IVC Course Code : 616

MEDICAL LAB TECHNICIAN (M.L.T)

First Year

(w.e.f. 2018-19)

Intermediate Vocational Course

Paper I : Biochemistry - 1

Paper II : Microbiology & Pathology

Paper III : Anatomy & Phisiology

STATE INSTITUTE OF VOCATIONAL EDUCATION, A.P.

BOARD OF INTERMEDIATE EDUCATION, A.P.

Smt. B. UDAYA LAKSHMI, I.A.S.

Commissioner & Secretary
Intermediate Education
ANDHRA PRADESH
GUNTUR.

S.I.V.E Co - Ordinating Committee

Sri P. Yerraiah, M,Sc., B.Ed.
Professor
State Institute of Vocational Education
Commissioner of Intermediate Education, Guntur

Sri P. Muralidhar, M,Sc., M.Phil..
Joint Secretary (Vocational)
Board of Intermediate Education,Guntur

Sri P. Seshu Narayana, M,Sc., B.Ed.
Reader
State Institute of Vocational Education
Commissioner of Intermediate Education, Guntur

Sri Dr. G.V.S.R. Murthy, M,Sc., Ph.D.
Lecturer
State Institute of Vocational Education
Commissioner of Intermediate Education, Guntur

DTP
Katari Ravi Kumar *B.Com, MCITP.*

Text Book Development Committee

Paper - I Biochemistry - 1

AUTHOR

Smt.P. Suneetha M.Sc
Junior Lecturer in MLT
Govt. Vocational Junior College
Guntur

Paper - II Microbiology & pathology

AUTHOR

Smt K.Kalpana, M.Sc.
Junior Medical Lab Technician
SRR & GVR Government Junior College
Machavaram
Vijayawada.

Paper - III Anatomy & Phisiology

AUTHOR

Dr. D. Kalyani, M.D,
Assiciate Professor
P.B.Siddardha Medical College
Vijayawada

EDITOR

Sri. Dr. N. Sarath Chandra Bose, M.Sc., Ph.D
Junior Lecturer in MLT
Government Junior College,
Guntur

ANNUAL SCHEME OF INSTRUCTION AND EXAMINATION FOR I YEAR

MEDICAL LAB TECHNICIAN COURSE

Part-A		Theory		Practical			Total	
		Periods	Marks		Periods	Marks	Periods	Marks
1.	General Foundation Course	150	50		-	-	150	50
2.	English	150	50		-	-	150	50
	Part-B							
3.	Paper-I Bio – Chemistry - I	135	50	Paper-I Bio – Chemistry - I.	135	50	270	100
4.	Paper-II Microbiology & Pathology	135	50	Paper-II Microbiology & Patholog	135	50	270	100
5.	Paper-III Anatomy & Physiology.	135	50	Paper-III Anatomy & Physiology	135	50	270	100
6.	OJT	-	-		365	100	365	100
	Total	**705**	**250**		**770**	**250**	**1475**	**500**

On the Job Training November and December

EVALUATION OF ON THE JOB TRAINING:

The "On the Job Training" shall carry 100 marks for each year and pass marks is 50. During on the job training the candidate shall put in a minimum of 90 % of attendance.

The evaluation shall be done in the last week of January.

Marks allotted for evaluation:

S.No	Name of the activity	Max. Marks allotted for each activity
1	Attendance and punctuality	30
2	Familiarity with technical terms	05
3	Familiarity with tools and material	05
4	Manual skills	05
5	Application of knowledge	10
6	Problem solving skills	10
7	Comprehension and observation	10
8	Human relations	05
9	Ability to communicate	10
10	Maintenance of dairy	10
	Total	100

NOTE: The On the Job Training mentioned is tentative. The spirit of On the Job training is to be maintained. The colleges are at liberty to conduct on the job training according to their local feasibility of institutions & industries. They may conduct the entire on the job training periods of I year and (450) II year either by conducting classes in morning session and send the students for OJT in afternoon session or two days in week or weekly or monthly or by any mode which is feasible for both the college and the institution. However, the total assigned periods for on the job training should be completed. The institutions are at liberty to conduct On the Job training during summer also, however there will not be any financial commitment to the department.

MEDICAL LAB TECHNICIAN

Paper - I

BIOCHEMISTRY - 1

INDEX

Chapter-1.

INTRODUCTION TO BIOCHEMISTRY.

Structure

1.1 Introduction to Bio Chemistry

1.2 Code of ethics for Medical Lab Technicians

1.3 Medical Lab Organization.

Learning objectives:

1. Student should understand the perspective of Bio chemistry & clinical biochemistry.

2.Student should learn and follow the code of ethics

3. The pattern of division of a lab into different sections and their organization should be observed and understood.

1.1 Introduction to biochemistry

Bio chemistry is the science of study of chemical reactions taking place in living matter. It explains the complex processes of life from the simplest organisms to complex organism .As it explains the physiological process ,it is also called ***Physiological Chemistry***. **Alexander Neuberg** is often referred to as Father of Bio chemistry.

Basic Bio chemistry deals with carbohydrates,lipids,proteins, amino acids etc.

Medical Bio chemistry is the study of bio chemical, metabolic and physiological processes.

Chemical biology is a discipline at the interphase of life sciences and physical sciences.

Analytical chemistry is a subdivision of chemistry dealing with qualitative and quantitative determination of chemical components.

Clinical Bio chemistry is the application part of Bio chemistry for the diagnosis of the clinical condition by determining various constituents of the body like blood/plasma/serum, urine, C.S.F, Sputum ,stools ,semen etc.Bio chemistry has applications in various fields- Medicine, Phar macy , Medical lab technology ,Biomedical engineering, Biotechnology, Nursing, Veterrnary, Dentistry, Agriculture, Dairying etc.

1.2 Code of Ethics for Medical Lab Technicians

Lab technicians should follow certain ethics and abide by the code of conduct to discharge his duties perfectly.

Punctuality:Lab worker should maintain punctuality in attending the duties assigned to him. In emergencies,punctuality saves the life of patient.

Promptness: lab worker should be prompt in his work,postponement of the work of the work delays diagnosis and thus the treatment.

1. **Accuracy**:Lab worker should be accurate in giving report.Guessing and assumptions should not be done in reporting.
2. **Confidentiality**: Clinical revealing should be maintained confidentially between lab technicianand Doctor.It should not be disclosed to the patient attendants or his relatives.
3. **Courtesy**: Courteous,kind and sympathetic approach should be followed towards the patient and his attendants.
4. **Obedience:** Technicians should be loyal and obedient to the superiors like Superintendent,pathologist, Micro Biologist,blood bank medical officer.
5. **Cordiality:** Technicians should maintain good relation and work in harmony with colleagues.
6. **Generosity:**Technicians should be generous and kind to the subordinates so as to extract their services promptly.
7. **Sincerity:** Technicians should maintain sincerity in the profession.Unlawful method of earning should not be followed.
8. **Cautiousness and Alertness**: Technicians should always be cautious and be acquainted with the safety procedures to be followed in the lab for preventing lab accidents and first -aid measures in case of lab accidents.
9. **Awareness:** Technicians should always be aware of the advancements through journals,books and literature so as to perform advanced methods.
10. **Maintenance of standards**: Technicians should maintain high standards in the work to elevate the status of the profession and reputation.
11. **Discipline:** Smoking,eating, drinking beverages should not be done in lab premises.Persons not connected with lab work should not be allowed into the lab. A white, cotton apron has to be worn by technicians.

1.3 Medical Lab Organization:

Medical Laboratory in big hospitals is divided into different specialties'. They are:

1. Clinical Pathology: It includes examination of the body fluids.
2. Haematology: It is the examination of blood cells and constituents.
3. Bio chemistry: It is the qualitative and quantitative examination of body fluids.
4. Micro Biology: It includes microbiological cultures, assays, staining, antibiotic sensitivity tests etc.
5. Histo-Pathology and Cytology:It includes processing and preparation of tissue specimen for microscopic examination.
6. Blood Bank and Immuno Haematology: In this collection and donation of blood takes place. Compatibility tests are done here before the blood is collected.

All the technicians working in a lab are called Medical Lab Technicians.

The following chart shows different members involved in a medical laboratory.

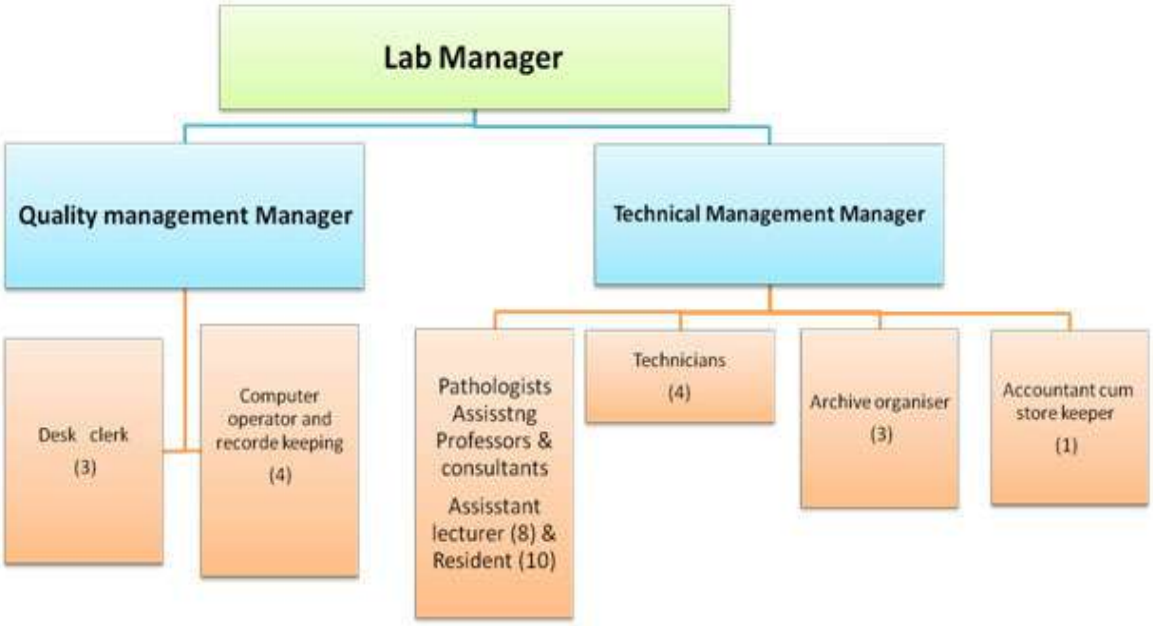


Fig 1.1 Different members of Medical Laboratory.

The following chart represents a medical laboratory lay out.

(please change diagram use blackand white photo

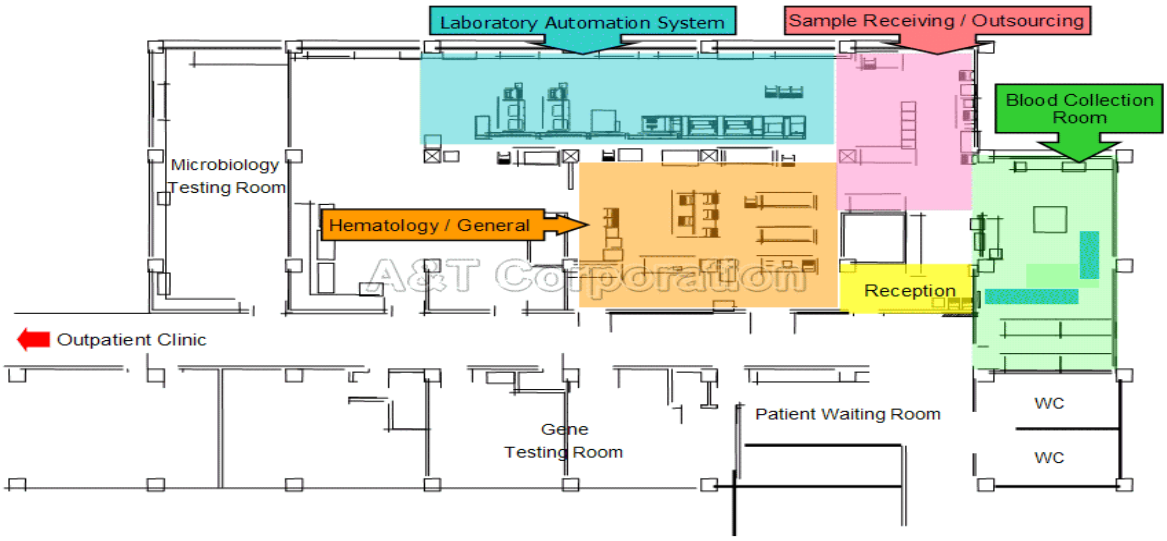


Fig 1.2 Medical Laboratory Layout

Many Accrediting Agencies like NABL,ISO,CRICIL,etc.are laboratories which provides formal recognition of competence of a laboratory with international criteria.

Conclusion:

Medical lab technicians should follow the code of ethics strictly.Medicine is the science of healing of body and mind.Medical lab technology is a part of it.Bio chemistry is needed in medical lab technology for the purpose of diagnosis.

Summary:

Medicine is the science of diagnosis,treatment or preventing thediseases that damage Body and mind.Bio chemistry is the science of study of bio chemical changes taking place in living beings.

A lab technician has to follow certain ethics and abide by the code of conduct.A lab technician should be punctual, prompt,accurate,courteous,loyal,sincere and maintain discipline.

A medical lab is divided into different specialities like Bio chemistry, Micro biology and serology, clinical pathology, blood bank and Immuno Haematology and histopathology and cytology.

Short answer type questions:

1. Define Biochemistry?
2. What are the fields of application Biochemistry?
3. Mention any two things of requisition in the code of conduct for lab technicians.

Long answer question:

1. Write an essay on the code of ethics for medical lab technicians.

Chapter -----2.

RECEPTION, REGISTRATION,MODELS OF STOCK REGISTERS, TYPES OF CHEMICALS AND LABORATORY SAFTY.

Structure:

2.1 Reception and Registration.

2.2 Different models of stock registers used laboratory.

2.3 Types of chemicals used in bio chemistry lab.

2.4 Safety measures to be taken in laboratory.

Learning objectives:

After studying this unit, the student is able to:

1. Understand the concept of reception and registration process in a laboratory and understand about lab registration form.
2. Student learn about the stock registers maintained in a lab.
3. Lab safety rules are understood.

2.1 Reception and Registration

When a person is ill, diagnosis begins with the physical examination by a doctor. There are various diagnostic tests to confirm a disease.Diagnostic tests ordered to be conducted are written over a format called lab order form.

Out- patient slip is given to out- patient and it contains the details of the patient which are entered into outpatient registration.

In patient is a patient whose clinical condition needs hospitalization and is given treatment by admitting the patient in the hospital ward.

Contents of lab order form:

Details of lab order form or lab request form should contain details such as hospital name and address , name of the patient , age, sex , date , O.P/ I.P. no; etc. are written on the upper column of the lab order form. Investigations to be done are written in the lower column of the lab order received.

Patient who receives lab order from doctor, hands over it to reception area lab technician in the reception. The technician will have the duties like entry of date and name ,sample collection, labelling and sorting of pathological samples like urine, blood, C S F, stools and other body fluids. In this form.

Reception:

Reception is the first point of contact with Laboratory Medicine where specimens requiring analysis are way they provide support to Bio Medical Scientists (BMS), clinical scientists and medical staff. Reception area lab assistant should possess good communication skills average computer skills and familiarity with medical terminology.

The lab order form is received in the lab, details are entered into lab register and samples are sent to different sections such as Haematology, Pathology, Biochemistry, Microbiology, immunology etc. Patient is instructed to collect the sample himself or technician collects the sample required from the patient. Collection container is labelled properly for identification. The specimen label contains details as name of the patient, O.P/I.P no. ,name of the tests, type of specimen etc.

Specimen reception is done not only in reception area, also in the doctor's chamber, O.P clinics, I.P clinics and other units of hospital , medical , surgical camps, epidemics investigation camps, patients home depending on the situation.

Fig 2.1 central specimen unit.

Specimen collection unit is also called central specimen unit (CSU) since it is the place where thepatient's samples from different wards,clinics, and other hospitals arrive, sorted and sent to related laboratories with relevant information.

Registration:

Registration is the process of entering the details of the order form into lab register. The data is entered into computer also. Separate lab software is also available for the purpose.

Lab register:

Register into which details such as date, I.P/ O.P.no.,name of the patient, age,sex, ward, nature of test,

name of the specimen, report findings are entered,is called a lab register.

After testing the specimen, findings of the tests are entered into the lab register, signed by the technician in the column concerned and report is submitted to the referring doctor confidentially.

Fig 2.2 Form for registration of in patient (case sheet)

Inner page of case sheet containing lab tests ordered is given below:

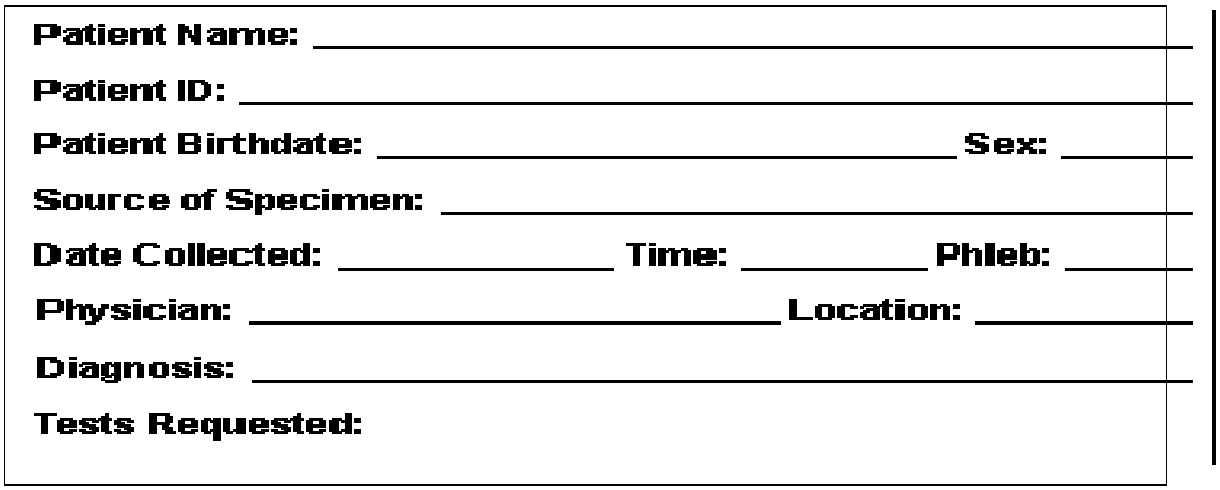


Fig 2.3Lab order form as the out- patient slip.

please keep another imaze

2.2 Different stock registers used in laboratories:

Stock registers is a record of goods purchased and/or stored in a laboratory. It is updating of addition of incoming stock. Stock registers are mainly of two types: they are-

(a) **consumable stock register and Non-consumable stock register**.

Consumable stock register: In this register, the products or commodities which are used up regularly and quickly are mentioned. Ex. Spirit, cotton, syringes, etc.

Non-consumable stock register: In this register, the products or commodities which are unaltered for long time and do not deteriorate for long time are mentioned. Ex. Centrifuge, Micro-scope, glass ware in lab, etc

Stock Register

Date	Name of the item	Opening stock	Stock received	Batch No.	Expiry date	Consumed	Wastage	Closing stock	Remarks	Signature

Fig 2.4 Format of stock register.

2.3 Types of chemicals used in biochemistry laboratory:

Some chemicals used in laboratory are toxic and corrosive. Corrosives are substances which can cause destruction of living tissues. Proper handling storage of chemicals and is necessary to prevent hazards. Chemicals are stored according to their physical and chemical properties. Types of chemicals are.

1. **Flammable chemicals**. Ex: Acetone, Ether, Xylene, Alcohol, etc.
2. **Corrosive chemicals**. Ex: concentrated acids, alkalies, phenol etc.
3. **Oxidizing chemicals**. Ex: Potassium dichromate, chromic acid, chlorites, etc.
4. **Explosive chemicals.** Ex: picric acid.
5. **Radioactive chemicals**. Ex: I-125, I-131, H-3.
6. **Carcinogenic chemicals**. Ex: Benzidine, O-toludine, Selenite, etc.
7. **Toxic chemicals**. Ex: Potassium cyanide.

2.4 Safety measures:

Students working in laboratory must have two major concerns to avoid accidents. They are:

(1) Safety and
(2) Efficiency in the laboratory work.

Students working in a bio chemistry laboratory must always be aware that the chemicals used are potentially toxic. Students must be familiar with general safety practices, facilities and emergency action.

Safety rules to be followed in laboratories:

1. Do not work alone in the laboratory.
2. Eating, drinking and playing in the lab is strictly avoided.
3. Wear gloves, aprons and safety glasses while handling dangerous substances.
4. Foot ware must completely cover the foot.
5. Visitors are not allowed into the lab.
6. Keep your work area and the common work areas clean.
7. Clean up spill or inform the instructor immediately about the spill so that he takes some immediate action.
8. Dispose the waste properly and in a timely manner according to the instructions provided in the lab manual.
9. Do not use earphones while working in the lab.
10. Replace the chemicals in the specified racks after the experiment is finished.
11. Do not pipet chemicals with your mouth.
12. Do not taste the chemicals because they are poisonous.
13. Do not heat, measure or mix the chemicals in front of your face.
14. Make sure test tubes containing chemicals reactions are pointed away from people.
15. A laboratory must always be equipped with a first aid kit.
16. In a potentially life threatening emergencies inform the instructor immediately and the person is rushed to the hospital.

Conclusion:

Reception is the first point of laboratory medicine. Registration is recording the things of lab request form into register .A biomedical laboratory maintains certain stock

registers to be maintained in the laboratory with details of equipment. Some chemicals used in labs are corrosive. Laboratory safety measures are to be followed by a student to perform practical safely.

Summary:

When a patient approach a medical laboratory, a lab order form is received at the lab, its lab register and after testing process is completed, the test findings are entered into lab register. The process of receiving the lab order form and collection of specimen from the patient is called reception. Entering the details of patient and test recordings into register is called registration.

Different stock registers are register maintained ina laboratory which contain all the details of the consumable and non-consumable equipment present in the lab. Corrosives are the substances which can cause destruction of living tissue. Several safety measures are to be followed by a student to perform the lab practical safely.

Short answer type questions:

1. What is lab order form?
2. Define lab register.
3. What is lab registration?
4. What is a stock register? Mention the different types of stock registers?
5. Define (a) out- patient (b) in-patient.
6. Define (a) Strong acid (b) Weak acid.
7. Define (a) Strong alkali (b) Weak alkali.
8. Write the properties of Hydrochloric acid.
9. Write the properties of sodium hydroxide.
10. What are corrosives? Mention some examples.
11. Name some chemicals used in laboratory.
12. Mention types of chemicals used in Bio chemistry lab.
13. Write some examples of carcinogenic chemicals.
14. Mention examples of Flammable chemicals.

Long Answer Type questions:

1. Write about reception and registration.
2. Mention the safety measures to be followed while working in a laboratory.

Activities:

1. Visit a hospital and observe in patient and out- patient registration.
2. Go to a lab and observe the process of registration and reception.

Chapter-3

Glassware and plastic ware used in Biochemical laboratory

Structure:

3.1 Glassware

3.2 Plastic ware: Brief outline.

3.3 Cleaning and drying of glass ware and plastic ware.

Learning objectives*:*

Student should-

1. Visit a chemistry, pharmacy and medical labs and get acquainted to different lab glassware and their uses.
2. Prepare a museum of glass ware with the help of fellow students and lecturer.
3. Learn to clean and dry the glass ware after the use.

3.1 Glass ware:

Types and composition:

Different glass and plastic containers are used in a laboratory for various purposes as measuring, transfer, mixing, boiling, filtration, etc. During these processes, they are exposed to chemicals contained in them. Glass used for manufacture of these glass ware must resist the action of chemicals like acids, alkalies etc. They must withstand the mechanical rigors during handling, operation and the effect of sudden changesof temperature during boiling, refluxing, etc.

(a) Composition of glass ware: The composition of glass is--

Pure silica, Sodium carbonate, Brokenglass, Boron oxide, Lead and Alumina.

- Boron glass acts as fusion agent.
- Reduction in the quantity of sodium ions renders the glass chemical resistance

- Without alkalies, melting the glass is expensive and difficult.
- Boron oxide reduces the temperature required for melting the glass.
- Trace quantities of lead gives clarity and brilliance.
- Alumina imparts hardness and increased resistance to chemical action and durability.

(b) Types of glass:Different types of glass are—

1. Borosilicate glass.
2. Treated soda lime glass.
3. Regular soda lime glass and
4. General purpose soda lime glass.

1. **Borosilicate Glass:** The glass ware generally used in laboratory is manufactured with Borosilicate glass. It is resistant to the action of chemicals and effects of sudden changes of temperature during boiling, refluxing etc. However it is not resistant to the action of hydro fluoric acid. This highly resistant glass is made by replacing alkali and alkaline earth cations by Boron /Aluminium and Zinc. It is chemically more resistant than soda lime glass. It is composed of --
    - Silica- 80.6%
    - Boron oxide- 12.6%
    - Sodium oxide- 4.15% and
    - Alumina -2.2%.

    Addition of boron reduces leaching action.

2. **Treated Soda Lime Glass:** Soda - lime glass subjected to the treatment by an atmosphere containing water vapour and Sulphur dioxide at elevated temperature is called as Treated Soda Lime Glass. This type of treatment is called as Sulphur treatment and it protects the glass from blooming or weathering.
3. **Regular Soda Lime Glass:** This is commercial glass. It is not subjected to Sulphur treatment. It has average to better than average resistance.
4. **General purpose soda lime Glass:** This is soda-lime glass used for general purpose.

Types of glass ware, their identification, application and use:

Types of laboratory glass ware:

(a) Graduated glassware.

(b) General glassware.

Graduated glass ware:

Glassware which have graduations on them and intended for purpose as containing, delivering etc. and which are used in accurate measurements for use in volumetric analysis are called as graduated glassware. There are two types of graduated apparatus in Great Britain –(1) class A and (2) class B

Class A apparatus is used in work of highest accuracy. Class B apparatus is used in routine work.

Different graduated glassware are:

1. Graduated flasks
2. Pipettes
3. Burettes
4. Weight burettes
5. Measuring cylinders
6. Hb tube and
7. Syringes.

1. **Graduated flasks:** They are also known as volumetric flasks. They are of two types-
   (a) To contain (TC) type: They are more accurate for quantitative work.
   (b) To deliver(TD) type

The graduated flasks has-

- Its bottom flat
- Is pear shape
- Has a long narrow neck
- A thin line etched around the neck. It indicates the volume it holds at definite temperature –usually 20 degrees C.

Fig 3.1Different graduated flasks.

Uses: They are used in making up final volume of standard solutions.

2. **Pipettes**: pipettes are two kinds :
   (a) Transfer pipettes
   (b) Measuring pipettes and
   (c) Specialized pipettes.

- **Transfer pipettes:** They are also called volumetric pipettes. It consists of a central bulb joined at the both ends to narrow tube. It has three parts:

  ---Upper suction tube.

  ---Central cylindrical bulb.

  ---Lower delivery tube.:

Fig 3.2 Transfer pipettes

Upper suction contains a mark etched around. When a liquid is pipetted up to this mark, liquid will be of the volume, the pipette is specified to contain. Lower tube is drawn out to a fine tip. Transfer pipettes are constructed with capacities of 1, 2, 5, 10, 20, 25, 50 and 100ml.

Uses: These pipettes are useful for delivering specific quantity of liquid. They are very accurate in quantitative work.

- **Measuring pipettes:** They are also called as graduated pipette. They do not contain a central bulb. There are three types of graduated pipettes.
    (1) **Type 1 graduated pipette**: They deliver required volume from top zero to selected graduation.
    (2) **Type 2 graduated pipette**: They deliver measured volume from selected graduation to the tip of delivery.
    (3) **Type 3 graduated pipette**: They are designed to remove selected volume of solution, thus to contain the capacity from tip of delivery to the selected graduation.

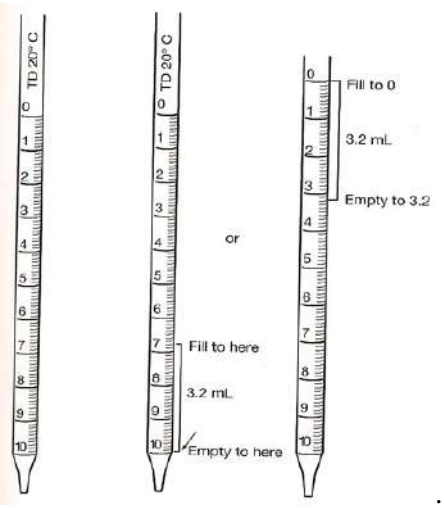


Fig 3.3 Image of measuring pipettes.

Uses: They can be used to deliver variable volume of liquid in the range of volume specified for the pipette. However they are not employed in accurate work where burette is preferred.

- **Specialised pipettes:**
  1. ***Serological pipettes***: serological pipettes are graduated pipettes used for pipetting serum, plasma, standard solutions, distilled water, etc. They are marked upto the tip 0.1 ml to 0.2 ml.
  2. ***Mohr pipettes***: Mohr pipettes are pipettes graduated above the tip, so that they can be used even when the tip is broken. They have similar application as serological pipettes but not that much accurate.
  3. ***Folin Oswald Measuring volumetric pipettes***: They are volumetric/transfer pipettes for measuring specific volume of liquid. They are useful in accurate quantitative works.
  4. ***Blood pipettes:*** They are:

(a) **R.B.C pipettes** used in Erythrocyte count. It contains a red bead in the central bulb. There are two marks etched around the lower tube. 0.5 to 1 below the bulb. 101 above the bulb. Rubber tube connected to the upper section of the tube aids in pipetting.

Blood is sucked up to 0.5 or 1 mark and diluted fluid is sucked up to 101 mark. Red bead helps in identification of the pipette and also in mixing of blood and diluting fluid. When clotting occurs in the lower delivery tube, a fuse wire is used to remove the clot.

Uses: It is used in R.B.C count.

(b) **W.B.C pipette** is T.C of Leucocytes. It is a pipette with a white bead in the central bulb. There are two marks etched around the lower delivery tube. 0.5 to 1 below the tube. 11 above the bulb. Rubber tube connected to the upper suction tube aids in pipetting.

Blood is sucked upto 0.5 or 1 mark .Diluting fluid is sucked up to 11 mark .White bead helps in identification and uniform mixing of blood with diluting fluid. Any clot in the lower delivery tube can be removed by a fuse wire.

Uses: It is used in 1.W.B.C. count 2.Sperm count

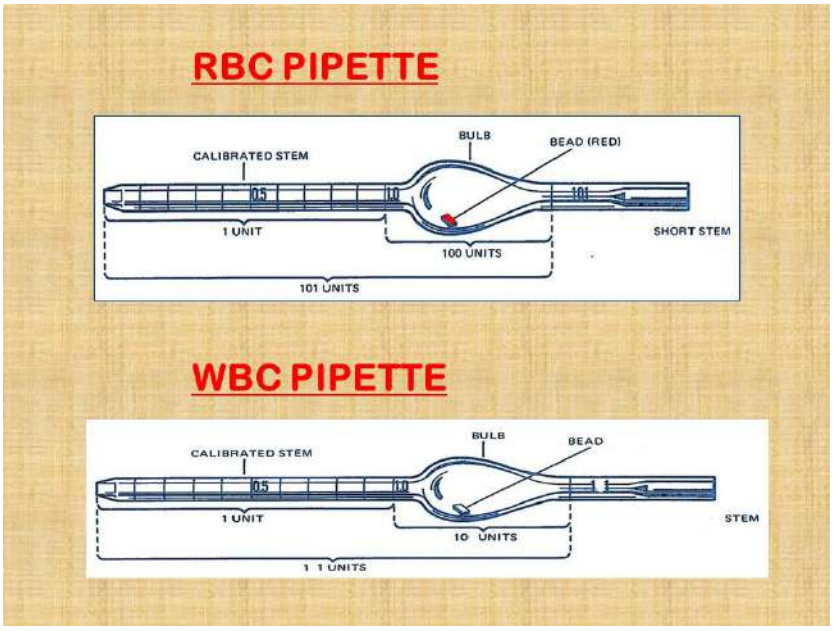


Fig 3.4 R.B.C pipette and W.B.C pipette.

(c) **<u>Hb pipette</u>**: This pipette is used in Hb estimation. It has no bulb. The mark etched around the tube is 20 micro liters (0.02ml) suction tube is attached at the top to aid in pipetting. Clot can be removed by a fuse wire.

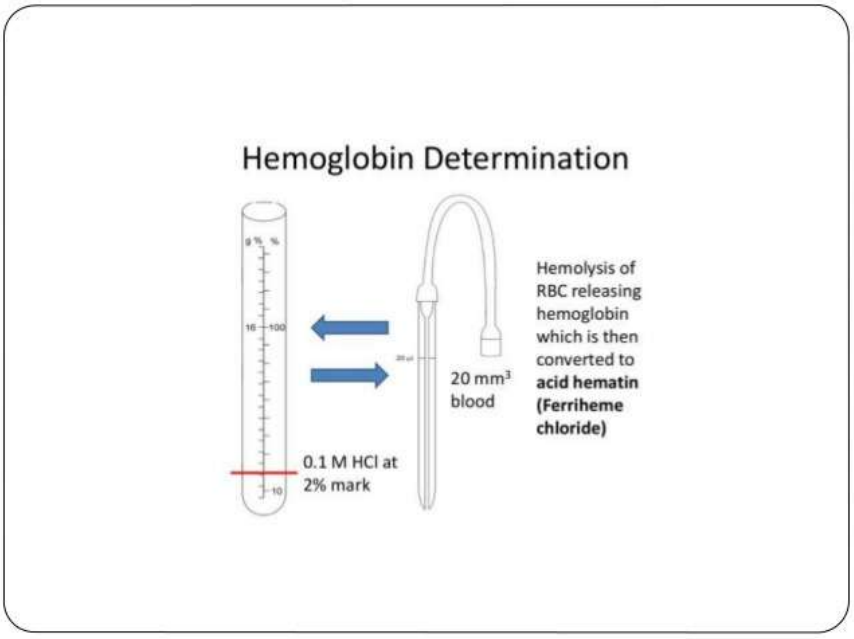


Fig 3.5 Diagram of a Hb pipette.

Uses: This pipette is useful in determining of Hb by Sahli's method and Cyanomethaenoglobin method.

(d) **E.S.R Pipette**: It is used in determination of erythrocyte sedimentation rate. It is a graduated with graduations from 0 to 200mm.

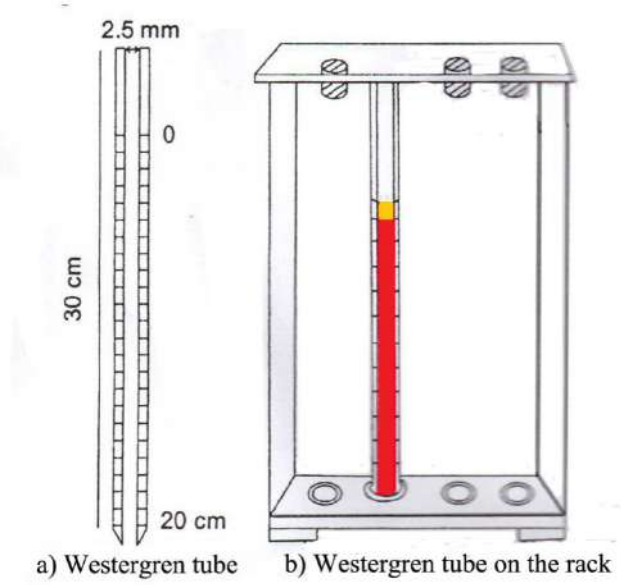


Fig 3.6 E.S.R pipette and E.S R stand.

Uses*:* It is used in E.S.R determination by Westergren's method.

3. **Burettes**: Burettes are long cylindrical tubes of uniform diameter. Bottom of the burettes is provided with stopcock. They are available from 5 to 100ml. There are two

classes of burettes-class A and class B.

Fig **3.7 Diagram of a burette.**

For smooth functioning of stopcock without friction or freezing, lubrication with pure Vaseline helps.

Uses: 1) They are used in titrations.

2) They are used in measuring and transfer of liquids.

4. **Weight burette**: They are used to measure the weight of the liquids, they transfer.

5. **Measuring Cylinders**: They are cylindrical in shape with flat bottom attached to provide base for resting vertically. They have pour points. There are graduations on the cylinder. They are available in capacities of 2 to 2000 ml. since the area of surface is more than in volumetric flasks, measuring is less accurate with these cylinders.

Fig 3.8Measuring cylinders.

(6) **Hb tube**: Hb tube is used in haemoglobin determination by Sahli's method. There 20 mark graduation on it up to 0.1N HCl is taken.

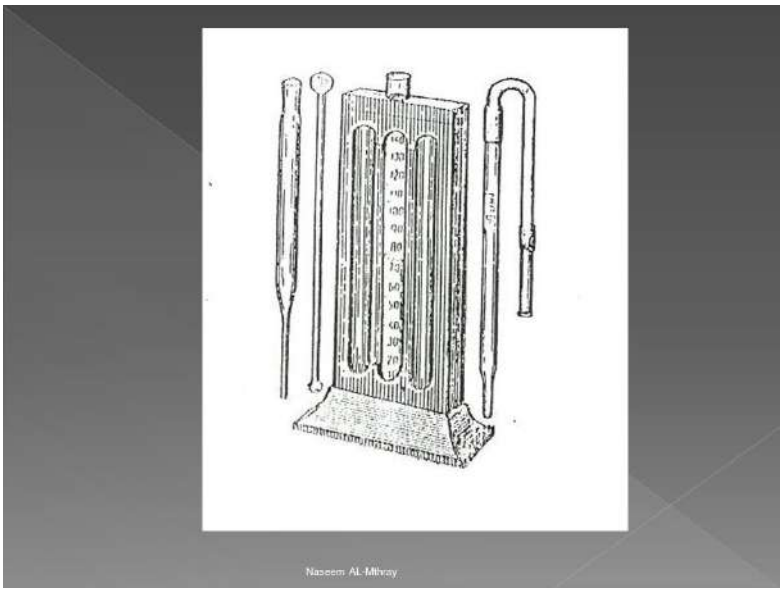


Fig 3.9 Hb tube in Hemocytometer.

(7) **Syringes:** Syringes are used in biochemical/ medical laboratories for the collection of venous and arterial blood specimens.

Fig 3.10 Image of a glass syringe.

General Glass Apparatus:

The general apparatus are:

1.Beaker 2.Flasks

3.Tubes 4.Separating funnels

5.Funnels 6. Condensers

7. Desiccators 8.Bottles

9.Wash bottles 10.Stirring rods

11.Weighing bottles 12.Watch glass

13.Clock glass 14.Weighing funnels

15.Glass slides

1. Beakers: Beakers are flat bottomed, broad based cylindrical vessels with a spout or pour point. They are available in capacities from 5 to 5000 ml. The most useful sizes are from 250 to 600 ml. pour point on the upper edge of beaker through which liquids can be transferred conveniently from the beaker into other container.

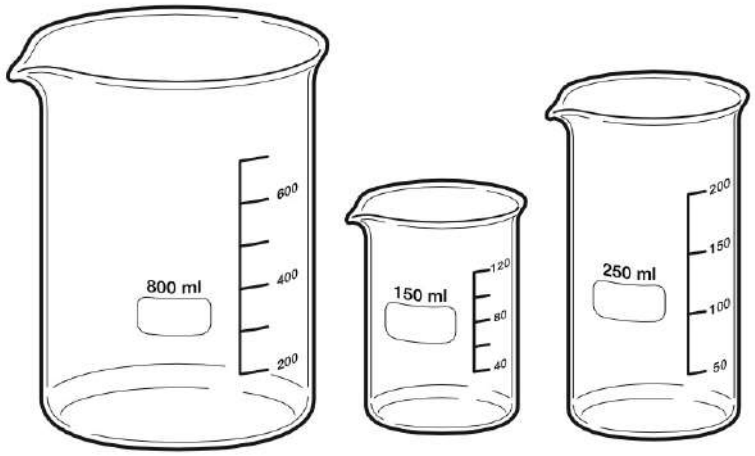


Fig 3.11 Different sizes of beaker.

Advantages of the pour point are:

- Convenience in pouring.
- Providing vent for steam during boiling.

- Providing convenience for protrusion of stirring rod when the beaker is covered.

Uses: 1.They are useful in preparation of solutions.

2. They are useful in boiling the solutions.

2.**Flasks:** Types of flasks – (a) Conical Flasks.

(b) Round bottomed flasks.

(c) Flat bottomed flasks.

(a) ***Conical flasks***: They are conical shaped with broad flat base. They are in different sizes ranging from 200-500 ml.

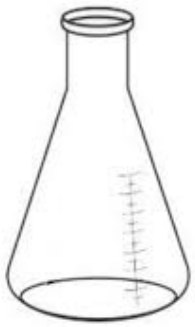


Fig 3.12 Diagram of a conical flask

Uses: 1.They are useful in conducting titrations.

2. They are also useful in boiling solutions. Their conical shape minimizes evaporation.

(b) ***Round bottomed flasks***: They have spherical bulb with a cylindrical neck. They can withstand high temperatures.

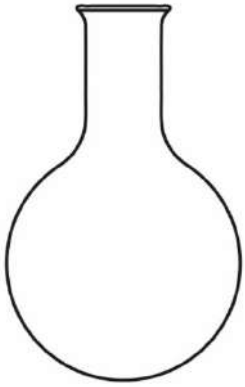


Fig 3.13 Diagram of round bottomed flask.

Uses: 1.They are used for making solutions.

2.They are used for boiling the solutions.

3. They are used in reflux condensations.

4. They are used for distillation purposes.

(c) ***Flat bottomed flask***: This is similar in shape to round bottomed flask except that it has flat bottom. It is used for boiling the solutions, liquids,etc.

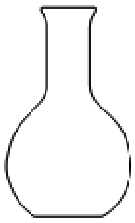
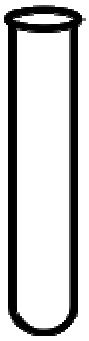


Fig 3.14Flat bottomed flask

3.***Tubes***: Different types of tubes are - (a) Test tubes (b) centrifuge tubes (c) Boiling tubes (d) Digestion tubes and (e) Nessler's tube.

(a) **Test tubes**: Test tube are two types--

1. *Test tubes with rim* are used when reagent is heated on a flame directly. Rim provides grim for holding with a test tube holder.



please verify fig no

2. *Test tubes without rim* are used for normal purpose.

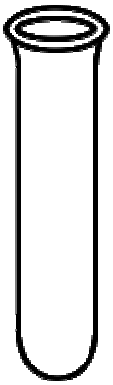


Fig **3.15(a) Test tubes without rim.** **Fig 3.15(b) Test tube with rim.**

They are available in different sizes. Commonly used test tubes are:

- Small size test tube have dimensions of 10 x 75 mm. They are used in qualitative testing procedures. They can also be used in place of centrifuge tubes.
- Medium size test tubes have dimensions of 15 x 125 mm. They find applications mainly in biochemical estimation.
- Big size test tubes have dimensions of 18 x 150 mm. They find application in heating directly on flame.

(b)**Centrifuge tubes:** They are similar in shape to the test tubes except that bottom portion is conical. They are useful for centrifugation and thus separation of solid from liquid in which it is insoluble.

Fig 3.16 Centrifuge tube.

(c)**Boiling Tubes:** They are bigger than tubes. They have similar shape as test tube. They are used for boiling small quantities of chemicals in qualitative testing procedures

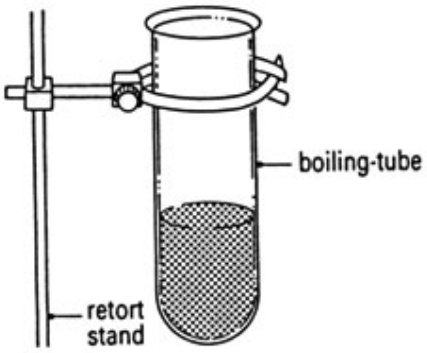


Fig **3.17 Boiling tube**.

(d)**Digestion Tube:** They are long narrow test tubes with slightly more than 50 ml capacity. They have graduations 25 ml. and 50 ml. etched around. They are used for converting organic matter into inorganic matter. This conversion is affected in the presence of digestion mixture which includes 50% sulphuric acid and 50% selenium dioxide.

Fig 3.18 Digestion tube

4. ***Separating Funnels***: It contains a conical chamber with a narrow neck into which a glass stopper fits. Conical chamber is attached to a narrow stem which has a stopcock. Stopcock helps in controlling delivery of the liquid contents taken in the chamber.

It is useful for the separation of immiscible liquids. For separation of immiscible liquids, stop cock is released until one liquid is delivered into. Another liquid remains in the chamber which can be delivered into another container.

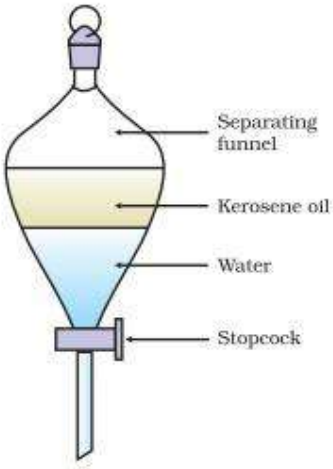


Fig 3.19 Diagram of a separating funnel

5. ***Funnels***: Funnels have conical top attached to a narrow stem. They are available in different sizes. Common sizes are 50, 65 and 75 ml. They useful in-
    a. Transfer of liquids from one container to another container.
    b. Separation of solid from liquid in which the solid is insoluble. This process is called filtration. Filtration is done with the help of a filter paper.

Fig 3.20 Drawing of a funnel

6. ***Condensers:*** condenser is a long narrow tube within a broad tube fused at the edges. It is arranged with inlet and outlet. It is useful in reflux condensation and distillation processes.

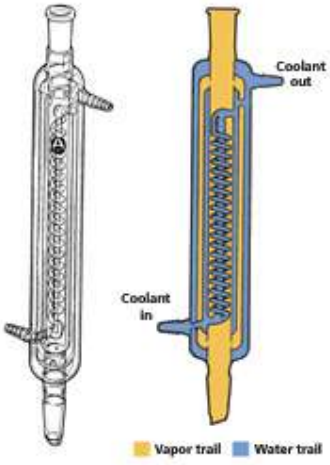


Fig 3.21Diagram of a condenser. **black and white diagram only**

7. ***Dessicators:*** Dessicator is a glass container. Dessicant substances are charged into the shallow dish at the bottom of the container. Substances to be protected from atmospheric moisture are taken in small containers and kept on a platform above the shallow dish and lid is closed. Examples of dessicants are Silicagel, Alumina, Calcium sulphate impregnated with cobalt salt.

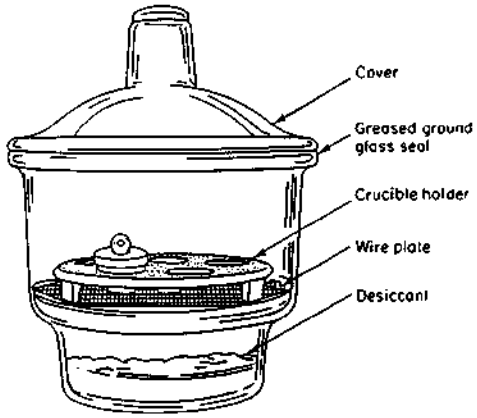


Fig **3.22 Diagram of a Dessicator.**

8. ***Bottles:*** Different types of bottles used in laboratory are—
   (a) **Reagent bottles**: They are cylindrical in shape with narrow neck fitted with stopper. They are available in different sizes ranging from 25-5000 ml. capacities. Amber colored bottles provide screening of U.V light present in the sunlight and prevent entry into the bottles. Thus photo sensitive chemicals like silver nitrate can be stored in such bottles.

Fig 3.23 Reagent bottle and Screw capped bottle.

(b)**Screw capped bottles**: They are available in 5-1000 ml. capacities. They are closed by screw caps made of metal or plastic. They store hygroscopic substances.

