl

V. Serum albumin:

Albumin is solely synthesized by the liver. It has a half-life of about 20-25 days, therefore, it is a good marker to assess chronic (and not acute)liver damage. Low serum albumin is commonly observed in patients with severe liver damage. It must, however, be noted that the serum albumin concentration is also decreased due to other factors such as malnutrition.

Functional impairment in liver is frequently associated with increased synthesis of globulins. Cirrhosis of the liver causes a reversal of albumin / globulin ratio (A/G ratio).

VI. Prothrombin time:

Prothrombin time measures extrinsic pathway of blood coagulation. Prothrombin is one of the factors of blood coagulation.

The liver synthesizes all the factors concerned with blood clotting. A decrease in the concentration of plasma clotting factors is found in the impairment of liver function. This can be assessed in the laboratory by measuring prothrombin time which is prolonged in patients with liver damage, compared to normal. The half-lives of clotting factors are relatively short (5-72 hrs.), therefore, changes in prothrombin time occur

quickly. Hence, this test is useful to assess acute as well as chronic liver damages; besides its help in the prognosis.

Vitamin K is required for the synthesis of blood clotting factors II, VII, IX and X. therefore, vitamin K deficiency can also cause prolonged prothrombin time which must be ruled out, before drawing conclusions on the liver functions. This is done by measuring prothrombin time before and after administration of vitamin K .Normal prothrombin time is 12 to 14 seconds. Severity of liver disease especially at end stage can be assessed by measurement of prothrombin time.

INR: INR is also measure of extrinsic pathway of coagulation. It is derived measure from prothrombin time .It is the ratio of patient's P.T. to normal (control) sample, raised to the power of the ISI value for the analytical system being used.

$$INR = (PT\ test / PT\ normal)^{ISI}$$

Normal value: 0.8 to 1.2

VII. Liver Biopsy:

Liver biopsy is direct examination of liver tissue obtained by various biopsy techniques. Liver biopsy is very specific diagnostic as well as prognostic tests. Biopsy can be FNAC, Ultrasound guided biopsy or CT guided or MRI biopsy. However tissue collected after biopsy will be received in the histopathology or cytology section of a clinical laboratory and not in biochemistry section.

Questions:

1) Enumerate various functions of liver.

2) Write the importance of liver function tests.

3) Describe role of serum enzyme levels in assessment of liver function.

4) Enlist various liver function tests .Describe any one in detail.

5) How can we differentiate between types of jaundice with the help of serum bilirubin level?

Chapter 17

Automation in Biochemistry

Objective: Student should be able to understand the meaning of 'Automation' and the types of auto analyzers and their uses.

Introduction

The modern biochemistry laboratory uses a high degree of automatiom. Many steps in the analytic process that were previously performed manually can now be performed automatically.

Automation in clinical laboratory is a process by which analytical instruments perform many tests with the least involvement of an analyst.

Advantages of Automation

1. Increase the number of tests done by one person in a given period of time.
2. Enable laboratories to process a much larger workload without a relative increase in manpower.
3. Allow a large number of tests to be performed with a small volume of sample.
4. Require very small amount of reagents thamn manual method which decreases the cost of consumables.
5. Minimize the variation in results from one individual to another (Coefficient of variation is reduced thereby increasing reproducibility)
6. Eliminate the potential errors of manual analysis such as equipment variation, calculation of results, etc.
7. Overall increase the production, quality and safety of a laboratory.

Automation may initially require high costs for purchasing the equipment, however it is economical in the long run due to the reduction in the manpower required to perform the tasks.

Types of auto Analyzers:

1. Continuous flow analyzers

- Liquids (reagents, diluents and samples) are pumped through a system of continuous tubing.

- Samples are introduced in a sequential manner, following each other through the same network of tubes. Series of air bubbles at regular intervals serve as separating media. The internal diameter of the tubing and the rate of flow determine the volumes of sample prior to mixing with the reagents and the turn around time of the result.
- An incubator is used to promote color development or the completion of enzymatic reaction.

Principle of detection:

- Detection is by measuring absorbency by spectrophotometer through a continuous flow cuvette (cell) at a certain wavelength.
- When there is no sample, the sampler probe is placed in distilled water to avoid blockages, clogging and precipitation.
- More sophisticated continuous flow analyzers use parallel single channels to run multiple tests on one sample.
- For single channel machines, results are plotted on a dot blot to check for possible systemic or random errors.
- For more sophisticated multi-channel machines, computers are used to store and analyze data and result may be reported to appropriate units via intranet.