(c) **Winchester quart bottles**: They are cylindrical with narrow neck. They are fitted with glass stopper. They are white or amber colored available in 2 liters capacity. They are useful for stocking reagent.

Drop bottles are bottles containing slotted glass stoppers. Slotted glass stopper help in deliver of stains and indicators in the form of drops.

Fig 3.24 Dropper bottle

9. ***Wash bottles***: Wash bottle is a flat bottom flask that deliver distilled water or other liquid for use in the transfer and washing of precipitates. It is fitted with a rubber bung with two holes. Tubes are fitted into the bottle and air is blown into bottle. It used for washing precipitates since plastic wash bottles cannot with stand organic solvents.

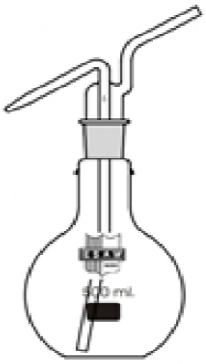


Fig 3.25 Glass wash bottle.

10. ***Stirring rods:*** They are made from 3-5 mm diameter glass rods cut into suitable length. It should be of such length that it should come 2-3 cm. out beyond the spout.

Fig 3.26 Glass stirring rod

Weighing Bottles: Bottles used for weighing chemicals are called as weighing bottles. They are used when substance is affected by atmosphere duringweighing. Chemical is placed in weighing bottles and weighed. Required quantity of substance is transferred into reaction vessel and then bottle is reweighed. Difference gives the weight of the chemical.

Fig 3.27 weighing bottle.

11. ***Watch glass***: Substance unaffected by atmosphere and which can affect balance pan are weighed using a watch glass.

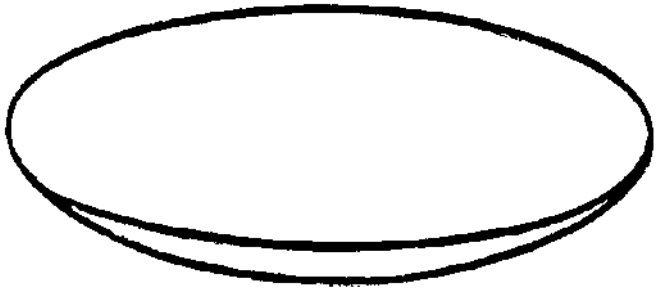


Fig 3.28 Watch glass.

12. ***Clock glass:*** clock glass is round flat piece of glass used for covering over a beaker can when a liquid and chemicals are heated in it. .

13. ***Weighing Funnel:*** Weighing funnel with scoop shaped end. After weighing on a balance, narrow stem of the funnel is inserted into the neck of the flask and the contents of the funnel are washed into flask with solvent/water from a wash.

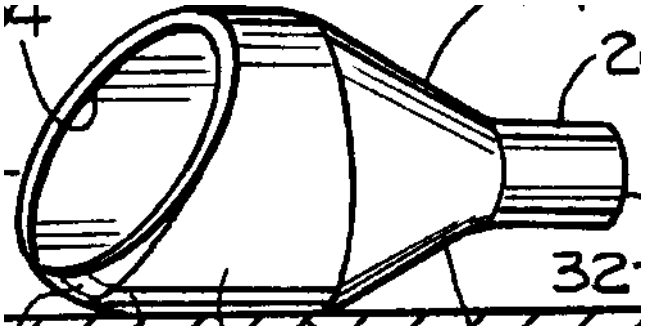


Fig 3.30 Weighing funnel. **Change fig no**

14. ***Glass slides***: Glass slides are flat rectangular pieces of glass. They are for microscopic examination of chemical substances in biochemistry.

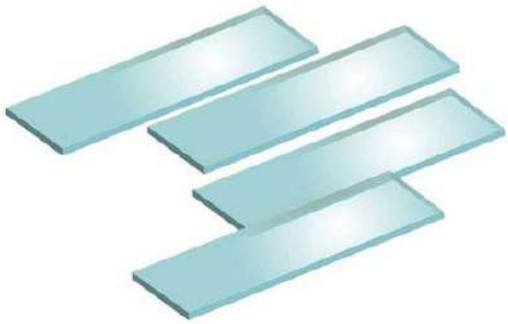


Fig 3.31 Glass slides

3.2 Plastic ware: Brief outline.

Materials used for making plastic lab ware: Different materials used for making plastic lab ware are:

--- Polyethylene.

---poly propylene.

---polyvinyl chloride

---polystyrene.

---poly amide(Nylon)

---poly carbonate and

---Tefflon.

Plastic is widely employed for lab ware like beakers, conical flasks, bottles, etc. They are cheaper than glass articles. Different plastic ware used in labs are:

1. ***Aspirator:*** It is also known as aspirator bottle which refers to a large cylindrical container used to store laboratory reagents. It is equipped with a tap or stopcock at the bottom for the delivery of the liquid.
2. ***Spatula:*** The term spatula refers to various small implements with a broad, flat, flexible blade used to mix. Spread, and lift materials from their containers. It has a long handle. Metallic spatulas also have been in use.

Fig 3.32 Plastic spatulas

3. ***Scoops:*** Scoops refers to small implements used to lift materials. Metallic scoops are also available.

Fig 3.33 plastic scoops

4. ***Lab wash bottles***: Also called Squeeze Bottles. A wash bottle has a screw-top lid with a nozzle and an internal dip to allow upright use. They are used to rinse various laboratory glass ware such as test tubes, flasks, etc. When hand pressure is applied to the bottle, the inside becomes pressurized and is forced out of the nozzle onto narrow stream of liquid. Wash bottles are used to fill with deionized water, distilled water, acetone, ethanol, etc.

Fig 3.34 plastic wash bottle.

3.3(a)Cleaning, drying, maintenance and storage of glass ware:

All the glass ware must be thoroughly clean and dry before using, otherwise results will be unreliable.

Test for cleanliness:

1. Fill the apparatus with distilled water.
2. With draw the water
3. Observe for broken films of water.

   Presence of unbroken film of water is indicates thorough cleanliness. Collection of drops indicates presence of grease and dirt.

Aims of cleaning:

1. **Chemical cleanliness**: Glass ware should be free from all chemicals.
2. **Removal of particulate matter**: Glass ware should be free of particles and fibres.
3. **Removal of pyrogens**: Pyrogens are the products of bacterial metabolism capable of increasing body temperature above the normal. Glass ware should be free of pyrogens.
4. **Removal of grease**: Glass ware should be free from grease.

Cleaning agents:

1. **Organic cleaning agents**: They may be strongly adsorbed on to glass. For this reason, cationic detergents which are strongly anti -bacterial cannot be used for bacteriological glass ware.
2. **Soap flakes and powders**: Precipitation of calcium and magnesium soaps takes place in hard water. Precipitated deposits on glass ware impairs brilliance. Water softener such as sodium meta- phosphate eliminates this problem.
3. **Inorganic cleaning agents:** These are used alone or with soaps which gives brilliance to glass ware. They are also easily removed from the surface of the glass ware. Ex: sodium hexa-meta phosphate.
4. **Chromic acid:** Chromic acid cleaning solution is made by dissolving 70 grams of sodium or potassium dichromate in 40 ml. of water using heat. Then it is diluted to I litre with conc. Sulfuric acid with constant stirring. Solution must never be added to acid. Gloves and rubber apron should be worn while preparing this solution.
5. **Mixture of conc. Sulfuric acid and fuming nitric acid**: This acid mixture is more efficient cleaning liquid.it used when the vessel is greasy and dirty. It must be handled with extreme caution.
6. **10% KOH in methylated spirit**: 100 grams of KOH is dissolved on 50 ml. of water and diluted to 1 litre with industrial methylated spirit. It is very effective degreasing agent. **Teepol:** it is mild and inexpensive detergent. It is used in cleaning pipettes and burettes. 1ml.stock solution is diluted with 30 ml distilled water for routine use.

Basic steps in cleaning:

Different steps in cleaning are-

1**.Soaking:** Apparatus are filled with and immersed in hot cleaning solution and left overnight.

2**. Brushing**: Hand brushing can be done. For bottles, brushing machines may be used.

3**. Rinsing**: Rinsing has to be done with water followed by rinsing with distilled water.

4. **Draining:** water remaining on the surface of the glass ware should be drained. Properly cleaned glass ware should be dried before usage. It may be done in a hot air oven.

Maintenance and Storage:

- Glass ware should always be maintained clean and dry. Old glass ware should be cleaned before using.
- After use also, the glass ware should be cleaned again with required chemicals.
- They should be stored in a cupboard meant for their storage carefully to avoid breakages.
- List of articles are maintained in a register.
- Old articles are to be replaced by new ones.

3.3(b) Cleaning, maintenance and storage of plastic articles:

Plastic articles require one or more of the following treatments,

1. Boiling in week detergent. Concentration of detergent should be about 1%.
2. Boling in dilute alkali. 1%sodium carbonate is enough.
3. Boling in dilute acid.1%HCL is enough.
4. Boling in solution of sodium hexa-meta phosphate or EDTA.

15 minutes boiling in each case is enough. It should be followed by washing in running water. It should be followed by boiling in distilled water. Finally three more rinse in distilled water should be given.

5. After cleaning, they should be drained.
6. They can be air dried or dried in in oven at 65 degrees for 15 minutes with vent open. Oven should be used if the container material is thermostable enough.
7. After these steps, they can be used in different procedures.
8. After usage in different procedures, they have to be cleaned suitably.
9. Then they have to be kept in cup board and cleaned as above before using them again.

Conclusion

Different glassware is widely used in a lab. Though plastic ware has some disadvantages, it is also coming into usage due to non-breakability& low expensiveness.

Summary

Different glass and plastic apparatus are used in a laboratory for various purposes as measuring, transfer, filtration, storage etc. Glass used for lab purpose must be resistant to the action of chemicals. They must with stand mechanical rigors during handling, effect of high temperatures and sudden changes in temperature. Borosilicate glass is of choice in lab ware.

Laboratory glass ware can be classified in to 1) Graduated glassware 2) General glassware. Graduated glassware include flasks, pipettes, burettes, etc. General glassware include test tubes, syringes, funnels, etc.

Glassware and plastic ware used in lab must be thoroughly cleaned and dried before use. Several cleaning agents are available. Different steps in cleaning are 1) soaking 2) Brushing 3)Rinsing 4) draining 5) Drying. Lab ware is to be maintained and stored properly.

MODEL QUESTIONS:

Short Answer Type Questions:

1. Write the composition of glass.
2. Mention types of glass.
3. Give the composition of borosilicate glass.
4. What is sulfur treatment?
5. Mention the types of lab glassware.
6. Mention the types of graduated apparatus on the basis of accuracy.
7. What are the types of graduated flasks?
8. Give the uses of a) volumetric pipettes b) pipettes.
9. What are different types of graduated pipettes?
10. What are serological pipettes?
11. Mention different types of blood pipettes.
12. What are weight burettes?
13. Write the uses of a beaker.
14. What is pour point?
15. Write the uses if conical flasks.
16. Differentiate between round bottomed flask and flat bottomed flask.
17. Give the uses of funnel.
18. What is a condenser?

19. Mention the use of a dessicators.
20. Mention the different types of bottles.
21. What is a weighing bottle?
22. What is a weighing funnel?

Long answer Questions:

1. Give the composition of glass. Write about types of glass.
2. What are the types of glassware? Write about pipettes.
3. Mention the types of graduated apparatus. Write about graduated flasks.
4. Name different general apparatus used in the lab. Write about a) Flasks b) separating funnels.
5. What are the aims of cleaning? Write the steps involved in cleaning.
6. Write about plastic ware.

Chapter 4

Basic lab operations

Structure

4.1 Centrifuges: Separation of solids from liquids

4.2 Weighing: Types of balances used, care and maintenance

4.3 Evaporation

4.4 Distillation

Learning objectives

1. Student should know the basic lab operations like centrifugation, filtration, evaporation, distillation and refluxing to become a good technicians.
2. Student should become proficient in these operations in the process of acquiring skills.

Introduction

A lab technician should have a knowledge in basic lab operations like Separation of solids from liquids by centrifugation, filtration using funnels, weighing, evaporation and distillation.

4.1 Centrifugation

Centrifuges are used in a lab to separate solids from liquids in which solids are insoluble in liquids. It is also used in the separation of immiscible liquids. Centrifuges are designed to accelerate the process of sedimentation by centrifugal force. In a biochemical laboratory, they are useful in separation of cells from plasma, precipitate from liquid, etc.

Principle:centrifuges work on the principle of centrifugal force to separate insoluble solid from liquid. Centrifugal force needed to affect the separation process is provided by mechanical energy.

Types of centrifuges:

1. Hand centrifuge
2. Electrical centrifuge
3. High speed centrifuge
4. Ultra- centrifuge

5. Refrigerated centrifuge

Construction of Electrical centrifuge: Electrical centrifuge is a device operated by an electric motor which can give up to 5,000 RPM. Two types of centrifuges are used in laboratories. They are:

a) Swing out head type.
b) Angle head type.

Parts of electrical centrifuge:

1. Rotor with tubes
2. Motor
3. Chamber to enclose the internal parts
4. Lid on the top to close chamber during centrifugation
5. A multistage speed regulator to obtain desired speed
6. Timer
7. Tachometer to read the speed
8. Graphite brushes to provide electrical contact to the rotor
9. Power switch.

Fig 4.1 centrifuge

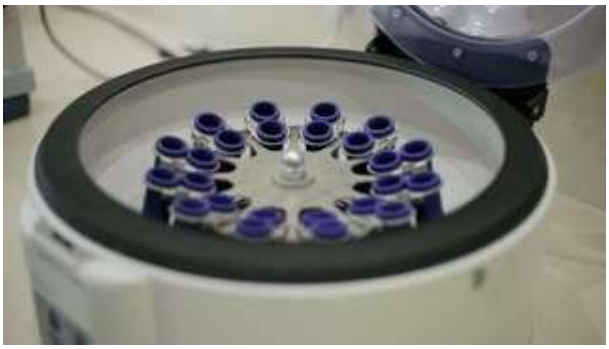


Fig 4.2 Rotor of centrifuge

Care and maintenance of electrical Centrifuge:

1. Place the centrifuge on a firm base.
2. Balance the tubes properly, otherwise tubes may be broken.
3. Increase or decrease the speed gradually.
4. Keep the lid closed during centrifugation.
5. Do not open the lid until the rotor comes to a stop.
6. Keep the chamber clean.
7. Cover the centrifuge with a cover, when not in use.
8. The graphite piece should be replaced by a new one, if it wears out.
9. Check the electrical connections at regular intervals of time.
10. Lubricate the moving parts at regular intervals of time.

Applications

It is useful in

1.Separation of insoluble solid from a liquid.

2.Separation of immiscible liquids.

In a medical laboratory, it has different applications. Some of them are-

1 .Separation of serum from clotted blood.

2 .Separation of plasma from cells.

3 .Separation of urinary sediment for predation for microscopic examination.

4 .Separation of precipitate or supernatant as required.

4.2 Weighing

Weighing is an important activity in a laboratory. Weighing of chemicals is required for preparation of reagents, standard solutions etc. Weighing for qualitative analysis can be approximate. Weighing requires balances. Accurate weighing can be done with analytical balances. Balance is one of the most important tools of an analytical chemist.

Different types of balances:

1. Simple balance.
2. Two knife single pan balance.
3. Top loading balance.
4. Electronic balance.
5. Miscellaneous:
   a) Torsion suspension balance
   b) Electro balance
   c) Torsion balance

Classification of balances according to capacity:

1 .Analytical balances:

Capacity: 150 to 200g. Accuracy upto 0.1 mg.

2 .Semimicro balances:

Capacity: 75 to 100g. Accuracy upto 0.01 mg.

3 .Micro balances:

Capacity: 10 to 30g. Accuracy upto 0,001 mg.

Simple Balance

It is also called as analytical balance, analytical 2 pan balance, chemical 2 pan balance.

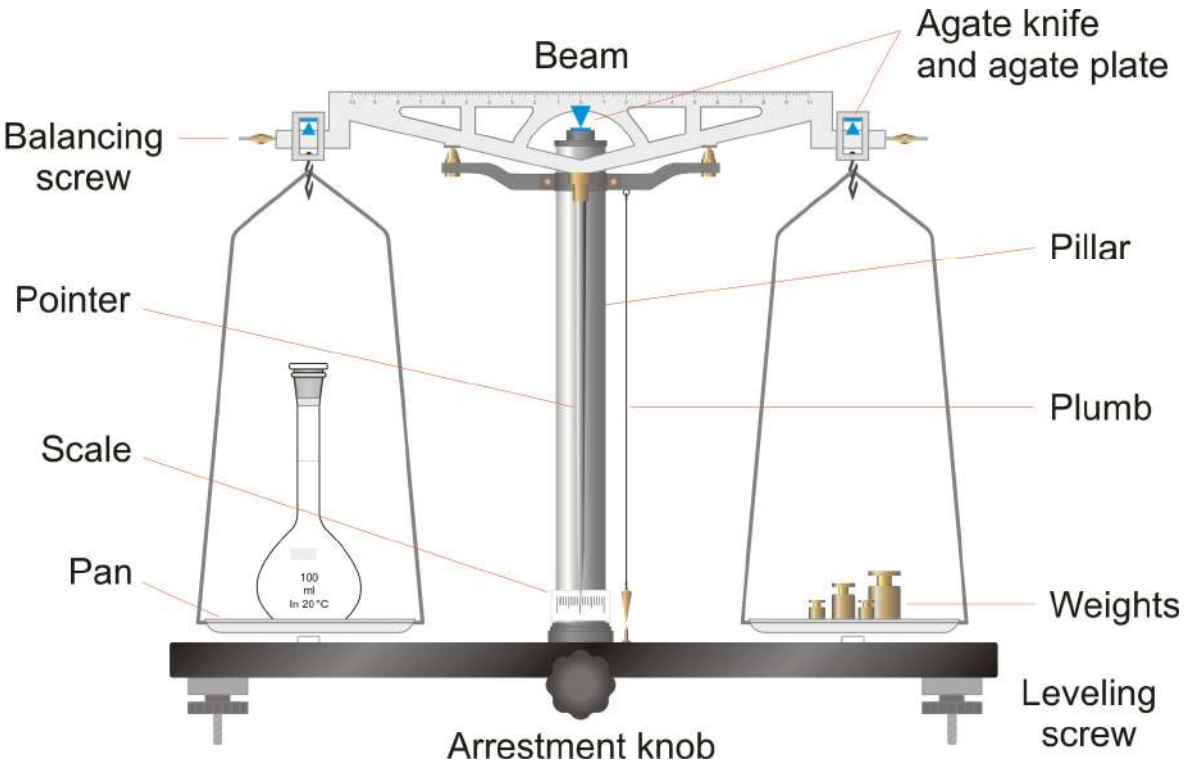


Fig 4.3 Simple balance

Components:

1. A beam
2. Knife edges and screw nuts
3. Stirrups
4. A pointer
5. Ivory scale
6. Rigid supports
7. Wooden platform with levelling screws
8. Handle
9. Central vertical pillar.

Operation:

1. Cleanthe pans of balance with camel brush.
2. Check the balance for equal sliding of the pointer on both sides by operating the handle lever.
3. Arrest the movement of the beam by operating the handle lever.
4. Place the sample to be weighed on the left pan and weights on the right pan.
5. Set the balance to move and check for equal movement of pointer on both sides of ivory scale. Arrest the movements of beam every time, a weight is removed or added to the pan.
6. Move the rider along the scale for adjustment of weight less than 10 mg.
7. When weighing is completed, arrest the beam and record the weight.
8. Clean any accidental spillage and close the balance case.

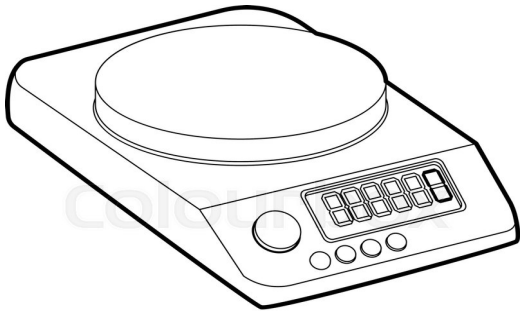
Care and maintenance:

1. The beam of the balance should be arrested when not in use, and also before adding or removing weights.
2. When not in use, the balance beam should be raised in order to protect the knife edges.
3. Doors of the balance must be closed whenever possible.
4. Pans should be maintained clean and dry.
5. The substance should not be weighed hot.
6. To release the balance, beam should be lowered gently.
7. Weights are to be kept in the pan with the help of a forceps.
8. The balance should be loaded with a weight greater than the maximum it is constructed to weigh.
9. Put a plastic cover over the wooden frame when not in use.

Uses: it is used to find out the mass of a substance in quantitative analysis.

Electronic balance

Electronic balance is modification of two knife single pan balance. In this type of balance, optical readout system is replaced by electrically operated measuring system.



balance

Fig 4.4 Electronic balance.

Components of electronic balance:

1. A null detector
2. Electromagnet
3. A read out device.

Operation:

1. Turn on its power supply.
2. Check digital display for zero reading.
3. If the reading is not zero, set it to zero.
4. Keep the substance to be weighed carefully on the balance.
5. Note the reading on the digital display.
6. If the substance being weighed is taken in weighing pan or watch glass, weight of empty pan or watch glass has to be substracted from total weight of substance along with pan or watch glass.

Uses: It is used for accurate weighing in quantitative determinations.

4.3 Evaporation

- Evaporation is the process of conversion of liquid into vapour at temperatures below boiling points.
- This technique can be used for purification of substances. Substances to be purified can be dissolved in a suitable solvent. Solvent on evaporation removes some of the impurities.
- Evaporation is allowed to take place by taking the solution in porcelain dish.

- Solvent on evaporation leaves purified substance in the dish.
- This process can be used for obtaining purest substances in volumetric analysis , recrystallization, etc.

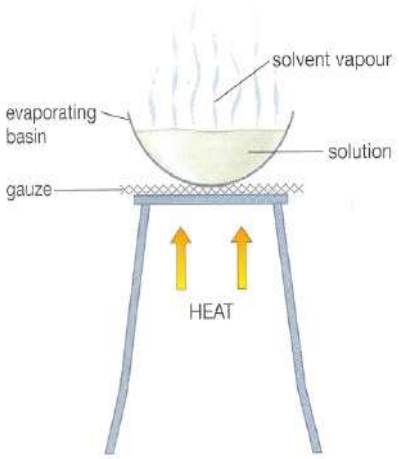


Fig 4.5 Process of evaporation

4.4 Distillation:

- Distillation is the process where in liquid is boiled and its vapors are condensed to collect the liquid. By this process, liquid is cleared off from the soluble impurities.
- Distillation is used in labs for the preparation of distilled water. It can be done in a using any of the following methods. a) Glass assembled apparatus.

b)All glass distillation apparatus

c)Stainless steel distillation apparatus.

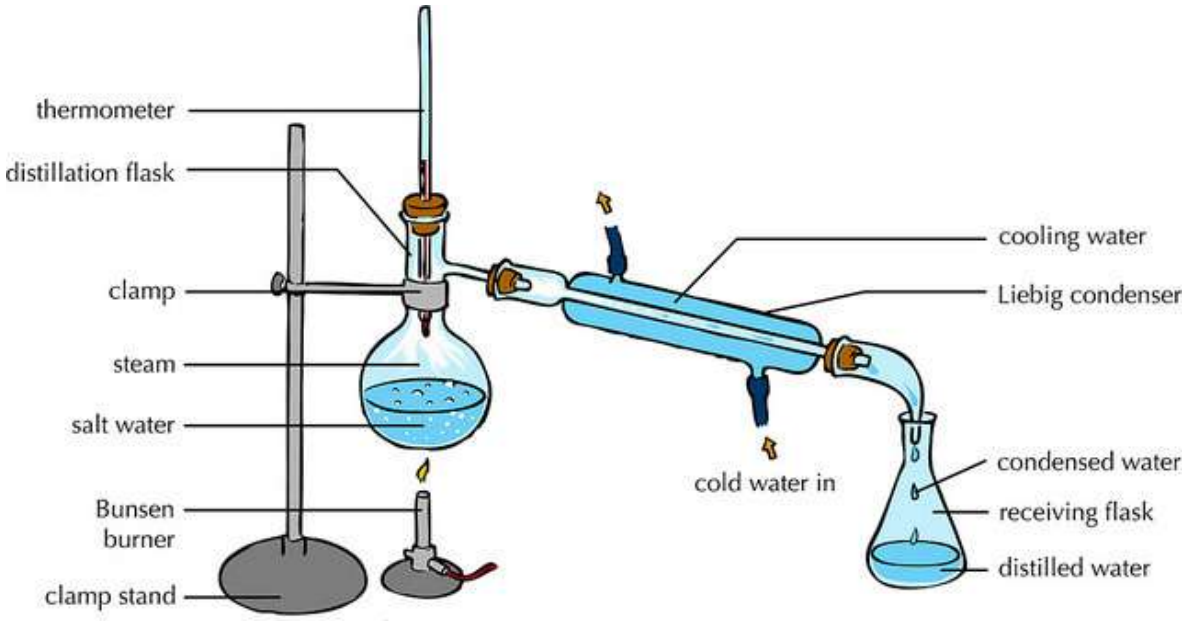


Fig 4.6 Distillation using glass assembled apparatus

Fig 4.7 All glass distillation apparatus

Process of distillation consists of:

1. Boiling of liquid forms vapors.
2. Condensation of the vapors in the condenser tube due to continuous cooling provided by continuous circulation of water.

3. Collecting the distillate. Distillate is the liquid formed by condensation of vapors of the boiled liquid.

Conclusion

A lab technician should have the basic knowledge of lab instruments such as centrifuge and weighing balance, their operation, care and maintenance.

Summary

Centrifugation, weighing, evaporation and distillation are some of the lab operations, a technician has to be perfect with. Centrifugation requires centrifuges, weighing is accomplished using different types of balances.

Evaporation helps purification. Distillation is useful for preparation of distilled water and purification. It requires distillation apparatus.

Model questions

Short Answer Type questions

1. Write the principle of centrifuge.
2. Mention different types of centrifuges.
3. What are the applications of centrifuges in a medical laboratory?
4. Define a) evaporation b) Distillation.
5. Name different types of weighing balances.

Long Answer Type Questions

1. What are the different types of centrifuges? Write about electrical centrifuge.
2. Write about simple balance and electronic balance.
3. Explain in detail about Evaporation and Distillation.

Chapter –5

Instrumental methods of Bio-chemical Analysis

Structure

5.1 Colorimeter

5.2 Spectrophotometry

Learning objective

1. Student should practice to operate the instrument
2. Student should know the application of these instruments.

Introduction

A lab technician should be well verse in basic lab operations like colorimeter and spectrophotometer, their working principal, care and maintenance.

5.1 Colorimetry

Colorimetryis the method of analysis of determination of concentrations by visual comparison of color intensities or by measurement of relative adsorption.

Colorimetersare the instruments used in colorimetric analysis for determination of concentrations by visual comparison of color intensities or by measurement of relative absorption.

Essential components of colorimeter:

1. Light source.
2. Wave length selector.
3. Filters.
4. Cuvette holders.
5. Photo cell or detectors.
6. Galvanometer.

There are two types of colorimetry. They are:

1. Visual colorimetry
2. Photo electric colorimetry

1. **Visual colorimetry**: visual colorimetry is the method of colorimetric analysis in which natural or artificial white light is generally used as light source and eye is used for color comparision.

Instruments used in Visual Colorimetry

Instruments used in visual colorimetry are called as comparators or colorimeter.

Name of the visual method	instrument/ apparatus used
1. Standard series method	1. Modified test tube rack 2 BDH Lovi bond Nessleriser mark 3 3 Lovibond 1000 comparator & Nessler tube.
2. Duplication method	Nessler tubes
3. Dilution method	Nessler tube
4. Balancing method	1 Hehner cylinders 2 Duboscq colorimeter.

Principles and Laws:

- When monochromatic light passes through a solution, some part of the light is absorbed by the solution and intensity of emitted light will be less than intensity of incident light.
- Laws explaining colorimetry are:

a) **Lamberts Law:** It was originally developed by Bouger and extended by Lambert. Hence called Lamberts-Bouger's law. It states that, when monochromatic light passes through a transparent medium, intensity of emitted light decreases exponentially as the thickness of the medium increases arithmetically.

It is expressed by,

$$le/lo = e^{-kt}$$

where, le= intensity of emitted light.

lo= intensity of incident light.

e =exponential.

K =constant

t= thickness of absorbing medium (length of the light path through the medium)

b) **Beer's Law**: Beer's law states that, when monochromatic light passes through a transparent medium, intensity of emitted light decreases exponentially as the concentration of the colored component in the medium increases arithmetically.

It is expressed by

$$\mathbf{le / lo = e^{-kct}}$$

or $\mathbf{\log\ lo / le = Kct}$

where, le = intensity of emitted light,

lo = intensity of incident light,

e = exponential

K = constant

c = concentration and

t = thickness (length of the light path through the absorbing medium)

2. **Photo electric colorimetry:** Photo electric colorimetry is the method of colorimetric analysis in which light of narrow wavelength within visible range is used and eye is replaced by photocell for comparison of color intensities. This is the advantage in photo electric colorimetry where color matching is done by photo cell and not by eye visibility which is more accurate.

Photo electric colorimeter uses light of wave length in the visible range i.e. 380-760 millimicrons only approximately as incident light.

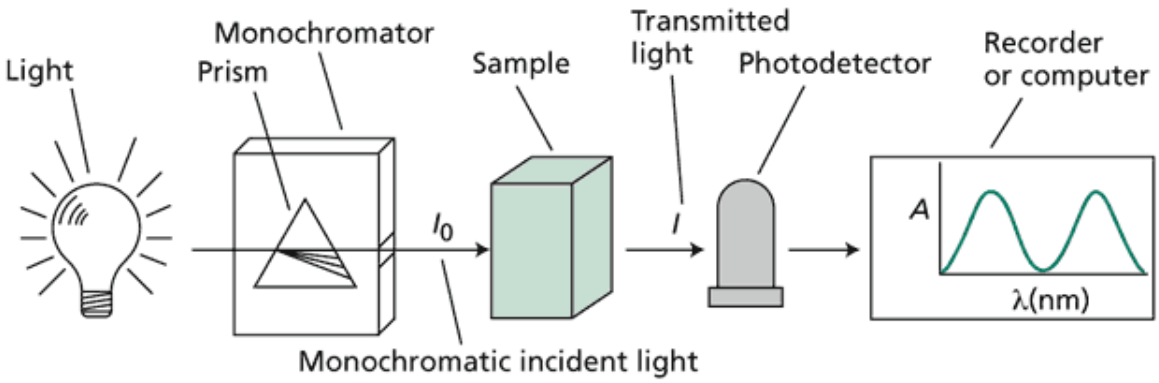


Fig: Components of photo electric colorimeter.

Principle and laws:

- When monochromatic light passes through a solution, some part of light is absorbed by the solution and the intensity of emitted light will be less than the intensity of incident light.
- This is in accordance with Beer-Lambert's law which states that, the intensity of emitted light decreases exponentially as-

a. The thickness of the absorbing medium increases and
b. Concentration of the colored component in the medium increases arithmetically.

Operation (Single cell photometer)

1. Take similarly treated test, standard and blank solutions.
2. Switch on the power supply to the colorimeter and the light source.
3. Set the colorimeter to zero optical density or 100% transmittance using blank at wavelength specified to determination.
4. Determine the optical densities of similarly treated test and standard solutions.
5. Calculate the concentration of unknown by using the formula-

$$\text{Concentration of unknown sample} = \frac{\text{Optical density of test}}{\text{Optical density of standard}} \times \text{concentration of standard.}$$

6. Set the colorimeter to zero optical density with distilled water and switch off the power supply.

Applications:

Photoelectric colorimeter is the most important tool in a biochemical laboratory.

1. It is useful in determination of components of biological fluids/serum/plasma, urine, CSF etc.
   EX: Blood sugar, serum cholesterol, serum uric acid, etc.
2. It is useful in pharmaceutical analysis, research and clinical studies.
3. Photometric titrations have been useful in locating end point in a titration. These titrations are called as photometric titrations, and have been used in several reactions.
   Ex: Amino acids, potassium permanganate, iodine, etc.

Care and maintenance:

1. Cover the photo electric colorimeter with a plastic cover, when not in use.
2. Keep the power supply and light source switched off, when not in use.
3. Check the sensitivity of galvanometer occasionally using a standard dichromate solution.
4. Proper filter and cuvette filled with distilled water is placed in their positions, before starting the colorimeter.
5. Do not keep the instrument near to a vibrating instrument and heating apparatus.

6.2 Spectrophotometry

Spectrophotometry is method of quantitative determination of concentration in ultra violet range (10-400nm), visible range (400-800 nm) and infrared range (0.8-1,000 microns) of electromagnetic spectrum.

Spectrophotometers are the instrumentsused in spectrophotometry. They are more precise than photo electric colorimeter.

Spectrophotometer differs from Colorimeter in thatit uses prism or diffraction gratings as monochromators. These separate the various wave lengths of radiant energy.

Principle and law:

- When monochromatic light passes through the solution, some part of light is absorbed by the solution and the emitted light is measured. Measurement of

absorptivity of the radiation by the components is to be determined in spectrophotometry.

- Beer – Lambert's law is followed in spectrophotometry.

Parts of spectrophotometer:

1. Light source.
2. Monochromators and slits.
3. Associated optics.
4. Cuvettes / sample holders.
5. Photocells.
6. Galvanometer.

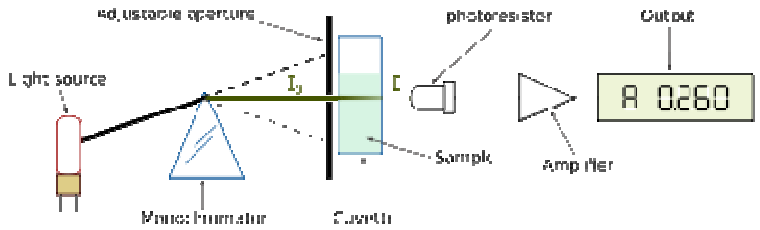


Fig: Components of a Spectrophotometer.

Types of spectrophotometers:

They can be divided into two groups-

1. Manual spectrophotometer.
2. Recording spectrophotometer.

Types of spectrophotometers based on wavelength of light-

1. Ultra violet spectrophotometer.
2. Visible spectrophotometer.
3. Infrared spectrophotometer.

Types of spectrophotometers on the basis of number of optical paths-

1. Single beam spectrophotometers consisting of one optical path.
2. Double beam spectrophotometers consisting of two optical paths.

Fig: Image of a spectrophotometer.

Operation

- Manual spectrophotometers are provided with different controls.
  They are wave length controls, slit width adjustment, dark current control, sensitivity control, readout, etc.
- Recording spectrophotometers also have switch control, scanning speed control, recording control.
- Wave length required for the estimation being done.
- Dark current control is for adjustment of zero transmittance.
- Sensitivity control is for adjustment of 100% transmittance.
- Balancing potentiometer is with a calibration scale forms read out.
- A spectrophotometer is operated using these contols.

Applications:

1. Visible spectrophotometry has wide range of applications. Ex: determination of salicylic acid, urea, glycine, etc.
2. UV spectrophotometry finds application in identification of Hydrocarbons, vitamins, steroids, hetero-cyclins and conjugated aliphatics. UV spectrophotometry is also used for identification of degradation products and for testing the purity in biological and pharmaceutical research.
3. Steroids, enzymes and many other substances can be determined by UV spectrophotometer.
4. Vitamin A can be assayed by measuring the absorbance at 334 millimicrons.

5. UV and visible spectrometry prove useful in elucidating of structures of organic compounds.
6. Spectrophotometry is useful in deciding constitution of compounds.
7. H^+ion concentration can be determined by using spectrophotometry by using suitable indicator.
8. UV spectrophotometry is used in determination of inorganic substances such as Lead in bone ash, Mg, As, Br, Cl_2, etc.

Maintenance and care:

1. Cover the spectrometer with a plastic cover, when not in use.
2. Keep the power supply and light sources switches off when not in use.
3. Put the proper filter and cuvette filled with distilled water in their positions before putting on the spectrophotometer.
4. Check the sensitivity of galvanometer occasionally using a standard dichromatic solution.
5. Maintain the instrument clean.
6. Do not keep the instrument nearer to vibrating instruments and heating apparatus.

Conclusion

Colorimetry is a method of quantitative analysis based on absorption of light transmitted through the solution of a substance in visible range.

Spectrophotometry is used to measure the intensity of light, or the wavelength that the specimen absorbs.

Summary

Colorimetry and spectrophotometry are important methods in instrumental analysis in any biochemical work.

Colorimeters use two types-a) visual colorimeter b) Photo electric colorimeter. Colorimetry Spectrophotometry is based on Beer-Lambert's law. Spectrophotometry is more accurate than colorimetry. It can be used in UV, visible, and infrared range.

Model questions:

Short Answer Type Questions

1. Define colorimetry.
2. Mention the types of colorimeters.
3. What is the difference between visual and photoelectric colorimetry?
4. Give Beer-Lamberts law.
5. Write any two applications of photoelectric colorimeter.
6. Write wavelength range of UV, visible and infrared rays.
7. Mention the difference between colorimeter and spectrophotometer.
8. Mention the types of spectrophotometers based on wavelength.

Long Answer Type Questions

1. Classify colorimetric methods and explain them.
2. Explain Beer-Lamberts law in detail.
3. Give the principle, construction, operation, care, maintenance and application of photoelectric colorimeter.
4. Give the principle, parts, operation, application, care and maintenance of spectrophotometer.

Chapter -6

Water, Chemicals and Related Substances.

Structure

6.1 Water

6.2 Purity of Chemicals

6.3 Corrosives

6.4 Hygroscopic Substances.

Learning Objectives

1. Student should have the knowledge of chemicals, their handling and storage aspects.
2. Properties of chemicals also should be known to the student for personal safety as well as fellow technicians safety.

6.1 Water

Formula: H_2o

Molecular weight: 18.02

Water is the most important constituent of all forms of life. It constitutes 70-90% of living forms. It is transparent bland liquid. Water is highly reactive. It has high melting and boiling point, heat of vaporization, heat of fusion and surface tension. The preparation of most reagents and solutions used in clinical laboratory requires pure water.

Purified water for laboratory use is prepared by any of the following process:

1. **Distillation**: Water purified by distillation is called distilled water. It is usually prepared by employing electrically heated glass distillation unit or stainless steel distillation unit.
2. **Ion-exchange:** Water obtained by percolation through Ion- exchange resins is called De- ionized water. Strong acid resins will remove anions from water. Commercially available units are Permutit, Elgastat, etc.
3. **Reverse osmosis:** In reverse osmosis, an aqueous solution is separated from pure water by semi-permeable membrane and sufficient pressure is applied to solution. This causes water to flow from aqueous solution in to pure water instead of pure

water in to aqueous solution. This principle of reverse osmosis is adopted in Milli-Q3 system of Millipore Corporation.

Fig 6.1 Distilled Water can.

Standards laid down for purified water: Indian pharmacopoeia prescribes limits for different chemicals and water. The pH of water is 4.5 to 7.0.

6.2 Purity of chemicals: Substances used in the analysis for diagnosis must be pure so as to get results with accuracy. Purity means state of not containing any impurities.

Grading of Reagents*:* Reagents supplied by the manufacturers are graded as per purity into-

1. **Analytical reagent quality (AR) or Anala** "R", a pure quality.
2. **Guaranteed reagent quality (GR),** a pure quality.
3. **General purpose reagent quality (GPR),** not that much pure as AR and GR.
4. **Laboratory reagent quality (LR).** This quality is used for preparing qualitative reagents such as Fehiling's solution, Seliwanoff's reagent, etc.
5. **A.C.S** reagents are the meeting the specifications of American chemical society.
6. **Primary standards,** are the analytical reagents of exceptional purity used for volumetric analysis.
7. **Reagents** are commercially available chemicals of highest purity for which American chemical society has not yet specified any requirement.
8. **Organic reagents (O.R),** are the reagents suitable for research application
9. **Purified chemicals**, are good quality chemicals where there are no official standards specified.

10. **U.S.P, are** Chemicals manufactured under current Good manufacturing practices (G.M.P), and are denoted as U.S.P.
11. **B.P,** are chemicals meeting the specifications of British pharmacopoeia denoted as B.P.
12. **I.P**, are chemicals meeting the specifications of Indian pharmacopoeia denoted as I.P.
13. **N.F**, are chemicals meeting the requirements of National Formulary denoted as N.F.
14. **C.P**, are chemically pure chemicals for general application denoted as C.P.

Sources of Impurities: Types and amount of impurities present in the chemicals depends upon several factors. Some of them are –

1. Raw materials employed in the manufacture
2. Method or process used in the manufacture
3. Chemical processes employed in the processes
4. Plant materials employed in the process
5. Container materials used for storage of final products
6. Decomposition during keeping
7. Adulteration.

Effects of impurities:

1. Impurities bring about changes in physical properties of substances like color, odour, taste, etc.
2. They may bring about chemical changes rendering the chemical reagent useless.
3. They act as interfering substances affecting the accuracy of the result.
4. Impurities may affect active strength of the substance influencing the result.

Test of purity: Tests for purity are tests for detecting impurities in the substances. Governing factor for these tests is to determine how much impurity is likely to bring about technical and other difficulties, when the substance is used in analysis.

6.3 Corrosives

Corrosive chemicals are those substances which can cause destruction of living tissues. Different chemicals of corrosive nature have to be handled by laboratory technicians. While handling these substances, they can cause corrosion, if they fall on any part of the body. A

technician should be well aware with measures of preventing accidents, first aid measures, precautions to be followed while handling with acids and alkalies and their storage aspects.

Accidents may happen by corrosives may be due to:

1. Spilling of splashes on the skin during handling.
2. Spilling of splashes in to eyes.
3. Swallowing on to mouth during pipetting.

Fig 6.2 Symbol showing corrosion.

Classification of corrosive substances: Corrosive substances can be classified onto categories on the basis of corrosiveness, they can cause. They are-

1. Substances with high degree of corrosiveness,
   (a) **Stong acids**: Stong acids are the substances which are ionised to the extent of 100%. Examples are Hydrochloric acid, Sulphuric acid, Nitric acid, etc.
   (b) **Strong alkalies:** Strong alkalies are those, which are ionised to the extent of 100%. Examples are Sodium hydroxide, Potassium hydroxide, Barium hydroxide, etc.
2. Substances with lesser degree of corrosiveness,
   (a) **Weak acids**: weak acids are those, which are ionised to the extent of less than 100%. Examples are Glacial acetic acid, Phosphoric acid, Nitrous acid, etc.
   (b) **Weak alkalies:** Weak alkalies are those, which are ionised to the extent of less than 100%. Examples are Ammonium hydroxide, zinc hydroxide, etc.
   (c) **Phenols and Cresols:** Phenols are aromatic hydroxy compounds in which OH group is directly attached to the Benzene ring. Examples are Phenols, Nitro phenol, Picric acid, etc.

Properties of some important chemicals:

1. **Hydro chloric acid**: (HCl):It occurs as colorless fuming liquid with pungent odour. It is miscible with water and alcohol. It is strong acid and attacks metals. It is to be handled carefully because it is corrosive to body parts like eyes, skin, mouth, lips and tongue. It is stored in well closed container.
2. **Sulfuric acid:(H_2SO_4)** Sulfuric acid is a colorless fuming acid. It has oily consistency. It is miscible with water. It is a strong acid and miscible with water. It is safe handled to avoid corrosion to the body parts like skin, eyes, mouth, etc. It is well closed container.
3. **Sodium hydroxide: (NaOH):** It is also called as caustic soda. It is available as pellets, flakes or sticks. It is extremely hygroscopic. It absorbs atmospheric carbon dioxide and partially converted to Sodium carbonate. It is freely soluble in water, alcohol and Glycerine. Heat is produced during dissolution. It is to be stored in air tight container.
4. **Potassium hydroxide: (KOH):** It is available as dry hard, brittle, white flakes, sticks or fused masses. It is highly hygroscopic and partially. Converts to potassium carbonate by absorbing atmospheric carbon dioxide. Since it is concentrated alkali, avoid spilling on skin, into eyes and swallowing in to mouth.
5. **Acetic acid: (CH3COOH):** It is also called glacial acetic acid. It is commercially available in strength of 45N. It is a colorless liquid soluble in water. It has oily consistency. It is stored in well closed container.
6. **Phenols and cresols:**
   (a) **Phenols:** phenols are colorless crystalline solids or liquids with characteristic odour. They darken in air due to oxidation. They are sparingly soluble in water. They dissolve readily in alcohol and ether. Phenols are weak acids. Ex: Hydroxy benzene(carbolic acid). They are hygroscopic. They produce blisters on skin.

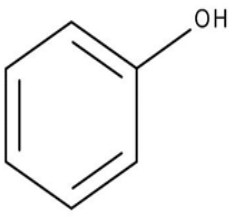
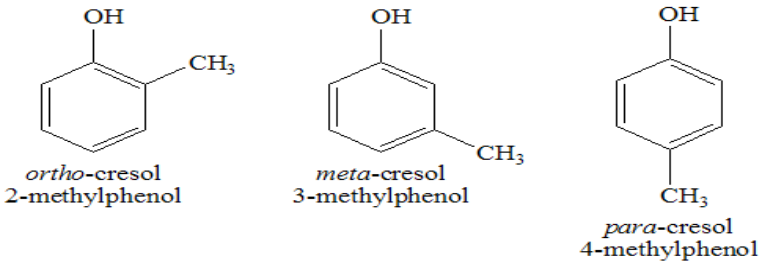


Fig **6.3 Hydroxyl benzene or phenol**

(b) **Cresols:** There are three forms of cresols. They are-Ortho "o", meta "m", and para"p" forms. They have similar properties as phenols. They are less toxic than phenols.

Fig 6.4**Structure of Ortho,meta and para forms of cresol.**



6.4 Hygroscopic Substances: Hygroscopy is the ability of a substance to attract and hold water molecules from the surrounding environment. This is achieved through either absorption or adsorption and the substance becomes physically changed by an increase in volume, stickiness or change of other physical properties. Water molecules gets suspended between the material molecules.

Ex: Aluminium Chloride, Conc. Sulfuric acid, Calcium oxide, Magnesium tri silicate, etc.

Deliquescent substances: These are the substances which absorb moisture from atmosphere and become aqueous solutions. The property of dissolving in water, absorbed from surrounding atmosphere is called deliquescence.

Ex: Sodium hydroxide, Calcium chloride, Potassium hydroxide, etc.

Both hygroscopic substances and deliquescent substances possess one property in common-absorption of water from the atmosphere.

Storage of hygroscopic substances: They are to be stored in sealed containers due to their affinity for atmospheric moisture.

Properties of some hygroscopic substance:

a) **Aluminium Chloride**: It is a hygroscopic salt. It is white in color, but turns to yellow color due to contamination with Iron chloride. It is prepared from Alumina. Alumina is mixed with charcoal and heated in atmosphere of dry chlorine gas to get anhydrous aluminium chloride. It is soluble in organic solvents like alcohol, ether, benzene, etc.

b) **Magnesium Tri Silicate**: It is slightly hygroscopic substance. It is white fine powder used as food additives. It is used in foods to absorb fats. It is odour less, taste less and is insoluble in water and alcohol. It is prepared by running a solution of sodium silicate into equimolar solution of magnesium sulfate or magnesium chloride. It is used as antacid in treatment of peptic ulcers.

Conclusion:

Purified water is used for quantitative determinations. Pure chemicals should be used for accuracy in results.

Summary:

Water is the most important constituents of all forms of life. Water is a good solvent. Purified water is used in laboratories. It is prepared by distillation, ion exchange, reverse osmosis, etc.

Chemical substances used in diagnostics should be pure to get results with accuracy. Reagents are graded into Anala R quality, guaranteed reagent quality GR, General purpose reagent quality GPR, laboratory reagent quality LR, quality according to purity. Qualitative work purest possible reagent i.e. AR. For qualitative work, laboratory reagent quality is enough.

Purity means freedom from impurities. Sources of impurities are raw material employed in the manufacture of reagents, method or process used in manufacture, chemical processes employed, plant materials used for storage, decomposition, adulteration, etc.

Corrosives are the substances which can cause destruction of living tissues. Strong acids, strong alkalies, weak acids, weak alkalies, phenols and cresols can cause corrosion in varying degrees. Hygroscopic substances are those which absorb moisture from atmosphere. Ex: sodium hydroxide, potassium hydroxide etc.

MODEL QUESTIONS.

Short Answer Type Questions

1. What water is used in laboratory?
2. How is purified water prepared?
3. What is ion-exchange?
4. Define reverse osmosis.

5. Define a) strong acids b) Weak acids.
6. Define a) strong alkalies b) weak alkalies.
7. Write the properties of HCl.
8. Write the properties of Acetic acid.
9. What is a hygroscopic substance?
10. Differentiate hygroscopic substance and deliquescent substance.
11. Give some examples of hygroscopic substances.
12. What are corrosives?
13. Write two effects of impurities.
14. What is purity of chemicals?

Long Answer Type Questions

1. Write about preparation and use of water on laboratory.
2. What are the sources of impurities? Discuss.
3. Explain in detail about Corrosives.
4. Give an account of the purity of chemicals.
5. Write in detail about strong acids and strong alkalies.
6. Discuss about hygroscopic substances.

------s

Chapter-7

Collection of Specimens

Structure

7.1 Blood

7.2 Urine

Learning objectives

1. Student should be able to collect different blood samples.
2. Student should also know about different urine specimens.
3. Student should also be knowing about the preservation methods of the specimen.

7.1 Blood (phlebotomy)

Phlebotomy is the surgical opening of a vein in order to withdraw blood. Specimens of blood are required to be collected for different diagnostic tests like hematological, biochemical, microbiological, serological aspects of diagnosis.

Types of blood Specimens

a) ***Random blood specimen*** is one which is collected at any time without any specific instructions to be followed by the patient before the collection.
b) ***Fasting specimen*** is one which is collected in morning after a night's fasting (12-16hours)
c) ***Post prandial specimen***is specimen collected 2 hours after taking lunch.

Forms of blood used in diagnostic tests

Blood is used for diagnostic testing in the form of-

1. ***Whole blood:*** It is used in blood cell count, differential counts, hemoglobin estimation, pH, lead, etc.
2. ***Serum:***It is used in determination of Amino acids, Free and esterified cholesterol, creatinine, copper, iron, enzymes, etc.
3. ***Plasma***: It is used in determination of Fibrinogen, Ascorbic acid, bicarbonates, chloride, etc.

4. ***Cellular parts:*** It is used in determination of Glucose-6PD, abnormal hemoglobin, etc.

Types of blood collection

Types of blood specimens based on routes of blood collection-

1. **Capillary blood**: Blood collected from capillaries**.**
2. **Venous blood:** Blood collected from veins.
3. **Arterial blood**: blood collected from arteries.

1) Collection of capillary Blood Specimen: Capillary blood is used when small volume of blood is needed.

Uses: capillary blood is used in blood cell counting procedures, hemoglobin estimation, blood grouping, bleeding time, clotting time, etc.

Quantity of blood collected: Volume up to 0.2 ml. can be collected. A skilled technician can obtained up to 1 ml. Amount up to 3 ml. can be collected by repeated expressions.

Sites of selection for capillary blood collection:

1. Tip of finger
2. Thumb
3. Lobe of an ear or great toe
4. Heel for infants.

Requirements:

1. Surgical needle or disposable hypodermic sterile needle or lancet.
2. Cotton
3. Spirit or povidone iodine
4. Thin tourniquet
5. Blood pipettes or slides or small tubes.

Fig 7.1: Blood lancets.

Fig 7.2: capillary blood collection.

Procedure

1. Rub the part with 70% alcohol or spirit.
2. Make a quick and good stab by using a disposable sterile lancet.
3. The cut should deep enough so that blood flows freely without squeezing the stabbed site.
4. Wrap a thin tourniquet at the base of the finger, if felt necessary.
5. By using a cotton the first drop should be wiped away and a separate fresh drop is used for the test.

Precautions

1. A free flow of blood is essential and blood collected by squeezing may lead to errors.
2. Pipettes used should be clean and dry since they ensure filling with ease.

1) Collection of venous blood specimen: When specimen of blood in more volumes than capillary blood is required, venous blood is preferred.

Uses: venous blood is collected for determinations like E.S.R, blood sugar, serum cholesterol, blood urea, etc.

Quantity of blood collected by venous route: Up to 10 ml. of blood is collected by veins. If a number of determinations are to be done, up to 20 ml. of blood can be collected.

Sites of selection for venous blood collection

1. Vein on hand or wrist
2. Antecubital area
3. Vein on the ankle
4. Vein foot.

Requirements

1. Rubber tourniquet or cuff of a sphygmomanometer.
2. Sterile syringe with a disposable needle
3. Spirit or ether
4. Cotton
5. Tubes for transfer the blood.

Procedure

1. Extend the arm of the patient.
2. Apply a tourniquet firmly a few inches above the elbow.
3. Clean the site of collection with cotton soaked in spirit and allow to evaporate.
4. Taking the arm of the patient in to grip with one hand and holding the skin at the site of collection with the thumb of another hand, penetrate the needle into vein by positioning the needle at 30^0 and 40^0 angle.
5. When the needle enters into the vein, withdraw the plunger slightly.
6. Release the tourniquet when blood appears in the barrel. This minimizes congestion.

7. Place a pad of cotton wool soaked on spirit on the site of needle in to the vein after drawing the required amount of blood.
8. Hold firmly for few minutes until blood stops.
9. Remove the needle from the syringe and transfer the collected blood into appropriate container.

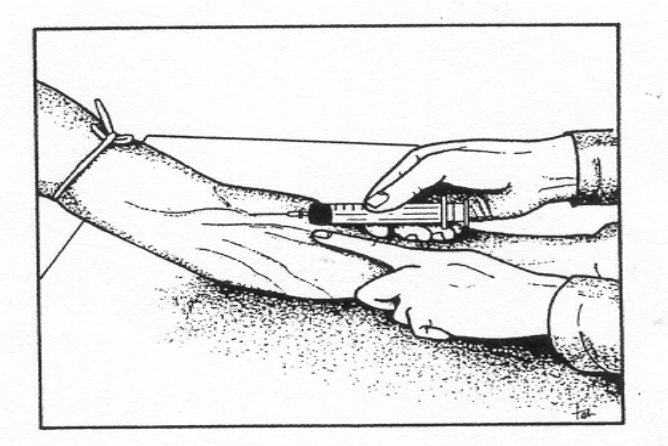


Fig 7.3: collection of venous blood.

Precautions

1. Tourniquet should be loosened once the needle has been inserted into vein.
2. Avoid the areas like healed burn areas and hematoma. Care is taken to prevent hematoma.
3. Follow some useful techniques when patient with difficult veins is to be handled, like making a fist, massaging the arm, choosing the other hand, etc.

2) Collection of arterial blood: venous blood and arterial blood are similar in composition except for a few differences such as O_2/CO_2 tensions. It is rarely examined. Arterial blood collection is painful and requires skill.

Uses: Arterial blood collectionis useful in measuring pH of arterial blood, partial pressure of O_2 and CO_2, oxygen saturation and bicarbonates.

Sites of selection for arterial blood

1. Radial artery
2. Brachial artery
3. Femoral artery.

Requirements

1. Betadine solution as anti-septic lotion.
2. 1% xylocaine as anesthetic drug
3. 20 or 21 gauge sterile needle.
4. 12 ml. heparinized syringe.
5. Cotton.

Procedure

1. Instruct the patient to take sitting position
2. Raise the wrist with a pillow and instruct the patient to extend the fingers downwards.
3. Palpate the artery, and rotate the hand back and forth until a good pulse is felt.
4. Clean the site of collection with antiseptic agent.
5. Anaesthetize the site of collection to reduce pain.
6. Puncture the artery and draw blood in pre heparinized syringe, by drawing the plunger. Care is taken not to pull needle out of artery.
7. After collecting 3-6 ml. of blood withdraw the needle.
8. Keep sufficient absorbent bandage over the site of puncture and apply pressure for about 2 minutes.
9. Expel air bubbles in the sample immediately.
10. Cap the syringe and rotate gently to cause proper mixing of blood with heparin.
11. Preserve by storing in the refrigerator, if it is not tested within 15 - 25minutes.

Precautions

1. Apply enough pressure at the puncture site and watch for bleeding.
2. Collection of arterial blood should be done without trauma.
3. Beware of air bubbles in the syringe as they will cause change of concentration of gases.

Novel blood collection system

Vacutainers: Vacutainers are one of the single use type novel blood collection system working by negative pressure. They are available with or without anticoagulants.They are of single use type.They are used to collect blood by venipuncture or by finger prick method, instead of conventional syringes and needles.

Parts of a vacutainer: The vacutainer system consists of-

1. Sterile single use blood collection needle.
2. A holder, used to secure needle during insertion into tube stopper and venipuncture.
3. A sterile vacutainer primary tube, an evacuated glass tube with rubber stopper containing vacuum.

Procedure

1. When needle is inserted at the site of collection, the rear cannula pushes through the rubber sleeve and punctures the rubber stopper.
2. This allows the vacuum in the tube to draw blood from the vein by negative pressure.
3. When one tube is withdrawn from the back of the needle, to collect blood in another container, the sleeves slides back into position and keeps the blood from flowing out through rear cannula.
4. When last tube has been filled, the entire system is removed from the patient's arm and the needle is disposed.

Precautions

1. While collecting blood, the arm of the patient is held downwards and blood is kept from contact with stopper. This will prevent back flow of blood from blood tubes into veins.
2. Only sterile tubes are used to avoid contamination.

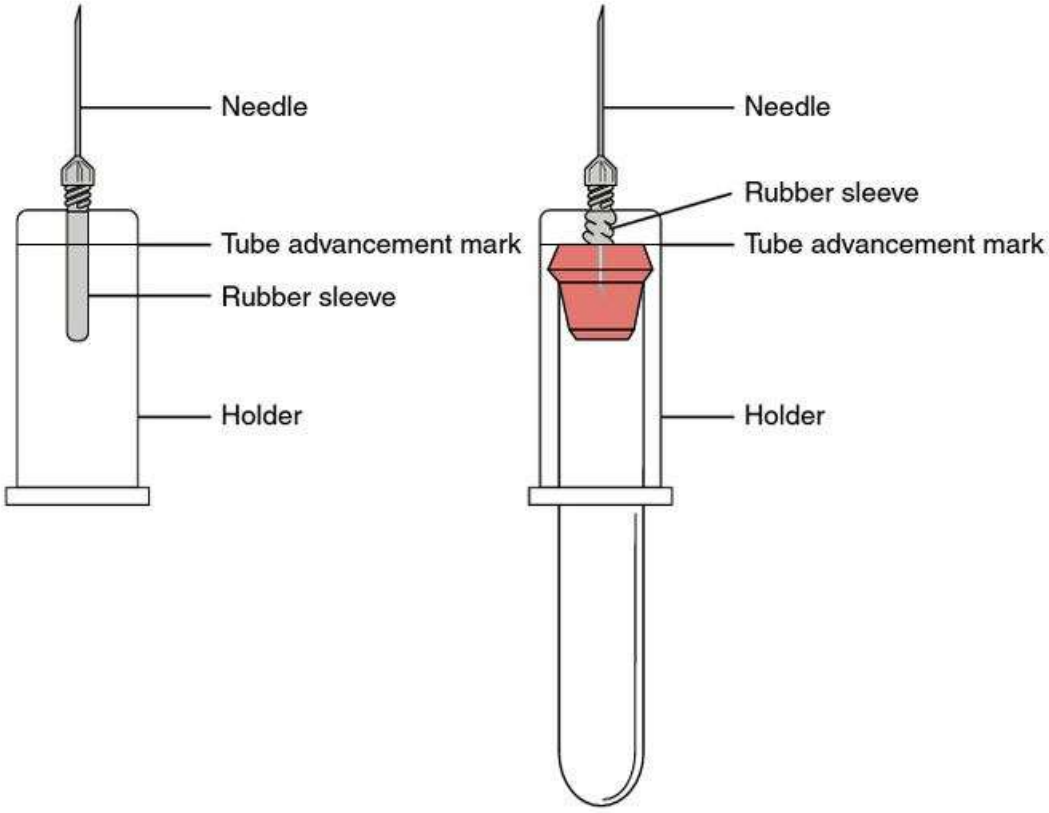


Fig 7.4: Diagram showing parts of a vacutainers.

Advantages of vacutainers

1. It eliminates the preparation of anticoagulant containing tubes
2. There is no processing of containers.
3. It minimizes hemolysis in specimens.
4. There is no possibility of blood spillage.
5. Since syringe is not required, there are fewer disposals. Only the needle is disposed after blood collection.

Preservation of blood

1. Blood is usually preserved at 2-4 ^{0} C in refrigerator.
2. ***Hematoma*** is clotting of blood***. Anticoagulants*** are used to avoid clotting of blood in some tests.

   Ex: a) Double oxalate, which is a mixture of 3 parts of ammonium oxalate and 2 parts of potassium oxalate.

b) Sodium citrate

c) Ethylene diamine tetra acetic acid (EDTA)

d) Sodium fluoride

e) Acid citrate dextrose (ACD)

f) Heparin.

3. ***Hemolysis***means destruction of red blood cells. It causes certain changes in the composition of the specimen. Measure to prevent hemolysis are-

a) Collection tubes must be clean and dry.

b) During collection, minimum amount of constriction should be applied to the arm.

c) Blood should be easily collected into collecting tubes after removing the needle.

d) Speed of centrifugation has to be controlled.

7.2 Urine

Collection of urine specimen is an important aspect in laboratories. It is used in physical, chemical and microbiological examination.

Types of urine samples

1. ***Single specimen:*** Urine specimen collected any time randomly is called as single specimen. It is called as single specimen. It is useful for qualitative analysis.
2. ***Fasting urine specimen:***Urine specimen collected after a fasting of 12-16 hours of overnight fasting is called fasting urine specimen.
3. ***Post prandial urine specimen:*** urine specimen collected 2 hours after lunch is called post prandial urine specimen.
4. ***24hours urine specimen:*** Specimen of urine collected in a period of 24 hours duration is called as 24hrs urine specimen. It is required in quantitative determination.

Method of collection of urine sample:

1. Collect specimen in dry, clean, sterile glass of plastic container which has wide mouth, spill free and has a screw cap top.
2. Label the specimen such as name of patient, type of specimen, ID number, date and time of collection.

Collection of 24-hours urine sample

a) Procedure:

1. Use a 2.5 to 5 liters capacity dark plastic or glass jar which contains 5 grams of sodium carbonate powder. This will adjusts the acidity of urine and helps in preservation.
2. A 24 hour urine collection has to be started at a specific time. Exact 24 hours of time is to be maintained.
3. For example, if 8 A.M is chosen in the morning, the bladder is emptied at that time and that urine is discarded. From that time on, add any urine that is passed into the jug. Recording of each urinated time is not needed. Urine passed during defecation in this 24 hours is also saved, otherwise it is incomplete.
4. During the collection, store the urine jug tightly capped in a refrigerator or an ice chest.
5. Exactly after 24 hours, i.e. at 8 A.M. on the next day morning, urine collection is ended by emptying the bladder into the jug for the last time.

b) Volume:

1. On an average diet and normal fluid intake, the volume of 24 hours urine specimen is between 1.2 to 1.5 liters.

2. In polyuria, the volume of 24 hours urine specimen is more than 2 liters.

3. In oliguria, the volume of 24 hours is reduced below 500 ml.

4. In Anuria, there is total suppression of urine.

c) Risks of 24 hours urine:

1. Forgetting to collect urine within time and going beyond the time resulting in excess collection.

2. Loss of urine from container by spilling.

3. Not keeping urine cold during collection period.

d) Preservation:

1. Freshly passed urine has to be examined. On standing, chemical changes take place. Bacterial growth may also take place. To avoid this it is to be placed in refrigerator at 2-8^{0}C.
2. Different chemicals are used as urinary preservatives.
   Ex: a) Conc HCl
   b) 2N HCl
   c) Acetic acid
   d) Meta phosphoric acid
   e) Formalin
   f) Boric Acid
   g) Thymol
   h) Toluol
   i) Chloroform.

Conclusion

- Specimen of blood and urine should be tested without delay.
- Labelling of the collected specimen is important for identification.
- If any delay is there for examining the urine, it has to be preserved.

Summary

Blood specimens are required for diagnostic tests. Random specimen, Fasting specimen are types of specimens. Different forms of blood used in testing are whole blood, plasma, serum and cellular parts. Different routes of blood collection are capillary, venous and arterial. Novel method is vacutainer method.

Urine specimen is of different types. 24 hours urine, single specimen urine, etc. several preservatives are used to preserve urine.

Model questions

Short Answer Type Questions

1. Mention different blood specimens.
2. What is a fasting specimens?
3. What do you mean by post prandial specimen?
4. Mention different routes of blood collection.
5. Mention some applications of capillary blood.
6. Name the different sites of capillary blood collection?
7. What is the quantity of blood collected by capillary method?
8. What is the quantity of blood collected by venous route?
9. Mention the sites of venous blood collection?
10. What is the angle of insertion of needle into vein?
11. How do you handle a patient with difficult veins?
12. Name the arteries used for blood collection.
13. What is a vacutainer?
14. Define anticoagulant.
15. Give some examples of anticoagulants.
16. Define the term hemolysis?
17. What is single urine specimen?
18. What is 24 hours urine specimen?

Long Answer Type Questions

1. Write about different types of blood specimens. What are different forms of blood used in diagnostic testing? Mention their uses.
2. Write about novel blood collection system.
3. Explain arterial blood collection.
4. Discuss different aspects of 24 hours urine specimen.
5. Explain venous blood collection.

Chapter-8

Solutions

Structure

8.1 Introduction

8.2 Types based on solute and solvent

8.3 Types based on method of expressing concentration and calculation.

Learning objectives

1. Students should prepare different solutions.
2. A student should practice various calculations.

8.1 Introduction

- *A **solution*** is defined as homogenous and monophasic mixture of two or more substance.
  Ex. 0.9% Sodium Chloride solution.
- A ***solute***is a substance present in minor proportion in a solution.
  Ex. Sodium Chloride present in 0.9% Sodium Chloride solution.
- A ***solvent i***s a substance present in major proportions in a solution.
  Ex: Water present in 0.9% sodium chloride.

8.2 Typesof solutions based on solutes and solvents.

Based on states of matter of solutes and solvents, solutions are of different types,

1. **Solid in liquid solutions:** Solid solute dissolved in liquid solvent is called solid in liquid solution.
   Ex: 10% sodium tungstate solution.
2. **Liquid in liquid solution**: Liquid solute dissolved in liquid solvent is called liquid in liquid solution.
   Ex: 2% Acetic acid solution.
3. **Gas in liquid solution**: Gaseous solute dissolved in liquid solvent is called gas in liquid solution.
   Ex:Aerated water.

4. **Solid in solid solution**: Solid solute dissolved In solid solvent is called solid in solid solution.

   Ex: Alloys.
5. **Liquid in solid solution**: Liquid solute dissolved in solid solvent is called liquid in solid solution.

   Ex: Hydrated salts such as $Na_2CO_3.10\ H_2O$
6. **Gas in solid solution**: Gaseous solute dissolved in solid solvent is called gas in solid solution.

   Ex: H_2 in palladium.
7. **Solid in gas solution:** solid solute dissolved in gaseous solvent is called gas in solid solution.

   Ex: Minute particles in inhaled air.
8. **Liquid in gas solution**: Liquid solute dissolved in gaseous solvent is called liquid in gas solution.

   Ex: Moisture in air.
9. **Gas in gas solution:** Gaseous solute dissolved in gaseous solvent is called gas in gas solute

   Ex: Air.

Methods of expressing concentration:

Different methods of expressing concentration are:

1. Percentage
2. Molarity
3. Normality
4. Molality
5. Formality
6. Mole fraction.

8.3 Types of solutions based on method of expressing concentration and calculations

Based on method of expressing concentration, different types of solution are:

1. Percentage solutions
2. Molar solutions
3. Normal solutions

4. Molal solutions

1) **Percentage solutions**: Solutions whose concentrations are expressed in percentage are called as percentage solutions.

There are four kinds of percentage solutions. They are:

a) Weight in volume percentage solutions. (W/V solution)

W/V solutions are solutions of solids in liquids, one part of solid solute by weight dissolved in liquid solvent to produce solution of 100 parts by volume is called as 1% weight in volume solution.

General formula:Solute – 1gand Solvent up to 100ml.give 1% W/V solution.

Calculations:

Example: Prepare 200 ml. of 0.9% W/V sodium chloride.

Answer: For 100 ml. --------0.9 grams of sodium chloride

For 200 ml. ---------?

$$\frac{0.9 \times 200}{100} = 1.8 \text{ grams}$$

1.8 grams of sodium chloride dissolved in enough water and diluted to 200 ml. with water will produce 200 ml. of 0.9% W/V sodium chloride solution (Normal saline)

Method of preparation:

1. Weigh accurately 1.8 grams of sodium chloride.
2. Dissolve in about 150 ml. of the water.
3. Examine for any dust/foreign particles. If necessary filter it using filter paper.
4. Dilute to 200 ml. volume with water in a graduated measuring jar.
5. Transfer into a clean and dry container and label it.

Example2: Prepare 250 ml. of 10% W/V sodium tungstate solution.

Answer: 100 ml. ---------- 10g

250 ml. ----------?

$$\frac{10 \times 250}{100} = 25 \text{ grams}$$

25 grams of sodium tungstate dissolved in enough water and diluted to 250 ml. with water will produce 10% W/V sodium tungstate solution.

Method of preparation:

1. Weigh accurately 25 grams of sodium tungstate.
2. Dissolve in approximately 168 ml. of water.
3. Examine for any dust/foreign particles. If present filter it using a filter paper.
4. Dilute to 250 ml. with distilled water in a graduated measuring jar.
5. Transfer into a clean dry container and label it.

b) Weight in weight solutions. (W/W solution)

W/W solutions are also percentage solutions of solids in liquids. One part of solid solute by weight dissolved in liquid solvent to produce solution of 100 parts by weight is called as 1% weight in solution.

General formula: Solute – 1g and solvent up to 100g will give 1% W/W solution.

c) Volume in weight solutions (V/W solutions)

V/W solutions are percentage solutions of liquids in solids. One part by volume of liquid solute dissolved in solid to produce solution of 100 parts by weight is called as 1% volume in weight solution.

General formula: liquid—1 ml. and solid up to 100g will give 1% V/w solution.

d) Volume in volume percentage solutions. (V/V solution)

V/V solutions are percentage solutions of liquids in liquids. One part by volume of liquid solute diluted to 100 parts by volume with liquid solvent is called as 1% volume in volume solution.

General formula: Liquid—1ml. and solid up to 100 ml. will give 1% V/V solution.

Calculations:

Example1: Prepare 50 ml. of 2% V/V acetic acid.

Answer: 100 ---- 2

50-----?

$$\frac{50 \times 2}{100} = 1 \text{ ml}$$

1 ml. of acetic acid, diluted to 50 ml. of water will produce 50 ml. of 2% V/V acetic acid solution.

Method of preparation

1. Take approximately 45 ml. of water into a measuring jar.
2. Add 1 ml. of acetic acid with stirring.
3. Make the volume to 50 ml.
4. Transfer to a clean and dry container and label it.

Example2: Prepare 150 ml. of 10% V/V formalin in normal saline.

Answer: 100 ml. ---------- 10ml.

150 ml. ----------?

$$\frac{150 \times 10}{100} = 15 \text{ ml.}$$

15 ml. of formalin diluted to 150 ml. with water will produce 150 ml. of 10% V/V formalin.

Method of preparation:

1. Take 15 ml. of formalin in a measuring jar.
2. Dilute to 150 ml. with water.
3. Transfer into a clean, dry container and label it.

2) **Molar solutions:** Molar solution is defined as a solution containing one mole of substances in one liter of solution.

Molarity: Molarity is number of moles of the solute per liter of solution. It is denoted M.

$$M = \frac{\text{Number of moles of solute}}{\text{Volume of solution in liters}}$$

Mole: Mole is defined as one gram molecular weight substance present in one liter solution.

Gram molecular weight: Molecular weight expressed in grams is called as gram molecular weight.

Example: One mole of H_2SO_4 = 98.078 grams of H_2SO_4 in one liter solution.

One mole of HCl = 36.5 grams of HCl in one liter solution.

Calculations:

Example 1:Prepare 75 ml. of 0.1 M HCl

Answer:

Molecular weight of HCl = 36.5

Gram molecular weight of HCl = 36.5 grams.

Weight of HCl required to make 1 liter of 1M HCl = 36.5 g.

Therefore, weight of HCl required to make 0.1M HCl =

1M ---------- 36.5 g

0.1M ----------?

$$\frac{0.1 \times 36.5}{1} = 3.65 \text{ g}$$

Weight of HCl required to make 75 ml. of o.1M solution =

1,000 ml ---------- 3.65 g

75 ml. ----------?

$$\frac{3.65 \times 75}{100} = 0.273 \text{ g}$$

Method of preparation:

1. Take accurately 0.273g HCl.
2. Add about 50 ml. of water gradually while stirring and cool.
3. Dilute to 75 ml. with distilled water.
4. Standardize and adjust molarity if necessary.
5. Mix thoroughly and store in a clean dry container.

Example 2: Prepare 100 ml. of 0.5M HCl with commercially available HCl.

(Specific gravity of commercially available HCl = 1.16. Percentage by weight = 36% W/W.)

Answer:

Molecular weight of HCl = 36.5

Gram molecular weight of HCl = 36.5g

Weight of HCl required to make 1 liter HCl solution = 36.5g.

Therefore weight of HCl required to make 1 liter of 0.5M HCl =

1M ---------- 36.5 g

0.5M ----------?

$$\frac{0.5 \times 36.5}{1} = 18.25$$

Weight of HCl required to make 100 ml. of 0.5M HCl =

1000 ml. ---------- 18.25 g

100 ml. ----------?

100 x 18.25

---------- 1.825 g

1000

Weight of HCl required to make 100 ml. of 0.5M HCl = 1.825g

Quantity of commercially available concentrated HCl required to make 100 ml. of 0.5M HCl =

36 g ---------- 100 g

1.825 g ----------?

1.825 x 100

---------- = 5.07 g

36

Specific gravity of HCl = 1.16

1.16g ---------- 1 ml.

5.07g ----------?

5.07 x 1

---------- = 4.37 ml.

1.16

Therefore, quantity of commercial concentrated HCl required to prepare 100 ml. of 0.5M HCl = 4.37 ml.

Method of preparation:

1. Measure accurately 4.37 ml. of commercially concentrated HCl.
2. Gradually add 75 ml. of distilled water by stirring and cool.
3. Dilute to 100 ml. with distilled water.
4. Standardize and adjust molarity if necessary.

5. Mix thoroughly and store in a clean and dry container.

Example 3: Prepare 50 ml. of 1M NaOH.

Answer:

Molecular weight of NaOH = 40.

Gram molecular weight of NaOH = 40 g

Weight of NaOH required to make 1 liter of 1M NaOH solution = 40 g.

Therefore, weight of NaOH required to make 50 ml. of 1M NaOH =

1000 ml. ---------- 40 g

50 ml. ----------?

$$\frac{50 \times 40}{1000} = 2 \text{ grams.}$$

Weight of NaOH required to make 50 ml. of 1M NaOH = 2 g.

Method of preparation:

1. Weigh accurately 2 g. of NaOH.
2. Dissolve about 40 ml. of distilled water.
3. Dilute to 50 ml. with distilled water.
4. Standardize and adjust molarity if necessary.
5. Mix thoroughly and store in a clean dry container.

3) Normal solution: Normal solution is defined as a solution containing one equivalent of substance in one liter of solution.

Normality: Normality is defined as number of equivalents of substance per liter of solution. It is denoted by N. It is expressed as:

$$N = \frac{\text{Number of equivalents of substance.}}{\text{Number of liters of solution.}}$$

$$N = \frac{\text{Number of milli equivalent of substances}}{\text{Number of milli liters of solution.}}$$

$$\text{Normality} = \text{Molarity} \times \frac{\text{Molecular weight}}{\text{Equivalent}}$$

Examples: Normality of sulfuric acid = 36 N.

Normality of HCl = 12N

Normality of glacial acetic acid = 45N.

Calculations:

Example 1: Prepare 500 ml. of 0.5 N HCl.

Answer:

Molecular weight of HCl = 36.5

Gram molecular weight of HCl = 36.5 g.

Equivalent weight of HCl = 36.5 g.

Weight of HCl required to make 1 liter of 1N HCl = 36.5 g.

Weight of HCl required to make 1 liter of 0.5 N HCl =

1 N ---------- 36.5 g.

0.5 N ----------?

$$\frac{0.5 \times 36.5}{1} = 18.25 \text{ g.}$$

Weight of HCl required to make 500 ml. of N HCl =

$$\frac{500 \times 18.25}{1000} = 9.125 \text{ g.}$$

Therefore weight of HCl required to make 500 ml. of 0.5 N HCl = 9.125 g.

Method of preparation:

1. Take accurately quantity of HCl equivalent to 9.125 g. of HCl.
2. Add gradually to approximately 450 ml. of distilled water with constant stirring and cool.
3. Dilute to 500 ml. with distilled water.
4. Standardize and adjust normality if necessary.

Example 2: Prepare 250 ml. of 0.5 N NaOH.

Answer:

Molecular weight of NaOH = 40

Gram molecular weight of NaOH = 40 g.

Equivalent weight of NaOH = 40 g.

Weight of NaOH required to make 1 liter if 1N NaOH = 40 g.

Weight of NaOH required to make I liter of 0.5 N NaOH =

$$\frac{0.5 \times 40}{1} = 20 \text{ g.}$$

Weight of NaOH required to make 250 ml. of 0.5 N NaOH =

1000 ml. ---------- 20 g.

250 ml. ----------?

$$\frac{250 \times 20}{} = 5 \text{ g.}$$

1000

Weight of NaOH required to make 250 ml. of 0.5 N NaOH = 5 g.

Method of preparation:

1. Weigh accurately 5 g. of NaOH.
2. Dissolve in about 200 ml. of distilled water.
3. Dilute to 250 ml. with distilled water.
4. Standardize and adjust normality if necessary.
5. Mix thoroughly and store in a clean and dry container.

4) Molal solutions: Molal solution is defined as solution containing one mole of solute in one kilogram of solvent.

Molality: Molality is the number of moles of solute per one kilogram of solvent. It is denoted by m.

$$m = \frac{\text{Number of moles of solute}}{\text{Weight of solvent in Kg.}}$$

Example: 1m HCL means 36.5 grams of HCl in Kg water.

5) Formality: It is the number of formula weight in gm. Dissolved per liter solution. When formula weight is equal to the molecular weight, formality is same as Molarity.

6) Mole Fraction: It is the ratio of number of moles of one of the components to the total number of moles of solute and solvent. It is denoted by X.

If n1 is number of moles of solute, n2 is number of moles of solvent, x1 is mole fraction of solute and x2 is mole fraction of solvent,

$$X1 = \frac{n1}{n1 + n2} \quad \text{and} \quad x2 = \frac{n1}{n1 + n2}$$

x1 + x2 = 1.

Conversion of mg % to m eq/ L:

$$\text{Conversion in m eq/L} = \frac{\text{mg. per 100 ml. x 10 x valency}}{\text{Molecular weight.}}$$

Conclusion

Solution are mixtures of more than one component. They are homogenous mixtures and monophasic.

Summary

Solutions are monophasic mixtures of two or more substances in which solute is dissolved in solvent. They are classified on the basis of states of matter of solute and solvent. Concentrations are expressed as percentage, molarity, normality, molality, mole fraction and formality. Percentage solutions are weight in volume (W/V) solutions, weight in weight (W/W) solutions, volume in weight (V/W) solutions and volume in volume (V/V) solutions.

Molarity is number of moles of substance present in one liter of solution. Normality is number of gram equivalents present in one liter of solution. Molality is number of moles present in one Kg. solvent. Mole fraction is ratio of number of moles of one of the components of the solution to the total number of moles of solute and solvent. Formality is number of formula weight in grams, dissolved in liter of solution.

Model Questions

Short Answer Type Questions

1. Define solution.
2. Define solute and solvent.
3. Give examples of a) Solid in liquid solution b) Liquid in liquid solution.
4. What is percentage solution?
5. Define molarity.
6. What is molar solution?
7. Define mole.
8. Name the different types of mole percentages.

9. What is meant by gram molecular weight?
10. Mention the relation between molarity and normality.
11. Define molality.
12. What is Molal solution?
13. Define formality.
14. What is mole fraction?

Long Answer Type Questions

1. Define solutions classify and give examples.
2. Explain the different methods of expressing concentrations.
3. Mention different types of percentage solutions. Write about weight in volume percentage solutions with an example.
4. Write about volume in volume percentage solutions and their preparation with an example.
5. Write about normal solutions giving one example.

Problems

1. Prepare 60 ml. of 0.2 M HCl.
2. Prepare 75 ml. of 0.5 M HCl from commercial concentrated HCl.
   (Specific gravity of commercial concentrated HCl is 1.16, percentage by weight is 36%)
3. How do you prepare 300 ml. of 0.2 M H_2SO_4 from commercially available concentrated H_2SO_4
   (Specific gravity of commercial concentrated H_2SO_4. Is 1.84 and percentage by weight is 95%)
4. Prepare 250 ml. of 0.1 N HCl.
5. Prepare 300 ml. of 0.5 N NaOH.

Chapter – 9

Carbohydrates and Lipids

Structure

9.1 Carbohydrates

9.2 Lipids

Learning Objectives

Student should

1. Get familiar with carbohydrates and quantitative tests of carbohydrates.
2. Know the clinical importance of lipids.

9.1 carbohydrates

Definition: They are defined as polyhydroxy aldehydes or ketones (or) substances which yieldpolyhydroxy aldehydes or ketones on hydrolysis.

Important functions:

- Humans and all animals except carnivores derive the major portion of their food calories from various types of carbohydrates in their diet.
- Most of the energy for metabolic activities of the cell in all organisms is derived from the oxidation of carbohydrates.
- Present In storage organs of plants in the form of starch.
- Present in liver and muscles of animals in the form of glycogen as important food reserves.
- They also serve as an important component of skeletal structures in plants in the form of cellulose.
- Also present peptidoglycan or murein layer in outer structures of bacteria.

Classification:

Carbohydrates may be classified into two major groups-

1. Sugars (sweet substances): They are classified into-
   a) Monosaccharides
   b) Oligosaccharides

2. Non-sugar (which are not sweet.)

1. Sugars:

a) Monosaccharides:

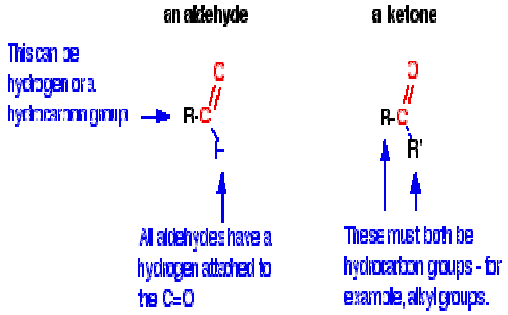
These consists of single polyhydroxy aldehyde or ketone unit which cannot be broken down to simpler substances on hydrolysis.

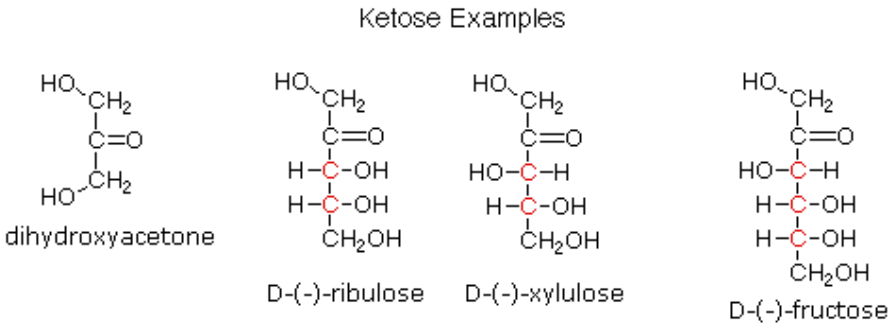
They are classified as:

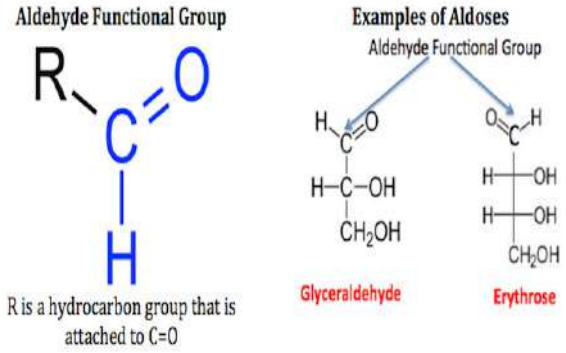
- **<u>Based on functional group:</u>**On the basis of functional group, monosaccharides are classified into:

1) ***Aldoses*** containing aldehyde as functional group.
2) ***Ketoses*** containing ketone as functional group.

Fig 9.1 structures of aldehyde and ketone groups.







- **<u>Based on number of carbons</u>**: On the basis of number of carbon atoms contained, monosaccharides can be classified into-

1) ***Trioses*** which contain 3 carbons atoms.
2) ***Tetroses*** which contain 4 carbons atoms.
3) ***Pentoses*** which contain 5 carbons atoms.
4) ***Hexoses*** which contain 6 carbons atoms.
5) ***Heptoses*** which contain 7 carbon atoms.
6) ***Octoses*** which contain 8 carbon atoms.

Each exists in two series:

Trioses: 1) Aldo trioses Ex: Glyceraldehyde.

2) Keto trioses Ex: Dihydroxy acetone.

Tetroses: 1) Aldo Tetroses EX: D-Erythrose.

2) KetoTetroses Ex: D-Erythrolulose.

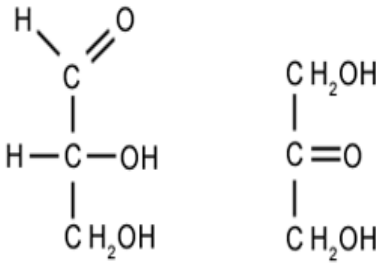
Pentoses: 1) Aldo Pentoses Ex: D-Ribose

2) KetoPentoses Ex: D-Ribulose.

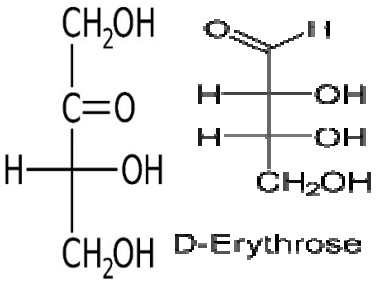
Hexoses: 1) Aldo hexoses Ex: D-Glucose.

2) Keto hexoses Ex: D-Fructose.

Fig 9.2 Structures of carbohydrates:

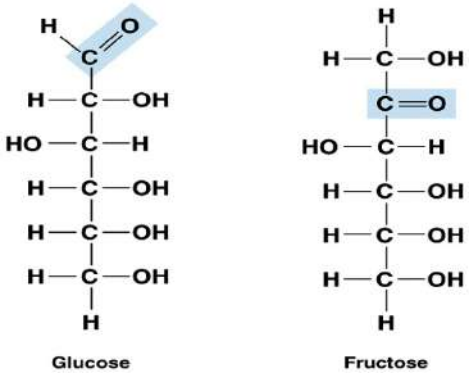


D-Glyceraldehyde Dihydroxyacetone



D-Erythrose

D-Erythrulose



Glucose Fructose

b) **Oligosaccharides:** Oligosaccharides are the carbohydrates which on hydrolysis yield two or more monosaccharides. They are further classified into:

1) Disaccharides.
2) Trisaccharides
3) Tetrasaccharides

1. ***Disaccharides:*** Disaccharides are the Oligosaccharides which on hydrolysis yield two monosaccharides.

Ex: Sucrose ----------> Glucose + Fructose

Maltose ----------> Glucose + Glucose

1. ***Trisaccharides:*** Trisaccharides are Oligosaccharides which yield three monosaccharides on hydrolysis.

Ex: Raffinose ----------> Glucose + Galactose + Fructose

2. ***Tetrasaccharides:*** Tetrasaccharides are Oligosaccharides which yield four monosaccharides on hydrolysis.

Ex: Stachyose ----------> Glucose + Frutose + 2 Galactose.

2. Non – Sugars:

They are usually taste less amorphous solids, insoluble in water and form colloidal suspension in water. On hydrolysis, they yield monosaccharide molecules.

Polysaccharides are further divided into:

a) ***Homo-polysaccharides***: They are polysaccharides made of single type of monosaccharide units.

Ex: Starch, Cellulose, Glycogen, Inulin.

b) ***Hetero-polysaccharides:*** They are polysaccharides made of different types of monosaccharide units.

Ex: Heparin, Hyaluronic acid, Chondroitin sulfate.

Tests to identify carbohydrates:

Qualitative tests: Following are testsused to detect different carbohydrates:

1. **Tollens's Test**: Reducing sugars on reacting with Ag^{+} (Ammonia complex) gives silver mirror.
2. **Benedict 's test:** Reducing sugars on reaction with Cu^{++}(citrate complex) give yellow-red precipitate.
3. **Fehling's Test:** Reducing sugars on reaction with Cu^{++} (tartrate complex) give yellow-red precipitate of Cu_2O.
4. **Salvinoff's Test for Fructose**: Fructose solution on addition of Salvinoff's reagent and boiling onwater bath for 5 minutes produces deep reddish color.

5. **O-Toluidine** Test for Galactose: Addition of O-Toluidine reagent to galactose solution and heating over boiling water bath for 10 minutes produces green color.
6. **Rubner's Test for Lactose:** Add 2.5 grams of Lead acetate solution to solution of Lactose and filter. Boil filtrate in another tube, add 1.5 ml. of NH_4OH and boil again for few seconds. Red color with red precipitate forms on cooling.
7. **Tauber's test for Pentose Sugars:** Addition of Tauber's reagent to Pentose sugar solution, boiling for 30 seconds and cooling develops pink to red color.
8. **Osazone Test for Carbohydrates**: Solution of carbohydrate taken in a dry test and acidified with in a dry test tube and acidified with a few drops of glacial acetic acid on addition of Osazone mixture should be placed in boiling water bath for 40 – 60 minutes. This tube should be cooled overnight and its deposit to be observed under microscope. Glucose forms yellow sheaves of slender needle like glucosazone crystals. Lactose forms close tufts of short fine crystals of lactosazone. Maltose forms clusters of broad bladed crystals of maltosazone.

9.2 Lipids

Definition: Lipids are cell components insoluble in water and soluble in non-polar solvents like acetone, ether, chloroform or benzene.

Biological Importance:

1. Lipids are used as energy storage component of the body. They are used during different metabolisms of the body.
2. Prostaglandins are a family of fatty acid derivatives. This lowers blood pressure and stimulates contraction of muscles.
3. Most membranestructure contain 40% of lipid. Membranes serve not only as barriers but also for binding certain enzymes and also acts as transport systems.
4. Gangliosides are the group of lipids present in gray matter of the brain. They function in the transmission of nerve impulses.
5. Acetic acid is used for synthesis of cholesterol. Cholesterol is precursor of many other steroids in animal tissues including bile acids, androgens- the male sex hormone, Oestrogen -- female sex hormone, Progesterone and Adreno-cortical hormone.
6. Some of the neutral lipids are found on the surface of erythrocytes and give them blood group specificity. They are partly responsible for the necessity of matching donor's and recipient's blood before transmission.

7. Cancer cells have characteristic glycosphingo lipids different from those in the normal cells.

Classification: On the basis of nature of products obtained on hydrolysis, lipids are mainly divided into three types. They are-

1. **Simple lipids**
2. **Conjugated lipids**
3. **Derived lipids**

1. **Simple lipids:** Simple lipids are esters of fatty acids with glycerol or fatty alcohols. Simple lipids are two types:
   - **a)** ***Fats and Oils:*** Fats and oils are esters of fatty acids with glycerol. Difference between fats and oils is, fats are solids at ordinary temperature whereas oils are liquids at ordinary temperature.
     Ex: Coconut oil, Arachis oil, olive oil, etc.
   - **b)** ***Waxes***: Waxes are esters of fatty acids with higher molecular weight monohydric alcohols or with sterols.
     Ex: Bees wax, Lanolin, Spermaceti, etc.
2. **Conjugated lipids:**Conjugated lipids are lipids conjugated with non-fatty prosthetic group. There are different types.
   - a) ***Phospholipids:***Phospholipids are lipids conjugated with phosphoric acid and nitrogenous component in the molecule.
     Ex: Lecithin, Cephalin, sphingomycins, etc.
   - b) ***Glycolipids:*** Glycolipids also called as cerebrosides are lipids containing sugar in glycosidic linkage with glycerol.
     Ex: Phrenoson, cerebron, Narvon, etc.
   - c) ***Sulpholipids***: They are lipids conjugated with sulfuric acid.
     Ex: sulfoquinovosyl diacylglycerol.
   - d) ***Lipoproteins:*** Lipids in the state of conjugation with proteins are called as lipoproteins. There are 4 types of lipoproteins: They are-
     - Chylomicrons
     - Very low density lipoproteins
     - Low density proteins
     - High density lipoproteins.

3. **Derived lipids:**Derived lipids are substances derived by hydrolysis of simple and compound lipids. Saturated and unsaturated fatty acids, sterols, alcohols, Glycerol's, etc. come under derived lipids.
   a) ***Saturated fatty acids:*** They are fatty acids that have no double bonds between the individual carbon atoms of the fatty acid chain. That is, the chain of carbon atoms is fully saturated with hydrogen atoms.

Ex: **Acetic acid – CH_3COOH**

Propionic acid-- C_2H_5COOH

   b) ***Unsaturated fatty acids***: Fatty acids containing one or more double bonds are unsaturated fatty acids. They can be sub-divided into different types based on number of double bonds.
      - **Oleic series**: Oleic series contain one double bond**. Ex**: Oleic acid
      - **Linoleic series**:Linoleic series contain two double bonds**. Ex**: Linoleic acid.
      - **Linolenic series**: Linolenic series contain three double bonds. **Ex:** Linolenic acid.
      - **Arachidonic acid**: It contain four double bonds.

   c) ***Sterols:*** They are derived lipids having cyclic structure obtained from nature. Steroids have cyclopentano per hydro phenanthrene ring. They are classified into different categories.
      - Sterols – cholesterol
      - Bile salts – Glycocholic and Taurocholic acids
      - Sex hormones – Testosterone, estradiol
      - Adreno cortical hormones – Corticosterone, cortisone.
      - Vitamins A, D, E and K
      - Cardiac glycosides – Strophanthin
      - Saponins – Digitonin.

   d) ***Essential fatty acids***: Fatty acids which cannot be synthesized in the body and have to be supplied through food are called as essential fatty acids. Ex: Linoleic acid is a precursor for the biosynthesis of arachidonic acid, which is not found in plants.

Clinical significance of lipids:

- Determination of lipids have diagnostic importance.

- Serum cholesterol is elevated in atherosclerosis, necrosis, and diabetes mellitus. It is decreased in hyperthyroidism, malabsorption and anemia.
- Elevation of plasma triglycerides indicates the risk of atherosclerotic diseases.
- Hyperlipidemias can be inherited trait or they can be secondary to nephrosis, diabetes mellitus, biliary obstruction and metabolic disorders of endocrine glands.

Conclusion

Carbohydrates are polyhydroxy aldehydes or ketones or substances which yield these on hydrolysis. Carbohydrates have significant role on living cells. Lipids are water insoluble components of cells. Both carbohydrates and lipidshave significant role in diagnosis.

Summary

Carbohydrates are polyhydroxy aldehydes or ketones. They have significant role in living cells. They are classified into monosaccharides, disaccharides, trisaccharides and tetra saccharides. Monosaccharides are further divided into trioses, Pentoses, hexoses, heptoses and Octoses. Non- sugars are polysaccharides which are divided into homopolysaccharides and heteropolysaccharides.

Quantitative tests for carbohydrates are Tollens's test, Benedicts test, Fehling's test, Salvinoff's test, O-Toluidine test, Rubner's test and Osazone test.