Uses:

1. Multi-channel machines are used for certain test profiles (e.g. liver function tests and lipid function tests).
2. Single channel machines may be used for frequently requested independent analysis e.g. blood glucose.

Disadvantages:

1. The machine does not allow test selection; all tests must be performed even if not requested.
2. The machine must run continuously even when there are no tests.
3. Because of the continuous flow, reagents must be drawn at all times even when there are no tests to perform; which results in reagent wasting. Therefore a good stock of reagents must be available to avoid system malfunction due to reagent depletion.
4. The instrument must be closely monitored all the time for air bubbles uniformity; reagent availability and tubing integrity and most important of all carry over problems.
5. Multi-channel machines are usually large in size and occupy large space.

2. **Centrifugal Analyzer**
    - Samples and reagents are added in a specially designed centrifugal type cuvette that has three main compartments (see fig).
    - Sample is added from the sample cup by auto-sampler into the sample compartment of the centrifugal cuvette.
    - The reagent probe into the reagent compartment of the centrifugal cuvette adds Reagent.
    - Both sample and reagents are allowed to equilibrate to the reaction temperature.
    - Mixing of sample and reagent occurs when the rotor holding the cuvette is spun at high speed (4000 rpm) and then sudden stop. The spinning causes the sample to be added to the reagent while the turbulence caused by sudden stop results in mixing of sample and reagent.
    - After mixing, the rotor is spun at 1000 rpm. The reaction mixture is pushed horizontally to the bottom of the cuvette.

Principle of detection:

It has clear transparent sides for spectrophotometric measurement.

Advantages:

1. Rapid test performance analyzing multiple samples. Batch analysis is a major advantage because reactions in all cuvettes are read simultaneously.
2. Requires small sample (as little as 2µL of plasma, serum, urine or whole blood).
3. Uses small reagent volumes (250 µL).
4. Can be programmed to carry out many different assay methods.

Disadvantages:

1. Only one test type can be performed each time.
2. The quality of cuvette and uniformity of detection window is crucial. Only reputable companies should be dealt with which adds to the cost of analysis.

3. **Discrete auto analyzers**

    Principle:

    - Non-continuous flow using random access fluid which is a hydrofluorocarbon liquid to reduce surface tension between

samples/reagents and their tubing And therefore reduce carry over.

- Discrete analyzers have the capability to run multiple tests one sample at a time or multiple samples one test at a time. They are the most versatile analyzers.
- Each sample is treated differently according to the tests requested and programmed by the operator:

  E.g. sample 1 glucose, urea, creatinine and electrolytes

  sample 2 total protein, albumin, calcium

  sample 3 triglycerides, cholesterol

  sample 4 bilirubin, ALT, AST, ALP

- These instruments are heavily dependent on electronic control.
- Sample is aspirated by the auto sampler from the sample cup and placed in the reaction cuvet. Samples are programmed or adjusted to reach a prescribed depth in those cups to maximize use of available sample.
- Mixing of sample and reagents may be achieved by several methods such as:

  a) Spinning of the cuvet at high speed followed by sudden stop.

  b) Introducing the reagent into the cuvet by jet action.

  c) Introducing air bubbles into the cuvet.

- The reaction chamber temperature is controlled for colour development or enzyme assay to proceed.
- The absorbency of the reaction in the reaction cuvet is read by a spectrophotometer, which is housed in the reaction chamber.
- Computer then calculates the results and produces it in printed format.
- Many of these machines have a Q.C system built in and automatically checks on the results of the Q.C samples to determine whether to accept or reject the results of the run.
- Kinetic rather than endpoint methodologies are used (minimize protein error and give more accurate results)
- Some of these machines have the ability to store or file patient results.

Uses:

Analytes that can be measured include; glucose, BUN, ammonia, bilirubin, uric acid, cholesterol, triglycerides, total calcium, total protein, albumin, creatinine, phosphorus, and serum enzymes e.g. Kodak Ektachem.

Advantages:

1. These machines are robust and produce reliable results with minimum problems.
2. These are high throughput machines that can analyze up to 75 samples in one go for single or multiple testing
3. Require little volume of sample and reagents.
4. Results are directly sent to the clinics via compatible computer system
5. Printers may be attached for printing data and error charts for the control samples

Disadvantages:

1. Are expensive to purchase
2. Produce a lot of waste
3. Are expensive to maintain
4. Since each sample is in a separate reaction container, uniformity of quality must be maintained in each cuvet so that a particular sample quality is not affected by the cuvet it is placed in.