Lipids are water insoluble components of cell. They are soluble in non-polar solvents like acetone, ether, chloroform, benzene, etc. They are classified into simple lipids, conjugated lipids and derived lipids. Essential fatty acids cannot be synthesized in the body, hence must be supplied through plant sources.

Model Questions

Short Answer Type Questions

1. Define carbohydrates.
2. Mention two important functions of carbohydrates.
3. What are trioses?
4. Name the number of carbon atoms in a) Tetroses b) Hexoses
5. What are monosaccharides?
6. Define Oligosaccharides.

7. Write the products of hydrolysis of sucrose and maltose.
8. What are homopolysaccharides? Give examples.
9. What are heteropolysaccharides? Give examples.
10. What is a lipid?
11. Define simple lipids.
12. Differentiate between fats and oils.
13. Give examples of phospholipids.
14. Give some examples of glycolipids.
15. What are Sulpholipids?
16. What are lipoproteins?
17. Mention different types of lipoproteins.
18. What are derived proteins?
19. Write the definition of saturated fatty acids?
20. What are unsaturated fatty acids?
21. What are essential fatty acids?
22. Name the lipids present in brain and nervous tissue.
23. Which lipids give blood group specificity?
24. Name the fat soluble vitamins.
25. What are sterols?
26. Mention the sterols which are synthesized from cholesterol
27. What are prostaglandins?
28. What is the function of plasma lipoprotein?

Long Answer Type Questions

1. Write about the classification of carbohydrates.
2. What are lipids? Classify them.
3. What are different tests used to identify carbohydrates.
4. Define derived lipids and classify them.
5. Write the important functions of lipids and carbohydrates.

Chapter -------- 10

Amino Acids and Proteins

Structure

10.1 Amino Acids

10.2 Proteins

Learning Objectives

1. Student should know the importance of amino acids and proteins.
2. Student should know the biological importance of amino acids and proteins.

10.1 Amino Acids

Definition: Amino acids are molecules containing an amino group ($-NH_2$), a carboxylic acid group (-COOH), and a side chain (R group) specific to each amino acid.

These organic compounds contain the elements hydrogen, carbon, nitrogen, oxygen and in some cases Sulphur. Hence they are also called as α – amino carboxylic acids (α- amino acids) due to attachment of amino group to carbon atom next to carboxyl group.

Important Functions

1. Amino acids are the building blocks of proteins.The primary functions of amino acids are to build and repair muscles. They are essential in the replacement of daily wear and tear.
2. Amino acids produce chemicals that allow our brains to function at its highest potential. **Glycine** and **Glutamic acid** are involved in the transmission of impulse in the nervous system.
3. **Glutamic acid**is useful in body building and fitness because of its ability to maintain muscle mass while trying to shed fat.
4. Many amino acid derivatives are hormones. **Adrenalin / epinephrine** is the hormone for fight and flight**. Norepinephrine** is an important neurotransmitter released from sympathetic neurons affecting the heart and it also increases the rate of contraction.
5. **Leucine** is an amino acid that provides ingredients for the production of components in the body are utilized for the production of energy.

6. **Arginine** forms intermediate products in urea synthesis. Arginine plays an important role in cell division, healing of wounds, removing ammonia from the body, immune function and release of hormones.
7. **Tyrosine** is a precursor for the formation of melanin, which is a pigment of hair, skin and eyes.

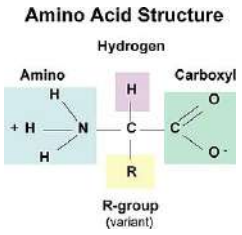


Fig 10.1: Structure of Amino Acid.

Classification

a) On the basis of capability/ non- capability of biosynthesis of amino acids in the body, they can be classified into-

1. Essential Amino Acids
2. Non- Essential Amino Acids

1. **Essential Amino Acids:** These cannot be synthesized in the body and hence must be supplied through diet by eating complete protein food of combination of vegetables. The **nine** essential amino acids include histidine, leucine, lysine, methionine, phenylalanine, tryptophan and valine.
2. **Non –essential amino acids:**Amino acids which can be synthesized in the body are called as non-essential amino acids. The **thirteen** non – essential amino acids are alanine, arginine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, hydroxyproline, proline, serine and tyrosine.

b) Amino acids classified based on number amino group and carboxyl groups:

1. **Mono amino mono carboxylic acids (Neutral amino acids**): They contain one amino acid group and one carboxylic group. Ex: Glycine, Alanine.
2. **Mono amino dicarboxylic acids**: They contain one amino group and two carboxyl groups.

   Ex: Aspartic acid, Glutamic acid.

3. **Di amino mono carboxylic acids:** They contain two amino groups and one carboxyl group.
   Ex: Lysine.

c) Amino acids classified on the basis of side chain:

1. Amino acids containing aliphatic side chain. Ex: Alanine.
2. Amino acids with side chains containing hydroxyl group. Ex: Serine.
3. Amino acids with side chain Sulphur atom. Ex: Cysteine
4. Amino acid containing aromatic rings. Ex: Phenyl alanine.
5. Amino acid with side chain pyrimidine. Ex: Proline.

Qualitative tests to identify amino acids:

1. **Solubility:** Amino acids are essentially soluble in water. The solubility vary from compound to compound. They may be soluble in water, dilute alkali or dilute acids.
2. **Ninhydrin Test:** Amino acids react with Ninhydrin to give violet color. This test is used for qualitative and quantitative identification of amino acids. Ninhydrin reagent is useful as detector in separation of amino acids in a specimen by paper chromatography.
3. **Stability to alkali:** Amino acids, unlike amides and volatile amines, do not evolve NH or alkaline vapors when boiled with alkali.
4. **Specific reactions for individual amino acids:**
   a) Xanthoproteic test
   b) Millan's test
   c) Hopkin's test.

10.2 Proteins

Definition: Proteins are high molecular weight polymers of a group of low molecular weight monomers of amino acids united by peptide bonds.

Important Functions:

1. They serve as structural elements. Collagen and elastin provide a fibrous framework in animal connective tissue. Keratin is the protein of hair, horns, nails, skin.
2. Some proteins act as storage components. Ovalbumin is used as an amino acid source of developing embryo. Casein is an important protein in milk.

3. Enzymes are proteins used in selective acceleration of bio chemical reactions like digestion and other metabolic reactions.
4. Insulin is a protein hormone produced by pancreas which regulates the concentration of sugar in blood of vertebrates.α
5. Some proteins have defensive function. Thrombin and fibrin are responsible for blood clotting and prevent loss of blood.
6. Defensive proteins are antibodies which help in protection against diseases.
7. Transport function is conducted proteins like-
   a) Hemoglobin – transport oxygen and carbon dioxide
   b) Myoglobin – transport oxygen in muscles
   c) Lipo – protein – transport lipids
8. Spiders and silk worms produce thick solution of protein fibroin which quickly solidifies into insoluble thread.

Classification

a) **Proteins are classified into** –

1. **Fibrous proteins:**
   - Fibrous proteins are polypeptide chains arranged in parallel along a single axis to yield fibers or sheets.
   - They are soluble in strong acids and alkalis, but insoluble in common solvents.

   Ex: Collagen present in tendons and bones.
   α-Keratin present in hair, nails, skin, etc.
   Elastin present in elastic connective tissue.
2. **Globular proteins:**
   - Globular proteins consists of polypeptide chains folded into compact spherical shapes.
   - They are soluble in water, dilute acids, dilute alkalis, etc.
   - Almost all enzymes, antibodies and many hormones are globular proteins. These proteins also serve function in transportation.

   Ex: Hemoglobin, Albumin, etc.
3. **Intermediates:**
   - Proteins resembling fibrous proteins in structure and resembling globular proteins in solubility properties fall into this category.

   Ex: Fibrinogen (plasma protein)

Myosin (muscle protein)

b) Another method of classification of proteins are-

1. **Simple proteins**: They are proteins which on hydrolysis yield amino acids only.

   Ex: Albumin or egg white

   Globulin or egg yolk

   Keratin of hair, nails, skin, etc.

   Collagen of bones and tendons

   Elastin of elastic connective tissue.

2. **Conjugated proteins:** They are simple proteins combined with non-prosthetic group. They include-
   a) ***Nucleoproteins:*** Proteins combined with nucleic acid as prosthetic group. Ex: DNA.
   b) ***Glycoproteins:*** Proteins combined with carbohydrates. Ex: Mucin (saliva).
   c) ***Lipoproteins:*** Proteins combined with lipids. Ex: Serum lipoproteins.
   d) ***Phosphoproteins***: Proteins combined with phosphoric acid. Ex: Casein of milk.
   e) ***Chromo proteins:*** Proteins combined with pigments. Ex: Hemoglobin.
   f) ***Metalloproteins:*** Proteins combined with metals. Ex: Ceruloplasmin (copper containing protein of serum.)
3. **Derived proteins:** They are the products resulting from hydrolysis by the action of acids, alkalis and enzymes.

Qualitative Tests to identify proteins

1. **Coagulation tests:** Proteins on heating, on exposure to U.V radiation, on reaction with acids undergo *coagulation i.e. precipitation*. When precipitation is irreversible.
2. **Color reactions:** Proteins give colored reactions with many reagents.
   a) ***Biuret test:*** Proteins on treatment with alkaline copper sulphate solution gives red or violet color. Proteins with 2 or more peptides linkages give this test.
   b) ***Millan's test:*** Addition of Millan's reagent to a protein solution gives white precipitate. This test is given by proteins yielding tyrosine on hydrolysis.
   c) ***Xanthoproteic test***: Protein on warming with concentrated nitric acid gives yellow color. This test is given by proteins containing amino acids with benzene ring.
   d) ***Ninhydrin test***: Protein on boiling with dilute aqueous solution of Ninhydrin produce violet color. This test is given by α-amino acids, proteins and dipeptides.

e) ***Hopkins-Cole test:*** Concentrated sulfuric acid when added down the side of a test tube containing protein and glyoxylic acid, violet ring appears between two layers.

Conclusion

- Amino acids build proteins.
- Proteins on hydrolysis yield amino acids.

Summary

Amino acids are building blocks of proteins in which an amino group is attached to carbon atom next to carboxyl group and hence also called as α- amino carboxylic acids. Essential amino acids are those which are not synthesized in the body and hence must be supplied through diet. Non-essential amino acids are those which can be synthesized in the body. Mono amino carboxylic acids contain one amino group and onecarboxyl group. Mono amino dicarboxylic acids contain one amino group and two carboxyl groups. Di-amino mono carboxylic group contains two amino groups and one carboxyl group Amino acids are classified based on the side chains. Side chains may be hydroxyl group, sulfur group, aromatic ring, etc. Amino acids are identified on the basis of solubility, Ninhydrin test, stability to alkali, etc.

Proteins are chemically polypeptides having variety of amino acids united by peptide bonds. Proteins are classified into 3 types. a) Fibrous proteins, b) globular proteins and c) intermediates. They are also classified into simple proteins, conjugated proteins and derived proteins. Proteins give positive result to coagulation test. They also give color reactions.

Amino acids and proteins have several important functions. They are principally responsible for replacing wear and tear of the body. Enzymes, hormones, hemoglobin, etc. are proteins.

Model Questions

Short Answer Type Questions

1. What are essential amino acids?
2. Write the definition of amino acids.
3. Name the amino acid responsible for transmission of impulses in the nervous system.
4. What are fibrous proteins?
5. What are glycoproteins?

6. Write about Ninhydrin test.
7. Name the amino acid with side chain Sulphur.

Long Answer Type Questions

1. Define amino acids. Give different classifications of amino acids.
2. What are proteins? Classify them.
3. Write the important functions of amino acids and proteins.

Write the qualitative tests for

Chapter – 11

Diagnostic tests

Structure

11.1 Serum Uric Acid

11.2 Serum Creatinine

11.3 Blood urea

11.4 Identification of Calcium in serum.

Learning objectives

1. Student should know about different biochemical estimations
2. Student should practice to calculate the concentrations using O.D values of different determinations in any medical lab.

11.1 Serum Uric Acid

Introduction: Uric acid is the end product of purine (nucleoprotein) metabolism. The serum uric acid level is often raised in gout. This test is used in the differentiating of gout from non-gouty arthritis.

Chemical structure: 2, 6, 8 trihydroxy purine.

Site of synthesis: Liver is the chief site of synthesis.

Normal value: Blood contains 2 - 7 mg. of uric acid for 100 ml.Daily output is 0.75 to 1 g.

Clinical significance: its value in blood is increased in-

1. Gout
2. Leukemia
3. Pneumonia
4. Arteriosclerosis with hypertension
5. Cardiac decompensation.
6. Uremia.

Determination of Serum Uric Acid

Principle: Uric acid reacts with phosphotungstic acid in alkaline medium to form a blue colored complex. Concentration of uric acid is directly proportional to the color intensity. Concentration of uric acid in serum is determined by comparing with similarly treated uric acid standard. Alkaline medium is provided by sodium carbonate.

Name of the method: Henry-Caraway's method.

Requirements:

1. Test tubes
2. Serological tubes
3. Centrifuge tubes
4. Colorimeter

Reagents:

1. **Sodium tungstate 10% w/v**
   a) 1o g/dl. Sodium tungstate : 50 ml.
   b) 2/3N, sulfuric acid : 50 ml.
   c) Orth phosphoric acid : 1 drop
   d) Distilled water : 800 ml.
   - Mix well and store at room temperature in an amber colored bottle.
2. **Sodium carbonate 10% w/v:**
   a) Anhydrous sodium carbonate : 10 mg.
   b) Distilled water : 100 ml.
   - Dissolve 10 mg. of anhydrous sodium carbonate in 75 ml. of distilled water and make to 100 ml. with distilled water. The reagent is stable at room temperature when stored in polythene bottle.
3. **Stock uric acid standard (100 mg %):**
   a) Lithium carbonate : 60 mg.
   b) Uric acid : 100 mg.
   c) Formalin : 2 ml.
   d) 50% Acetic acid : 1 ml.
   e) Distilled water : 100 ml.

- Heat about 80 ml. of distilled water in 250 ml. beaker to 60^0C. Add 60 mg, lithium carbonate and mix well. Add 100 mg. uric acid and mix thoroughly. Add 2 ml. formalin and then, slowly with shaking add 1 ml. acetic acid. Mix well and make the final volume to 100 ml. by adding distilled water. Store in amber colored bottle at 2—8^0C.

4. **Working uric standard (5 mg %):**
   a) Stock uric acid standard solution : 5 ml.
   b) Distilled water : 100 ml.
   - Dilute 5 ml. of stock uric acid solution to 100 ml. with distilled water.
5. **Stock phosphor tungstic acid reagent:**
   a) Sodium tungstate (molybdate free): 50 mg.
   b) Ortho phosphoric acid : 40 ml.
   c) Distilled water : 400 ml.
   - Mix and reflux gently for two hours. Cool and make final volume 500 ml. store at 2 – 8^0C in an amber colored container.
6. **Working phosphotungstic acid solution:**
   a) Stock phosphotungstic acid : 5 ml.
   b) Distilled water : 100 ml.
   - Dilute 5 ml. of stock phosphotungstic acid to 100 ml. volume with distilled water.

Specimen: Serum (or plasma).

Wave length: 660 nm (Red filter)

Procedure:

1. Take three test tubes and label them as T, S and B representing Test, Sample and Blank respectively.
2. Take the reagents as follows:

Reagent	T	S	B
Distilled water	3.5 ml.	3.5 ml.	4 ml.
Serum	0.5 ml.	--- ----	
Uric acid (standard)	----	0.5 ml. ----	

Sodium tungstate 10% w/v	0.5 ml.	0.5 ml.	0.5 ml.
Sulfuric acid	0.5 ml.	0.5 ml.	0.5 ml

This step gives protein free serum filtrate and similarly treated standard and blank.

3. Mix well and centrifuge after five minutes.
4. Take the supernatant into test tubes as follows and mix well.

	T	**S**	**B**
Supernatant	3 ml.	3 ml.	3 ml.
Sodium Carbonate	1 ml.	1 ml.	1 ml.

5. Set the colorimeter to 100% transmission with blank at 660 nm wave length (red filter).
6. Determine the O.D. of the test and standard.
7. Determine the concentration of serum uric acid with formula.

$$\text{Concentration of serum uric acid} = \frac{\text{O.D. of Test}}{\text{O.D. of standard}} \times 5 \text{ mg/ dl}$$

Normal value: 2 to 7 mg / dl.

11.2 Serum Creatinine

Introduction: Creatinine is the waste product of creatinine (nucleoprotein) metabolism.

Creatinine level raises in the body from spontaneous break down of creatinine phosphate.

Clinical Significance: Serum creatinine is increased in renal failure. Increased serum creatinine indicates renal disease. Elevation values are also observed in certain conditions like congestion heart failure, shock and mechanical obstruction of the urinary tract.

Normal values: Serum creatinine ranges from 0.6 to 1.5 mg/ dl. Daily output is 1.5 to 3 gm.

Determination of Serum Creatinine

Principle: Creatinine reacts with picric acid in alkaline medium to form a reddish yellow complex, intensity of which is directly proportional to the concentration of creatinine in the specimen and can be measured at 520 nm (green filter).

Name of the method: Alkaline- picrate method (Jaffe's reaction)

Specimen: Serum (or plasma)

Requirements:

1. Test tubes
2. Serological pipette
3. Volumetric flask.
4. Test tube stand
5. Centrifuge tubes
6. Centrifuge.
7. Colorimeter.

Reagents:

1. **Picric acid reagent:** 0.91 gm/dl (0.04 M)
2. **Sodium hydroxide**: 10 gm/dl
3. **Working creatinine standards**: 1 mg/dl, 5 mg/dl and 10 mg/dl.
    - These standards are prepared in 0.01 N hydrochloric acid by using stock creatinine standard 100 mg/dl. They are stable at 2 -- 8^0C.
4. **Alkaline picrate solution**:
    - It is prepared by mixing 4 parts of picric acid reagent and 1 part of sodium hydroxide. This reagent is stable for one day.

Specimen: Serum (or plasma)

Wave length: 520 nm (green filter)

Procedure:

1. Prepare protein free blood filtrate as follows
   - Distilled water – 3 ml.
   - Serum -- 1 ml.
   - 2/3 N H_2SO_4 ---- 0.5 ml.
   - 10% Sodium tungstate solution --- o.5 ml.

   Mix all the contents, centrifuge and take the supernatant.
2. Pipette it into the tubes labelled as T, S and B, representing test, sample and blank as follows

Reagent	T	S	B
Distilled water	3 ml.	3 ml.	3 ml.
Filtrate	2 ml.	---	-----
Working standard	----	2 ml.	-----
Alkaline picrate	1 ml.	1 ml.	1 ml.

3. Mix all the contents and keep at room temperature for 20 minutes.
4. Set the colorimeter to 100% transmission with blank.
5. Determine the O.D. of test and different standards.
6. Determine the concentration of unknown using the following formula-

$$\text{Concentration of serum creatinine} = \frac{\text{O.D of test}}{\text{O.D of standard}} \times \text{Concentration of standard mg/dl.}$$

Normal values:

Males : 0.9 to 1.5 mg/dl

Women : 0.8 to 1.2 mg/dl.

11.3 Blood Urea

Introduction: Urea is a main excretory product of protein catabolism. It is water soluble. Determination of blood urea is significant in assessment of kidney functioning.

Structure:

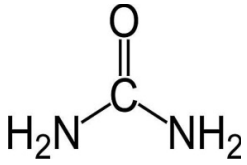


Fig 11.1: Structure of Urea

Site of formation: Formation of urea takes place in liver in the ureotelic organisms by sequence of reactions called as Urea cycle or Krebs – Hanseleitcycle.

Clinical significance: Elevated levels of blood urea are observed in pre-renal, renal and post renal conditions. Diabetes Miletus, dehydration, cardiac failure, etc. are pre – renal conditions. Kidney diseases are renal conditions. Enlargement of prostate, stones in urinary tract, etc. are post-renalconditions. Blood urea is decreased in severe liver diseases, pregnancy, malnutrition, etc.

Normal range: Average of 30 mg. of urea will be present per 100 ml. of blood. Average of 30 gm. Of urea is excreted through urine in 24 hours in an adult.

Determination of Blood Urea

There are different methods of determination of blood urea. They are:

1. Diacetylmonoxime method (DAM).
2. Enzymatic method/ Berthelot reaction method.
3. Titration method

1. **<u>Diacetylmonoxime method (DAM):</u>**

Principle: Urea reacts with Diacetylmonoxime in hot acidic medium and in the presence of thiosemicarbazide and ferric ions to form a pink colored compound which can be measured on a green filter (520 nm)

Requirements:

1. Test tubes

2. 10 ml. pipette
3. 0.1 serological pipette
4. 100 ml. measuring cylinder
5. Water bath
6. Stop watch
7. Colorimeter.

Reagents:

1. DAM – TSC reagent:

a) Diacetylmonoxime : 1 g.
b) Thiosemicarbazide : 0.2 g.
c) Sodium chloride : 9 g.
d) Distilled water : 1 liter

- Dissolve Diacetylmonoxime in 600 ml. of distilled water. To this solution dissolve thiosemicarbazide and sodium chloride. Dilute it to 1 liter with distilled water. This stable at room temperature for one year.

2. Acid reagent:

a) Ortho-phosphoric acid : 10 ml.
b) Sulfuric acid : 60 ml.
c) 10% aqueous ferric chloride solution : 1 ml.
d) Distilled water : 1 liter.

- Add Ortho-phosphoric acid and sulfuric acid of 750 ml. of distilled. Cool and add ferric chloride solution. Make it to 1 liter with distilled water. This reagent is stable at room temperature for one year.

3. Stock Urea Standard Reagent (1% w/v):

a) Dry urea : 1 g.
b) 0.2% w/v benzoic acid aqueous solution : 100 ml.

- Dissolve urea in 75 ml. of benzoic acid solution and dilute to 100 ml. with benzoic acid solution. This is stable at room temperature for one year when refrigerated.

4. Urea working standard (50 mg/dl.):

a) Stock urea standard : 5 ml.
b) 0.2% benzoic acid aqueous solution : 100 ml.

- Dilute stock urea standards to benzoic acid solution. This is to be freshly prepared for each batch of determination.

Sample: Blood

Wave length: 520 nm (green filter)

Procedure:

1) Prepare protein free blood filtrate (T) as follows-
   a) Blood : 0.1 ml.
   b) 2/3 N Sulfuric acid : 0.2 ml.
   c) 10% Sodium tungstate solution : 0.2 ml.
   d) Distilled water : 3.5 ml.
   - Mix all the contents and centrifuge after 5 minutes. Take supernatant in a test tube and label as test (T).
2) Give similar treatment to 0.1 ml. of urea working standard, take the supernatant in a test tube and label as standard (S).
3) Give similar treatment to 0.1 ml. of distilled water, take the supernatant in a test tube and label it as blank (B).
4) Label the tubes as T, S and B and take the reagents as follows:

Reagent	**T**	**S**	**B**
Supernatant	2 ml.	2 ml.	2 ml.
DAM – TSC reagent	3 ml.	3 ml.	3 ml.
Acid reagent	3 ml.	3 ml.	3 ml.

5) Mix the contents and plug the tubes with cotton.
6) Heat in a boiling water bath for 15 minutes and cool.
7) Set the colorimeter to 100% transmission using blank at 520 nm wave length.
8) Determine the O.D. of standard and test.
9) Determine the concentration of blood urea using the formula:

$$\text{Concentration of blood urea, mg/dl.} = \frac{\text{O.D. of test}}{} \times \text{concentration of standard}$$

O.D. of standard

Normal value: 15 – 45 mg/dl.

Blood urea

Blood urea Nitrogen, mg/dl. = ----------

2.15

2) Enzymatic method (Berthelot reaction method):

Principle: The procedure is based on Berthelot reaction. Urease splits into ammonia and carbon dioxide. Ammonia and carbon dioxide reacts with phenol in the presence of hypochlorite to form Indophenol, with which alkali gives a blue colored compound.

Requirements:

1. Test tubes
2. 1.0 ml, 5.0 ml., 0.1 ml. graduated pipette
3. Water bath
4. Colorimeter.

Reagents:

1. **Urease reagent**:
   a) Urease : 1000 units
   b) Phosphate buffer : 100 ml.
   - Dissolve urease in 75 ml. of phosphate buffer and dilute to 100 ml. with phosphate buffer, pH 7.0 (0.05 M). It is stable at 2 – 8^0C for 3 months.
2. **Phenol reagent:**
   a) Phenol : 5 g.
   b) Sodium Nitroprusside : 0.025 g.
   c) Distilled water : 500 ml.
   - Dissolve phenol and Sodium nitroprusside in 450 ml. of distilled water and dilute to 500 ml. with distilled water. It is stable at 2 – 8^0C for 3 months.
3. **Hypochlorite Reagent:**
   a) Sodium hypochlorite : 0.21 g.
   b) Sodium hydroxide : 2.5 g.
   c) Distilled water : 500 ml.

- Dissolve the reagents in 450 ml. of distilled water and then make it to 500 ml. with distilled water. It is stable for 3 months at 2 – 8^0C.

4. Standard urea nitrogen 20 mg/dl.:

a) Urea : 42.8 g.

b) Saturated benzoic acid : 100 ml.

- Dissolve urea in 75 ml. of benzoic acid and make it to 100 ml. using benzoic acid. This is stable for 3 months at 2 – 8^0C.

Sample: Serum or heparinized plasma.

Wave length: 546 nm. (Green filter)

Procedure:

1. Take 3 test tubes and label them as T, S and B representing test, standard and blank respectively.
2. Take reagents in the tubes are taken as follows-

-- **Reagents**	**T**	**S**	B
Urease reagent	0.5 ml.	0.5 ml.	0.5 ml.
Serum/plasma	0.02 ml.	----	---
Standard urea nitrogen	-----	0.2 ml.	-----

3. Mix and keep at 37^0C in a water bath for 10 minutes.
4. Now add the reagents as follows-

Reagent	**T**	**S**	**B**
Phenol reagent	1 ml.	1 ml.	1 ml
Hypochlorite reagent	1 ml.	1 ml.	1 ml.

5. Mix the contents and keep the tubes in water bath again at 37^0C for 10 minutes.

6. Add 5 ml. of distilled water to each tube and mix thoroughly.
7. Set the colorimeter to zero O.D. with the help of blank at 546 nm wave length.
8. Determine the O.D. of test and standard.
9. Determine the values of blood urea nitrogen and blood urea using the formulae—

$$\textbf{Blood urea nitrogen} = \frac{\textbf{O.D. of test}}{\textbf{O.D. of standard}} \times \textbf{20 mg/dl.}$$

Blood urea = Blood urea nitrogen x 2.14

Normal values:

Blood urea nitrogen = 5 – 21 mg/dl.

Blood urea = 11 – 45 mg/dl.

11.4 Identification of Calcium in Serum

Introduction: The calcium of the blood is confined to plasma. Serum calcium is used to screen a range of conditions related to bones, heart, nerves, kidneys and teeth. The test may be done patients having parathyroid disorder, malabsorption or thyroid problem.

Normal range: Total serum calcium ranges from about 9 to 11 mg/dl.

Clinical significance: Decrease serum calcium values are found in hypoparathyroidism, rickets, etc. A fall in serum calcium can occur in acute pancreatitis and renal diseases. Increased serum calcium values are observed in hyperparathyroidism, more intake of vitamin D and multiple myeloma.

Determination of Serum Calcium:

Principle: Calcium reacts directly with cresolphthalein complexion (CPC) reagent containing dimethyl sulfoxide and 8 – hydroxylquinoline. Since magnesium reacts with CPC, the addition of 8 – hydroxylquinoline eliminates the interference from magnesium.

Requirements:

1. Test tubes
2. 100 ml. graduated cylinder
3. 100 ml. beaker

4. 10 ml. graduated pipette
5. Serological pipette
6. Stop watch
7. Colorimeter.

Reagents:

1. **Calcium reagent 1:**
   a) Cresolphthaleincomplexion: 40 mg.
   b) Concentrated hydrochloric acid : 1.0 ml.
   c) 8 – Hydroxylquinoline : 2.5 g.
   d) Dimethyl sulfoxide : 100 ml.
   e) Glass distilled water : 900 ml.
      - Mix all the contents and finally make it to 1 liter by using glass distilled water. The reagent is stable at room temperature for 3 months.
2. **Calcium reagent 2:**
   a) Potassium cyanide : 500 mg.
   b) Dimethylamine : 40 ml.
   c) Glass distilled water : 960 ml.

   Mix the contents thoroughly and adjust it to 1 liter by using glass distilled water. It is stable at room temperature for 3 months.
3. **Calcium standard 10 mg/dl. (5.0 meq/liter):**
   a) Calcium carbonate : 25 mg.
   b) Hydrochloric acid : 50% (v/v)
      - It is prepared with 25 g. calcium carbonate hydrochloric acid 50% v/v. This reagent is stable at 2 – 8^0C.
4. **EDTA** : 4.0 g/dl.

Specimen: Serum or heparinized plasma.

Wave length: 575 nm (yellow filter)

Procedure:

1. Prepare fresh working reagent by mixing equal volumes of calcium reagents 1 and 2. The color of the reagent should be light purple.

2. Take 3 test tubes and mark them as T, S and B indicating test, standard and blank respectively.
3. Take reagents as follows-

Reagent	**T**	**S**	**B**
Working reagent	6.0 ml.	6.0 ml.	6.0 ml.
Serum/heparinized plasma	0.05 ml.	-----	------
Standard 10 mg/dl.	-----	0.05 ml.	------
Distilled water	-----	------	0.05 ml.

4. Mix the contents thoroughly and keep at room temperature for exactly 10 minutes.
5. Set the colorimeter to zero O.D. at 575 nm wave length (yellow color) using blank.
6. Read the intensities of test and standard.
7. Calculate serum calcium using the formula-

$$\text{Serum calcium} = \frac{\text{O.D. of test}}{\text{O.D. of standard}} \times 10 \text{ mg/dl.}$$

Normal range: 8.5 – 10.5 mg/dl.

Conclusion

Different diagnostic tests are useful for assessing clinical condition of a patient. Different tests help to diagnose different diseases.

Summary

Determination of blood urea is significant in assessment of renal functioning. Different methods of determination of blood urea are Diacetylmonoxime method, enzymatic method and Titration method. Determination of serum uric acid is significant in determination of Gout, Leukemia, etc. It is determined by Henry- caraway method. Determination of serum creatinine is significant in the diagnosis of renal functioning, cardiac functioning, etc. It is

determined by alkaline picrate method. Serum calcium is determined to screen conditions related to bones, heart, teeth, etc.

Model Questions

Short Answer Type Questions

1. What is uric acid?
2. Give the normal values of serum uric acid.
3. Write the clinical significance of determination of serum uric acid.
4. Give the principle of determination of serum uric acid by Henry- Caraway method.
5. Write the composition of stock uric acid reagent (100 mg/dl).
6. How do you prepare 5 mg/dl working serum uric acid solution from stock uric acid standard solution?
7. Write the clinical significance of serum creatinine determination.
8. Write the composition of alkaline picrate reagent.
9. Give the principle of serum creatinine determination by alkaline picrate method.
10. Mention the normal values of serum creatinine for male and female.
11. What is urea? Give its chemical structure.
12. Write the principle of determination of blood urea by Diacetylmonoxime method.
13. Mention the wave lengths selected for determination of blood urea by
    a) Diacetylmonoxime method b) Berthelot reaction method.
14. Write the principle of determination of blood urea by enzymatic method.
15. Name the enzyme reagent used in blood urea determination by Berthelot reaction method and give its composition.
16. What is the normal value of serum calcium?
17. Write the clinical significance of serum calcium determination.

Long Answer Type Questions

1. How do you determine serum uric acid by Henry- Caraway method?
2. Describe the determination of serum creatinine by alkaline picrate method.
3. Explain the method of determination of blood urea by Diacetylmonoxime method.
4. Write the Enzymatic method of determination of blood urea.
5. Explain the method of determination of serum calcium.

Chapter – 12

Vitamins and Minerals

Structure

12.1 Vitamins

12.2 Minerals

Learning Objectives

1. Student should get familiar with types of vitamins, daily requirement and deficiency diseases.
2. Student should get the knowledge of minerals, their sources, daily requirement and related deficiency diseases.
3. Student should conduct a survey of deficiency of different vitamins and minerals in his locality to have a view over the vitamin and mineral deficiency situations.

12.1 Vitamins

Definition: A vitamin is an organic compound required as a nutrient in small amounts by an organism.

Classification: Depending on solubility property, vitamins are classified into two types. They are:

1. **Water soluble vitamins**: There are nine water soluble vitamins. Eight B vitamins and vitamin C.
2. **Fat soluble vitamins**: There are four fat soluble vitamins. Vitamins A, D, E and K.

Water soluble vitamins:

The nine water soluble vitamins are:

1) Thiamin (Vitamin B_1)
2) Riboflavin (Vitamin B_2)
3) Nicotinic acid or Niacin (Vitamin B_3)
4) Pantothenic acid (Vitamin B_5)
5) Pyridoxine (Vitamin B_6)
6) Biotin(Vitamin B_7 or Vitamins H)
7) Folic acid (Vitamin B_9)
8) Cyanocobalamin (vitamin B_{12})

9) Ascorbic acid (Vitamin C)
10) Miscellaneous.

1) **Thiamin (Vitamin B_1):** It is also called aneurin. It occurs in living cells in its active coenzyme form- Thiamin pyrophosphate (TPP). It participates in decarboxylation of α-Keto acids and forms Acetyl coenzyme A.

Dietary Sources: This vitamin is adequately available in many natural foods. It is available in the germ of cereals, brans, egg yolk, yeast extracts, peas, beans and nuts, pork, liver, heart, kidney, etc.

Daily requirement: Adults: 1 – 1.5 mg. per day.
Children: 0.7 – 1.2 mg. per day.

Deficiency diseases: Deficiency of this vitamin leads to loss of appetite, gastrointestinal disturbances, muscular weakness, pain in arms and legs, decrease in blood pressure. Severe deficiency affects entire nervous system leading to Beriberi.

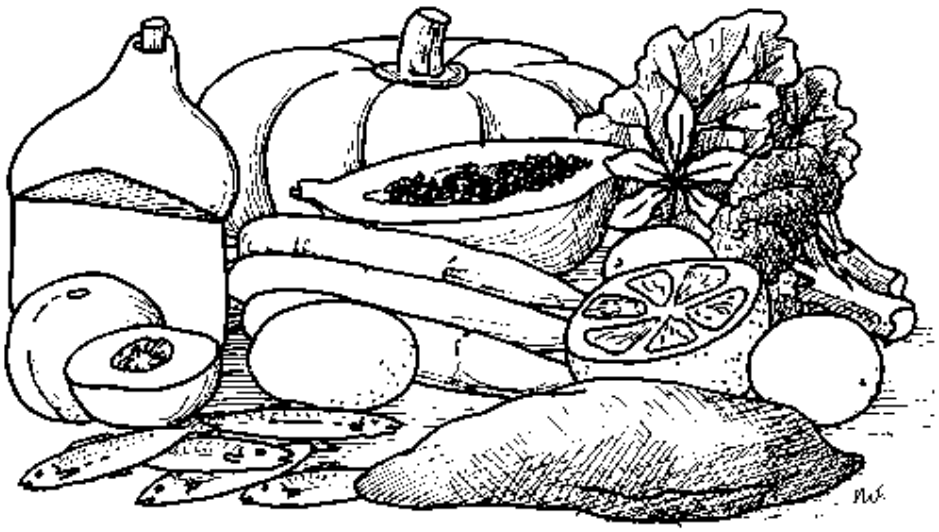


Fig 12.1: Sources of Vitamin B1

2) **Riboflavin (Vitamin B_2):** It is also called as Lacto Flavin. It is heat stable, light sensitive yellow crystalline solid.

Dietary sources: Liver, milk, cheese, almonds, leafy green vegetables, kidneys, yeast, legumes, tomatoes and mushrooms are good sources of vitamin B_2.

Daily requirement: Adults: 1.2 – 1.7 mg. per day

Pregnant and Lactation females: 1.5 – 2.0 mg. per day.

Deficiency diseases: Deficiency of Riboflavin in man causes inflammation of tongue and lining of mouth cheilosis–cracking of lips and corners of mouth, dry and scaling skin. The eyes may also become itchy, watery and sensitive to bright light.

Fig 12.2: Sources of vitamin B2

3) **Nicotinic Acid or Niacin (Vitamin B_3):** It is called as Pellagra preventing factor. It was first prepared by oxidation of Nicotine. After 1913, it was isolated from yeast.

Dietary sources: Niacin is found in variety of foods including liver, chicken, beef, fish, cereal, peanuts, dates, broccoli, carrots, nuts and legumes. It is synthesized from tryptophan, which is found in meat, fish, dairy and eggs.

Daily requirement: Adults: 15 – 20 mg. per day.

Children: 10 – 15 mg. per day.

Deficiency diseases: severe deficiency of niacin in diet causes disease called pellagra, characterized by diarrhea, dermatitis and dementia. Common psychiatric symptoms are anxiety, fatigue, poor concentration, restlessness and depression.

Fig: 12.3 Vitamin B3 foods

4) **Pantothenic acid (Vitamin B_5):**This is structural component of coenzyme A. its function is to serve as carrier of acyl groups in enzymatic reactions.

Dietary sources: The rich sources of pantothenic acid are eggs, cereals, legumes, meat, pork, yeast, milk, etc.

Daily requirement: Children: 2 – 4 mg. per day

Adults, pregnant and lactating women: 5 – 10 mg. per day.

Deficiency diseases: Clinical cases related to deficiency if this vitamin is very rare. Symptoms of deficiency are due to impaired energy levels leading to irritability, fatigue and apathy.

5) **Pyridoxine (vitamin B_6):**Pyridoxine is biologically converted into pyridoxal and pyridoxine. Active coenzyme forms Pyridoxal phosphate and Pyridoxine phosphate which take active part in amino acid metabolism.

Dietary sources: The important food sources of this vitamin are milk, fish, meat, eggs, banana, cabbage, corn, whole grain, etc.

Daily requirements: Adults: 2.2 – 2.2 mg. per day.

Pregnant and lactating women**:** 2.5 mg. per day.

Deficiency diseases: In some infants, its inadequate supply in diets lead to epileptic like seizures. This deficiency of this vitamin leads to hypochromic anemia.

Fig 12.4 Vitamin B6 Rich food

6) **Biotin**:Biotin is made by intestinal bacteria. It is necessary for the production of fatty acids and the metabolism of fats and amino acids. It plays an important role in the citric acid cycle by which energy is released during aerobic respiration.

Dietary sources:The sources of biotin are eggs, liver, kidney, milk, tomatoes, grains, nuts, string beans, spinach and grass.

Daily requirements: Adults: 100 – 300 µg. per day**.** Minimal requirement of this vitamin is not established because quantity of vitamin provided by bacteria cannot be determined.

Deficiency diseases: Biotin deficiency is caused by prolonged feeding of raw egg white. Raw egg white contains a protein called Avidin. It has the property of binding with biotin and preventing its absorption from intestine.

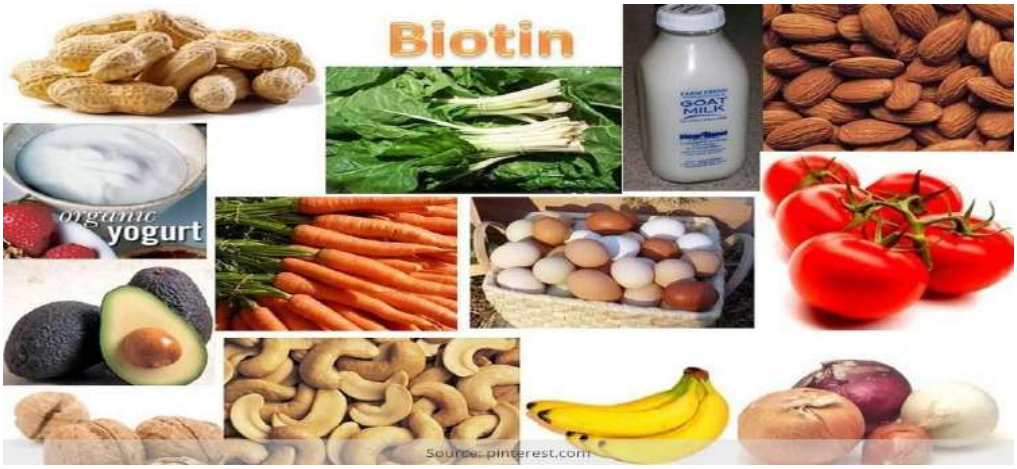


Fig 12.5: Sources of Biotin

7) **Folic acid (Vitamin B_9):** It is a pteridine derivative synthesized by intestinal bacteria. It is rich in leafy vegetables.

Dietary sources: Leaves and foliage of spinach, whey, mushrooms, liver, yeast, bone marrow, soya beans and fish. In moderate amounts, it is also present in fruits like oranges, bananas, etc. It is synthesized by bacteria.

Daily requirement: Adult: 100 µg. per day.

Pregnant women**:** 300 µg. per day.

Lactation women: 150 µg. per day.

Deficiency diseases: Folic acid deficiency leads to impairment in the biosynthesis of purines and pyrimidines. This leads to diarrhea, depression, confusion, and during pregnancy, fetal neural defects and brain defects.

Fig 12.6: Sources of Folic acid.

8) **Cyanocobalamin (Vitamin B_{12}):**It is a vitamin containing cobalt. It is essential for the growth of epithelial cells. It is required in the formation of myelinated nerve fiber, blood, and fatty acid synthesis and energy production.

Dietary sources: the rich sources of this vitamin are milk, fish, liver, kidney, pork, egg, chicken curd, etc.

Daily requirement: Adults: 3µg. per day.

Children: 0.5 – 1.5 µg. per day.

Pregnancy and lactating women**:** 4µg. per day.

Deficiency diseases: deficiency of this vitamin causes pernicious anemia and peripheral neuritis. It is essential for normal maturation and development or erythrocytes. Deficiency of this vitamin leads to neurological disorder rand degeneration of peripheral nervous tissue causing numbness, tingling in fingers and toes.

Fig 12.7: sources of vitamin B $_{12}$

9) **Ascorbic acid (Vitamin C):**It is a vitamin which is found in citrus fruits, involved in the repair of tissues and the enzymatic production of certain neurotransmitters. It is important for proper functioning of immune system. It works as antioxidant.

Dietary sources: Citrus fruits like orange, lemon, etc. tomatoes, potatoes, cabbage, papaya, amla, etc.

Daily requirement: Children: 45 mg. per day

Adults: 80 mg. per day

Pregnant women: 110 mg. per day.

Deficiency diseases: Deficiency of this vitamin results in dry and rough skin. It also results in increased rate of infections, sore joints and bones and scurvy.

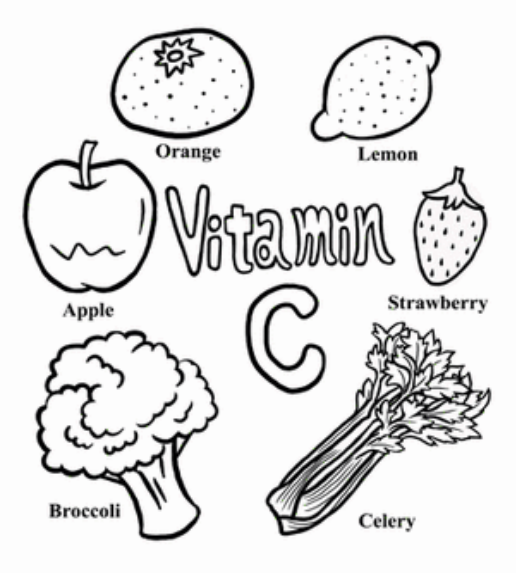


Fig 12.8: Sources of vitamin C

10) **Miscellaneous vitamins:** They are compounds which function like vitamins.

a) **Choline**:

- It is a component of phosphor-lecithin involved in membrane structure and lipid transport.
- Choline is present in liver, chicken, cauliflower, spinach, peanuts and almonds.
- Daily requirement is 425 mg. per day in adults. In pregnant women about 550 mg. is required.
- Choline deficiency include liver diseases and neurological disorders.

b) Lipoic acid:

- It is also called as acetate replacement factor. It is also called as Protogene.

- Lipoic acid is found in all foods like kidneys, heart, liver, broccoli, etc.
- Lipoic acid is involved in dicarboxylic reactions.

c) Inositol:

- It is a carbohydrate, but does not belong to any specific sugar.
- It is required for synthesis of phosphatidylinositol, constitute of cell membrane. It also acts as a lipotropic factor.
- It is found in cereals, high bran content, beans, fruits, etc.

Fat soluble vitamins:

These are soluble in fat. Bile salts facilitate their absorption through GIT. The fat soluble vitamins are:

1. Vitamin A
2. Vitamin D
3. Vitamin E and
4. Vitamin K.

1) **Vitamin A**: It is useful for maintenance of integrity of epithelial tissue. It is necessary for healthy skin and general growth also.

Dietary sources: yellow vegetables and fruits, cod liver oil, Shark liver oil, etc.

Daily requirement: Children: 1500 – 5000 I.U.

Adults: 5000 I.U.

Deficiency diseases: Xerophthalmia, Keratomalacia, etc.

Diseases caused by excess Vitamin A: Sluggishness, head ache, roughening of skin, etc.

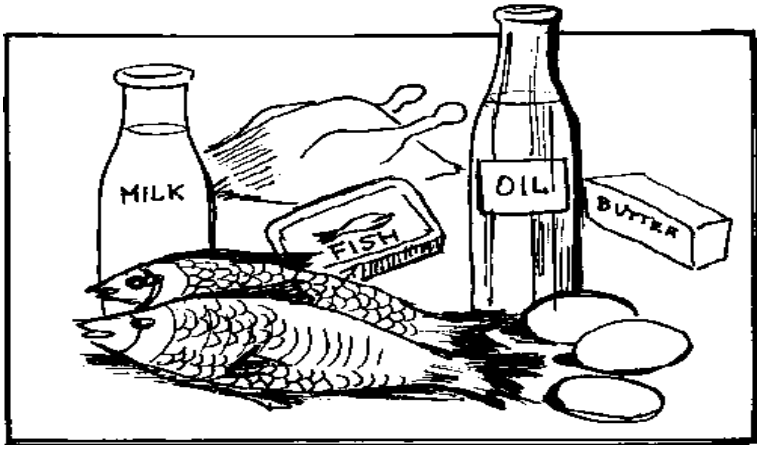


Fig 12.9: Sources of vitamin A

2) **Vitamin D:**They are sterols. It is essential for increasing absorption of calcium, magnesium, etc.

Dietary sources: Liver of fish, eggs, milk, butter etc.

Daily requirement: 200 – 400 I.U.

Deficiency diseases: Rickets in children and osteomalacia in adults.

3) **Vitamin E**: It is also called Tocopherol.

Dietary sources: Eggs, meat, liver, fish, corn oil, cotton seed oil, etc.

Daily requirement: 25 – 30 mg. per day.

Deficiency diseases: Hemolysis, anemia, hepatic necrosis, etc.

Fig 12.10: sources of vitamin E

4) **Vitamin K:** It is essential for coagulation of blood. It catalyze the synthesis of prothrombin by liver.

Dietary sources: Green leafy vegetables, cauliflower, carrots, milk, etc.

Daily requirement: Average diet contains quantities adequate for adults.

Deficiency diseases: Its deficiency is not reported in healthy individuals. Its deficiency is reported in infants and new born with mother's diet of low quantities of vitamin K. its deficiency causes hemorrhagic conditions.

Fig: sources of vitamin K

Fig 12.11: Vitamin K rich food

12.2 Minerals

Definition: Minerals are substances found in food that are essential for growth and health. They are supplied through diet.

Classification: They are classified into-

1. Macro elements(Principal elements)
2. Trace elements.

1) ***Macro elements (Principal elements)***: They are seven in number. They are Sodium, Potassium, Calcium, Phosphorous, Magnesium, Chlorine and Sulphur.

a) **Sodium:** It is the major cation of extracellular fluid. It is required for maintaining – neuromuscular function, heart beat initiation, cell permeability and normal water balance.
Dietary sources: Bread, cheese, table salt, carrots, cauliflower, etc.
Daily requirement: Adults: 5 – 15 g. per day.
Deficiency diseases:In Hyponatremia, lower concentration of Sodium in blood than normal leads to dizziness, lethargy, malaise. In Hypernatremia, higher values are present which leads dehydration.

b) **Potassium**: Itis the major cation of intracellular fluid. It is useful for maintaining cardio-muscular activity, osmotic pressure, acid - base balance, and water retention and protein biosynthesis in ribosomes.
Dietary sources: Chicken, beef, liver, banana, etc.
Daily requirement: About 4 g. per day.

Deficiency diseases:Deficiency of potassium is called hypokalemia, which causes injury to myocardium and kidneys, etc. Condition of excess of potassium is called hyperkaliemia, which causes renal failure, severe dehydration and Addison's disease.

c) **Calcium**: It is essential along with Phosphorous for the formation of bones and teeth. Ionic calcium is essential for coagulation of blood, nervous excitability and neuro – muscular transmission.

Dietary sources: Milk, cheese, egg, cabbage, cauliflower, etc.

Daily requirement: 1 – 1.2 g. per day.

Deficiency diseases: Tetanus, rickets, osteomalacia, osteoporosis, renal rickets, etc.

d) **Phosphorous**: It is essential along calcium for formation of bones and teeth.

Dietary sources: Milk, cheese, egg yolk, meat, etc.

Daily requirement: 240 – 1200 mg. per day.

Deficiency diseases: Low values leads to rickets, renal rickets, hyper - parathyroidism.

e) **Magnesium**: 70% of body's magnesium content is combined with calcium and phosphorous.

Dietary requirement: Milk, eggs, cabbage, etc.

Daily requirement: 100 – 300 mg. per day.

Deficiency diseases: Deficiency leads to depression, muscular weakness, convulsions, etc.

f) **Chlorine**: It is essential as chloride of sodium for acid base balance. Chloride ion is also essential for water balance, osmotic regulation, production of HCl, activation of amylase.

Dietary sources: Sodium chloride.

Daily requirement: 5 – 20 mg. per day.

Deficiency diseases:Diarrhea, sweating and vomiting.

g) **Sulphur**: It is present in Sulphur containing amino acids, cysteine and methionine.

Dietary sources: Cysteine and methionine.

Deficiency diseases: Increased levels are observed in renal impairment, pyloric and intestine obstruction, leukemia, etc.

2) ***Trace elements:*** They are required in small quantities. On the basis of essentiality, they can be classified into-

a) Essential trace elements: Iron, Iodine, Copper, Cobalt, Fluorine**.**

b) Possibly essential elements: Nickel, Tin, Vanadium, etc.

c) **Non-essential elements:** Aluminium, Boron, Lead, Mercury, etc.

a) Essential trace elements:

1. **Iron:**Total iron present in normal adult body is about 5 g. It is present in hemoglobin, myoglobin and plasma.

Dietary sources: Liver, heart, kidney, fish, egg, etc.

Daily requirement: 10 – 18 mg. per day.

Deficiency diseases: Anemia.

Symptoms due to excessiveness: Siderosis, nutritional Siderosis and hemochromatosis.

2. **Iodine**: It is required for biosynthesis of Thyroxine and Tri iodothyronine.

   Dietary sources: sea foods, vegetables, etc.

   Daily requirement: 100 – 200 µg. per day.

   Deficiency diseases:Goiter in adults, Cretinism in children.

3. **Copper:** It is required for normal functioning of metabolism along with other nutrients.

   Dietary sources: Sea food, whole grains, legumes, lemons, etc.

   Daily requirements: 1 – 3 mg. per day.

   Deficiency diseases: Illnesses that reduce digestion, loss of appetite, anemia, etc.

4. **Cobalt:** Cobalt is an important micronutrient in our body. It is present in liver. It helps in blood formation. It is utilized in the synthesis of vitamin B_{12}.

   Dietary sources: Liver, meat, clams, goat milk, oysters, apricots, etc.

   Daily requirement: 5 – 8 mcg. Per day.

   Deficiency diseases: Its deficiency results in lack of vitamin B_{12} which leads to pernicious anemia and nervous system disorders.

5. **Fluorine:** It is essential for development of teeth and bones.

   Dietary sources: Drinking water.

   Daily requirement: Drinking water containing 1 – 2 ppm. Of fluoride.

   Deficiency diseases: Dental caries in children.

   Symptoms due to excessiveness: Dental fluorosis, hyper calcification of bones, etc.

Minerals	
Amounts Per Serving Size 339 g **(1 Cup)**	
Calcium	20.3 mg
Iron	1.4 mg
Magnesium	6.8 mg
Phosphorus	13.6 mg
Potassium	176 mg
Sodium	13.6 mg
Zinc	0.7 mg
Copper	0.1 mg
Manganese	0.3 mg
Selenium	2.7 mcg
Fluoride	23.7 mcg

Fig 12.12: Table showing minerals acquired from one cup of food.

Conclusion

Vitamins are organic substances essential for life. They are classified into

1. Water soluble vitamins
2. Fat soluble vitamins

Minerals are of two types:

1. Macro elements
2. Trace elements.

Summary

Vitamins are organic substances essential for life. They are classified into –1) Water soluble vitamins and 2) fat soluble vitamins. Water soluble vitamins are Thiamin, Riboflavin, nicotinic acid, pantothenic acid, pyridoxine, biotin, folic acid, cyanocobalamin, lipoic acid, etc. Fat soluble vitamins are vitamins A, D, E and K.

Minerals are also important for the body. They are two types-1) Essential elements and 2) Trace elements.

Macro elements are seven. They are sodium, potassium, calcium, phosphorous, magnesium, chlorine and Sulphur. Trace mineral elements can be classified into essential, possibly essential and non-essential elements.

Model Questions

Short Answer Type Questions

1. Write the sources of vitamin A and D.
2. Mention the daily requirements of A and D vitamins.
3. Write the deficiency diseases of vitamin A.
4. What are the deficiency diseases of vitaminC?
5. Mention the deficiency diseases of vitamin K and E?
6. What are macro elements?
7. Write the functions of Sodium.
8. What is hyperkaliemia?
9. Name the conditions of hyponatremia.
10. How is chlorine essential for our body?
11. Give the symptoms that occur due to excessiveness of Fluorine in water.

Long Answer Type Questions

1. Classify vitamins and minerals.
2. Write about fat soluble vitamins.
3. Discuss various water soluble vitamins.
4. Write a note on macro elements or principal elements.

Chapter - 13

Blood Glucose Levels

Structure

13.1 Blood Glucose

13.2 Determination of Blood Glucose or Blood sugar.

Learning Objectives

1. Student should know about different biochemical estimations.
2. Student should practise to calculate the concentration using O.D. values of different determination in any medical.
3. Student should conduct a survey in the surroundings to get view of types of blood sugar and increasing cases in children and elders.

13.1 Blood Glucose or Blood Sugar

The end products of carbohydrates in food are glucose, galactose, fructose and pentose. The major function of carbohydrate in metabolism is as fuel to be oxidized and provide energy for metabolic activities. The main blood glucose is α-β-D glucose and is added in blood by the following ways:

1) Absorption from the intestine.
2) By glycogenolysis.
3) By gluconeogenesis.

From the blood circulation, glucose is reduced by;

1) Conversion of liver glycogen.
2) Conversion to tissue glycogen.
3) By synthesis of fats.
4) Synthesis of lactose and glycoprotein.

Because of these processes, the blood glucose levels remain constant up to 100 mg/dl.

Diabetes mellitus:

Definition: Diabetes mellitus is a chronic disease due to disorder of carbohydrate metabolism.

Conditions leading to diabetes mellitus:The blood sugar level remains steady between 70 - 100 mg/dl during the 24 hours and following food intake, it rises up to 140 – 150 mg/dl. ***Insulin***is a hormone produced by Beta cells of Islets of Langerhans of Pancreas. It increases utilization of glucose by tissues. When there is absent or deficient secretion of insulin by Beta cells of Islets of Langerhans, glucose cannot be utilized by the cells properly resulting in its increased level in blood which is the characteristic condition of diabetes mellitus.

Hyper glycaemia:When the fasting blood glucose level is more than 120 mg/dl, the condition is called Hype glycaemia, which is the characteristic condition of diabetes mellitus.

Hypo glycaemia: When fasting blood glucose levels fall below 72 mg/dl, the condition is called Hypo glycaemia. It is most common when associated with treatment of diabetes.

Glycohemoglobin:Glycohemoglobin is the type of hemoglobin seen in red cells in patients with chronic diabetes. These are represented as HbA1a, HbA1b and HBA1c which comprise about 1.6%, 0.8% and 4% respectively of the total hemoglobin. These are collectively measured as***HbAabc or HbA1***. HbA1 levels reflect carbohydrate imbalance than GTT or fasting glucose concentration. Normal range of HbA1 is 5 – 8 %.

Types of diabetes mellitus:The new classification system identifies four types of diabetes mellitus. They are –

1. **Type 1 diabetes mellitus:** It is characterized by Beta cell destruction caused by autoimmune process, usually leading to absolute insulin deficiency. Over 95% of persons develop this before the age of 25. This is seen in persons having a family history of type 1 diabetes mellitus.
2. **Type 2 diabetes mellitus**: It is characterized by Insulin resistance in peripheral tissue and an insulin secretory defect of beta cells. This is the most common form of diabetes mellitus and is highly associated with a family history of diabetes, older age,obesityand lack of exercise.

3. **Other specific types of diabetes mellitus**: Types of diabetes mellitus of various known etiologies are grouped together to form the 'other specific types of diabetes mellitus'. This group includes persons with genetic defects of beta cells function.
4. **Gestational diabetes:**Women who develop type 1 diabetes mellitus during pregnancy and women with undiagnosed asymptomatic type 2 diabetes mellitus during pregnancy are classified with gestational diabetes mellitus. This gets resolved in most women after delivery but they have increased risk of developing type 2 diabetes mellitus later in her life.

Detection of diabetes mellitus: Determination of blood sugar is important in diabetes mellitus. Blood sugar is determined by the following methods:

a) **Fasting blood sugar (FBS):**The blood sample is collected after the patient fasts for 12 hours or overnight.
b) **Post-prandial blood sugar (PPBS):** Blood is collected one and half to 2 hours after the intake of rich carbohydrate meal.
c) **Random blood sample (RBS):** Blood is collected at any time, regardless the time of food intake.

13.2 Determination of Blood Glucose.

Blood glucose is determined mainly by two tests. They are:

1. GOD – POD method.
2. GTT method.

1) GOD – POD method: This is the enzymatic method to determine blood glucose.

Principle:

Glucose undergoes oxidation by the action of the enzyme glucose oxidase to give gluconic acid.

$$\text{Glucose} + H_2O + O_2 \xrightarrow{\text{GOD}} \text{gluconic acid} + H_2O_2$$

H_2O_2 formed in this reaction is cleaved into water and oxygen by the action of peroxidase enzymes.

POD

H_2O_2 ----------> $H_2O + ½ O_2$

Nascent oxygen formed in this reaction reacts with 4 amino phenazone to give pink colored compound.

Color intensity of this compound is directly proportional to the concentration of glucose in plasma/serum. Concentration of glucose in plasma/serum can be calculated by comparing with the intensity of color developed with similarly treated standard.

Wave length: 530 nm.

Specimen: Fluoride plasma / serum. (It is to be collected within 30 minutes after collection of blood.)

Reagents:

1. **Buffer – Enzyme reagent:**
   a) Glucose oxidase (GOD) : 650 units
   b) Peroxidase (POD) : 500 units
   C) 4 – Amino phenazone : 20 mg.
   d) Sodium azide : 30 mg.
   e) Phosphate buffer (M/0) : 100 ml.
   - Dissolve all the contents in 100 ml. of M/10 phosphate buffer.
2. **Phenol reagent:**
   a) Phenol : 1 g.
   b) 0.1 N HCl : 1 liter.
   - Dissolve phenol in 750 ml. of 0.1 N HCl and dilute to 1 liter with 0.1 N HCl.

Procedure:

1. Take 3 test tubes and label them as T, S and B representing test, standard and blank respectively.
2. Pipette the reagents into the tubes as follows-

S No.	Reagent	T	S	B
1.	Glucose reagent	3 ml.	3 ml.	3 ml.
2.	plasma/serum	0.02 ml.	---	----
3.	Glucose Standard	---	0.02 ml.	----
4.	Distilled water	---	---	0.02 ml

3. Mix and keep at 37^0C for 15 minutes or at lab temperature for 3 minutes.

4. Determine O.D. at 530 nm. Wave length.

5. Determine plasma glucose concentration using the formula-

$$\text{Plasma / Serum glucose} = \frac{\text{O.D. of the test}}{\text{O.D. of the standard}} \times \text{concentration of standard (100 mg/dl)}$$

Normal values: 70 – 120 mg/dl on fasting.

2) GTT method: In some circumstances, a glucose tolerance test is significant to diagnose the unknown cases of diabetes mellitus. Assessment of glucose tolerance of an individual is called glucose tolerance test (GTT).

Preparation of the patient: Patient should be kept on balanced diet containing 300 g. of carbohydrate per day for three days prior to the test. Patient should be on fasting for 10-14 hours before reporting to the laboratory. Coffee or tea without sugar can be allowed in the morning. Patient is restricted from smoking or chewing tobacco till the analysis is over.

Specimens

1. Fasting blood specimen.

2. Fasting urine specimen.
3. Post glucose blood specimens for fasting urine specimen negative cases in the interval of 30 minutes (4 0r 5 specimens) in adults and children.
4. For pregnant patient, blood samples are obtained every hour for 3 hours. Timing is begun when the patient begins to drink the glucose solution.
5. One post prandial blood specimen for fasting urine specimen positive cases.
6. Post glucose urine specimen for unknown diabetics after each blood specimen (at least 2 specimens)

Procedure

1. Collect fasting blood and urine specimens.
2. Test the fasting urine specimen by Benedict's qualitative method.
3. If the fasting urine specimen is positive for glucose, collection of post prandial blood specimen is enough.
4. If the fasting urine specimen is negative for glucose, adults are then given a drink containing 75 g. of glucose. Children are given ideal 1.75 g/kg body weight up to 75 g. Pregnant patients are given 100 g. of glucose. This quantity of glucose is given by dissolving in about 300 ml. of cold water. Risk of vomiting may be avoided by addition of lemon juice to the glucose solution.
5. Collect 4 or 5 post glucose blood and urine specimens at ½ an hour intervals.
6. Determine glucose content in each blood specimen and test qualitatively each sample of urine for sugar and ketone bodies.

Interpretation

1. **Normal glucose tolerance:**
    - Concentration of glucose in fasting blood specimen will be in the normal range.
    - Maximum blood glucose level is reached in the first or second post glucose specimen.
    - Blood glucose reaches normal within 2 hours after oral glucose administration.
    - Urine sugar is absent all throughout.

2. **Decreased glucose tolerance**:
    - Values in fasting blood specimens are high. Post glucose/ post prandial values are higher. Return of these values to normal is delayed.
    - Urine specimens are positive for Benedict's qualitative test.
3. **Increased glucose tolerance**:
    - Fasting blood glucose may be below limits. Only a small raise may be observed in blood glucose.

Case study of Normal Response:

S. No.	Time	Blood Glucose (mg/dl)	Urine sugar
1.	Zero hour	80	Negative
2.	½ hour	130	Negative
3	1 hour	140	Negative
4	1 & ½ hour	100	Negative
5	2 hours	80	Negative

1. After administration of oral glucose solution, zero hour blood glucose level is normal.
2. Half an hour specimen shows raise in glucose level to 130 mg/dl.
3. One hour blood specimen shows maximum raise to 140 mg/dl.
4. One and half hour blood specimen shows a fall of glucose level to 100 mg/dl.
5. Two hours sample shows further fall to 80 mg/dl.
6. It shows that, there is maximum raise in blood glucose within 1 and ½ hour and has fallen down to normal and it equals zero hour sample at the end, and urine sugar is negative throughout.

Conclusion

Study of disease and its related diagnostic tests are useful for assessing the clinical conditions of a patient.

Summary

Insulin is the hormone secreted by beta cells of islets of Langerhans in Pancreas which maintains blood glucose levels. Raise of blood glucose is termed as diabetes mellitus. Normal blood glucose is 75 – 120 md /dl. Hyper glycaemia is raise in blood glucose and hyper glycaemia is fall of blood glucose levels. There are 4 types in diabetes mellitus. Type 1, type 2, other specific type and gestational diabetes.

Blood glucose determination is an important in diagnosis and assessment of diabetes mellitus. Fasting blood, post prandial blood and random blood is collected from patient to diagnose diabetes mellitus, depending on the requirement. GOD – POD method is important method to determine blood glucose. Glucose tolerance test (GTT) is significant in the diagnosis of unknown cases of diabetes mellitus.

Model Questions

Short Answer Type Questions

1. What is diabetes mellitus?
2. How is Glucose added in to the blood?
3. From blood circulation, how is glucose reduced?
4. Write the normal values of blood sugar?
5. Mention the different types of diabetes mellitus.
6. Define a) Hyper glycaemia b) Hypo glycaemia.
7. Name the hormone which helps to maintain blood glucose levels normal. Where is it secreted?
8. What is Glyco hemoglobin?
9. Write the normal range of HbA1.
10. Mention the different types of blood samples collected to determine blood sugar.
11. What is fasting blood specimen?
12. Give the principle of blood sugar determination by GOD – POD method.
13. What is GTT?
14. Give the list of specimens required for glucose tolerance test.

15. While giving glucose to patient during GTT, how do you avoid vomiting?
16. What is normal glucose tolerance in GTT?
17. How do you interpret decreased glucose tolerance in GTT?

Long Answer Type Questions

1. What is diabetes mellitus? Explain the types of diabetes mellitus and types of blood samples collected to determine blood glucose.
2. Write in detail about GOD – POD method for the determination of blood sugar.
3. In which conditions GTT is preferred? Write in detail about GTT.

--

MEDICAL LAB TECHNICIAN

Paper - II

MICROBIOLOGY & PATHOLOGY

INDEX

UNIT 1

History of Microbiological Scientists

Structure

1.0 History of Medicine

1.1 Antony Von Leeuwenhoek

1.2 Robert Koch

1.3 Edward Jenner

1.4 Joseph Lister

1.5 Louis Pasteur

1.0 History of Medicine

Comparision of Indias Vedic sages (Ancient Indian Hindu Scientists) with Western and Europian scientist.

Birds eye view about medical lab technician course.

This contains the following steps.

Subjects and their definition

Role of Medical Lab Technician

On the Job Training (OJT)

Apprenticeship Training

Jobmela(Employement)

Birds Eye view about medical Lab Technician Course

1. Subjects and their defition

1. Biochemistry in both 1st and 2nd year.
2. Microbiology and pathology in 1st year and Microbiology in 2nd year.
3. Anatomy and Physiology in 1st year and Pathology in 2nd year.

Biochemistry : Biochemistry is the science of study of biochemical reactions taking place in living matter.

Microbiology : The branch of science dealing wit hthe study of microorganisms in called Microbiology.

Anatomy : The branch of science dealing with the study of structures of human body is called as Anatomy.

Physiology : The branch of science dealing with the study of functions of human body is called physiology

Pathology : The branch of science dealing with the study of diseases is called pathology.

2. Role of Medical lab Technician

Medical lab technician collect samples and performs tests to analyze body fluids, tissues and other substances.

A medical lab technician searches for basic clues to the absence, presence, extent and causes of diseases. This skilled individual is responsible for performing lab tests efficiently and accurately for high quality patient care.

Medical lab technicians are playing a vital role in the diagnosis and prevention of disease. Medical lab technician perform less complex tests and less lab procedures.

Medical Lab Technician may

- Collect specimens at specimen collecting centers
- Prepare specimens and operate automated analysers
- Perform manual tests in accordance with detailed instructions.
- Work under the supervision of concerned dept heads or lab managers.

Medical lab Technicians should have the following other skills

- Good analytical judgement and the ability to work under pressure
- Utmost attention and care is essential for med lab technicians because small differences or changes in test substances or numerical readouts can be crucial to a diagnosis.
- Mannual dexterity (skill in performing tests) and normal color vision are highly desirable.
- Because of wide spread use of automated lab equipment, computer skills are important.

3.On the Job Training

Both MLT 1st and 2nd year students will undergo OJT in the month of December every year, under the supervision of concerned class incharge lecturer for 30 days in the government hospitals or recognized well organized medical diagnostic center (preferably in government hospitals).

4. Apprenticeship Training

The 2nd year MLT students will be given apprenticeship training for one year in the various medical laboratories in the district head quarters and in the surrounding places of that zone after the announcement of their 2nd year results, under the chairmanship of Board of apprenticeship Training (BOAT) Chennai with the assistance of SIVE staff and guidance. This apprenticeship training is a must for every MLT 2^{nd} year student to get a job a government hospitals and primary health centers.

5. JOB Mela (Employment)

The board of apprenticehsip training (BOAT) Chennai officers will conduct JOB mela every year in the month of June at zonal level in the state with the assitance of SIVE staff at a single stretch. Every MLT pass out 2nd year student should and must attend the JOB mela after completing their apprenticeship training. Only apprenticeship training completed fresh students will be given importance in the JOB Mela.

Medical lab technician's course has a wide range of job potentiality and talented, hard working, worthy students flourish like any thing and even pass out students also settle easily in their life.

1.1 Antonyvan Leeuwenhoek

Bacteria, and other microorganisms, were first observed by 'Antonie van Leeuwenhoek' in 1676 using a single-lens microscope of his own design. He doing so Leeuwenhoek made one of the most important discoveries in biology and initiated the scientific fields of bacteriology and microbiology. He is the first microbiologist.

Fig. 1.1 Antony Van Leeuwenhoek

He is best known for his work on the improvement of the microscope and for his contributions towards the establishment of microbiology.

1.2 Robert Koch

Robert Koch was born on December 11th 1843 in Germany. Robert Koch was the Father of medical microbiology and discovered the Bacteriology. Robert postulated the germ theory, has seperated the Anthrax Bacillus, tuberculosis, and Vibrio cholera. Robert Koch introduced the staining techniques. Robert Koch got the Noble Prize in Medicine in 1905. Robert Koch was died on 27th May 1910.

Fig. 1.2 Robert Koch

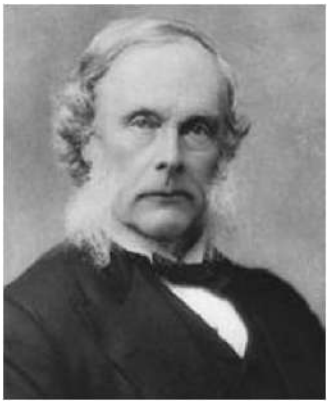
1.3 Edward Jenner

Edward Jenner was born on 17th May 1749 in London. Jenner used to study his natural surroundings. Edward Jenner was the Poineer of SmallPox Vaccine. Jenner is also sometimes called as the "Father of Immunology". Edward Jenners Works gave the life to many than any others Works. Edward Jenner has prepared the Cow Pox Vaccine to protect the smallpox. Edward Jenner was died on 26th January 1823.

Fig. 1.3 Edward Jenner

1.4 Joseph Lister

Lister was born on 5th April,1827 in United Kingdom.Lister was Famous for surgical sterile techniques.Lister used the Carbolic Acid in antiseptic surgery



.

Fig. 1.4 Joseph Lister

Lister confirmed Pasteurs conclusions(to eliminate the microorganisms responsible for gangrene by filtration, exposure to heat or exposure to chemical

solutions) by conducting experiments on his own and developed the antiseptic techniques for Wounds. Lister died on February 10th 1912.

1.5 Louis Pasteur (Father of Modern Microbiology)

Louis Pasteur is often referred to as the "Father of Microbiology" (along with scientist Robert Koch) for his contributions to discerning the cause and prevention of disease. He is best known for inventing the process of pasteurization, creating the first vaccine for rabies, and the germ theory of disease. Louis Pasteur was born on December 27th 1822 in France (Dole). He was a French Chemist and microbiologist.Pasteur was the first microbiologist to prepare the vaccine for the rabies and anthrax. Experiments of Pasteur supported the germ theory of disease. Pasteur invented the pasteurisation and sterilisation techniques. Pasteur is the one of the main founders of microbiology together with Ferdinand Cohn and Robert Koch.Pasteur explained about Fermentation theory.Pasteur worked in the fields of chemistry,mostly notably assymetry of certain crystals on the molecular basis.Pasteur died on September 28th 1822 in France.

Fig. 1.5 Louis Pasteur

Key Terms

Microbiologist: the person who is engaged with scientific study of topics of microbiology

Microscope : Instrument/tool used to observe the objects and the microorganisms which can not be seen with our naked eye.

Immunology : The branch of science dealing with the study of immunity to infection.

Spermatozoa : The male sex cell of animal/human being that fertilizes with the egg/ovum.

Bacteria : A group of microorganisms most of which can cause diseases

Acid Fast : Not readily decolourised by acids after staining

Bacilli : The rod shaped bacteria is called as bacilli

AFB : Acid Fast Bacillus

Vaccine : A substance prepared from the microorganisms it self and when injected onto the body provides a immunity against a disease is called as vaccine.

Smallpox : A serious disease which forms vrious layers or blisters on the skin and leaves the permanent scars.

Germ : A microorganism which causes disease/a part of an oorganism developing into a new one.

Pasteurisation : The process of heating and cooling to destroy the germs present in the milk

Fermentation : The process of undergoing a chemical change by the action of yeast or bacteria.

Infection : The process of causing and producing a disease to a person or animal by a germ or bacteria

Surgery : The process of medical treatment which involves cutting open the body and repairing or removing the parts.

Short Answer Type Questons

1) Name the scientists who contributed their services to the Microbiology field.
2) Mention any two contributions of Louis Pasteur
3) Write the contribution of Robert Koch and and Edward Jenner in MicroBiology
4) Write the contribution of Joseph Lister in the MicroBiology.
5) Mention the contributions of Edward Jenner and Joseph Lister in MicroBiology.
6) Mention the contributions of Robert Koch and Antony Van Leeuwen hoek.
7) Mention the achievements of Joseph Lister and Louis Pasteur in Microbiology.

Long Answer Type Question

a) Write thecontributions of following scientists in Microbiology
   - Antony Van Leeuwen Hoek
   - Louis Pasteur
   - Joseph Lister

b) Mention the contribution of following scientists in Microbiology
   - Robert Koch
   - Edward jenner
   - Louis Pasteur

UNIT 2

Microscopy

Structure

2.1 Principle, Working and maintenance of Compound microscope.

2.2 Principle of Fluorescent microscope and Dark field microscope

Introduction

A Compound microscope is used to produce an enlarged and well defined object images which can not be seen with our naked eyes.The degree of enlargement is called as magnification.With the invention of Microscopes,it has become easy for the Microbiologists and Pathologists in identifying the microorganisms and blood components and slide preperations for finding out the abnormal condition of a patient.With the use of compound Microscope,the morphology of bacteria,both Gram positive and Gram negitive bacteria, can be known.Various pathological slides were screened and observed with the help of Compound Microscope and results were given.With the help of Flouroscent Microscope, Mycobacteria can be identified.Very slender organisms like Spirochaetes can be identified with the help of Dark field Microscope.

2.1 Compound Microscope

Principle and Working

The Microscope is used to Visualise Stained and Unstained Microrganisms like Bacteria and Fungi and to identify them. The Microscope magnifies the image of the Object to be visualised through it. The laboratory Microscopeprovides a magnification of

40(scanner) x100(low power) x 400(High power) x 1000 (Oil immersion)

The total magnification is obtained by multiplying the magnification of the objective with that of eyepiece.

Total Magnification= Magnification of eye piece x Magnification of the objective.

Parts of the Microscope

The Microscope consists of

a) Stand
b) Mechanical adjustments
c) Optics or the Lens.

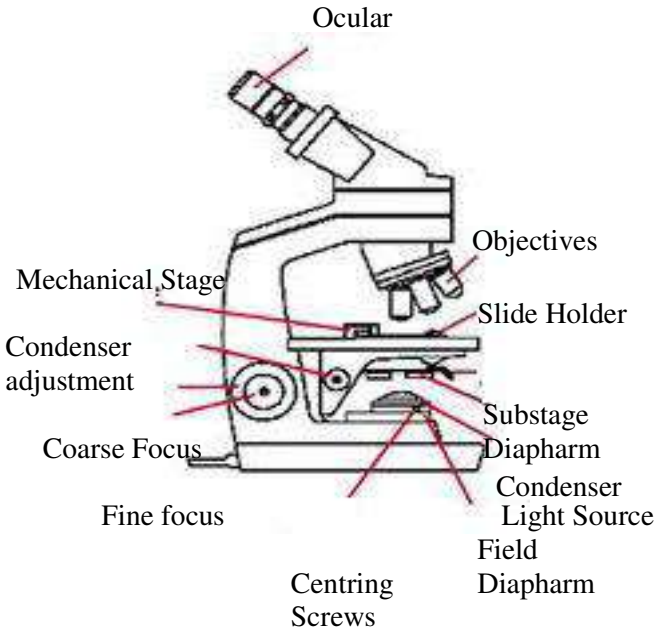


Fig.2.1 Mono-Ocular Microscope

BINOCULAR

1. Stand

The Stand consists of

- Tube-Supports objective and eye piece
- Body-Gives support to the tube
- Arm-The Microscope is held with the arm.It gives correct height and angle to the body and the tube.
- The stage has a pair of spring clips to hold the microslide.
- The sub-stage holds the condenser lense with the iris diaphragm and a holder for fitting.
- The horse shoe shaped foot which supports the body of the microscope

2. Mechanical Adjustment

The different adjustments are

- Coarse adjustment
- Fine adjustment
- Inclination
- Condenser adjustment
- Aperture adjustments
- Stage

Coarse Adjustment: Controlled by a pair of large knobs, one on each side of the body. It helps to move the tube with its lenses.

Fine Adjustment: Necessary for high power lences and oil immersion lenses. It is controlled by two smaller knobs on each side of the body.

Draw Tube: Used for adjusting distance between objective and eye piece lence

Inclination: Arm is tilted upon the foot by a finger.

Condenser Adjustments: The condenser is focused by rotating a knob below the stage.

Aperture Adjustments: This is done by iris diaphragm.

Stage: It has the knobs for the to and fro, side to side movements of slidesover the stage.

3. Microscope Optics

The following are the different parts of the microscopic optics

- Monocular microscope
- Binocular microscope
- Eye piece
- Objective
- Numerical aperture
- Oil immersion objectives
- Condenser and Iris
- Mirror
- Light

Monocular Microscope

Simple compound microscope is the example for this.This has only one eye piece

Working of the Compound Microscope

This constitutes the following two steps

I. Setting of the Microscope
II. Focussing the object

I. Setting of the Microscope

This again consists of the following steps

- Positioning of the microscope
- Fitting the accessories of the microscope.
- Positioning the lamp (if electric illumation is to be used)
- Preliminary adjustment of the mirror.
- Centring the condenser.
- Adjustment of the diaphragm.
- Adjustment of eye pieces.

Positioning of the Microscope

- Microscope should be placed on a firm level bench or on a piece of heavy cloth.The ideal height of the platform is 2 ft 8 in.
- The bench should be placed near the window,if day light is to be is used.
- Place the microscope away from the window,if electricillumination is to be used.

Fitting the Accessories of the Microscope

- In a clock wise Direction, fit the objectives into the revolving nosepiece.
- Put the eye pieces in proper place.
- Fit the condensor under the stage.
- Fix the mirror on the foot

Positioning the lamp(if electric illumunation is to be used)

- Facing the mirror,keep the lamp about 20 cms infront of the mirror
- At about an angle of 45 degrees fix the mirror

Preliminary adjustment of the mirror

- Use the plain side of the mirror
- To the maximum,open the iris diaphragm
- Next raise the condenser
- Then on the top of the condenser,place a piece of thin white paper
- To get the image of the bulb in the exact center of light circle,adjust themirror

Centering the Condenser

- On the stage (without condenser) place slide preparation.
- Open the iris diaphragm after lowering the condenser.
- With lowest power objective, examine through eye piece. Bring the object into focus
- Raise the condenser slowly until the edges of light ring circle are in sharp focus and then close the diaphragm.
- Adjust the position of the mirror if necessary in such a manner that light circle is in exact center of the bright area surrounded by the dark zone.
- Then adjust the condensers centring screws accordingly.
-

Adjustment of the Diaphragm

- First open the diaphragm completely
- Look down the tube after removing the eyepie
- Until the light circle on the objective takes up only 2/3 of the surface,slowly close the diaphragm.
- For each objective repeat this procedure.

Adjustment of Eyepieces

- Select the eyepiece(X5orX6)
- If binocular is used,the distance between the pupils should be adjusted

II. Focussing the Objective

Focussing the objective consists of the following steps

- Use of lowpower objective
- Use of high power objective
- Use of oil immersion objective

Use of low power objective(X5orX10)

- To the lowest position,adjust the condenser
- Until the objective is just above the slide preparation,lower the objective
- Until a clear image is seen in the eye piece,using the coarse adjustment

Use of high power objective(X40)

- About half way down,adjust the condenser.
- If already the object is set to a low power objective,until a blurred image appears on the field,raise the objective very slowly by using the coarse adjustment.
- Using the fine adjustment,bring the image into focus.for sufficient illumination,raise the condenser.

Use of oil immersion objective(X100)

- To the uppermost position, adjust the condenser.Open the iris diaphragm fully
- On the dry stained slide preparation,place a small drop of immersion oil
- Until the objective is in contact with the oil,lower the objective.

Care and Maintenance of the Microscope

This involves the following steps.

- Requirements: Tissue paper, Xylene, Soft cloth, Petrolium jelly, Plastic cover,a fine paint brush.
- Cleaning the objectives
- Cleaning the eyepieces
- Cleaning the condenser and mirror
- Cleaning the support and the stage

Precautions

- When microscopic examination is carried out under oil immersion lens,Cedar wood oil is widely used
- Since paraffin oil or mineral oil often enters the objective,it should not be preferred.
- When microscope is not in use, dont leave the slides on it. Dont touch the lenses at any time.
- Cover the microscope with pastic cover when it is not in use and put the lower objective in the focussing position

2.2 Fluorescent Mircoscope and Darkfield Microscope

2.2.1 Fluorescent Microscope

Principle

This microscopes works on the principle of fluorescence.This involves the use of ultrvoilet lightwhich strikes the fluoroscent substances and visible light is emitted.Then the material stained with dye is visible.

This method causes illumination of microorganisms which are stained with fluoroscent dyes,which converts ultravoilet light into visible light by lengthening their wavelength.

Fig. 2.3 Fluoroescent microscope

This procedure is used to visualise the Mycobacteria against dark back ground. Special filter is used to remove all unwanted fluoroscent light.The second filter is placed above the eye piece to prevent damage to the eye by UV light.

Equipment : This microscope consists of the following parts

- A fluoroscent lamp
- A blue filter(primary filter)
- A yellow filter(secondary filter)
- An immersion dark ground condenser
- A non fluorescent immersion oil eg. liquid paraffin oil

Uses

- Identification of microorganiosms-Mycobacteria
- Fluoroscent antibody techniques-bacteriology and parasitology
- Histopathology
- Cytogenetics

Short Answer Type Questions

- Write the principle of compound microscope
- Write the principle of fluoroscent microscope
- Wha it is the use of numerical aperture in compound microscope?
- Write the uses of fluoroscent microscope
- Write the parts of fluoroscent microscope
- Define the objective and mention the various objectives of compound microscope

Long Answer Type Questions

- Write the construction, care and maintenance of compound microscope.
- Write about the Fluoroscent microscope.
- Draw the neat diagram of compound microscope and locate the variousParts of it.

UNIT 3

Sterilisation

Structure

3.1 Introduction
3.2 Classification of Sterilisation

3.1 Introduction

Microorganisms are ubiquitous.Since they cause contamination, infection, and decay, it becomes necessary to remove or destroy them from materials and from areas. This is the object of sterilisation.The process of sterilisation finds application in microbiology for prevention of contamination by extraneous organisms,in surgery for maintenance asepsis, in food and drug manufacture for ensuring safety from contaminating organisms and in many other situations. In todays dialy life any where across the globe, sterlisation is playing an imporatant role and gaining prominance day by day, because of health awareness among the people. Sterilisationhas wide range of applications right from diary, food industies to biological products.Sterilisation also playing an important role especially in the pharmaceutical industries,which is having direct link with the human beings.

Sterilization: Sterilisation is defined as the process by which an article, surface or medium is freed of all microorganisms either in vegetative or spore state .

Disinfection: Disinfection means the destruction of all pathogenic organisms or organisms capable of giving rise to infection.

Chemical disinfectants which can be safely applied to skin or mucous membrane surfaces and are used to prevent infection by inhibiting the growth of bacteria is called as antisepsis

Antisepsis: The term antisepsis is used to indicate the prevention of infection, usually by inhibiting the growth of bacteria in wounds or tissues.

Bactericidal agents: The agents which are used to destroy and kill the bacteria completely are called as the bactericida agents.

Bacteriostatic agents: The agents which are used to prevent the multiplication of bacteria by stopping the growth of bacteria are called as the bacteriostatic agents.

3.2 Classification of Sterilization

The various agent used in sterisation can be classified as follows;

Physical Agents

- Sun light
- Drying
- Dry Heat-Flaming,Incineration,Hot air oven

- Moist Heat-Pasteurisation,Boiling under normal pressure,Steam under pressure
- Filtration-Candle filters,asbestos disc filters,membrane filters
- Radiation.
- Ultrasonic and sonic vibrations

Chemical Agents

- Phenol and Phenolic compounds
- Alcohols
- Aldehydes
- Halogens
- Dyes
- Acids and Alkalis

Gaseous Agents

- Ethylene oxide
- Beta-propiolactone

3.2.1 Physical Agents

1. Sunlight: Sun light possesses appreciable bactericidal activity and plays an important role in spontaneous sterilisation that occurs natural cinditions.The action is primarily due to its content of ultraviolet rays. Bateria suspended in water are readily destroyed by exposure to sunlight. This is one of natural methods of sterilisation in cases of water in tanks, rivers and lakes.

2. Drying: Moisture is essential for the growth of the bacteria. Drying in air has therefore, deleterious effect on many bacteria. Susceptibility to drying varies with different bacteria and also with the conditions under which they are exposed to drying

Dry Heat Sterilisation-Heat is the most reliable method of sterilization

a) Flaming:

Fig. 3.1 Electric Gas Burner

Inoculating loops or wires,points of forceps and spatulas are held in Bunsen flame till they become red hot for sterilising them.If the loops contain infective proteinaceous material,they should be first dipped in chemical disinfectants before flaming to prevent spattering. Scalpels,needles,mouths of culture tubes,glass slides,cover slips etc,could be passed a few times through the bunsen flame without allowing them to become red hot.

(b) Red Heat: By exposing the materials to be sterilised till they get become as red hot to the flames of bunsen burner.

(c) Incineration: Through the process of burning,the conversion of material to be sterilised into ashes is called as Incineration.This is an excellent method for rapidly destroying materials such as soiled dressings,animal carcasses,bedding and pathological material.

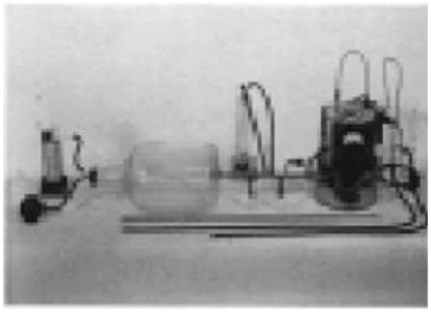


Fig. 3.2 An incinerator and aerosol chamber for testing

(d) Hot Air Oven

Fig. 3.3 Hot Air Oven

Construction and Working: This is the most widely used method of sterisation by dry heat.A holding period of 160^{o} C for one hour is used. Hot air oven is used to sterilise glassware,foreceps,scissors, scalpels, all glass syringes, and swabs. Some pharmaceutical products such as liquid paraffin, sulphonamides, dusting powder, fats, greases etc.
The oven is usually heated by electricity, with heating elements in the wall of chamber and it must be fitted with a fan to ensure even distribution of air and elimination of air pockets.It

should not be overloaded.The material should be arranged in a manner that allows free circulation of air in between.

Glassware should be perfectly dry before being placed in the oven.Test tubes,flasks,etc should be plugged with cotton wool.Other glassware such as petridishes and pipettes should be wrapped in kraft papper.For cutting instruments such as those used in ophthalmic surgery, a sterilising time of 2 hours at 150o is recommended.The oven must be allowed to cool slowly for about 2 hours before the door is opened,since the glassware may get cracked by sudden or uneven cooling.

4. Moist Heat Sterilisation

a) Temperature below 100^0C

For Pasteurisation of Milk

Boiling of milk to kill the pathogens present in the milk is called as the pasteurisation of milk. The temperature employed is either 60°C for 30 minuteswhich is alled as the holder method or 72 °C for 15 to 20 seconds followed by cooling quickly to 13 °C or lower is called as flash process.

Fig. 3.4 Pasteurisation Equipment

By these processes all nonsporing pathogens such as mycobacteria, Brucellae, and salmonae are destroyed.

(b) Inspissation

Fig. 3.5 Laboratory Inspissator

Heating at 80-85^{o}C for half an hour on three successive days in an inspissator to kill the bacteria is called as Inspissation.With this media such as Lowenstein Jensens and Loefflers serum are rendered sterile.

(c) Tyndallisation: The process of exposure of media containing sugars or gelatin to 100^{o}C for 20 minutes on three successive days is called as tyndallisation or intermittent sterilisation.
The principle is that the first exposure kills all vegetative bacteria,and if any spores present, being in a favourable medium, will germinate and be killed on sunsequent conditions

Autoclave

Principle

The principle of autoclave or steam steriliser is that water boils at which its water vapour equals that of the surrounding atmosphere

Construction and Working

The autoclave consists of a vertical or horizontal cylinder of gunmetal or stainless steel, in a supporting sheet-iron case. The lid or door is fastened by screw clamps and made air tight by an asbestos washer. The autoclave has on its lid or upper side a discharge tap for air and steam, a pressure gauge and safety valve that can be set to blow off at any desired pressure. In this autoclave,material for sterilisation is exposed to 121^{o}C for 15 to 20 minutes at 15 lbs pressure per square inch. Saturated steam heats the article to be sterilisedby releasing the latent heat. On condensation 1600 ml of steam at 100^{o}C and at atmospheric pressure condenses into 1 ml of water and liberates 518 calories of heat. The condensed water ensures moist conditions for killing bacteria. The air is poor conductor of heat and must be removed from chamber. The contents must be so packed that free circulation of steam occurs.Autoclave is used to sterilise the culture media,rubber goods,syringes and dressings.

Fig. 3.6 Auto Clave

3.2.2 Chemical Agents

Disinfection: Disinfection means the destruction of all pathogenic organisms or organisms capable of giving rise to infection

Mode of action

The chemical substances act as bactericidal agents as under:

- Coagulation of bacterial protoplasm
- Disruption of cell membrane by chemical substances. They may alter physical and chemical property of cell membrane.Thus results in killing or inhibiting the bacterial cell.
- Oxidation or burning out the bacterial protoplasm
- By affecting bacterial enzymes or coenzymes systems,thus causing interference of bacterial metabolism.

Chemical Disinfectants

The chemicals which are used for killing the organisms responsible for causing the infection are called as the chemical disinfectants.

1. Phenol and Phenolic compounds

Phenol(carbolic acid) is a powerful microbicidal substance.

Mode of action: The lethal effect of phenols is due to their capacity to cause cell membrane damage,thus releasing cell contents and causing lysis.

Uses: Phenolic disinfectants derived from coaltar are widely used as disinfectants for various purposes in hospitals.Lysol and cresols are active against a wide range of organisms.

2. Alcohols

Ethyl alcohol and Isopropyl alcohols are most frequently used.

Mode of Action: Alcohols act by denaturating the bacterial proteins.These have no action on spores and virusses. To be effective,these must be used at a concentration of 60 to 70 percent in water.

Uses: Isopropyl alcohol is used to disinfect thermometer.Methyl alcoholis effective againsat fungal spores and is used for treating the cabinets and incubators affected by them.Alcohols are mainly used as skin antiseptics.

3. Aldehydes

Formaldehyde and glutaraldehyde are the two aldehydes mainly used.

Mode of action: Formaldehyde is active against the amino group in the protein molecule.In aqueous solutions, it is bactericidal and sporicidal and also has lethal effect on viruses.

Glutaraldehyde has an action similar to that of formaldehyde.It is specially effective against tubercle bacilli,fungi and viruses.It is less toxic and irritant to the eyes and skin than formaldehyde

Uses: Formaldehyde is used to preserve anotomical species and for destroying anthrax spores in hair and wool.Glutaraldehyde is used to disinfect endoscopes.

4. Halogens

Mode of action: Iodine compounds act as bactericidal agents with a moderate activity against spores.These are active against the tubercle bacilli and a number of viruses.Clorine and hypochlorites are also bactericidal in activity.

Uses: Iodine compounds are used as skin disinfctants.Clorine and its compounds are also used as disinfectants for many years in water supplies, swimming baths, and food and diary industries.

5. Dyes

The substsnces which are used to impart colour to some thing and at the same time having the bacteriostatic and bactericidal activity are called as the dyes.Aniline and Acridine dyes belong to this category.

Mode of action: Both the aniline and acridine dyes in high dilution act as bacteriostatic and are having low bactericidal activity.These are more active against gram positive than the gram negitive bacteria. The aniline dyes in use arebrilliant green, malachite green, and crystal voilet.

Uses: The aniline and acridine dyes are extensively used as skin and wound antiseptics

6. Acids and Alkalies

Inorganic acids like boric acid and chromic acid,organic acids like benzoic acid,salicylic acid are used as effective disinfectants.

3.2.3 Gaseous Agents (Gaseous Methods of Sterilisation)

Ethylene oxide and beta propio lactone are used as gaseous agents

Fig. 3.9 An Ethylene Oxide Sterilisation Gas Chamber

Key Terms

Microorganisms: The organisms which we can not see with our nakedeye.
Pathogens: The microorganisms capable of causing diseases
Sterilisation: The process by which an article,surface,or medium is freed of all microorganisms either in vegetative or spore state
Disinfection : The process of destruction of all pathogenic organisms
Antisepsis : The process of prevention of infection usually by inhibiting the growth of bacteria.
Bactericidal Agents : The agents used to stop the multiplication of bacteria completely by killing all the bacteria.
Bacteriostatic Agents : The agents used to prevent the multiplication of bacteria by keeping the bacterial growth to a standstil.
Dry Heat Sterilisation : The sterilisation which utilises the only dry heat
Incineration : The process of converting ,materials to be sterilised ,directly into ashes, through burning
Hot Air Oven : The instrument used to sterilse the glasswares, foreceps, scalpels, all glasssyringes, swabs, etc by dry heat at 160^{o}C for 1 hour
Moist Heat Sterilisation : The sterilisation which involves the utilisation of steam
Psteurisation of Milk : Boiling of the milk by holder method(60 ^{o}C for 30 min) and flash process(72 ^{o}C for 20 sec)
Insipissation : The process of sterilising the media in an in an insipissator on three successive days at 80 to 85 ^{o}C for 30 minutes
Tyndallisation : The process of sterilising the media containg sugars or gelatin at 100 ^{o}C for 20 minutes on three seccessive days
Autoclave : The instrument used to sterilise the culture mediqa,rubber goods,syringes and dressings at 121 ^{o}C for 15 to 20 minutes
Filtration : The process of sterilisation utilised to sterilise the heat sensitive antibiotic solutions, serum and carbohydrate solutions used in the preparation of culture media
Radiation : Emission of subatomic particles (electrons)in the form of energy which is used to kill the bacteria.
Ionising Radiation : Emission of electrons in the form of energy by Ionisation.
Chemical Disinfectants : The chemicals which are used to kill all pathogenic organisms
Ethylalcohol : The chemical which is used as skin antiseptic
Isopropylalcohol: The chemical which is used to disinfect the thermometer and also used as skin antiseptic
Aniline&Acridine dyes: The dyes which are used as skin and wound anti septics.