Review Questions:

1. Define Automation? What is the need for automation in clinical laboratory?
2. Describe the various advantages of automation.
3. Describe the working, used advantages and disadvantages of continuous flow auto analyzer.
4. Describe the working, used advantages and disadvantages of centrifugal auto analyzer.
5. Describe the working, used advantages and disadvantages of discrete auto analyzer.

Chapter 18

Blood Gas Analyzer

Objective - The student shall understand the importance of blood gas analysis and functioning of blood gas analyzer.

Definition

Blood gas analysis, also called arterial blood gas (ABG) analysis, is a test which measures the amounts of oxygen and carbon dioxide in the blood, as well as the acidity (pH) of the blood.

Importance

An ABG analysis evaluates how effectively the lungs are delivering oxygen to the blood and how efficiently they are eliminating carbon dioxide from it. The test also indicates how well the lungs and kidneys are interacting to maintain normal blood pH (acid-base balance).

Parameter Blood Gases

1. **pH:**This is alogarithmic expression of hydrogen ion concentration the acidity or alkalinity of the blood.

   The normal human arterial pH is 7.4. Any pH below this is acid, and any pH above it is alkaline. There is a narrow range of pH values (7.35 to 7.45) that the human body.

2. **PCO_2**: This value is measured directly by the CO_2electrode. An increased PCO_2 Is often the result of acute, chronic or impending respiratory failure, whereas a decreased PCO_2 is the result of hyperventilation stimulated by a metabolic acidosis or hysteria and severe anxiety reactions. The normal arterial PCO_2 is 40 mmHg.
3. **PO_2**: The partial pressure of oxygen in the blood is measured directly by electrode. The normal acceptable range is roughly between 85 and 100. An increased PO_2 is usually the result of excessive oxygen administration that needs to be adjusted downwards on such results. A decreased PO_2 is often the result of any number of respiratory or cardiopulmonary problems.

Theory of operation

PO_2Electrode

The PO_2 electrode basically consists of two terminals (1).The cathode, which usually made of platinum (negatively charged) and (2) the anode, which usually made of silver– sliver chloride (positively charged). How does this unit measure PO_2 in the blood sample? As shown in Fig.19.1, the electricity source (battery or wall electricity) supplies the platinum cathode with energy (voltage of 700 m V).

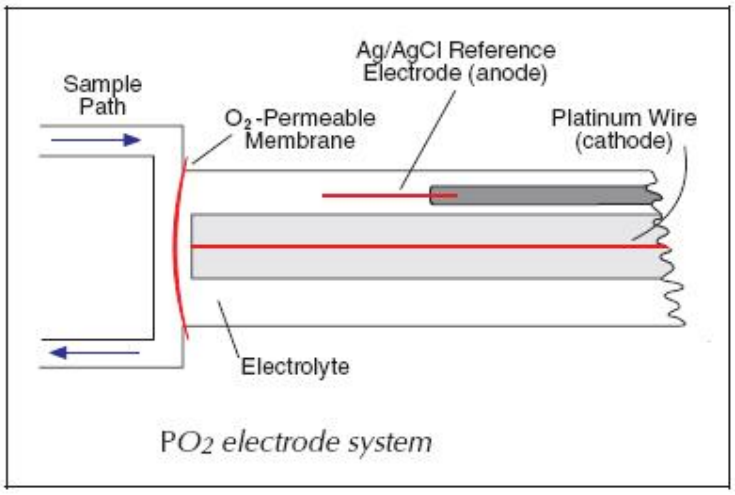


Figure 18.1 O2 Electrode

This voltage attracts oxygen molecules to the cathode surface, where they react with water. This reaction consumes four electrons for every oxygen molecule reacts with water and produces four hydroxyl ions. The consumed four electrons, in turn, are replaced rapidly in the electrolyte solution as silver and chloride react at the anode.

This continuous reaction leads to continuous flow of electrons from the anode to the cathode (electrical current). This electrical current is measured by using an ammeter (electrical current flow meter). The current generated is indirect proportion to the amount of dissolved oxygen in the blood sample, which in direct proportion to PO_2 in that sample.

pH Electrode

The pH electrode uses voltage to measure pH, rather than actual current as in PO_2 electrode. It compares a voltage created through the blood sample (with unknown pH) to known reference voltage (in a solution with known pH). To make this possible, the pH electrode basically needs four electrode terminals (Fig. 19.2), rather than two terminals (as in the PO_2electrode). Practically, one common pH-sensitive glass electrode terminal between the two solutions is adequate. This glass terminal allows the hydrogen ions to diffuse into it from each side. The difference in the hydrogen ions concentration across this glass terminal creates a net electrical potential (voltage). A specific equation is used to calculate the blood sample pH, using the reference fluid pH, the created voltage, and the fluid temperature.