Short Answer Type Questions

- Define sterilisation
- Define Disinfection
- Define chemical disinfectants
- What are the articles sterilised in hot air oven
- How many methods are there in moist heat sterilisation?
- What is Incineration?
- Define pasteurisation of milk.
- What is Tyndallisation?
- Define Bactericidal agent and disinfectant
- Define Bacteriostatic agent and Inspissation.
- Mention the two agents of gaseous sterilisation.
- What are the articles sterilised by the Autoclave?

Long Answer Type Questions

- Define sterilisation and mention the classification of sterilisation.
- Describe the dry heat sterilisation method briefly.
- Explain in detail about Hot air oven and Incineration
- Write the classification of sterilisation and briefly describe the moist heat heat sterilisation.
- Explain in detail about the Autoclave and pasteurisation of milk.

UNIT 4

Glassware & Biomedical Waste

Structure

4.1 Introduction

New glassware needs special attention because it may contain

- Resistant spores present in the straw and other packing material
- It may give off alkali, which may interfere with the bacterial growth.

Hence it should be placed in 10% HCL overnight, washed in tap water or distilled water and autoclaved Cleaning lab glassware is not as simple as washing the dishes at home.Some cleaning basics have to be learnt while cleaning the glassware.Some times detegents like LIQUINOX OR ALCONOX are used, which are specially designed for cleaning the glassware.But these detergents are not always preferable for cleaning the glassware.Care should be taken while cleaning the special glassware.

Remove the stoppers and stopcocks when they are not in use.Otherwise they may freeze in place.The glassware was sterilised using one of the methods of strilisation.The contaminated material either clinically infective material or inoculated culture media must be disposed, otherwise it will become as health hazard to the people. Biomedical waste consists of solids, liquids, sharps,and lab waste that are potentially infectious or dangerous and considered as biowaste. Biomedical waste differs from other types of hazardous waste such as industrial waste. Biomedical waste comes from biological sources or is used in thediagnosis, prevention or treatment of diseases. Biomedical waste producers include,hospitals,health clinics,nursing homes, medical research labs, physicians' offices,dentists, and home health care. Biomedical waste is handled by placing in specially-labelled bags and containers for removal by biomedical waste transporters. It is most important to know clearly about the cleaning,drying and sterilisation of glassware. And at the same time we have to dispose the contaminated materials like clinical infective material and inoculated culture media other wise,both if not disposed poses greater health hazards to the public.So the goverrnment should strictly frame the amendments regarding this i.e. in disposing the contaminated material from all the medical labs,hospitals,health clinics,nursing homes,medical research labs,physicians offices ,dentists and home health care.

4.2 Cleaning of Glass ware

Basics of the cleaning of the glassware

It is generally easier to clean glassware if we do it in the right way.Sometimes detergent like Liquinox or alconox is used in some special cases to clean the glassware,but always detergent and tapwater is not preferable to clean the glassware.We have to rinse the glassware with proper solvent,then finish up with a couple of rinses with distilled water,followed by final rinses with the deoionised water.

4.2.1 Cleaning of glassware for general laboratory use

Glass ware with discarded culture are placed in 3% lysol after use are transferred to boiling soap solution containing tubercle bacilli or spore bearing organism or must be autoclaved.The discarded slide is boiled for 1hour in 5% soap solution in tap water or distillled water.The glassware is cleaned with test tube brush in hot and cold water.Finally the glass ware is allowed to drain and dried in hot air oven or climate

Cleaning of glassware for biochemical work:

Remove any grease with petroleum and then wash with warm tap water.Place in dichromate sulphuric acid cleaning solution for 12 to 24 hours. Remove,wash with hot water 4-5 times and distil water twice

4.2.2 Cleaning Special Glassware

Cleaning of Pipettes

- If pipettes are contaminated with infected material,discard the used pipettes into 3% lysol solution and leave until it is washed in tap water.
- If necessary keep overnight in dichromate-sulphuric acid cleaning fluid
- Wash with tap water or an antimagnetic pipette washer
- Connect the pipette to a water pump by rubber tubing and draw through distil water followed by acetone.Finally pump air until the internal surface is dry.

Cleaning of Volumetric Flasks

- In some cases we may need to soak the glass ware overnight in soapywater.
- Clean volumetric flasks using warm soapy water.
- The glass ware may require scrubbing with a brush
- Rinse with tap water followed by 3-4 rinses with deionised water

Cleaning of glass ware used for organic chemistry

- Rinse the glassware with appropriate solvent
- Use deionised water for water-soluble contents
- Use ethanol for ethanol-soluble contents,followed by rinses in deionized water
- Rinse with other solvents as needed,followed by ethanol and finally with deionised water.
- If the glassware requires scrubbing,scrub with a brush using hot soapy water, rinse thoroughly with tap water, followed by rinses with deionised water

Cleaning of the glassware containing the common lab chemicals:

Water Soluble Solutions:Rinse 3-4 times with deionised water then put the glassware away(e.g, Sodium chloride or sucrose solutions)

Water Insoluble Solutions:Rinse 2-3 times with ethanol or acetone, rinse 3-4 times with deionised water,then put the glassware away.In some situations some other solvents need to be used for the initial rinse(e.g. Solutions in hexane or chloroform)

Strong Acids:(concentrated HCL or H_2SO_4)Under the fume hood,carefully rinse the glassware with copious volumes of tap water.Rinse 3-4 times with deionised water,then put the glassware away

Strong Bases: (e.g. 6M NaOH or concentrated NH_4OH)Under the fume hood,carefully rinse the glassware with copious volumes of tap water.Rinse 3-4 times with deionised water,then put the glassware away.

Weak Acids: (e.g.acetic acids solutions or dilutions of strong acids such as 0.1M or 1M HCL or H_2SO_4) Rinse 3-4 times with deionised water before putting the glassware away

Weak Bases: (e.g.0.1M and 1M NaoH and NH_4OH) Rinse thoroughly with tap water to remove the base,then rinse 3-4 timres with deionised water before putting the glassware away

4.3 Drying of Glasware

Removal of water and even the moisture completely from the glass ware is called as the drying

- If glassware is to be used immediately after cleaning and must be dry,rinse it 2-3 times with acetone.
- This will remove any water and will evapourate quickly
- Some times we can apply vaccum to evapourate the solvent

or.

- Glassware should be cleaned thoroughly
- Excess of water should be drained
- Articles should be arranged in the hotairoven and door closed
- Power supply to the hotairoven should be turned on
- Vent should be opened and temperature should be set to 65oC
- After reaching 65^{o}C article should be kept for 15 minutes and dried
- After completion of drying,power should be turned off.
- After the internal temperature reaches the room temperarure,door should be opened
- Article should be unloaded from the oven carefully

4.4 Sterilization of Glassware

- The process by which the glassware is freed of all microorganisms either in vegetative or spore state is called as the sterilisation of glassware.
- Mouths of culture tubes,glass slides,coverslips are sterilised by passing a few times through the bunsen flame without allowing them to become red hot.
- Glassware, all glass syringes are sterilised by the hotairoven.Glassware should be perfectly dry before being placed in the oven.Test tubes, flasks etc should be

plugged with the cotton wool.Other glassware such as petridishes and pipettes should be wrapped in kraft paper

- Glass syringes are also sterillised by the autoclave
- Glass syringes are also sterilised by the ionising radiation.

4.5 Disposal of Contaminated material

(i.e.clinical infective material and inoculated culture media):

It is necessary to dispose the contaminated material immediately from the site to prevent the health hazards.It is most important to dispose the contaminated material from the hospitals,health clinics,nursing homes,medical research laborarories,offices of physicians,home health care,to protect the public from narious unwanted diseases.

4.5.1 Disposal of Clinical Infective Material

- Various color bins/buckets should be used for collecting the clinical infective material
- Care should be taken while collecting the clinical infective material,which otherwise pose threat to health of an individual collecting the clinical infective material
- The clinical infective material may include the sample specimens of HIV, malignant tumours, STD diseases, etc,so must be handled carefully
- The material should be disinfected with suitable chemical disinfectants to prevent the infection in some cases and some times the infective material should be burnt.
- The clinical infective material should be taken to a far away place and disposed
- The disinfected material can also be burried by digging a pit.
- The color bins/buckets should be cleaned and disinfected properly before reusage.

Fig. 4.1 Disposal of clinical infective material

4.5.2 Disposal of Inoculated Culture Media

Culture : The artificial growth of microorganisms is called as culture.

Culture Media : The media consisting of carbon ,nitrogen,oxygen,sources material and inorganic electrolytes to support and enhance the growth of the microorganisms is called as the culture media.

Inoculation: The process of transfer of bacterial specimen using inoculating loop wire onto the culture media asceptically is known as Inoculation.
While handling the preserved cultures and stock cultures,care should be taken to prevent the spread and transmission of diseases
Inorder to prevent the spread and transmission of diseases,the inoculated culture media should be disposed properly
Inoculated culture media should be treated properly before disposal
The inoculated culture media should be autoclaved at temperature of 121^{o}C for 15 minutes or 115^{o}C for 30 minutes
Then this material should be taken away to a far off place and disposed
The clinical infective material and inoculated culture media are placed in buckets and autoclaved seperately at 121 ^{o}C for 15 minutes.
After autoclaving the plates, tubes, other glassware is washed and dried in the hotairoven. The slides also should be boiled, washed and dried in hotairoven.

4.6 Handling and Disposal of Biomedical Waste

Fig. 4.2 Biomedical waste comprising plastics and biological waste

Biomedical waste(BMW): BMW consists of solids, liquids, sharps,and laboratory waste that are potentially infectious or dangerous and considered as biowaste. BMW must be properly managed to protect the general public, specifically health care and sanitatin workers who are regularly exposed to BMW as an occupational hazard . BMW comes from biological sources during the diagnosis, prevention,or treatment of diseases.common producers of BMW include hospitals, health clinics, nursing homes, medical research laboratories, offices of physicians, dentists, and home health care.

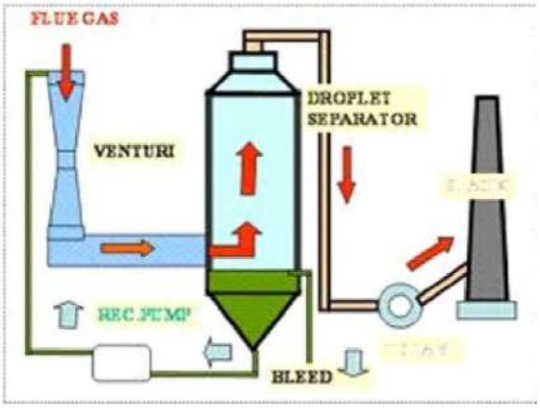


Fig. 4.3 Biomedical Waste Treatment and Disposal

Handling BMW

Sorting of medical waste in hospital is the first step in handling the BMW
At the site where it is generated,BMW is placed in specially-labled bags and containers for removal by BMW transporters
The person handling the BMW should wear disposable latex hand gloves.
Discard the gloves immediately after use.

Fig. 4.4 Biomedical Waste Management and Handling

- The person should wear mask covering the nose and mouth
- The person should wear a cap over the head
- The person should wear foot protectives
- The person should wash his hands with soap and warm water after handling BMW
- The person also should wash all areas of his body with soap and water that he thinks may have come into contact with BMW,even if he is not sure that his body actually touched the BMW
- Keep all sores and cuts covered
- The person should wear an apron or another type of cover to protect his clothes from contact with the waste
- Promptly clean and disinfect soiled,hard-surfaced floors by using a germicidal or bleach solution and mopping up with paper towels

Disposal of Biomedical Waste

Biomedical waste consists of solids,liquids, sharps and lab waste that are potentially infectious or dangerous and are considered as biowaste.It must be properly managed to protect the general public, specifically health care sanitation workers exposed to the biomedical waste Biomedical waste comes from the biological sources during the diagnosis, prevention and treatment of various diseases.Common producers of biomedical medical waste include hospitals, health clinics, nursing homes, medical research labs, offices of physicians and dentists. Householdwaste biomedical waste usually consists of needles and syringes from drugs administered at home(such as insulin) soiled wound dressings, disposable gloves other cloths that have come in to contact with bodily fluids.

- Colour bins/bags should be used for collecting the biomedical waste.
- The biomedical waste should be disinfected with suitable chemical disinfectants to prevent infection.
- The biomedical waste should be taken away to a far away place and disposed
- The biomedical waste which is disinfected can also be burried by digging a pit and filled with soil.
- The used disposable needles should be made into pieces by cutting
- The used syringes should be destroyed
- Biomedical waste after autoclaving should be disposed
- The biomedical waste like swabs,dressings,cotton plugs and dressings should be disinfected,disposed or dried and burnt.
- Dispose the needles lancets,syringes in a plastic soda pop bottlewith a cap

Short Answer Type Questions

- Mention the lab glassware
- How do you clean the glassware containing water soluble solutions?
- How do you clean the pipettes?
- How do you clean the glassware containing strong acids?
- How do you clean the volumetric flasks?
- How do you clean the glassware containing strong bases?
- Mention the chemical used in drying the glassware.
- Define biomedical waste

Long Answer Type Questions

- Write about the cleaning of glassware.
- Define Biomedicalwaste and write about the handling of Biomedicalwaste
- Write about the disposal of Biomedicalwaste

UNIT 5

Bacteria

Structure

5.1 Introduction

With the discovery of microscope only,the presence of bacteria was found. Before that the scientists only assumed that uncurable and illness of the patients was due to some diseases and also assumed that,those diseases are also due to some organisms. All these assumptions were cleared after the invention of microscope, which is playing a crucial role in identifying bacteria.Staining techniques is also paying an important role in identifying the bacteria.

5.2 Morphology and Classification of Bacteria

Bacteria are classified into 7 groups depending on their shape:

1. Cocci-(Kokhos means berry)-These are spherical in shape
   - Bacilli-(Baculus means rod)-These are rod shaped cells
   - Vibrios-These are comma shaped,curved rods possessing vibratory motility
   - Spirilla-These are rigid spiral forms
   - Spirochaetes(Coiled hairs)-These are thin flexuous spiral forms
   - Actinomycetes-These are branching filamentous bacteria.When seen in tissues they appear like radiating rays of the sun(sunray appearance).This is due to the rigid cell wall.
   - Mycoplasma - These are bacteria with defective cell wall.This may be due to spontaneous mutation or due to penicillin administration.Such cells are called as protoplasts,spheroplasts or L forms.

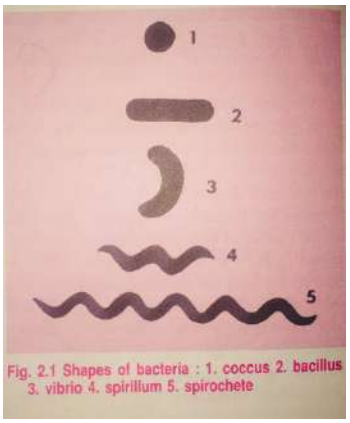


Fig. 2.1 Shapes of bacteria : 1. coccus 2. bacillus 3. vibrio 4. spirillum 5. spirochete

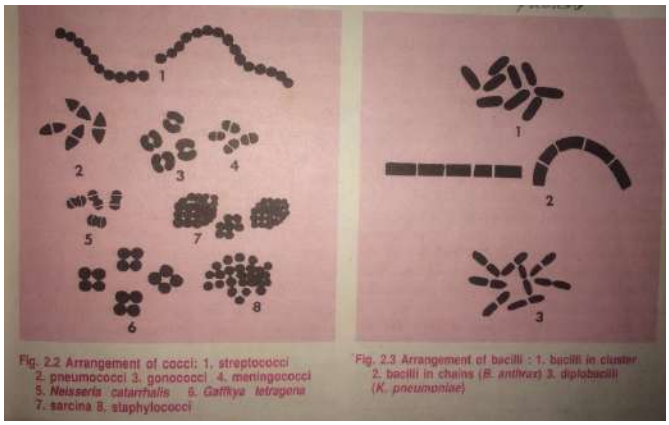


Fig. 2.2 Arrangement of cocci: 1. streptococci 2. pneumococci 3. gonococci 4. meningococci 5. *Neisseria catarrhalis* 6. *Gaffkya tetragena* 7. sarcina 8. staphylococci

Fig. 2.3 Arrangement of bacilli : 1. bacilli in cluster 2. bacilli in chains (*B. anthrax*) 3. diplobacilli (*K. pneumoniae*)

Arrangement of Bacterial cells

1) Cocci in pairs-Diplococci
2) Cocci in chains-Streptococci
3) Cocci in clusters-Staphylococci
4) Cocci in groups of four- Tetrads
5) Cocci in pockets of eight –Sarcina
6) Bacilli in chains- Streptobacilli
7) Bacilli in chinese letter pattern- Coryne bacteria

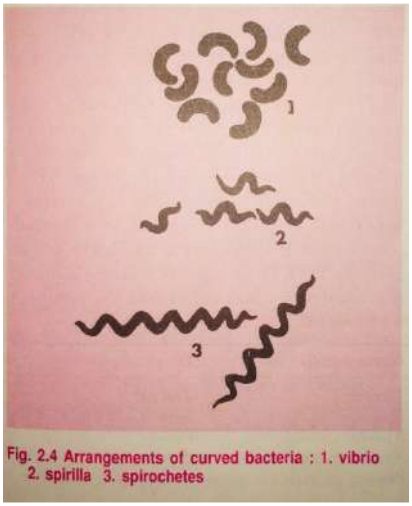


Fig. 2.4 Arrangements of curved bacteria : 1. vibrio 2. spirilla 3. spirochetes

Structure of Bacterial cell : (Bacterial Anatomy)

Like other living cells,bacteria possess protoplasm,cytoplasmic membrane, cellwall. Some intracellular and extracellular structures may be present. Under certain conditions of growth the cell is enclosed in a viscid layer known as capsule.Chemically it is a polysaccharide except Anthracis where it is a polypeptide.

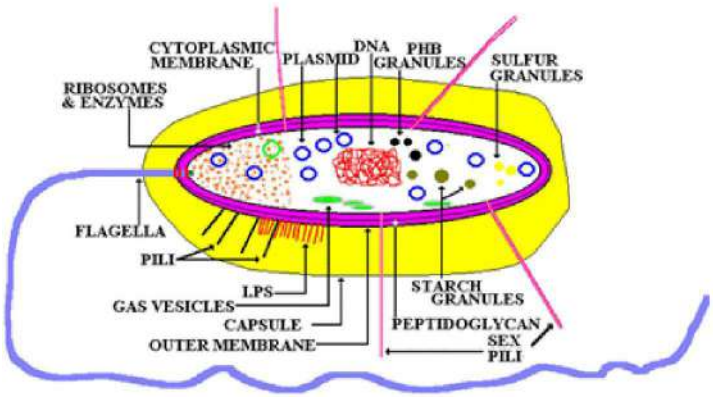


Fig. 5.1 Bacterial Cell Structure

Some bacteria carry filamentous structures protruding from the cell surface,which form the organs of locomotion.These are called as flagella.

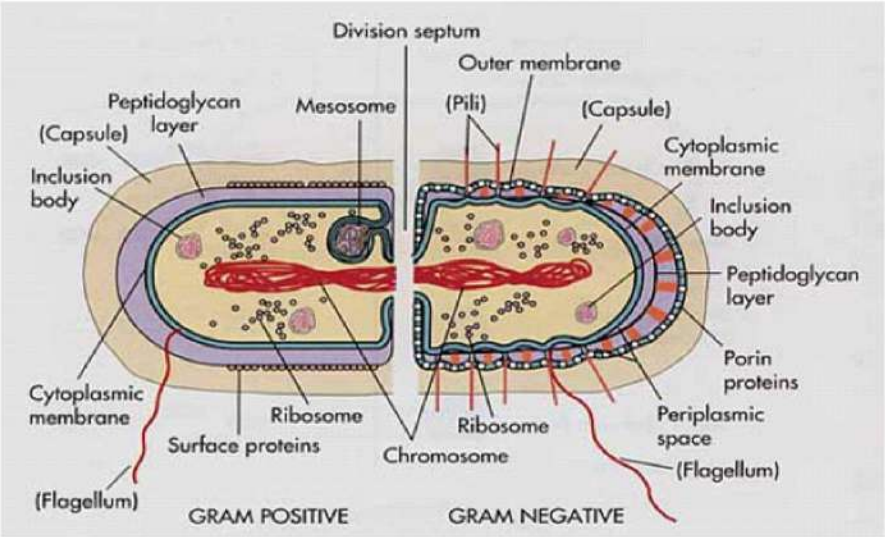


Fig. 5.2 Gram Positive and Gram Negative Bacteria Structure

Fimbriae : These are the organs of adhesion cellwall:

Cellwall is about 10 to 25 micro mtrs in thickness.It maintains the shape and rigidity of bacterial cell. It can not be seen by direct microscope.it can be demonstrated by reaction by Electron microscopy and reaction with specific antibody.Chemically it is made up with mucopeptides(peptidoglycon), N-acetyl glucosamine and N-acetyl muramic acid molecules cross linked by peptide chains.

The cellwall of Gram positive bacteria has simpler chemical nature than Gram negitive bacteria.

Cellwall carries bacterial antigens,which are important in virulence and immunity. The cellwall of Gram negative bacteria has lipopolysaccharide which are associated with endotxic activity. LipidA is responsible for this.

The outermost layer of gram negative has a proteinaceous outer membrane called as porins They form diffusion channels for small molecules.Cellwall synthesis is inhibited by lysozyme.

Cytoplasmic membrane: This is 5 to 10 micro mtrs in thickness.It allows the to and fro movement of metabolites from the cell.

Cytoplasm

This contains ribosomes,mesosomes, inclusions and vacuoles.It is a viscous watery sloution.It stains uniformly with basic dyes.

Ribosomes

These are the centeres of protein synthesis.

Mesosomes

These are the primary sites of respiratory enzymes.They coordinate nuclear and cytoplasmic division

Nucleus

This is seen by the electron microscope. It contains a double stranded DNA arranging in the form of a circle measuring 1000 micro mtre length. Bacteria possess extranuclear genetic elements consisting of DNA called as Plasmids and Episomes. Bacterial nuclei can be demonstrated by acid or ribonuclease hydrolysis and subsequent staining for nuclear material. Bacterial nuclei have no nuclear membrane or nucleolus.

Capsule and Slime layer

Many bacteria secrete a viscid material around the cell surface.When this viscid material is organised into a sharply defined structure, it is known as capsule.When the material not forms a defined structure but releases loose secretion,then it is called as a slime layer.capsules which are too thin and seen under light microscope are called as microcapsules.

Capsular material is antigenic in nature and may be demonstrated by serological methods.

Flagella

The organs of locomotion of bacterial cells are called as flagella.Each flagella has a hook,filament and a basal body. They are 3 to 20 micro mtrs long and possess a protein called flagellin. Flagella have antigens and antibodies which are useful in serodiagnosis. Flagella of different genera of bacteria will have the same chemical composition but are antigenically different. Flagellar antibodies are not protective but are useful in serodiagnosis.

Arrangement of Flagella

1. Peritrichous : Flagella may be arranged all around the cell
2. Polar : Flagella are arranged at both ends of the cell
3. Monotrichous : The polar flagella may be single as in cholera vibrio
4. Lophotrichous : Polar flagella may be in tufts as in spirilla
5. Amphitrichous : Flagella are present at both the poles

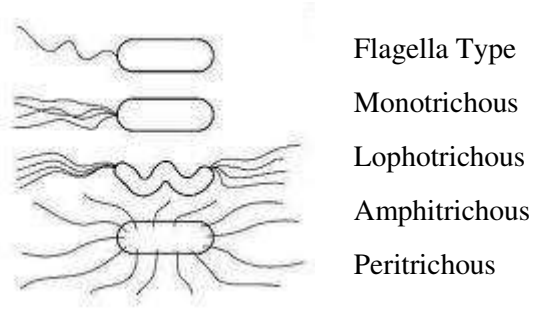


Fig 5.3 Arrangement of Flagella

Bacterial Spore

The highly resistant resting stage of bacteria is called as bacterial spore.Each bacterium forms one spore which on germination forms single vegetative cell. Bacterial spore formed inside the bacteria are called as the endospores.Spores are not a method of reproduction. They are formed due to depletion of nutrients in adverse conditions.

The bacterial spore has a core and a nuclear body surrounded by a spore wall. Again the spore wall is surrounded by the spore cortex,which in turn is enclosed by a multilayered tough spore coat. The shape and the position of the spore are characteristic for each species.

Types of Spores

1. Central or equatorial
2. Subterminal
3. Terminal
4. Oval or Spherical
5. May or may not distend the bacterial cell

Spores are resistant to boiling for prolonged periods.Spores are destroyed by autoclaving at 121degreeC for 15 minutes.

Sporulation helps bacteria to survive for long periods under unfavourable conditions.Spores germinate to form the vegetative bacterium.

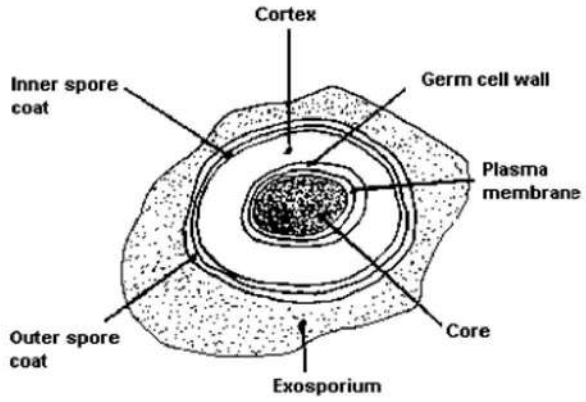


Fig. 5.4 Bacterial Spore

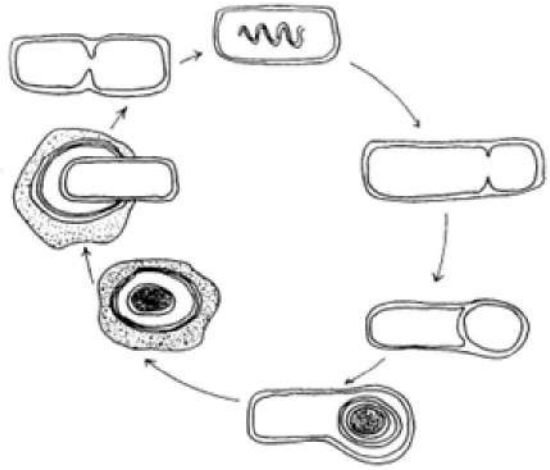


Fig. 5.5 Cycle of Spore Formation and Germination

Key Terms

Aerobes : Bacteria which requires oxygen for their growth
Anaerobes: Bacteria which do not require oxygen for their growth
Obligate anaerobes: Bacteria which die on exposure to oxygen
Microaerophilc bacteria: Bacteria which grow in the presence of low oxygen tension
Cocci: Spherical shaped bacteria
Bacilli : Rod shaped bacteria
Spore : Resistant resting stage of bacteria
Endospore: The spore formed with in the bacteria

Short Answer Type Questions

- Write the two functions of cellwall
- Mention two important functions of nucleus
- Write about ribosomes
- Write about Flagella
- Name the types of flagella
- What is spore? Name the type of spores.
- What is Cocci & Bacili?
- Expand DNA & RNA

Long Answer Type Questions

- Describe the morphological classification of bacteria
- Draw the neat diagram of bacteria and mark the different parts of it
- Explain about the bacterial spore and its types
- Write about the following
  a)Nucleus b) Mesosomes c) Cellwall

UNIT 6

Micro Biological investigation

Structure

6.1 Introduction

Diagnosis of bacterial diseases should preferably be made by direct method e.g., by finding of bacteria in smears of specimens or by isolation of the organisms by culture. Indirect methods are also helpful in many cases.

Succesful diagnosis depends on the selection of specimen, its time of collection in proper way followed by quick transport of the specimen to the laboratory. If delay in transport is inevitable,it should be refrigerated and sent in cold condition or be collected and sent in transport media.

6.2 Collection of Specimen

The following general rules may be followed

Time of Collection : Specimens particularly for culture should be collected before any anti-microbial agents have been used.If antibiotics have already been started,information should be given to the microbiologists particularly when blood for culture is wanted.Stage of the disease when the sample is collected is also very important for succesful diagnosis.

Site of Collection : The specimen should be from those sites where the suspected organismsare most likely to be present with as little external contamination as possible.

Amount of Specimens to be collected : A sufficient quantity of the specimen should be collected in proper container for complete examination.

A strict precaution should be taken during collection so that the inner sides of the container are not soiled by any sort of external contamination.

Labeling: The specimen should be properly labled with particulars of the patient e.g.name,age,address,along with patient's signs and symptoms and other relevant information including type of investigations asked for.

Transport of specimen: All specimens should be sent to the lab quickly where it should be processed without delay. If delay is inevitable,the samples may be kept in refrigerator before they can't be transported to the lab.

Methods Of Collection Of Clinical Specimen For Micro-Biological investigation

Sputum: Coughed sputum instead of saliva is collected in a wide mouthed sterile container.In case of children who cannot cough out sputm but swallow it, a gastric aspirate may be taken instead of sputum.

Urine: For diagnosis of acute urinary tract infection mid stream morning sample of urine is advised.The extermal genitalia is washed with soap and water and when the patient voids

urine the first and the last part of urine are avoided and only the mid stream urine is collected in a wide mouthed sterile container.

Swab: A swab is cotton tipped applicator stick contained in a cotton-plugged test tube.Specimens are collected by swabs,preferably from deeper tissues avoiding the surface.

Stool: The stool sample is collected in a sterile container and send to the lab without delay.If delay is inevitable help of transport media is taken.Eg.V.R fluid for cholerae,Cary Blair transport media for pathogenic members of the enterobacteriacae family.

Blood : It is collected from vein and kept undisturbed till serum is seperated.Serological test can be done with that serum. Understrict sterile conditions about 5-10ml of blood is drawn by vein puncture and transferred to blood culture bottle containibg 50ml of 1% glucose broth.The dilution of blood should be 1 in 50 to 1 in 10.Various anticoagulants may be added.

Cerebrospinal Fluid : Under strict aseptic procedure the Cerebrospinal fluid is usually collected by lumbar puncture in 3 test tubes.One is sent for biochemical test,second for culture and third used for staining micriscopical examination for total and differential cell count.

Aspiration : Abscess and closed wound pus can be collected by aspiration from lesions by a sterilized syringe and needle.

Sputums Petroffs method of Concentration

This is the most widely employed method.The sputum is incubated with an equal volume of 4% sodium hydroxide at 37^{o}C with frequent shaking till it becomes clear,on an average for 20 minutes.It is then centrifuged at 3000rpm for 30 minutes and the sediment neutralised with 0.1 NHCL and used for smear,culture and animal inoculation.

Key Terms

- Clinical Specimen : The sample collected for the detecting the presence of pathogen.
- Sputum : The sample consisting of mucus coughed out from the lungs but not from the salivary glands.
- Swab : A pad used for cleaning a wound or taking liquid from the body for testing.

Short Answer Type Questions

- How do you collect sputum for microiological investigation?
- Write about procedure for collection of urine ?
- What is swab and mention its prominence?
- How do you collect the blood specimen for microbiologcal investigation?
- Write about the petroff's method of sputum concentration
- Name different clinical specimens for microbiological investigation?

Long Answer Type Questions

- What are the general rules followed while collecting the specimens?
- How do you collect various clinical specimens for microbiological investigation?

UNIT 7

Processing of Clinical specimen

Structure

7.1 Preparation of Direct Smear and Staining
7.2 Different techniques of Inoculation
7.3 Hanging drop preparation and its use
7.4 Preparation and Inoculation of various media

7.1 Preparation of Direct Smear and Staining

Clinical specimens collected when converted into thin films on the glass slide either with a slide or with a match stick(in case of sputum) is called as a smear.And the prepared smear is subjected to related staining. For the staining refer staining chapter.

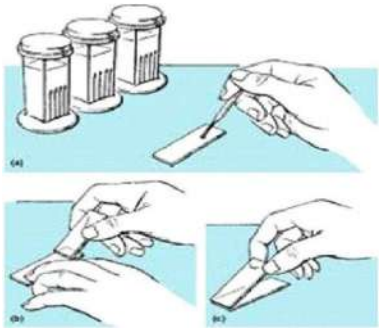


Fig. 7.1 Preparation of Direct Smear

Collection Of Specimen : Specimens are taken with two sterilized swabs,which are rubbed on the affected site under direct vision by using the tongue depressor. One swab is used for making smears and the other for culture. For collection of clinical specimen refer the collection of clinical specimen for microbiological inestigation

Smear Examination

Prepared smears are stained by the following methods and examined under oil immersion lens.

I. Gram's Stain: Useful for detection of streptococcus, staphylococcus, haemophilus, corynebacterium, etc.

II. Alberts Stain: For demonstration of metachromatic granules of C.diptheriae,though confirmation of toxigenic C.diphtheriae cannot be done on this basis only.

7.2 Different Techniques of Inoculation For Isolation Of Bacteria

- The streak culture(surface plating)
- The lawn or carpet culture
- The stroke culture
- The stab culture
- Pour plate culture
- Liquid cultures

The Streak Culture: This method is routinely employed for the isolation of bacteria in pure culture from clinical specimens.With platinum loop,one loopful of the specimen is transferred onto the surface of a well dried plate,on which it is spread over a small area at the periphery. The inoculum is then distributed thinly over the plate by streaking it with the loop in a series of parallel lines, in different segments of the plate. The loop should be flamed and cooled in between the different sets of the streaks. On incubation growth may be observed.

The Lawn or Carpet Culture Culture: Lawn cultures are prepared by flooding the surface of the plate with a liquid culture or suspension of the bacterium.Alternatively,the surface of the plate may be be inoculated by applying a swab soaked in the bacterial culture or suspension. The lawn culture provides a uniform surface growth of the bacterium and is useful for bacteriophage typing and antibiotic sensitivity testing.It may also be employed when a large amount of growth is required in solid media as in the preparation of bacterial antigens and vaccines.

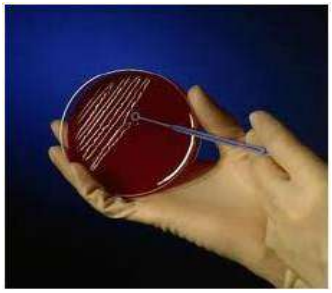


Fig. 7.2 Streak Plate Culture

The Stroke Culture: These cultures are made in tubes containing agar slopes(slants) and is employed for providing a pure growth of the bacterium for slide agglutination and other diagnostic tests.

The Stab Cultures: These cultures are prepared by puncturing with a charged long,straight wire into a suitable medium such as nutrient gelatin or glucose agar.

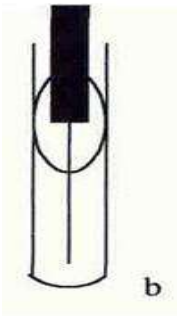


Fig. 7.3 Stab Culture

Stab cultures are employed mainly for demonstration of gelatin liquifaction and oxygen requirement of bacterium under study.They are also used in the maintenance of ssstock cultures.

Pour Plate Culture: Tubes containing 15ml of the agar medium are melted and left to cool in a water bath at 45-50^{o}C.Appropriate dilutions of the inoculum are added in 1ml volume to the molten agar, mixed well,and the contents of the tube poured into a sterile petridish and allowed to set. After incubation,colonies will be seen well distributed throught the depth of the medium.The pour plate method gives an estmate of the viable bacterial count in a suspension and is the recommended method for quantitative urine cultures.

Liquid Cultures: Liquid cultures in tubes,bottles or flasks,may be inoculated by touching with a charged loop or by adding the inoculum with pipettes or syringes.Large inocula can be employed in liquid cultures and hence this is the method adopted for blood culture and for sterility tests.

The major disadvantage of liquid culture is that it does not provide a pure culture from mixed inocula.

This is essential for the identification of the organism by performing different tetsts, e.g.staining,biochemical tests,motility test,serological tests.

Isolation of Micro-Organism

Isolation can be done by inoculating the specimen on solid media or liquid media.

Methods of Inoculating in Solid Media: If any ordinary media or an enriched media is used, the surface of media is dried well by keeping it in an incubator.For this the lid of the petri-dish is placed looking upwards and the plate containing media are kept in slanting way inside the lid keeping the media surface looking downwards obliquely.

A platinum loop is well sterilized by keeping it vertically on a flame and making it red hot and allowed to cool.The loop is touched with well mixed specimens and rubbed on one side of dry media in a little area.The loop is again sterilized and the plate is rotated for 90. The loop is rubbed 3-4 times on media touching a little part of media of previously inoculated side and non-inoculated area.Again the plate is rotated and the above process repeated.

7.3 Hanging Drop Preparation

Aim : To study the morphology and motility of bacteriain the given suspension.

Requirements : Cavity slide,Coverslip, Vaseline, Microscope and the Bacterial suspension to be examined.

Procedure

- Vaseline is applied to the four corners of a clean coverslip.
- Using a sterile loop,a loopful of the given suspension is placed on the center of the coverslip.

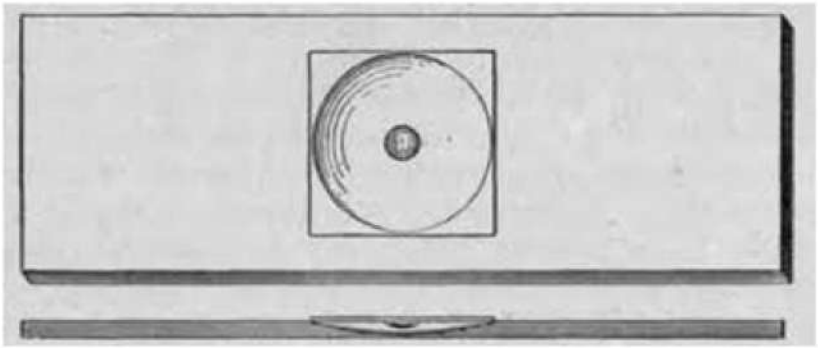


Fig. 7.4 Top View

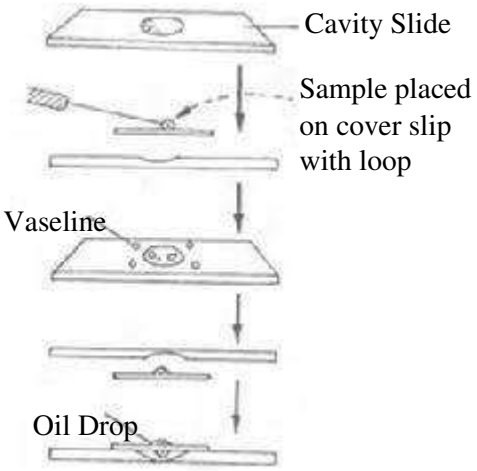


Fig. 7.5 Cavity Slide Preparation

- A cavity slide is inverted over the coverslip so thet the drop of suspension is in the center of the cavity.
- The slide is quickly and carefully turned over so that the cover slip is on the top with the drop hanging in the cavity.
- The microscope is adjusted for reduced light by lowering the condenser and using the concave mirror.
- The edge of the drop is focussed under low power.The microscope is then turned to high power to observr the morphology of the bacteria.
- The shape and motility of the bacteria in thr given suspension are observed.

Short Answer Type Questions

- How do you prepare the direct smear?
- What is inoculation?
- What is the use of hanging drop preparation?
- Explain about the following:
  a) Streak culture b) Lawn or carpet culture c) Stroke culture d) Stab culture
  e) Pour plate culture f) Liquid cultures

Long Answer Type Questions

- Write about the preparation of direct smear and staining.
- Write about the different techniques of inoculation for isolation of bacteria
- Write about the Hanging drop preparation and its use

UNIT 8

Staining Techniques

Techniques

8.1 Introduction

Live bacteria do not show much structural detail under the light microscope due to lack of contrast. Hence it is customary to use staining techniques to produce colour contrast. Bacteria may be stained in the living state. However, many dyes are toxic and kill the cell on staining. This type of staining during which the cell is killed is known as supravital staining. Non-toxic staining during which the cells retain their viability is known as vital staining. Routine methods of staining bacteria employ drying and fixation of smears-procedures that kill them. Bacteria have an affinity for basic dyes due to acidic nature of their protoplasm. Staining techniques in common use in bacteriology are the following

- Simple staining
- Grams staining
- Zeihl Neelson staining
- Negative staining

Fig. 8.1 Simple Staining

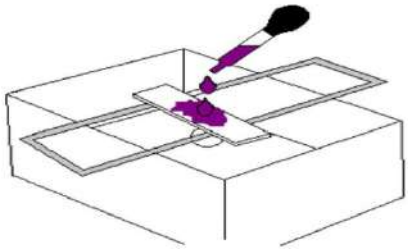


Fig. 8.2 Method of Simple Staining

8.2 Simple Staining

Definition : The staining which uses the simple staining reagents is called as the simple staining.

Composition : (Composition and Preparation)

Loefflers Methylene blue reagent orDilute Carbol Fuschin reagent

Procedure

1. Take the clean grease free microscopic slide
2. Mark the specimen
3. Mark 2 lines in the centre of the slide for preparing smears.
4. Smears are made on the opposite side of the line.
5. Loop is sterilized by flaming till it becomes red hot. Allow it cool near the flame
6. Place a loop full of emulsion in the centre of lines and make a uniform smear
7. Strerlize the loop and keep it a side
8. Smear is allowed to dry in air
9. Fix the smear by passing through flame 3-4 times
10. The slide is placed on the rack and is flooded with Loefflers methylene blue or Dil.Carbol Fuschin
11. The stain is allowed to act for 3 minutes for methylene blue and 30 seconds for Dilute.Carbol fuschin respectively
12. The slide is then washed with distilled water and gently blotted to dry
13. A drop of cedar Wood oil/Liquid parrafin is placed on the smear
14. The microscope is adjusted for increased light by raising the condenser,and slide is examined with oil immersion objective using the plane mirror.

Result

- Only the presence of bacteria can be detected by this staining.
- The bacteria can not be differentiated by this staining.

8.3 Grams Staining

The staining technique which differentiates bacteria into gram positive and gram negative bacteria is called as the gram staining

Reagents (Composition and Preparation)

1.Crystal voilet stain

Solution A

i) Crystal voilet : 2g
ii) Ethyl alcohol : 20ml

Solution B

i) Ammonium oxalate : 0.8g
ii) Distilled water : 80ml

Mix solutions A and B. Keep for 24 hours and filter. Store in an amber coloured dropping bottle.

2. Grams Iodine solution

(i) Iodine : 1.og
(ii) Potassiun iodide : 2g
(iii) Distilled Water : 100ml

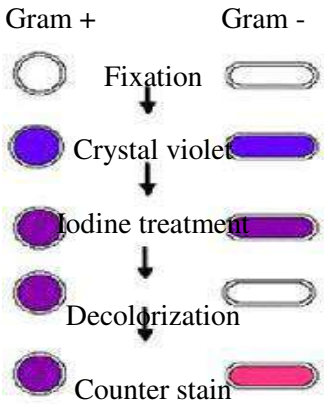


Fig. 8.3 Steps of Gram Staining

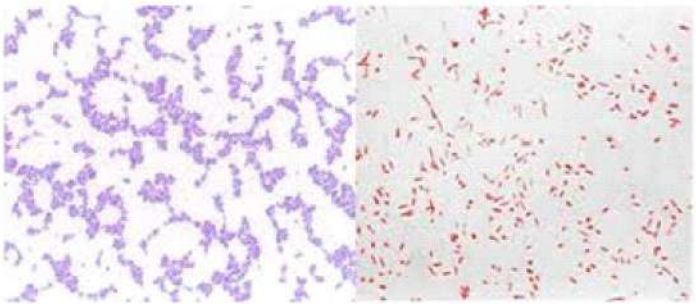


Fig. 8.4 Gram Positive & Gram Negative Bacteria

Store in amber coloured bottle

3. Decolouriser

Mix 95% alcohol and acetone in equal proportion.Store in a white dropping bottle.

4. Safranine solution

a) Safranine O : 0.34
b)Absolute alcohol : 10ml
c)Distilled water : 90ml

Dissolve the saffranine in alcohol and then dilute it with distilled water.Filter and store in an amber coloured dropping bottle.

Procedure

1. Primary Staining

The fixed smear is covered with gentian voilet for 1 minute and washed with water.

Gentian voilet is basic dye which combines chemically with the cytoplasm of the bacterial cell.It renders all organisms voilet

2. Mordanting
It is then covered with Grams Iodine,kept for 1 minute and washed with water.

Grams iodine fixes basic dyes to the smear,and thus acts as a mordant

3. Decolourisation
The smear is covered with alcohol for a few seconds and is washed withwater immediately Alcohol acts as a decolouriser,Gram positive organisms retain the primary dye while Gram negative organisms get declourised

4. Counter Staining
The smear is then covered with Dil carbol fuschin,kept for 1 minute and washed with water. Using filtr paper the slide is gently blotted to dry

A drop of Cedar wood oil/Liquid paraffin is placed on the smear.
The microscope is adjusted for increased light by raising the condenser and the slide is examined under the oil immersion objective using the plane mirror.

Result
Gram positive organisms remain as voilet,while Gram since negative organisms are in decolourised state take up the counter stain and turn pink.

Gram Positive – Violet color

Gram Negative- Pink color

8.4 Zeihl Neelsons Staining
This staining technique is used to detect the presence of Acid Fast bacilli. In this sputum smear is used. The organisms such as Mycobacterium tuberculosis and Mycobacterium leprae are extremely difficult to stain by ordinary methods because of the lipid containing cellwalls. Thats why this staining technique was used to stain the acid fast bacilli.

This staining consists of two methods

- Hot staining method
- Cold staining method.

<u>I. Ziehl Neelson Hot staining method</u>

This staining technique was used to detect the presence of M.Tuberculosis
Specimen-Sputum
Reagents(composition and Preparation)
1.Stock carbol fuschin staining solution:

<u>Solution A</u>

(i) Basic fuschin powder :3g
(ii) 95%v/v ethyl alcohol :to 100ml

Dissolve the powder in alcoholby using mortar and pestle.If necessary heat carefully in aboiling watrbath

<u>Solution B</u>

50 % w/v phenol solution
Mix both the solutuin A and B before using

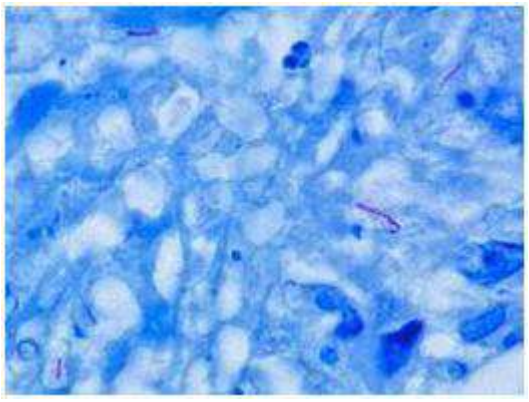


Fig. 8.5 Mycobacterium Tuberculosis with Hot Staining Method

2. Working carbol fuschin staining solution:

Mix 10ml of solutionA with 90 ml of solutionB.Keep at room temperature overnight,filter and store in amber coloured dropping bottle.

3. 20%v/v Sulphuric acid
4. Methylene blue counter stain

(i) Methylene blue: 0.3g
(ii) Distilled water: 100 ml

Dissolve the powder in distilled water,filter and store in amber coloured dropping bottle

Procedure

- Prepare the smear with sputum and fix it on a glass slide by passing it over a bunsen flame for few seconds
- Place the heat fixed slide on the staining rack or rods and flood the smear with working carbolfischin stain.
- Heat gently by bunsen burner flame,until steam raises.Avoid boiling and continue heating for about 5 minutes. Do not allow the stain to dry on the slide.Add more stain if necessary
- Wash the stain off the slide with water and continue rinsing until until the water that runs off is colourless
- Declourisation : Cover the slide with 20% sulphuric acid for about oneminute
- Counter staining:Cover the slide with methylene blue stain for one minute
- Wash with tap water,allow ware to drain, and allow it to dry in air or blot carefully
- Microscopic examination:
- Observe the slide under lower power objective and then observe under oil immersion objective.

Results

Acid Fast organisms : Bright red bacilli on blue back ground
Other organisms : Dark blue

II. Zeihl Neelsons Cold Staining Method

This staining technique was used to detect the presence of Mycobacterium leprae.