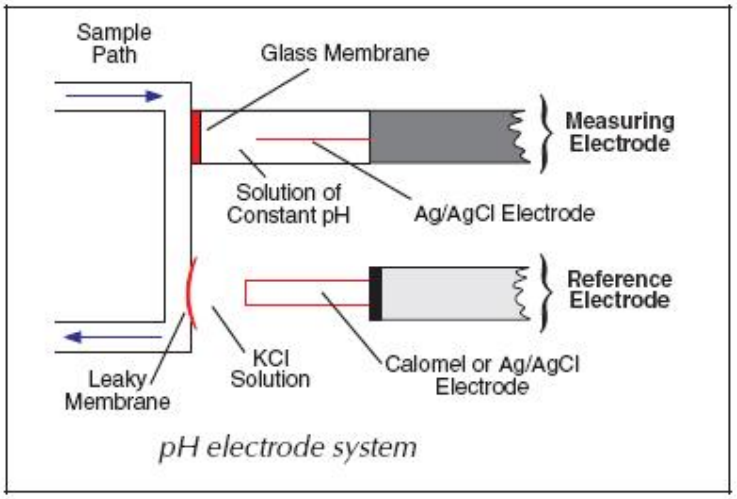


Figure 18.2 pH Electrode

PCO_2 Electrode

The PCO_2 electrode is a modified pH electrode. There are two major differences between this electrode and the pH electrode. The first difference is that in this electrode, the blood sample comes in contact with a CO_2 permeable membrane (such as Teflon, Silicone rubber), rather than a pH-sensitive glass (in the pH electrode), as shown in (Fig.6). The CO_2 from the blood sample diffuses via the CO_2 permeable (silicone) membrane into a bicarbonate solution.

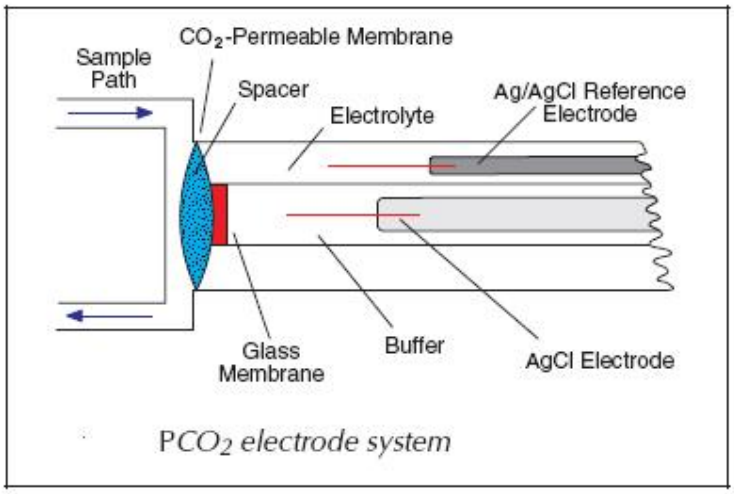


Figure 18.3 CO2 Electrode

The amount of the hydrogen ions produced by the hydrolysis process in the bicarbonate solution is proportional to the amount of the CO_2 diffused through the silicone membrane. The difference in the hydrogen ions concentration across the pH-sensitive glass terminal creates a voltage. The measured voltage (by voltmeter) can be converted to PCO_2 units. The other difference is that the CO_2 electrode has two similar electrode terminals (silver–silver chloride). However, the pH electrode has two different electrode terminals (silver–silver chloride and mercury mercurous chloride)

Figure 18.4 Blood Gas Analyzer

Interpretation

1. pH value of Human blood is 7,35 -7,45
   If it is less than 7,35 there is Acidosis
   If it is more than 7.45 there is Alkalosis

2. PO2 - The normal value is 80-100 mm of Hg
   If it is less than 80 there is Hypoxaemia.
   If it is more than 100 there is Hyperoxaemia.

Review Questions:

1. What is ABG? What is its clinical significance?
2. How the blood is collected for ABG?
3. Give the details of operation of Arterial Gas Analyzer.