Fig. 8.6 Myco Bacterium Leprae with Cold Staining Method Reagents

Composition and preparation :Same as for the previous a hot staining technique

Specimen : Skin smears from visible lesions

Procedure

- Flood the fixed smear with working carbol fuchsin stain.Wait for 12-15 minutes without heating.
- Wash the smear with running tap water.
- Decolourize with 5% sulphuric acid for one minute.
- Counterstain with methylene blue for one minute.
- Wash with water, drain and blot dry.
- Observe under low power objective and examine under oil immersion objective.

Result

- M.Leprae:Bright red bacilli on blue background.
- Macrophage cells:Blue

8.5 Negative Staining

The staining technique which is used to demonstrate the bacterial capsule in which the bacteria will never get stained is called as Negative Staining

Fig. 8.7 Bacterium Prepared by Negative Staining

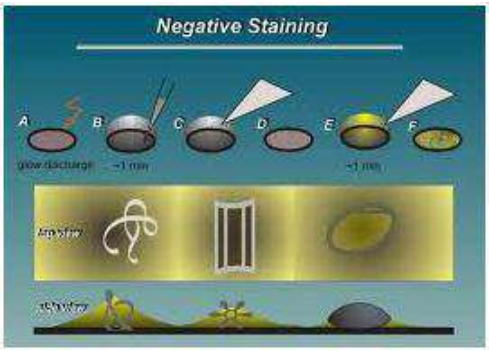


Fig. 8.8 Negative Staining Scheme

Reagents: 10gm/dl nigrosine solutiom.
Sample :A given culture.

Procedure

- Transfer asepitcally a loopful of culture on a clean and dry slide.
- Mix it with loopful stain.
- Make smear by using a glas slide.
- Allow it to dry slowly at room temperature.
- Observe under oil immersion lens.

Results

- Background:Bluish Black
- Organisms:Colourless (hollow bodies)

Key Terms

Staining : The process of using a dye or chemical to impart colour to the bacteria in order to see the important components of the bacteria.

Simple Staining : With this presence of bacteria can be known by using simple dye

Gram Staining : With this Bacteria are classified into gram positive and Gram negative bacteria.

Negative Staining : In this bacteria remains unstained.Bacterial capsules and spirochaetes can be observed with staining.

Ziehl Neelson Staining : With this Acid Fast bacilli can be observed

Short Answer Type Questions

- What is the importance of simple staining?
- Mention the colours of Gram positive and Gram negative bacteria?
- What are the steps involved in gram staining?
- Give two examples for Acid Fast Bacilli?
- How do you detect Mycobacterium leprae?
- Name the reagents used in Gram staining?
- Define staining?
- What is negative staining?
- What is the color of AFB?

Long Answer Type Questions

- Explain about the Gram staining technique.
- Explain about the Ziehl neelson hot staining method
- Write about the Simple staining and Negative staining
- Explain about the Ziehl Neelson Cold staining method

UNIT 9

Culture media

Structure

9.1 Introduction

It is essential to grow the organisms from infected material to identify the cause of infection.Only after growing them and isolating them in pure culture,it is usually possible to identify them.For studying their characteristics as well,it is necessary to culture them.

The majority of bacteria to be studied are pathogenic. Hence to obtainsuitable growth of bacteria,the culture media should approximate to the composition and reaction of the tissues and body fluids in which these bacteria grow. No single medium can satisfy all the requirements.The food requirements of bacteria vary with their natural environments and the particular role they play in nature.They derive their energy by oxidation and by decomposition of food material such as proteins and carbohydrates

It was Louis Pasteur who first introduced the use of complex media Blood,chicken and meat broth were in use.All were liquid media and though growth could be obtained, growth characteristics and purity of cultures could not be made.Robert Koch introduced solid media. On the suggestion of Frau Hesse, Robert Koch Introduced agar agar as a base for preparing solid media. Peptone, Blood, Serum, Yeast extract are the other ingredients

A Culture medium can be defined as an artificially prepared substance containing various microbial nutritional elements at optimum pH,which will allow growth of most of the microorganisms.

Classification of Media

A. Media have been classified into various ways according to the consistency.
   a) Liquid Media
   b) Solid Media
   c) Semi-Solid-Media

Liquid Media

Liquid Media are used for particular sub-culturing and bio-chemical tests like motility test,sensitivity test.However,in liquid media,the growth usually does not exhibit special characteristic appearence.

When there is more than one type of organism,they cannot be seperated by growing in liquid media.

Solid Media

Growth usually shows special characteristic appearance that helps in identification of the organism.

Micro-organism can be separated with certainty from mixtures by growth in solid media.

Semi-Solid Media: It enables motile organisms to spread. So a motile organism can be separated from a non-motile organism.

9.2 Simple media or Basal media

Simple media or Basal media contains only the basic requirements for growth of bacteria

a) Peptone water
b) Nutrient agar
c) glucose broth

Peptone water Compostion

Peptone: 5 g
Sodium chloride:5g
Water: 500 ml

Fig. 9.1 Growth on Basal Media

Preparation: Dissolve the ingredients in warm water.Adjust the PH to 7.4-7.5 and filter.Distribute 5ml in test tube and autoclave at 121^{o}C for 15 minutes

Uses

For subculturing the gram negative bacillifor sugar fermentation tests
For Indole test

Nutrient Agar	
Composition	
Peptone	5.0g
Beefextract	3.0g
Sodium chloride	8.0g
Agar	15.0g
Distilled water	1000ml

Preparation:

- Place the solid ingradients(except agar) in one litre conical flask
- Add about 8ooml of distilled water
- Dissolve the ingradients by using a glass rod.If necessary heat the solution to dissolve the chemicals
- Adjust to Ph7.3 by using 1N sodium hydroxide or 1N hydrochloric acid
- Add agar and boil the solution for 1 or 2 minutes
- Make volume 1000ml by adding distilled water.
- Distribute in conical flasks(100ml),bottles and tubes.Sterilize by autoclaving at 121degreeC for 15 minutes.

Uses

- Used as basic culture medium in the preparation of blood agar and other media
- Also used in the form of slope medium to subculture pathogens isolated on carbohydrate containing media
- Used to maintain Cultures of control organisms in semisolid form and in solid form

Glucose broth:
Composition:

peptone --10g

Meat Extract --10g

Sodium chloride--5g
Glucose-------
- 10g

Water------- 1000ml

Preparation

- Mix the ingradients and dissolve them by heating .Adjust the PH to 7.5-7.6. Distribute 5ml in test tubeand autoclave at 121degreeC for 15minutes

Uses:

- Supports the growth of microorganisms that do not have special nutritional requirements
- To maintain the stock cultures of control strains of bacteria

a) Enriched Media

- Blood agar medium
- Loefflers serum slope
- Chocolate agar medium
- Blood agar medium:

Composition:

Nutrient agar	-	500ml
Sterile defibrinated blood	-	25ml

Preparation

Fig. 9.2 Growth on Blood Agar Media

Preparation

- Prepare and sterilise glucose broth(refer preparation of glucose broth)
- Add sterile serum and mix well
- PH of the medium should be adjusted to 7.0-7.4
- Dispense 2.5ml amounts in test tubes
- Inspissate at 75degreeC for 1 hour in a sloped position
- Inspissate the next day for further 1 hour at 75degreeC

Uses

This medium is mainly used to culture the corynebacteriun diphtheriae to show the volutin granules.

Chocolate Agar medium

Composition:

Nutrient agar	100 ml
Sterile sheep blood	10 ml

Preparation:

Melt 100ml nutrient agar,cool it in a water bath at 75degreeC.Add 10ml sterile sheep blood. Mix blood and agar by gentle agitation from time to time,until it becomes brown in 10 minutes.

Uses

Used to grow Gonococci, H.influenza, Pneumococci.

Fig. 9.3 Growth on Chocolate Agar (Enriched Media)

9.4 Differential Media

Mac Conkeys Agar Medium

Composition:

Peptone	20g
Sodium Taurocholate	5g
Agar	20g
Water	1000ml
Lactose(10%)	100ml
Neutral red solution(2% in 50% Ethanol)	3.5ml

Preparation:

- Dissolve peptone and taurocholate in water by heating and cool, adjust PH to 7.5
- Add agar and dissolve it
- Adjust PH to 7.5
- Add Lactose and neutral redwhich should be well shaken before use Autoclace at 115oC for 15minutes
- Pour the medium into plates
- It should be reddish brown(15ml per plate)

Fig. 9.5 Lactose +ve & -ve Bacteria on Maconkeys Agar Medium

Uses

- Used for differentiating lactose ferminting bacteria from non-lactose fermenting bacteria
- Media for biochemical reactions.

9.9 Anaerobic Media

Anaerobic organisms which can not grow in the presence of oxygen can be grown in suitable media by using oxygen free environment, in which reducing substances are added to remove oxygen and also to maintain anaerobic atmosphere.

- Robertsons cooked meat medium
- Thioglycolate broth

Robertsons cooked meat medium

Compostion

Fresh bullock heart	500g
Water	500ml
Sodium hydroxide	1.5ml

Preparation:

Mince the meat,Place alkaline boiling water and simmer for 20 minutes to neutralise the Lactic acid.Drain off the liquid.While still hot,press the minced meat in a cloth and dry completely.Place the dried minced meat in big test tube to a depth of 2.5cm.And add Nutrient broth in such a way the broth column will be atleast 1cm above the meat particles.

Fig . 9.21 Robert Son Cooked Meat Media

Now autoclave these tubes at 121oc for 20 minutes.A tall column meat is essential because conditions are anaerobic only when there are meat particles.

Uses:

Used to culture anaerobes in blood

(ii) Thioglycolate broth:

Compostion:

Yeast extract powder	5.0g
Tryptone	15.0g
Glucose	5.5g
Sodium thioglycolate	0.5g
Sodium chloride	2.5g
L-Cystine	0.5g
Methylene blue or Resazurine	0.001g
Agar	0.5g
Distilled water	100ml

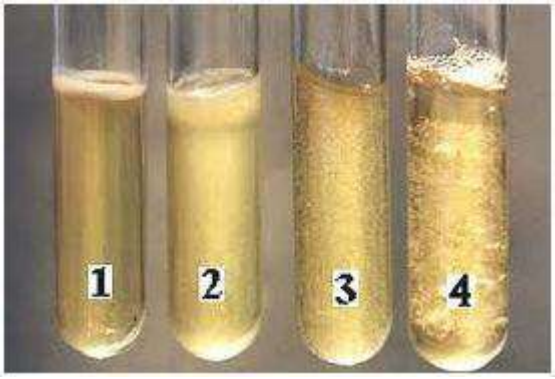


Fig. 9.22 Growth in Thioglycolate Medium

Preparation:

Heat the solid ingradients in distilled water until clear solution is obtained. PH should be adjusted to PH7.2. Dispense in 10ml amounts in test tubes and sterilise by autoclaving at 121oC for 15 minutes. Store at room temperature in a cool and dark place.

Uses:

Used to culture anaerobes in blood

9.8MediaforBiochemicalReactions

Basedonbiochemicalreactionsthebiochemicaltestswereperformed using variousmedia, thesebiochemicaltestwhichinturnidentifiesthevarioubacteria.

1) IndoleTest

MotolityIndoleureaMedium:

Composition:

Tryptone	30.0g
Potassiumdihydrogenphosphate	1.0g
Sodiumchloride	5.0g
Agar	4.0g
0.25%v/vphenolred	2ml
Distilledwater	900ml
20g/dlurea(indistilledwater)	

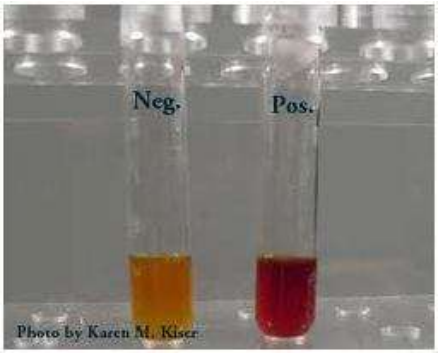


Fig.9.11IndoleProductioninIndoleTest

Preparation

- Placethedryingradientsinaconicalflaskandaddabout900mlofdistilled water.
- Boiltillthecontentsaredissolved.
- Coolto50-55^{o}Candmakevolume 1000mlbyadding20g/dlureasolution.
- PHofthesolutionshouldbeadjustedto 6.0-7.3.
- Dispensein95mlamountsinscrewcapbottles.
- Autoclaveat121^{o}C for15minutes.
- Dispenseasceptic allyinpetridishesaftercoolingto about50-55^{o}C.

Uses:

UsedtodifferentiateEnterobacteriaspecies

2)**<u>Voges-ProskaurTest</u>**

Glucosephosphatepeptonewatermedium:

Composition:

Peptone	5g
Dipotassiumhydrogenphosphate	5g
Glucose	5g
Distilledwater	1000ml

Preparation :

Dissolvetheingradientsindistilledwaterandadjustthe PH7.6.Dispense themediumin5mlamountsintoatesttubeandauto-claveat121°Cfor 15 minutes.

Uses

UsedtoassistinthedifferentiationofEnterobacteria

2) MethylRedTest

Glucosephosphatepeptonewatermedium:

Composition

Peptone	5g
Dipotassiumhydrogenphosphate	5g
Glucose	5g
Water	1000ml

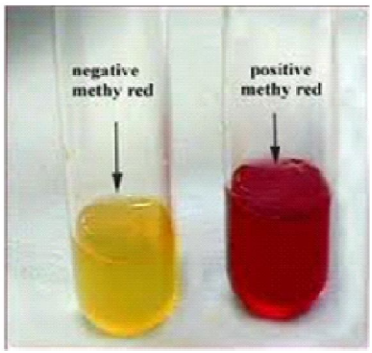


Fig.9.13MethyleRedTestwith+ve&-veresult

Preparation

Dissolvetheingradientsindistilledwaterandadjust pH to 7.6.Dispense themediumin5mlamountsinatesttube.Auto-clavethemediumintesttubesat 121°Cfor15minutes.

Uses

ThistestisperformedtodifferentiatetheEntrobacteria

4)CitrateUtilisationTest

SimmonsCitrateMedium

Composition:

Sodiumchloride	5.0g
Magnesiumsulphate	0.2g
Sodiumcitrate	2.0g
Dipotassiumphosphate	1.0g
Ammoniumdihydrogen phosphate	1.0g
Agar	20.0g
0.4%w/vbromothymolblue	4.0ml
Distilledwater	1000ml

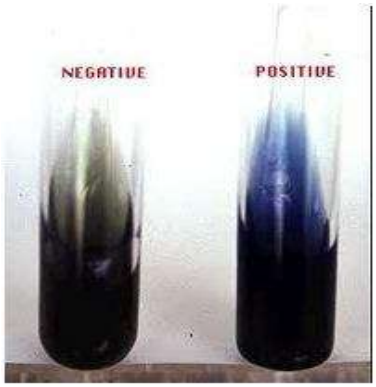


Fig.9.14CitrtateTestwithPositiveandNegativeresult

Preparation

Dissolvetheingradientsindistilledwaterbygentleheat.PHofthemedium shouldbeadjustedto6.9.Dispensein5mlamountsincottonplugged tubes. Sterilizebyauto-clavingat121°Cfor15minutes.Allowtosolidifyinslanting posotion.

Uses:

UsedtoidentifythepathogenicbacteriasuchasEnterobacteria,Serraria, and Klebsiella.

5)**UreaTest**

MotilityIndoleUreaMedium

Forcomposiotion,preparationandusesreferIndoletest

6)**OxidaseTest**

Requirements

i) Oxidasereagent:

1.0g/dltetramethyl-p-phenylenediaminedihydrochlorideindistilledwater(it shouldbepreparedfreshifitappearsblueincolour)

ii) **FilterPaperStrips**

Fig.9.15Leftsidewith+vetest&Rightwith-vetest(Ureasetest)

Fig.9.16Oxidase+veTest

Uses

Usedtohelpintheidentificationoftheorganismswhichproduceoxidase enzyme

7)**CatalaseTest**

NutrientAgarSlope:

Composition

Peptone: 5.0g

Beefextract: 3.0g

Sodiumchloride :8.0g

Agar: 15.0g

Distilledwater :1000ml

Fig.9.17CatalasePositiveTest

Preparation

1. Dissolveallthe ingradientsindistilledwaterbyheatingandallowthemto coolbykeepingthedispensedtesttubesinslantingposition.
   10vol Hydrogen peroxide 1ml

Uses:

Usedtodifferentiatecatalaseproducingbacteriafromnon-catalase producingbacteria.

8) NitrateReductionTest

i) Nitratebroth:

Composition:

Potassiumnitrate 0.2g

Peptone 5.0g

Distilledwater 1000ml

Preparation

Dissolveallthe ingredientsindistilledwater.AdjustthePHto7.4.Dispense themediumin5mlamountsintesttubeandsterilizeby autoclaving at121oCfor 15minutes.

TestreagentispreparedwithsolutionA(SulphanilicAcidin5nAcetic Acid)solutionB(AlphanaphthylAminein5nAceticAcid)beforeuse.Add1 mlofthisreagenttothetestculture.

Formationofredcolourindicates+vetestotherwise –vetest

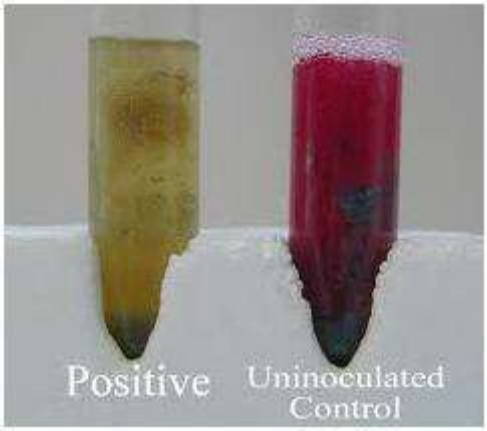


Fig.9.18NitrateReductionTestResults

Uses

ThistesthelpstodifferentiatemembersoftheEnterobacteriaceaethat producetheenzymenitratereductase,fromGramnegativebacteriathatdonot producetheenzyme.

9) PhenylalanineDeaminationTest

PhenylalanineAgarMedium

Composition:

YeastExtract	:3g
DL-Phenylalanineor	:2g
L-Phenylalnine	:1g
Disodiumhydrogenphosphate	:1g
Sodiumchloride	:5g
Agar	:12g
Distilledwater	:1000ml

Preparation:

Dissolveallthe ingredients in distilled water, if required by heating. Distribute and sterilize by auto clavingat121oCfor15minutes.Allowtosolidifyintubesas longslopes

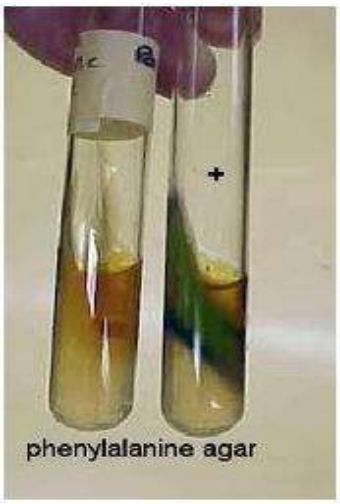


Fig.9.19GreenColourwith+vetest,Otherwise-vetest

Uses

Usedmainlytoassistintheidentificationof Proteus species

10) GelatinLiquifactionTest

NutrientGelatinMedium

Composition:

Peptone	10.0g
Beefextract	3.0g
Gelatin	120.0g
Distilledwater	1000ml

Preparation

Dissolvethesolid ingredientsin100mlofdistilledwaterbyheating.Dispense in7mlamountsinscrewcaptesttubes.Sterilizebyautoclavingat121^{o}C for 15 minutes

Uses

Thistestisperformedintheidentificationoforganismssuchas PseudomonasandVibriocholerae

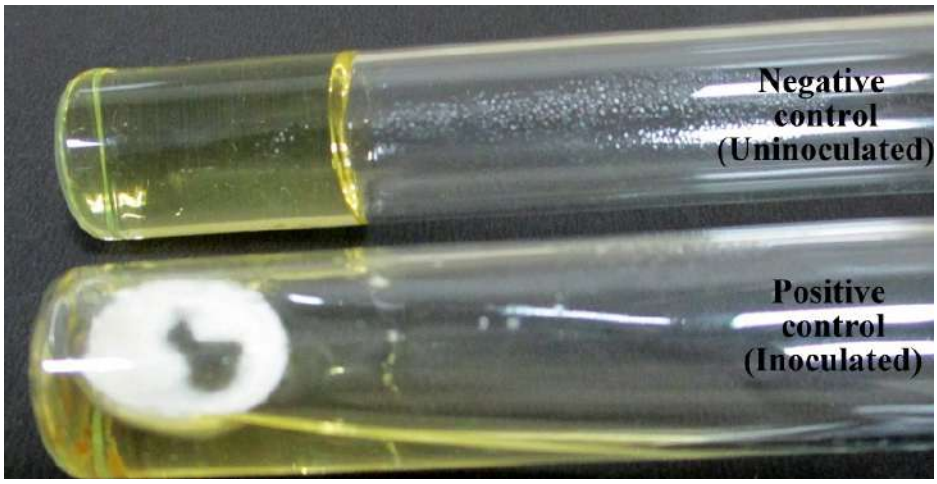


Fig.9.20Tubewith –vetestinthebottomandwith+vetestattopside

Short Answer Type Questions

- Define culture media
- Give the examples for basal media
- Write the composition of peptone water
- Define Enriched media
- What is an enrichment medium?
- Write the examples for enriched media
- Define differential media
- Give the examples for blood culture media
- What is an anaerobic media?
- Write the media for biochemical reactions
- Give the example for fungal media
- Write the examples of ANAEROBIC MEDIA
- Expand IMViC tests

Long Answer Type Questions

- Define Basal media and write composition,preparaton and uses of any two basal media

- Write the composition,preparation and uses of Macconkeys agar medium
- Write the compostion,preparation and uses of Robertsons cooked meat medium
- Write the classification of culture media
- Explain in detail about enriched media with examples

PATHOLOGY

PATHOLOGY

Introduction

The branch of Biological science which deals with the study of diseases nature,its cause and symptoms is known as Pathology.

There will be structural and functional alterations,after patient being infected with microorganism or if there is any abnormality in the normal human being.The symptoms will be produced due to the disturbances in the normal functions of the effected cells of the body which leads to the illness of a person.Pathology deals with the study of disturbed functions and how the effect,how they arise,how they progress and how they effect other cell systems.

Fig. 1 Rudolf Virchow, Father of Pathology

UNIT 10

URINE ANALYSIS

Structure

10.1 Introduction

Analysis of urine physically,chemically and microscopically and even the collection of urine sample is called as the urine analysis.Collection of urine sample also plays an important role.Sterilised and clean containers are used for the collection of urine samples.After collection of the urine sample physical examination of urine sample is the next step in urine analysis.Presence of albumin in urine can be detected carefully with boiling test for albumin.Chemical examination of urine plays crucial role in urine analysis which has prominant role in diagnosing the disease.Ketonebodies,bilesalts,bilepigments and blood presence in urine can be detected by various tests.Microscopic examination of urine is the last step of the urine analysis.Cells,Casts,Crystals and atypical cells can be detected after preparaing the slide preparation from the centrifuged urine sample i.e taking the sediment of urine sample after centrifugation.

10.2 Collection of Urine Samples

Types of Urine Samples

Types of urine samples: a)Single specimen b) 24 Hrs urine specimen

Single Specimen: Urine specimen collected any time randomly is called as Single urine Specimen. It is useful in qualitatives analysis.

24 hrs Urine Specimen: Specimen of urine collected in a period of 24 hrs duration is called as 24 hrs Urine Specimen.It is required in quantitatives determination. A big container is required for collection.Preservative is needed to prevent any charges. Sometime, say 8.am has to be selected in the morning.At that time bladder has to be emptied and urine passed has to be discarded.Subsequently pased urine specimens up to same time in the next morning have to be collected.Container has to be covered and kept in a cool place during this period.

Labelling & Preservatives

Labelling: Labelling of sample plays an important role in medical laboratories.Samples have to be labelled after collection to enable their identification during testing and reporting.Eventhough the volume of sample is more and if it is not labelled due to forgetfullness it is useless.Proper numbering should be marked on the label seeing the previous samples.After labelling on the sample,the same numbers should be noted in the records with patients name.this will avoid the confusion in the identification of patients sample.

Preservatives: The chemicals are used to preserve the urine specimens are called as Preservatives.

Freshly passed urine has to be examined.On standing,chemical changes take place.Bacterial growth also further causes chemical changes.If urine is to be kept prior to examination,it has to be stored at 2-8`C in refregirator.

Different Urinary Preservatives are

1) Conc.HCL	2) 2N HCL	3) Acetic Acid	4) Metaphosphoric Acid
5) Formalin	6) Boric Acid	7) Thymol	8) Toluol
9) Chloroform			

10.3 Physical Examination

- General Appearance
- Quantity (Volume)
- Odour
- Colour
- Specific Gravity

- PH (Reaction)
- Albumin

1. **General Appearence:** Normally freshly voided urine is usually clear, slightly cloudy occasionally.But when it is allowed to stand a faint cloudiness develops due to the settlement of pus cells. RBC's, epithelial cells and mucus. It is allowed for a long time settlement. Then the sediments will increase due to the precipitation of various salts and whole urine will become cloudy with bacterial growth.

Abnormal Findings (Clinical Significance)

Abnormal cloudness develops due to the settelements of phosphates, urates, urinary deposits and occasionally chemicals.

Report: Urine is clear / Cloudy / Turbid with or without sediment.

2. **Quantity (Volume):** Children - infants : 100 - 500ml. / 24-hrs

1- 8 years : 500 - 1000ml. / 24-hrs

Usually 1000 ml. to 1600 ml. volume of urine will be excreted by a normal person per 24-hrs in adults.

Abnormal Findings (Clinical Significance)

1. If volume of urine is more than 500ml, it indicates poly urea,diabetes mellitus,diabetes insipidus.
   If volume of urine is less than 200ml,then it indicates oliguria,aneuria,and renal conditions.
3. **Odour:** Normally urine has Aromatic Odour.

Abnormal Findings(Clinical Significance)

It is ammonical/pungent in cystitis and in urinary retention due to presence of ammonia.Fruity odour is due to presence of ketone bodies in urine

4. **Colour:** Normal urine appears as amber to yellow colour.Colour of the urine is due to urochrome.

Abnormal Findings(Clinical Significanse)

- Cloudy urine is due to phosphates,proteinuria,pus and chyluri.
- Uniform opalescence is due to bacterial growth.
- Smoky colour is due to haematuria.
- Turbideness of urine is due to fat droplets,chyluria and pus.
- Red urine is due to haemaglobinuria and myoglobinuria.
- Deep yellow to brown or greenish brown urine is due to bile pigments namely bilirubin.
- Orange red or orange brown urine containing haemoglobin.Other causes are homogentisic acid and melanin.
- **Specific Gravity**: The presence of various solutes in the urine changes the specific gravity of urine.Specific Gravity depends up on the cincentration of various solutes in the urine.

Specific Gravity of the urine can be measured with:

1) Urinometer 2) Refractometer 3) Can be tested with dip sticks
4) Osmometry:

This method gives the most accurate assessment

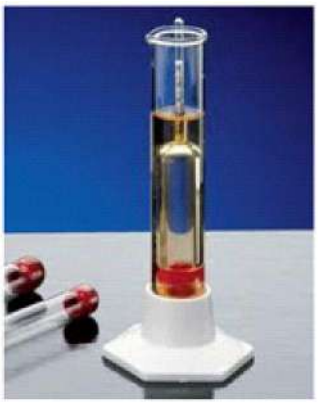
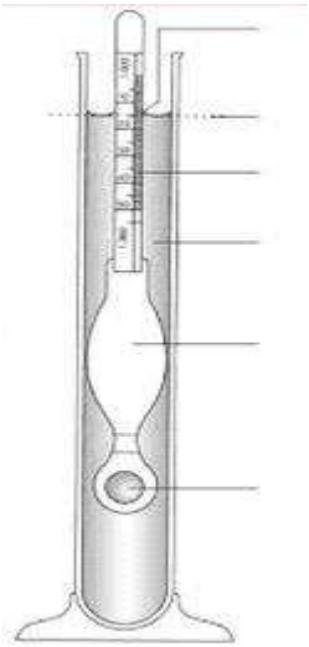


Fig. 10.1 Urinometer



Meniscus

Reading Level

Specific gravity scale

Urine in cylinder

Hydrometer

Mercury bulb

Measurement of specific gravity of urine with urinometer:

Take the urine sample into a beaker up to nearly to full.By means of filterpaper remove the froth of bubbles.Float the urinometer,so that it does not touch the bottom or sides of the beaker.Take the reading from the bottom of the meniscus,If the urine amount is less, dilute the urine to raise the volume upto 70-80ml,take the reading and multiply the last 2 digits by the dilution factor

Normal values: 1.003-1.060

Fig. 10.2 Urinometer in detail

Abnormal findings (clinical significance)

High specific gravity values are found in

Diabetes Mellitus
Fever
Acute Nephritis
Excessive sweating

Low specific gravity values are found in

Chronic Nephritis
Diabetes Nephritis

6. Reaction:(PH)

Reaction indicates the PH of the urine.PH of the urine can be measured with the PH meter and indicated by litmus papers.

Normal Findings:

PH range 5-7(slightly acidic)
To confirm the test,blue and red litmus papers should be dipped in urine.
If blue litmus paper turns to red colour then the urine is acidic in nature.
If red litmus paper turns to blue colour then the urine is alkaline in nature.
Usually specific collected urine shows PH value of 6.0

Fig 10.3 Urine PH meter

Abnormal Findings (Clinical Significance)

PH of urine <4.7 is more acidic

PH of urine>7.5 is alkaline

7. Albumin(Protein): There are 5 main proteins found in the urine.

1) Serum or plasma globulin 2) Serum or plasma albumin
3) Mucus or mucin 4) Haemoglobin
5) Bence jones protein.

There are three main albumin proteins tests found in the urine.Those were
i) **Boiling Test for Albumin (Coagulation test or heat and acetic acid test)**

Procedure: If the collected urine (patients) sample looks turbid, filter it and if it is alkaline make it slightly acidic to litmus by adding few drop of 3% acetic acid. Fill the urine into a clean dry test tube upto 1/3 rd from top portion of the test tube. Boil the urine present in the top one third portion test tube over a spirit lamp. If any cloudiness appears add a few drops of 3% of acetic acid. If the cloudiness appears while the tube is being heated but disappears when it reached the boiling point Bence Jones protein is present. If the cloudiness disappears on addition of 3% acetic acid,it indicates the presence of phosphates in urine. If the cloudiness still remains after adding acetic acid, then it is due to the presence of the album in urine sample. If the urine remains clear no albumin is present.

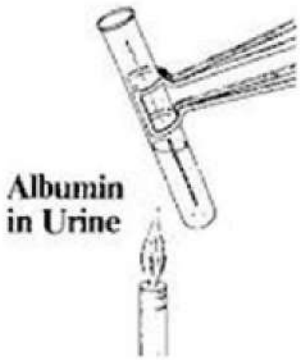


Fig. 10.4 Boiling Test for Albumin

Normal Findings: Albumin is absent in normal urine.

Abnormals Findings: Presence of albumin in urine indicates the nephritis or kidney damage.State whether there is only a faint haziness (+),a cloud (++),a dense cloud(+++) or a definite flocculent precipitate(++++) to give a rough idea of the quantity of albumin present.

Clinical Significance: Urine generally contains 2-8 mg. of proteins,when their quantity increases to 150 mg.,one can conclude that filtration of urine in the kidney is improper and the condition is referred to as proteinurea or albuminurea. By these diseases like myelo and pyelo nephritis,diseases of

urinary tract can be diagnosed. Proteinurea reveals the irregular functioning of the kidneys. Diseases like glomerullo nephritis,toxemia during conceptions and diabetic nephropathy can be identified through this test.

(ii) Sulphosalicylic Acid Test for Albumin(Precipitation of Protein)

Procedure: Take a clean test tube and to it add 5ml. of clear urine and then to it add 10 drops of 20% sulphosalicylic acid.

Report: If protein is present there will be a white precipitation increasing with the amount of protein.

(iii) Nitric Acid Test for Albumin (Hellers Test)

Procedure: Take a clean test tube and it to add 2 to 3 ml. of nitric acid and then gently float equal volume of clear urine.

Report: Presence of white ring at the junction indicates the presence of albumin.

10.4 Chemical Examination

1. Sugar (Glucose) i) Benedicts Qualitative Test ii) Fehlings Test

- Ketone Bodies (Acetone Bodies)
- Bile Salts.
- Bile Pigments
- Blood in Urine
- Strip Method
- Albumin

Sugar (Glucose): Presence of sugar in urine is detected by Benedicts Qualitative Test & Fehlings Test.

Benedicts Qualitative Test

Principle: In this method the cupric ion is reduced to cuprous oxide.

Procedure: Take 5ml. of Benedicts qualitative reagent and subject it to boiling,and then add 7-8 drops oe urine with the help of pipette.Sugar condition can be decided basing on the colour developed.Sometime colour cannot be change then it might have to be reboilrd or deep cooled.Conclusions are to drawn on thorough examination.

Report: A Bluish white precipitate,a light green colour or no change is recorded as negative.

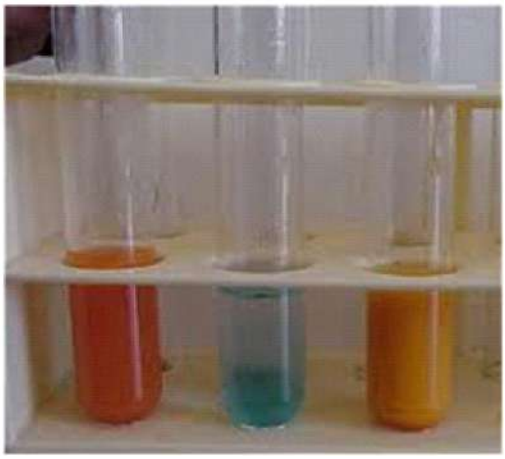


Fig. 10.5 Benedicts Qualitative Test

A greenish colour with a little yellow deposit is reported as a trace(+).Green yelloowish precipitate indicates sugar presence(++).Presence of orange colour in urine indicates glucose(+++).Presence of brick red in urine indicates presence of sugar in urine(++++).

(ii) Fehlings Test

This test can be also used for detecting the presence of sugar in urine.

Procedure: Take a dry clean test tube and to it add 1ml. each of solution - A and solution-B and then add 6ml distilled water mix and boil this solution present in the test tube. Add urine, a few at a time to the solution,but should not boiled now.The volume of urine should not be more than volume of the reagent.

Report: If the sugar is present a heavy red or yellow precipitate appears.

Clinical Significance: Glycosuria with Hyperglycaemia is indicated in diabetes mellitus,other endocrinedisorders,pancreatic disease,CNS dysfunction,massive metabolic derangement.hypoglycaemia indicates the presence of los levels of glucose in urine.

2. Ketone Bodies(Acetone Bodies)

Ketone Bodies are the products of incomplete fat metabolism and thgeir presence is indicative of acidosis(ketosis).Ketonuria is commonly seen in uncontrolled diabetes mellitus.

The ketone bodies are 1) Acetone. 2) Acetoacetic acid. 3)Beta-hydroxy bityric acid.

The presence of Ketone bodies in human body can detected by tests.
i) Rothera's Test ii) Gerhard's Test

Rothera's Test: Acetone and Aceto-acetic acid react with sodium nitroprusside in the presence of alkali to produce a purple coloured complex.

Sodium Nitroprusside is decomposed to Sodium Ferro Cyanide,Sodium Nitrite and Ferric Hydride in an alkaline solution. These chemicals are strong oxidising agents.thye ammonium sulphate acts as a buffer maintaining the alkalinity within the range at which complex has a purple colour.

Rothera's Mixture

Ammonium Sulphate - 99 parts

Sodium Nitroprusside - 1 part Grind and Mix well

Procedure: Take 5ml. of urine in a test tube, Saturate it with crystals of ammonium sulphate. To it add 2 drops of freshly prepared sodium nitroprusside reagent.

Mix well, overlay with about 14ml. of concentrated ammonium hyderoxide.A positive shows the appearence of a reddish purple ring at the interface within 1mt. and 30sec. A brown ring is not a positive reaction.

Interpretation

- No ring or brown ring-negative.
- A faint pinkish-purple ring appearing slowly.trace.
- Narrow dark purple ring.-++
- Wide dark purple ring appearing rapidly-++++

Clinical Significance: Presence of ketone bodies is observed in diabetes mellitus and starvation.

Gerhard's test:

Take a clean dry test tube and add about 5ml. of urine & 10% of ferric chloride solution precipitate will be formed.Filter it to the filtrate add o0ne or two drops of 10% ferric chloride solution.

Result: Formation of a red colour in test tube indicates the presence of ketone bodies in the patient. This is 20 times less sensitive than Rothera's test.

Fig. 10.5 Detection of Ketone Bodies by strips

3. Bile Salts (Hays Test)

Sodium glycocholate and sodium taurocochlate are called as Bile Sallts.These are helpful in the emulsification of the fat globules.(Fat Digestion).

Principle: Bile Salts reduce the surface tension.

Procedure: Fill the urine sample up to the 3/4th height of a clean beaker sprinkle little dry sulphur powder over the surface of about 1/2 inch height.

Result: If the Bile salts are present sulphur particles will sink.In normal urine sulphur practices will float.

4) Bile Pigments

Liver secretes bile juice.It contains yellow green substance bile pigment(bilirubin).In some hepatic diseases,bile enters to the blood and is eliminated through urine. The presence of bile pigment bilirubin can be detected by four tests.

- Fouchets Test (Harrison's Spot Test)
- Smith's Test
- Gmelin Test
- Lugols Test.

i) **Fouchet's Test(Harrison's Spot Test)**

This test is used for detecting the bile pigments in urine.

Principle: After precipitation of the bile pigments by Barium Chloride it is oxidized by acids to derivatives like biliverdine(green) and bilicyanine(blue).

Procedure: To about 2ml. of acid urine add 1ml.of 10% of barium chloride. Mix and wait for few minutes,for the precipitate ti appear(if there is no precipitate add 1 or 2 drops of saturated ammonia sulphate solution).Filter through a filter paper and add a drop or two of Fouchet's reagent(Trichloracetic acid and ferric chloride).

Result: A positive reaction is indicated by a blue or green colour.This test is more sensitive than the iodine or nitric acid ring test.the sensitive of the test is 0.05 to 0.01 mg/100ml. of urine.

Clinical Significance: Bile pigments are increased in obstructicve jaundice and hepatitis.

(ii) Smiths Test

This is another test for detecting the presence of bile pigments in urine.

Procedure: Take 3ml. of urine in a test tube,over the urine layer add equal amount of diluted tincture iodine.

Result: A bright green ring develops at the junction of the liquids if bile pigment is present.

(iii) Gmelin Test

This is also another test for detecting the presence of bile pigments in urine.

Procedure: Take about 2ml. of Nitric acid in a test tube.Add vertically urine at the centre portion of acid layer in a test tube.

Result: A green or blue ring indicates the presence of bile pigments.

(iv) Lugol's Iodine Test

Procedure: Take 4ml. of urine in to a test tube and add 4 drops of lugol iodine solution and stir it.Afterwards observe the colour in th etest tube.

Result: Yellow or brownish yellow colour formation indicates the absence of bile pigments in urine.

Formation of green colour indicates the presence of bile pigments in the urine.

Pale Green:(+)

Intence Green:(++)

5. Blood In Urine:(Haematuria)

The presence of blood in urine is known as Haematuria.

The presence of blood in urine or haematuria can be detected by the following tests.

Benzidine Test (Occult Blood Test)
Paper Strips

Benzidine Test(Occult Blood Test)

Principle: The peroxidase activity of haemoglobin decomposes Hydrogen peroxide and the liberated oxygen oxidises the benzidine.

Procedure: To 2ml. of urine (previously boiled and cooled) and 1ml. of clear benzidine solution(benzidine powder and glacial acetic acid),mix well,add 1ml.of freshly prepared 3% hydrogen peroxide and mix.

Result: A deep blue colour indicates presence of haemoglobin in urine.
Clinical Significance: The appearence of green or blue colour within 5 minutes.indicatesthe presence of blood or haemoglobin or myoglobin in the urine.

(iii) Paper Strips

Blood present in urine reacts with the peroxide-orthotoluidine reagent of paper strip to produce a blue colour.

This indicates the presence of blood in urine.

Microscopical examination:

Introduction

Identification or detection of normal or abnormal components like cells, casts, crystals, mucus threads, parasites and bacteria using microscope is known as Microscopic Examination.

First collect the urine sample(clear,fresh morning specimen).Obtain urinary sediment by centrifusing urine at 3000 rpm for 5 minutes.Withdraw the clear supernatant fluid,place a drop of the sediment on a glas slide and cover it with a cover slip.Examine first under low power objective,then observe under high power objectives of a microscope.Vary the high intensity for screening casts. If protein is present, look for casts R.B.C;S pus cella and epithelial cells.

- Cells
- Casts
- Crystals
- Atypical Cells
- Mucuc Threads

- Yeast Cells
- Bacteria
- Spermetozoa
- Parasites & Parasiticova

Urates

a - Calcium, Magnesium and potassium (mostly amorphous)

b - Ammonium (Spherical)

c - Sodium (thorn - apple forms)

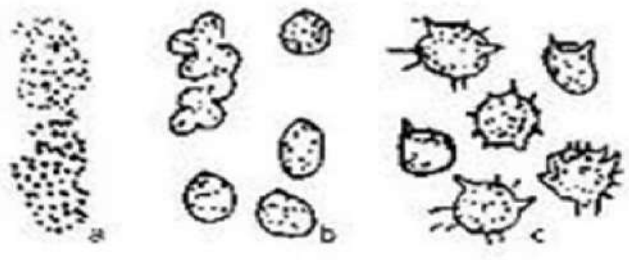


Fig. 10.7 Microscopial examination of Urine

(I) Cells

(i) RBC'S

Under high power objective of a microscope RBC appears as pale disks. More than one RBC for high power field is abnormal.The red blood cells swell up and broken down in diluted urine.The red cells may show crenated margings.

Clinical Significanse: Increased red blood cells are found in (a) Polynephritis (b) Renal Stones (c) Cystitis (d) Polycythemiavera

Decreased red blood cells are found in Anemias.

(ii) WBC

Normal values 0-5 per high power field.

Clinical Significance: Large number of WBC'S indicates bacterial infection of urinary tract.

Increased white blood cells are found in Leucytosis,Leukacmia.

Decreased white blood cells are found in openia.

(iii) Epithelial Cells

These cells have a single rounded nucleus Squamous epithelial cells present in urine in moderate numbers have no pathological importance.

Clinical Significance: Presence of all other epithelial cells indicates pathological condition.

(iv) Renal Tubular Epithelial Cells

Unstained cells have almost the same size as that of a neutrophill but contain

a large round nucleus.Oval fat bodies are these cells containing fat globules,the nucleus,then,is not visible.

(v) Blader Epithelial Cells

Unstained cella are larger than renal tubular cells,have a round nuclear and vary in size depending on depth of origin in transitional.

(vi) Squamous Epithelial Cells

These are unstained large flattened cells with abudant cyptoplasm and a small round nucleus.The cell may be folded or rolled.

Casts

Urinary casts are formed in the lumen of the tubules of the nephrons.
The following casts are observed after microscopical examination of urine sediment covered with coverslip on a glass slide.

- Hyaline Casts
- Granular Casts
- Waxy Casts
- Epithelial Casts
- Cylindroids
- Fatty Casts
- Fibrinous Casts
- Pus Casts
- Pseudo Casts
- Blood Casts

Crystals

Generally many of the crystals which are found in the urine have little clinical significance although they may be found in calculus formation,metabolic disorders and in regulation of medication.

(a) Crystals Present In Acidic Urine

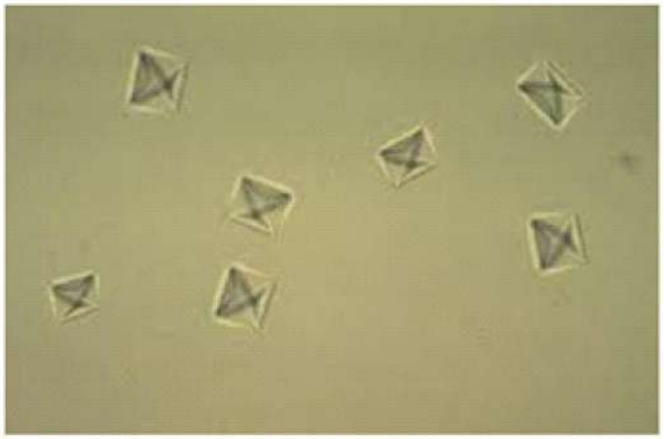


Fig. 10.8 Crystine Crystals present in the Neonates Urine

- Uric Acid Crystals
- Calcium Oxalate Crystals
- Amorphous urate crystals
- Sodium urate crystals
- Calcium sulphate crystals
- Hippuric acid crystals
- Cysteine crystals
- Tyrosine Crystals
- Leucine Crystals
- Cholestrol Crystals
- Sulphur Crystals

b) Crystals Found In Alkaline Urine

- Triole Phosphate (Ammonium Magnesium Phosphate) Crystals
- Amorphous Phosphate Crystals
- Calcium Carbonate Crystals
- Ammonium Biurate Crystals
- Calcium Phosphate Crystals

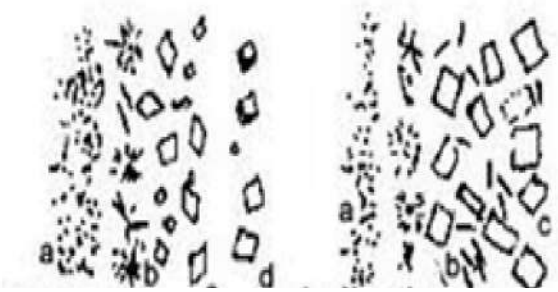


Fig. 10.9 Microscopic Examination of Urine

Atypical Cells

- Mucus Threads
- Yeast Cells
- Bacteria
- Spermatozoa

Key Terms

Sediment: The solid portion whch gets settled at the bottom of the testtube after centrifugation of urine

Microscopic Examination: Identification oe identification of normal or abnormal components using microscope is known as microscopic examination of urine.

Pus cells: A yellowish liuid formed in certain infections consisting of tissue fluid,bacteria and WBC

Alveolar: Pertaining to lungs

Chronic: Persisting for a long time

Cystinuria: Excretion of cystine in urine

Leucocytosis: Increased levels of WBC count

Leucopenia: The condition in which leucocytes count is decreased

Leukaemia: Abnormal in WBC count with immature cells among them

Physical Examination: The tests which does not require any kind of chemical substances is called as chemical examination.

Diabetes Mellitus: Lack of insulin secretion

Diabetes Insipidus: Lack of secretion of Anti diuretic hormone

Calculi: Related to stones and Kidneys

Haematuria: Excretion of blood in urine

Aneuria: Stoppage of urine formation or excretion

Centrifuge: Instrument used to separate solid substances from liquid mixture by centrifugal force

Glycosuria: Presence of sugar in urine

Hypoglycaemia: Low levels of sugar in urine

Hyperglycaemia: High levels of sugar in urine

Ketonuria: Excretion of ketone bodies in urine

Mucus: The viscid fluid secreted by mucous glands

Nephritis: Inflammation of nephron.

Chemical Examination: The test performed with chemicals to detect/ identify the presence of normal or abnormal components of human body is called as the chemical examination

Albumin: It is a variety of protein found in animal,vegetable matter,which is soluble in water and coagulates on heating

Albuminuria: Excretion of albumin in urine

Benedicts Qualitative Test: Test used to detect the presence of sugar in urine

Ketonuria: Excretion of ketone bodies in urine

Short Answer Type Questions

What is granular cast and mention about its clinical significance?

Write notes on parasites and parasitic ova present in urine.

Write about clinical significance of tyrosine crystals

Explain about mucus threads

Mention the names of different casts

Define microscopic examination of urine

What is leukaemia?

What is the normal urine out put or volume of urine in 24hrs in adults?

What are the causes of oliguria?

Define oliguria

What are the causes of polyuria?

Define polyuria and aneuria

In which conditions urine will appear as red urine and deep yellow?

What is the odour of normal urine?

What is the normal PH of urine?

What is the use of urinometer?

What is the specific gravity of normal urine.

What are the bile salts and mention their use?

Name the bilepigments

What is the significance of benzidine test?

Define ketonuria

Mention the names of ketonebodies

What is the importance of Hays test?

Write the names of bilesalts

What is the significance of Fouchets test?

Define Haematuria

What is Diabetes mellitus?

Mention the names of bilepigments

Define chemical examination

Long Answer Type Questions

Mention the different types of casts in urine

Write notes on various types crystals present in urine

Explain in detail about the cells present in urine and about its clinical significance

Write notes on physical examination of urine

Write about the Benedicts qualitative test and Fehlings test

Explain about the boiling test for albumin

Write about the following tests

a) Rotheras test b) Hays test c) Fouchets test

Define Haematuria and write about benzidine test

Write briefly about the chemical examination of urine by strip method

UNIT 11

REAGENTS USED IN PATHOLOGY

Structure

11.1 Introduction

The term Reagent can be applied in particular to any chemical compound or mixture of compounds,usually in solution,employed in chemical analysis or for the detection of biological constituents.A solution is a combination of two substances a solute and a solvent.The dissolved portion is called as solute and the substance present in relatively greater proportions in the solution is called the solvent.

Various types of reagents and solutions used in a pathological laboratory Are

a) Normal solutions b) Molar solutions c) Percent solutions d) Buffered solutions e) Indicators f) Primary standards g) Other complex reagents

Note: All the reagent bottles should be labelled appropriately

11.2 Preparation of Reagents, Principles and Interpretation

The following are the various reagents used in pathological laboratory

- Haemoglobin diluting fluids
  - 0.1N Hydrochloric acid
  - Drabkins reagent
- Benedicts solution
- RBC diluting fluid
- WBC diluting fluid
- 4.0g/dl EDTA solution (anticoagulant for completeblood count)
- Absolute eosinophil diluting fluid(Hinglemans solution)
- Blood banking reagents.
- Semen diluting fluid
- Cytology and Histopathology Fixtures

Haemoglobin Diluting Fluids

a) 0.1N HCL(200ml):

Method of Preparation

Take accurately quantity of HCL equivalent to 0.73 g of HCL

Add gradually to approximately 150ml of distilled water with constant stirring and cool

Dilute to 200ml with distilled water

Standardise and adjust normality if necessary

Principle

Brown coloured acid haematin is produced from haemoglobin,when blood is added to 0.1N HCL. After dilution the resulting or final colour formed is compared with standard brown glass reference blocks of a sahlis haemoglobinometer.

I**nterpretatio**n

0.1 NHCL is used in the estimation of Hb by Sahlis acid haematin method Decreased Hb values indicates anaemia.High Hb values are observed in congenital heart disease,in polycythemia vera and in emphysema

b) Drabkins reagent:

Sodium bicarbonate : 1.og

Potassium cyanide : 0.2g

Potassium ferricyanide : 0.2g

Distilled water : 1000ml

This reagent is stable in polythene container at 2-8^{0}C

To prepare the Drabkin's Solution, reconstitute one vial of the Drabkin's Reagent with 1000 ml of water.

Principle

When blood is mixed with Drabskins reagent containing potassium cyanide and potassium ferricyanide,haemoglobin reacts with ferricyanide to form methaemoglobin which is converted to stable yanmethaemoglobin(HiCN) by the cyanide.The intensity of colour is proportional to haemoglobin concentration and it is compared with a known cyanmethaemoglobin standard at 540nm

Interpretation

Haemoglobin: It is normally cinfined to the corpuscles but may be present free in the plasma(haemoglobinaemia)under conditions when there is appreciable intravascular haemolysis e.g,

In severe haemolytic anaemia or malaria
In septicaemia(blood poisoning)due to haemolytic streptococcus
As a result of transfusion with incompatible blood

Benedicts Reagent Method of Preparation

One litre of Benedict's reagent can be prepared from 100 g of anhydrous sodium carbonate, 173 g of sodium citrate and 17.3 g of copper(II) sulfate pentahydrate. It is often used in place of Fehling's solution.

Dissolve 173gm of sodium citrate with 100gm of sodium carbonate anhydrous in about 600ml of distilled water and gently heat it in 1000ml beaker or flask.In another beaker dissove 17.3g of copper sulphate in 100ml of distilled water and pour it into the carbonate citrate solution with constant stirring.Transfer to a 1000ml volumetric flask quantitatively wasing the beaker

with distilled water.Mixwell and bring the volume to 1000ml.When it cools to room temperature with disrilled water.If it is not clear, filter.

Principle

When sugars containing free aldehyde or keto groups are treated with Benedicts reagent,sugars are converted to enediols.Enediols are powerful reducing agents.

They reduce cupric ions to cuprous ions,which is responsible for change in colour of the Benedicts reagent.

Benedicts solution is a chemical solution used to detect the presence of glucose and other reducing sugars. Medically, it is used to test the urine of diabetics

Interpretation

This test is used in clinical practice to detect the reducing substance present in the urine.It is a semiquantitative test.When sugars are present at different concentrations,different colours are developed,which is interpretated and reported as follows.

Colour	Approximate amount of Sugar	Reported as
Green	0.5%	+
yellow	1.0%	++
orange	1.5%	+++
Red	2.0%	++++

RBC Diluting Fluid

Formal citrate sloution

Trisodium Citrate : 3 gm

Formalin : 1 ml

Distilled water : 99ml

Preparation

Trisodiumcitrate is dissolved in few ml of distilled water and to that formalin is added and finally volume is made to 100ml with distilled water.

b) Hayems Fluid

Sodium chloride 0.5 gm

Sodium sulphate 2.5 gm

Merecuric chloride 0.25gm

Distilled water 100ml

Peparation

All the contents are dissolved in few ml of distilled water and finally the volume is make up with 100 ml distilled water

Principle

The blood specimen is diluted to 1:200 with the RBC diluting fuid and cells are counted under high power(40 x objective) by using a counting chamber.The number of cells in undiluted blood are caluclated and reported as the number of red cells per cmm of whole blood

Interpretation

The RBC is a count of the number of red blood cells per cubic millimeter of blood. In response to hypoxia, the hormone erthyropoietin, secreted by the kidneys, stimulates the bone marrow to produce red blood cells. The formation of red blood cells is known as erthyropoiesis.

Normal red blood cell values at various ages are:
Adults: (males): 4.6 - 5.9 million
(Females): 4.2-5.4 million
Pregnancy: Slightly lower than normal adult values
Newborns : 5.5 - 6 million
Children : 4.6 - 4.8 million

4. WBC Diluting Fluid

Composition:

Glacial acetic acid	: 2.0ml
1%(w/v)gentian voilet	: 1.0ml
Distilled water	: 97ml

Preparation

Both the components are dissolved in few ml of distilled water and volume is made to 100ml. This solution is stable at room temperature(25+ r – 5^{o}C.A pinch of thymol may be added as preservative.

Principle

The glacial acetic acid lyses the red cells while the gentian voilet slightly stains the nuclei of the leucolytes.The blood specimen is diluted to 1:20 in a WBC pipette with diluting fluid and the cells are counted under the low power of the microscope by using a counting chamber.The number of cells in undiluted blood are reported per cubic mm of whole blood.

Interpretation

Total WBC: 4,500 - 10,000 millions per cmm

The numbers of leukocytes changes with age and during pregnancy.

On the day of birth, a newborn has a high white blood cell count, ranging from 9,000 to 30,000 leukocytes. This number falls to adult levels within two weeks.

The percentage of neutrophils is high for the first few weeks after birth, but then lymphocyte predominance is seen.

Until about 8 years of age, lymphocytes are more predominant than neutrophils.

In the elderly, the total WBC decreases slightly.

Pregnancy results in a leukocytosis, primarily due to an increase in neutrophils with a slight increase in lymphocytes.

Leukocytosis, a WBC above 10,000, is usually due to an increase in one of the five types of white blood cells and is given the name of the cell that shows the primary increase.

Neutrophilic leukocytosis	= Neutrophilia
Lymphocytic leukocytosis	= Lymphocytosis
Eosinophilic leukocytosis	= Eosinophilia
Monocytic leukocytosis	= Monocytosis
Basophilic leukocytosis	= Basophilia

6. Platelet Diluting Fluid

Compositiion

Sodium Citrate	: 3.8gm
Formalin	: 0.2ml
Brilliant cresyl blue	: 0.1gm
Distilled water	: 100ml

Preparation

Sodium citrate is dissolved in little amount of distilled water.To this formalin is added.Brilliant cresyl blue is also dissolved in little amount of distilled water, above two portions are added to one another and the final volume is made with distilled water to 100ml. Filter this fluid each time before use.

Principle

Blood is diluted with the diluting fluid to give a dilution of 1:200 and the cells are counted in the improved Neubauer chamber.

Interpretation

- Normal Results
- 150,000 - 400,000 platelets per microliter (mcL).

Increased Values of ESR

Are found with all diseases associated with a modification of the plasma proteins like globulin, albumin and fibrinogen. ESR shows especially high values in

- Tuberculosis
- Leishmaniases
- Malignant condition
- Hepatic Amoebiasis
- Acute and Chronic Inflammation
- 4.0g/dl EDTA (Ethylene Diamine Tetra Acetic Acid)
- EDTA - 4gm
- Distilled water - 100ml

Preparation

EDTA (Ethylene Diamine Tetra Acetic Acid)

4gm of EDTA is dissolved in distilled water and solution is prepared.

Principle

It acts as powerful calcium chelating agewnt.The calcium in blood is bound in an unionised and forms soluble complex with EDTA.

Interpretation

As the results are indirectly correlated with the following tests,refer the following tests in the previous topics.

Tests performed with EDTA are

- Haemoglobin
- WBC count
- RBC count
- PCV determination
- ESR byWintrobes method
- Platelet count *Differential WBC count

Absolute Eosinophil Diluting Fluid (Hinglrmans solution)Composition

The most commonly used diluting fluid is Hingleman's fluid. Hingleman's solution

Yellow Eosin – 0.5 g – stains eosinophil granules

95% phenol – 0.5 ml
Formalin – 0.5 ml – fixes the cells
Distilled water – 99 ml – lyse the RBCs

Preparation

Dissolve all the components in few ml of distilled water and made up to

100ml volume with distilled water.

Principle

Blood is diluted with a special diluting fluid which lyses the RBCs and stains the eosinophils red. These cells are counted under low power objective using Levy chamber with improved Neubauer ruling.

Interpretation

Normal values

40-440/cu mm (micro liter) i.e. 0.04 - 0.44 x 109/L

4.6.6 Eosinophilia - Increase in the absolute eosinophil count beyond the upper limit of normal (> 440 cells / cu mm)

Causes for Eosinophilia

Allergic Diseases: Bronchial asthma, Hay fever. In asthma - eosinophil count correlates with pulmonary performance and also indicates the adequacy of steroid therapy.

Skin Disorders: Eczema, atopic dermatitis.

Parasitic infections: Hook worm, Filariasis, Trichinosis, Cysticercosis.

Infectious diseases: Scarlet fever.

Semen Diluting Fluid

Semen diluting fuid is used in sperm counting

Composition

Semen Diluting Fluid

Sodium bicarbonate – 5 gm.

Formalin – 1 gm

Distilled water – 99 ml.

Preparation

Both the solid and liquid ingradients are dissolved in few ml of distilled water and made to 100ml with the distilled water.

Principle

Sodium bicarbonate provides basic medium to semen by keeping it as lively onFormalin acts preservative by restoring the natural condition of the semen

Water is used for diluting purpose

Interpretation

Sperm Vitality

Normal Observation

1. Spermatozoa Head caps :Light blue
2. Nuclear posterior : Dark blue
3. Bodies and tails : Red or pink
4. Spermatozoa size : 50-70 microns
5. Head size:3-6microns x 2-3 microns.

Low sperm counts are observed when there is supression of endogenous gonadotrophin production by exogenous estrogens or androgens or by anabolic agents.If sperm count is less than 60millions/ml,then there are less chances for fertility.

Normal sperm count is 100-150 millions/ml

Abnormal forms of Spermatozoa

Giant head spermatozoa
Pin head spermatozoa
Immature spermatozoan(spermatid)
Constricted head spermatozoa
Double tail spermatozoa
Amorphous form spermatozoa
Acute tapering form spermatozoa

Short Answer Type Questions

- Write the principle of Drabkins reagent.
- Write the interpretation of Drabkins reagent.
- Write the principle of Benedicts solution.
- Write the interpretion of Benedicts solution.
- Write the composition and preparation of RBC diluting fluid.
- Write the principle of RBC diluting fluid.
- Wite the interpretation of RC diluting fluid.
- Write the principle of WBC diluting fluid.
- Write the interpretition of WBC diluting fluid.
- Write the principle of 4g/dl EDTA solution.
- Write preparation of Absolute eosinophol diluting fluid.
- Write the principle of Absolute eosinophil dilting fluid.
- Write the interpretition of Absolute eosinophil diluting fluid.
- Write preparation of semen diluting fluid.
- Write principle of semen diluting fluid.
- Write interpretition of semen diluting fluid.
- Write interpretition of double oxalate mixture.

Long Anwer Type Questions

Write preparation,principle and interpretition of the following regents

(a) 0.1N HCL (b) Drabkins reagent (c) Benedicts solution

RBC AND WBC diluting fluids

Write the preaparation,principle and interpretition of following reagents

(a) 3.8g/dl Trisodium citrate (b) 4.0g/dl EDTA solution

(c) Absolute eosinophil diluting fluid (d) Reticulocyte dliuting fluid

UNIT 12

Sputum Analysis

Structure

12.0 Introduction

Matter which is expectorated from the lungs is known as the sputum.The analysis of sputum for finding out normal and abnormal constituents of a patient by physical,chemical and microscopical examination using laboratory techniques is known as sputum analysis.Normal sputum is colourless,watery and odourless tracheobronchial secretion.It is a constant mixture of plasma,mucin,electrolytes and water.When this secretion mixture passes through the lower and upper respiratory tract,the secretory mixture contaminated with cells removed from tissues in layers,nasal,and salivary gland secretions and normal bacterial flora of the oral cavity.Only sputum specimen is used to detect the presence of Mycobacterium tuberculosis bacteria and Mycobacterium leprae bacteria by Ziehl neelsen hot and cold staining methods.Physical examination also helps inidentification and in the diagnosis of certain diseases concerned to Lungs.

The lower respiratory tract has been kept sterile by

Alveolar Macrophage System

Mucociliary System: Early morning specimen or the entire 24-hr specimen should be collected into a sterile wide moth glass bottle and closed with a screw cap.

12.1 Physical Examination

Quantity: The amount varies from disease to disease.

Volume: 24 hrs volume sputum specimen should be collected and measured from the patients with chronic bronchitis,lung abscesses and bronichal asthma.

Abnormal Findings:Decreasing sputum volume indicates the normal state of the person.Increasing sputum volume indicates abnormal state of the patient.

Consistency And Appearence: Normal sputum is colourless water

and opalascent.Based on consistency and appearence,sputum can be classified in to the following types,which indicates abnormal of patient.

(i) Serous Sputum (ii) Mucoid Sputum(tough Sticky) iii) Purulent Sputum iv) Bloody Sputum. Ex : Seropurulent, Mucopurulent.

iv) C o l o u r: Normal sputum is clear and colouless.

Fig 12.1 Sputum Collection

Fig. 12.2 Physical Examination of Sputum

Abnormal Findings: Yellow coloured sputum of the patient indicates the pus&epithelial cells as seen in pnemonic process.Greenish coloured sputum of the sputum patient indicates pseudomonas infection.Rust coloured sputum of the patient indicates the decomposition of haemoglobin as seen in pneumococcal pheumonia or pulmonary gangrene.Bright red coloured sputum of the patient indicates recent haemorrhage,pulomonary infraction,breakdown of a blood vesselpulomonary tuberculosisBlack coloured sputum of the patient is due to inhalation of dirt.Coal,dust or due to the decomposition of anthracotic tissue.

Odour: Normal odour has no odour.

Abnormal Findings: Sputum with putrid odour of the patient indicates lung abcess bronchiectasis and gangrene of the lung.

Sputum with sweetish odour of the patient indicates pulomonary tuberculosis with cavities, bronchomoniliasis and bronchiectasis.

Sputum with cheesy odour of the patient indicates the necrosis of malignant tumours and perforating empyemas.

12.2 Preparation or Making a Sputum Smear

Sputum should be collected from the patient in to sterile container.From the sterile container a portion of sputum has been transferred on to the glass slide.With the help of a match stick or thick sterile needle and sputum has been made in the form of a film and covered with a cover slip and observed under the microscope.

12.3 Staining of Sputum Smear

Sputum smear on the glass slide has been dried in the air.After that sputum smear has been fixed by passing over a fame of a spirit lamp.After fixation of the sputum smear,sputum smear should be subjected to the following stainig techniques.

(i) Gram staining (ii)Acid Fast Staining (iii)Wrights staining

(iv) Buffered Crystal Voilet Staining (v) Paps staining

Fig. 12.3 Gram Stained Sputum Smears

(i) Grams Staining Of Sputum Smear

Christian gram has been discovered the Grams Staining Technique.By this technique the presence of gram +ve or gram -ve bacteria in a sputum smear can be identified.

Required Reagent

- Crystal Voilate Solution
- Grams Iodine Solution
- Ethyl Alcohol
- Safranine Solution

Staining Procedure

First take a clean dry slide and prepare sputum smear then dry it in air.After that mix the sputum smear on glass slide by passing over a flame of a spirit lamp.After fixation crystal voilate solution is added to the smear and after one minute wash with tap water.After that add gram iodine solution to the sputum smear and after one minute remove the grams iodine solution.After that decolourise the sputum smear on glass slide with 95% alcohol for 20 or 30 seconds.After that alcohol is removed from the slide by washing with tap water.After that again counter stain the sputum smear on glass slide with safranin solution.After that safranin solution is removed from the sputum smear of glass slide,dry the sputum smear in air and first observe under low power objective and secondly under high power objective of simple microscope.

Result: Gram +ve bacteria appear as in voilet colour.

Gram -ve bacteria appear as in pink colour.

Acid Fast Staining For Sputum Smear(Ziehl Neelsen Stain Method): Sir Ehrich had been discovered the Acid Fast Stainig. Mycobacteriumtuerculosis has been identified with Ziehl neelsen hot stain technique by examining sputum after acid fast staining under sample microscope.

Ziehl Neelson Hot Stain Method

Required Reagents

Carbol Fuchsin Solution.
Working Carbol Fuchsin Solution.
Stock Carbol Fuchsin Solution.
20% Sulphuric Acid.
3% Mythelene Blue Solution.

Procedure

Prepare the sputum smear on glass slide,dry in air and fix by passing through a flame of bumsen burner.Keep the sputum smear present on the gkass slide on a rack or on glass rods of a tray.Then stain it with working carbol fuchsin.Afterwards heat the slide withy sputum smear over a bunsen burner flames till the fumes comes out of the sputum smear.Afterwards wash it with water.Next counter stain the glass slide with sputum smear with methylene blue.Afterwards wash it with tep water and dry it in air.After then observe or examine under microscope.

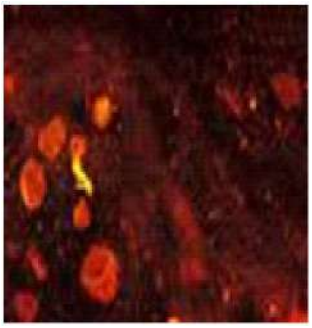


Fig. 12.4 Mycobacterium Tuberculosis in Sputum Sample

Result: Acid Fast organisms appears as red bacilli on blue black ground.

Ziehl Neelson Modified Method or Cold Stainig Technique:

By these technique mycobacterium leprae can be identified.

Required Reagents

- Stock Carbol Fuchsin Solution.
- Working Carbol Fuchsin Solution.
- 3% Mythelene Blue Solution.
- 5% Sulphuric Acid.

Procedure: Prepare the Sputum Smear as usual using glass slide and then stain it with working carbon fuchsin for15 minutes.And then wash it with to water.Then decolourise the sputum smear with5% sulphuric acid. Afterwards counter stain with methylene blue for 1 minute.Wash the sputum smear slide with water,dry it and observe under microscope.

Result: Acid fast bacili appear as bright red bacili on blue background.

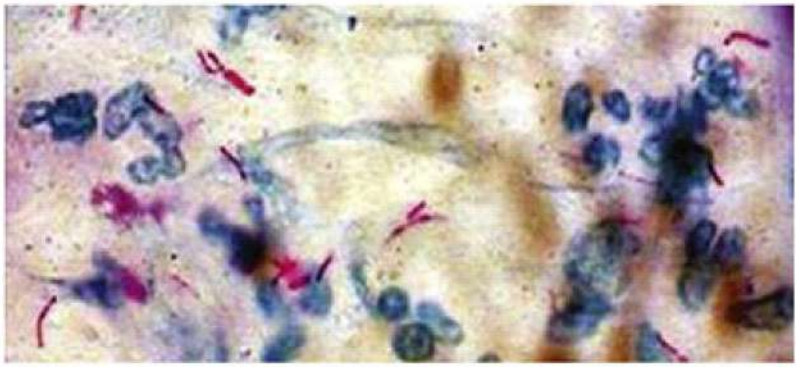


Fig. 12.5 Sputum specimen showing AFB

Wright Staining Of Sputum Smear: The presence of blood cells in sputum can be detected with the help of wrights stain.

Buffered Crystal Voilet Stainig: The presence of epithelial cells in sputum can be detected with this stainig technique.

Paps Stain: The stainig technique is useful for studying cytology of sputum.

12.4 Mounting

The covering 0f sputum specimen taken onto the glass slide with a coverslip for for diagnostic purposes by examining microscopically is called as mounting.

In certain cases for mounting the specimen mouting media are used in special situations.

12.5 Microscopical Examination of Sputum Smear

The detction or identification of normal or abnormal components of a sputum stained smear using microscope is known as Microscopical Examination of Sputum Smear.

Procedure: Transfer sputum on to clean glass slide and prepare smear and cover it with cover slip.Then observe under simple microscope.When ever you observe sputum smear under simple microscope,normally the following abnormal components can be detected. Those are Elastic Fibres

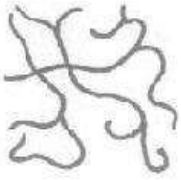
Abnormal Findings

The presence of elastic fibres in sputum smear indicate destruction of lung tissue,whether from grangrene or abcess.These elastic fibres can be detected with the number of a wet cover slip preperations.

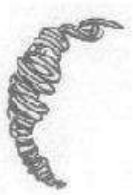
Bronchial casts

Heart Failure Cell

Myelin globules



Elastic Fibers



Curshmann's Spiral

Fig. 12.6 Sputum Examination Microscopic Findings

(ii) Curschmans Spiral

Abnormal Findings: These structures are sometimes found in asthmatic patient.But their exact nature is not known.

(iii) Charcot Leyden Crystals

Abnormal Findings: The presence of these crystals in sputum smear indicate the asthma.

(iv) Pigment Cells

Many mono nuclear cells containig pigments may be found in the sputum.These cells may show dark brown blood pigments or black carbon pigments.

(v) Sulpher Granules

The presence of sulphur granules indicates Actinomycosis of the lungs.

vi) Bronchial Cast

In such conditions fibrinous cast may form inside the bronchial tree and may be found in the sputum as branching Structures.

Cellular Structure

From granular degeneration pus cells may be observed in sputum smear.

Red Blood Cells

Abnormal Findings : The presence of Eosinophil cells is sputum smear indicates asthma of a patient.

(ix) Parasites:

Abnormal Findings: The presence of hooklets ajnd still often of fragments of the laminated Octocyst of echnicoccus hydatid indicate hydatid disease of the lung.

(x) Asbestosis Bodies

Abnormal Findings: The presence of asbestosis in sputum smear indicate the asbestosis.

Short Answer Type Questions

- Define sputum.
- Define sputum analysis.
- What is the volume of sputum?
- Write the types of sputum.
- What is the clinical significance of volume?
- What is the colour of sputum?
- Write the odour of sputum.
- What is the clinical significance of colour?
- What is the clinical significance of odour?
- .How do you prepare sputum smear?
- Abbreviate AFB.
- Give examples for AFB.
- Mention the methods of staining of sputum smear.

Long Answer Type Questions

- Write down the physical examination of sputum.
- How will examine sputum smear microscopically?
- Write down the procedure of Ziehl neelsen hot staining method
- Explain about the Ziehl neelsen cold staining method

UNIT 13

Semen Analysis

Structure

13.1 Introduction

The secretion from the testicles and accessory male sex Organs.ex.Prostate containg spermatozoa is known as semen.Spermatozoa is suspened in seminal plasma.The seminal plasma activates the spermatozoa to a greater motility.The analysis of semen plays an important role in finding out the functioning of gonads..Semen because of containing the sperms which are playing prominant role in forming zygote after getting fertilised with ovum.Many of the childless male individual wont come forward in giving semen for analysis.

Nothing wrong is there in knowing the reason behind not bearing (getting) child.It is not only the responsibility of male individual to consult the doctor to know reason behind not bearing(getting) the child and at the same time the female individual also should consult the doctor to know the reason. Both the married couple should consult the doctor without hesitation for finding out reason.If there is no possibilty of conceiving,then the couple should either adapt a child or should approach the test tube baby center.

The couple should not feel shy regarding this.So many organs are getting replaced,transplanted in the todays human beings existing life.So in the same way the childless couple can approach the test tube care center if all the doors are closed.Research is going on in preparing the artificial ovum and sperm from the human cells in a congenial environment. Once this is achieved then childless couple can have ready made ova and sperm. If these are prepared from childless individuals then it will be a great achievement in the medical technology. I hope India too will try in this direction in the quest of new achievements in the field of medical technology in the coming future. Lets hope with positive attitude.

Composition of Semen

Semen is viscid,neutral or slightly alkaline and pale yellow coloured due to its flavin ocntent. Approxmately 60% of semen volume is derivede from the seminal vescicles ,which are also the major source of the the high fructose

content of semen.The other constituents of semen are potassium, critic acid, ascorbic acid, ergothionine and phosphoryl choline.

13.2 Collection of Specimen

Before collecting the semen sample the person should not participate in intercourse up to three days.After three days the person can collect the semen specimen.In clinical pathological laboratory the specimen is collected by masturabation.

Another Method: In home patient can collect semen in to wide mouth clean bottle powder free in dry. In patients home by coitus interruptus he can collect the semen in to the wide mouth container.The specimen should be delivered with in 30 minutes to the laboratory.

Fig. 13.1 Semen collection from animal

Storage: The semen specimen should be examined immediately after collection.If it is necesary to store,it should be kept at room temperature but do not store in the refregirator.

Couselling Before Collection

The Individual from which the semen was collected should be given counselling before collection due to lack of knowledge regarding the purpose of semen collection.Guide lines and way of collecting the semen should be explained by the technician(coitus interuptus or by masturbation).

The individuals should be made psychologically alert strong before the collection of semen.The individual should feel comfortable in giving semen.They should have awarenesss regarding the semen collection.

13.3 Physical Examination

(a) Volume

Measure the semen immediately after collection.From few drops to 5ml. of semen should be present.Normally volume of semen is in between 3-4ml.If semen volume is below 1.5ml. then the semen volume should be considered as below normal.

(b) Viscosity

Viscosity of semen should be observed by pouring from a pastuer pipette drop by drop.The semen specimen has normal viscosity,if semen is poured drop by drop.

(c) Liquefacation

Semen should be converted into liquid state with in 30 minutes.

The motility of spemetozoa is stopped if the semen did not convert in to l;iquid state.i.e. motility of spermetozoa is restricted,if semen remains highly viscous.

(d) Reaction

The pH of semen always remains in the alkaline side.The normal value is 7.2 to 8.9.

(e) Motility

Take one drop of semen om to the glass slide and cover with a coverslip and observe under low power and high power objective.

If spermetozoa is absent in the semen then that condition is called as Azoospermia.

If semen contains few spermetozoa which have motility then that conditions called as Oligozoospermia .

If semen contains spermetozoa which has no motility then that condition is called as Necrozoospermia .

Fig. 13.2 Sperm Insemination

(f) Colour

It is opaque,white or grey white coagulum.

(g) Odour

Musty or Acrid

13.4 Microscopic Examination of Semen

Study of Motility of Sperms

Transfer a small drop of liquified semen in a glass slide and cover it with a cover slip.

Observe the cover slip preperation under the high power objective with reduced illumination.

Count the number of sperms which are actively motile out of the total count of 200.

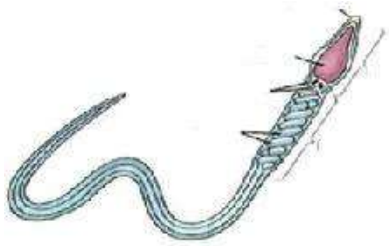


Fig. 13.3 Sperm Structure

Calculate the percentage of sperm showing actual progressive motion.

Observe the slide after 2 hours,3 hours and 6 hours. Care should be taken to present drying of he sperm.

Observe for pus cells,epithelial cells and or the other findings.

Determination of Sperm Count

After liquefaction gently mix the specimen.

Draw semen up to the 0.5 mark of a WBC pipette.

Draw the semen diluting fluid up to 11 mark and mix well.

Load the Neubauer chamber and allow the sperms to settle for about 5 minutes.

Count the sperms in the four corner squares(as in WBC count)

Calculation: Sperms /ml. of semen = (Sperms counted in four Squares x 10 x 20 x 1000) / 4

Normal sperm count = 100 to 150 millions/ml.

Determination of Morphology of Sperms

Semen after (liquefacation) getting liquified transfer a drop of semen on to a clean glass slide and prepare a smear.

Dry the semen smear in air and heat very gently to fix.If necessary remove the mucus by dipping semen smear in semen diluting fluid and then in buffer distilled water.

Using Leishman stain,stain the smear or stain in 0.25% aqueous basic fuchsin for 5 minutes.

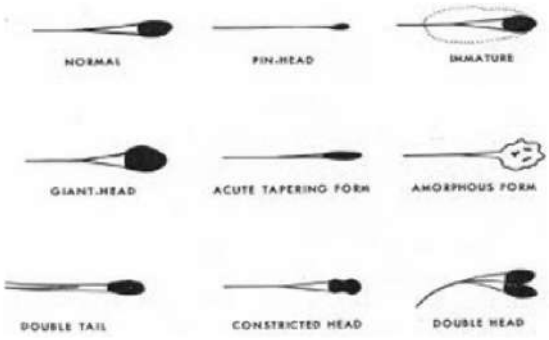


Fig. 13.4 Morphology of Sperms

NORMAL OBSERVATIONS	ABNORMAL FORMS OF SPERMS
1. Spermetozoa Head Caps: Light Blue.	Giant head Spermatozoa Pin head Spermatozoa Immature Spermatozoa
2. Nuclear Posterior: Dark Blue.	Constricted Head spermatozoa Double tail Spermatozoa
3. Bodies And Tails: Red Or Pink.	Double head Spermatozoa Amorphous Form spermatozoa
4. Spermetozoa Size: 50-70 Microns.	Acute tapering form spermatozoa
5. Head Size: 3-6 Microns X 2-3 Microns.	

Clinical Significance

Low sperm counts are observed when there is supression of endogenous gonadotrophin production by exogenous estrogens or by anabolic agents. Hypothyrodism and hyper thyrodism oligozoospermia. Trauma infections,irradiation and antimitotic chemotherapy can damage the testes. These patients often have oligospermic or azoospermia. Loss of libido,testicular atrophy and azoospermia can result from a pituiatary and hyper thalamic turnor. If sperm count is less than 60millions/ml. these are less chances for fertility.Normal sperm count 100-150 millions/ml.

Conclusion

Many of the individuals feel discomfortable in consulting the doctor regarding childlessness. Many factors play role in effecting either in the release of ovum in right time or presence of fully motile spermatozoa. Especially in India manyindivduals feel shy and won't consult the doctor.

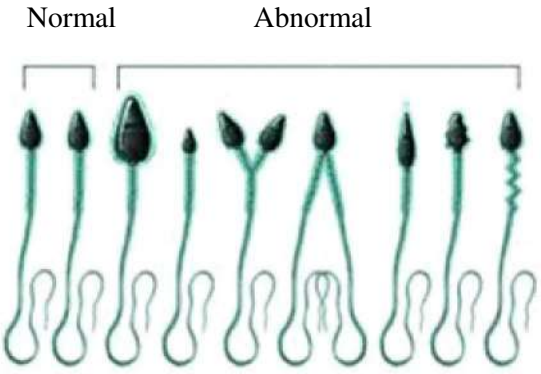


Fig. 13.5 Abnormal sperm Morphology

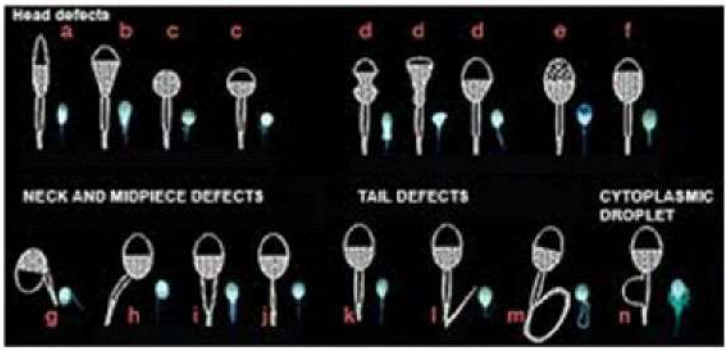


Fig. 13.6 Abnormal forms of Human Sperm Etozoa

Some times with minor problems also the individuals may not bear the child. And the collection of semen by both the methods (masturbation and coitus interuptus) are not easy methods of collecting the semen.With minor surgeries also the problems of women can be solved. So the individuals in the quest of children should approach or consult the doctor.Some times the persons (society) surrounding the individuals and his family members also prick them with harsh words.

The society should change their attitude towards the individuals not bearing the child.Any how Sputum analysis plays ultimately an important role in knowing the status of sperms present in the semen.

Key Terms

Semen : The secretion from the testicles and accessory male sex organs ex. prostate containing spermatozoa is the semen

Seminal plasma : The fluid portion in which sperms are suspended

Semen analysis : Analysis of semen to know about the spermatozoa condition

Masturbation : The process of stimulating the individuals genital organ with hands for the collection of semen

Coitus Interuptus : The process of collecting the semen by interupting sexual intercourse

Azoospermia : The condition in which spermatozoa is absent in semen

Oligozoospermia : The condition in which semen contains few spermatozoa which have motility

Necrozoospermia : The condition in which the semen contains few spermatozoa which has no motility

Liquefaction : The process of conversion of semen from solid state to lquid state

Short Answer Type Questions

Define semen

How do you collect semen specimen

What is Masturbation?

What is the normal volume of semen?

What is the PH of the semen?

What is the odour of semen?

Write the composition of semen

What are the various parameters present in physical examination of semen

Mention the normal sperm count

What is the PH of semen

Long Answer Type Questions

How will you examine different parameters of a semen by physical examination?

Write notes on microscopic examination of ssemen

Explain about the determination of morphology and sperm count.

UNIT 14

Body Fluids

Structure

14.1 Introduction

The commonly examined body fluids in the pathological laboratory are

1.Serous fluids such as

(a) Pleural (around the lungs)

(b) Pericardial (around the heart)

(c) Peritoneal fluids (around the abdominal and pelvic cavities).

2.Synovial fluids(around the joints).

14.2 Peritonial Fluid

Definition: The double serous membrane which covers the organs present abdominal cavity and pelvic cavity is known as peritonial cavity.The fluid present in the peritonial cavity is known as peritonial fluid.Generally peritonial fluid contains less than 100ml.

Normal composition of Peritonial Fluid

Peritonial fluid appears as clear or pale yellow in colour. Peritonial fluid do not consists of albumin. The pH of peritonial fluid is 7.4.Peritonial fluie consistitutes alkaline phosphatase,ammonia,cholestrol, glucose, lactic acid, lactate dehydrogenous and white blood cells.

Collection of Peritionial Fluid (Specimen)

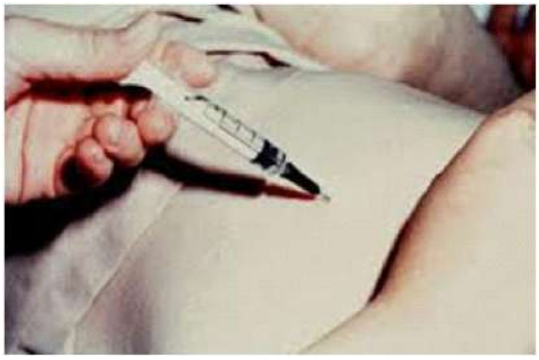


Fig. 14.1 Peritoneal Fluid Collection

The specimen is collected in fluoride oxolate tube, EDTA tube and plain tube. The peritonial fluid is collected into 3 asceptic tubes as follows:

Add 5ml of peritonial fluid into a testtube containing 15mg of fluoride oxolate.This is used for determination of protein and sugar.

Add 5ml of peritonial fluid into a testtube containing 15mg of EDTA.This is used for microscopic examination.

Add 5ml of peritonial fluid into a plain tube which is used for bacteriological tests.

Diluting Fluid and Labelling

Diluting Fluid

If the specimen is clear do not dilute it.Charge neubauer chamber directly by the specimen.If the specimen is turbid then dilute it by using saline.Acetic acid present in WBC diluting fluid may cause turbidity by reacting with the high protein content of the fluid.

Labelling

Labelling of sample plays an important role in medical laboratories. Samples have to be labelled after collection to enable their identification during testing and reporting. Eventhough the volume of sample is more and if it is not labelled due to forgetfullness it is useless. Proper numbering should be marked on the label seeing the previous samples. After labelling on the sample,the same numbers should be noted in the records with patients name.this will avoid the confusion in the identification of patients sample

Physical Examination of Peritoneal Fluid (Clinical Significance)

Peritoneal fluid is turbid in the following conditions.

(a)Appendicitis
(b) Pancreatitis

(c) Infected intestine

(d) Ruptured bowel due to the trauma

Peritoneal fluid is pale yellow in colour or ambered colour in the following.

(a)Hepatic vein obstruction
(b) Cirrhosis
(c) Nephrotic syndrome
(d)Congestive heart failure

Peritoneal fluid is greenish in colour in the following conditions.

(a)Perforated intestine
(b) Perforated gall bladder
(c) Appendicitis
(d)Perforated duodenal ulcer.

Peritoneal fluid is milky white in colour in the following conditions
(a)Parasitic infections
(b) Nephriotic Syndrom
(c) Carcinoma
(d)Lymphoma

Peritonial fluid is bloody in the following conditions.

a)Haemorrhagic Pancreatitis

b) Ruptured Liver

Microscopical Examination of Peritonial Fluid

In peritonial fluid total leucocyte count more than 500/cmm or RBC count more than 10,000/cmm are considered as abnormal.Increased total leucocyte count,mainly neutrophils in peritoneal fluid indicate acute peritonitis from any cause.

14.3 Pericardial Fluid

The fluid which is present in double membranous sac which covers the heart is known as Pericardial Fluid.

Normal Composition of the Pericardial Fluid

Pericardial fluid appears as pale yellow col;oured one and is clear.Volume of pericardial fluod is 20 to 50 ml. normally.If volume pf pericardial fluid exceeds 200ml., that condition should be considered an abnormal one.Pericardial fluid constitutes glucose and lactate dehydrogenase.

Collection of Pericardial Fluid (or Pericardial Fluid Aspiration)

Aspirate pericardial fluid into 3 sterile tubes.Use EDTA tube for gross examination and for microscopic examination.Plain or heparinised tube should be used for microbiological examination.

Heparinised tube is used for chemical examination.

Collection of Pericardial Fluid

Note: Pericardial fluid aspiration should be done under CT scan guidance.

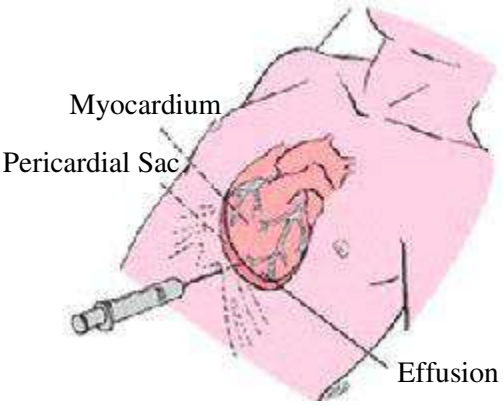


Fig. 14.2 Collection of Pericardial Fluid

Diluting Fluid and Labelling

Diluting

If the specimen is clear do not dilute it.Charge neubauer chamber directly by the specimen.If the specimen is turbid then dilute it by using saline.Acetic acid present in WBC diluting fluid may cause turbidity by reacting with the high protein content of the fluid.

Labelling

Labelling of sample plays an important role in medical laboratories.Samples have to be labelled after collection to enable their identification during testing and reporting.Eventhough the volume of sample is more and if it is not labelled due to forgetfullness it is useless.Proper

numbering should be marked on the label seeing the previous samples.After labelling on the sample,the same numbers should be noted in the records with patients name.this will avoid the confusion in the identification of patients sample

Clinical Significance

Pericardial fluid may be clear,cluody grossly bloody,milky or similar to gold point.

Increased amounts of normal appearing pericardial fluid may be found in
(a) Congestive Heart Failure
(b) Earky Stages of Inflammation
Cloudy appearence may be associated with
a)Septic/nonseptic inflammation
b) Chronic effusions of any etiology

c) Myxedema
d) Idiopathic
e) Post myocardial infraction syndrom.

Blood tinged pericardial fluid is seen in traumatic tap but it clears on aspirating more fluid.

Grossly Bloody Fluid may be caused by

(a) Post myocardial infraction syndrome
(b) Post pericardiectomy syndrome
(c) Tuberculosis
(d) Rheumatoid arthritis
(e) Systemic lupus erythematosus
(f) Metastatic Carcinoma
(g) Bacterial pericarditis

Milky Pericardial May Be Due to the

(a) True chylopericardium
(b) Bacterial
(c) Fungal
(d) Tuberculosis
(e) Rheumatoid Pericarditis
(f) Myxedema

Microscopial Examination of Pericardial Fluid

Total and differential counts are done as CSF. Increased leucocytes with more neutrophils indicates bacterial pericarditis but this condition may also seen in viral pericarditis.A high percentage of lymphocytes suggests tuberculosis pericarditis.

Microbiological Examination of Pericardial Fluid

Cultures for bacteria,fungi and tuberculosis should be performed in all effusions of unknown ethiology.

14.4 Pleural Fluid

The fluid which is present in the cavity between the layers sorrounding the lungs is called as PLEURAL FLUID.

Normal Composition of Pleural Fluids

Pleural fluid is clear and slightly amber in colour.The pH of pleural fluid is 7.4 and specific gravity of pleural fluid is 1.016.The volume of pleural fluid

isless than 25ml.Pleural fluid consisits, cholestraol, glucose, lactate dehydrogenase, total protein and White Blood Cells.

Collection of Pleural Fluid

Collect pleural fluid in to 3 sterile EDTA tubes and label the test rubes.Use the first test tube for culturing and Grams Staining. Use the remaining two test tubes for cell counts, differential counts, total protein, glucose and cytology.

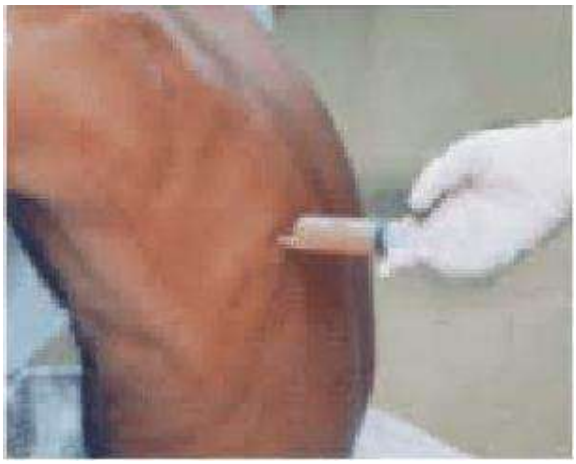


Fig. 14.3 Pleural Fluid Collection

Diluting Fluid and Labelling

Same as in Peritoneal fluid

Clinical Significance

Haemorrhogic pleural fluid can be observed in the following conditions

(a) Intra Pleural Malignancy
(b) Pancreatitis
(c) Pulmonary Infraction
(d) Pleural Infection
(e) Closed Chest Trauma
(f) Tuberculosis
(g) Hepatic Cirrosis

Microscopial Examination of Pleural Fluid

Follow the procedure of total count and differential count of CSF for pleural fluid also. A WBC count of more than 1000/cmm.or over 50% of neutrophills suggests inflammation(septic or nonseptic).

Sometimes lymphocytic effusion may be seen in

(a) Cardiopulmonary Disease
(b) Cirrhosis
(c) Infectious Mononucleosis
(d) Subacute Bacterial Pulmonary Infection

14.5 Cerebrospinal Fluid

Definition : The fluid whih is formed by selective dialysis of the plasma by the choroid plexus of the ventricles of the brain is known as CSF.CSF is present in the cavity present between the layers that sorrounds the brain in the skull and the spinal chord in the spinal column.The volume of CSF in adults is 150ml.

Collections of CSF

The CSF specimen should be cillected by a specially trained technician,a physician or a nurse. The sterile lumbar puncture needle is inserted between the fourth and fifth lumbar vertabrae to a depth of 4-5 cm. After collection of CSF specimen the fluid is collected through the needle in to two test tubes.

In to first test tube about 0.5ml. or few drops of CSF is collected.

In to second test tube about 3-5ml. of CSF is collected.

Note :1. The specimen in the test tube no.1 is used for bacterial culture.
.2.Specimen second test tube is centrifuged.
3.Supernatent liquid is used for biochemical tests such as glucose, protein, globulin and chlorides.
4.Use the sediment for grams staining,acid fast staining and differential leucocyte count after preparing a smear

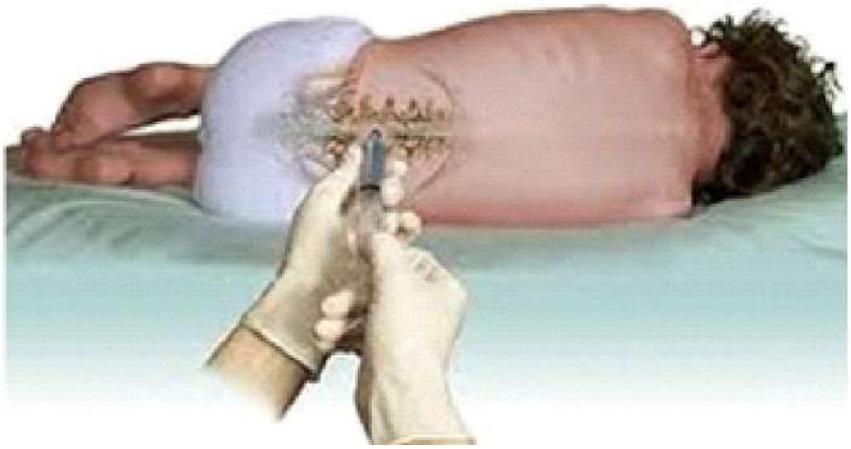


Fig. 14.4 Collection of CSF

Normal Composition of CSF

Colour : CSF has no colour i.e. colourless.

The pH of CSF is 7.3 -7.4

Appearance : CSF appears to be clear

CSF will not form clot on standing.

Specific Gravity : 1.003 - 1.008

Total Solids : Urea,Globulns,Sugar,Uric acid and Proteins etc.- 1.70g/dl.

Volume : Volume of CSF is 100-150 ml.

Routine Examination of CSF : It should be performed by

- Physical examination
- Microscopic examination
- Chemical examination

Physical Examination

Collect the specimen and note down the observations for the following aspects.

Colour : CSF has no colour in normal condition.

Appearence :CSF appears to be clear in normal condition.

Presence Of Blood : This will be observed in cerebral haemorrhages, **meningitis.**

Presence of clot or fibrin web in CSF take place due to blood brain barrier disturbance.

pH: The pH of CSF is 2-10.5

Microscopic Examination

Requirements: 1. Fuchs rosenthal counting chamber or improved neubaur counting chamber with cover slip. 2. Glass slides 3. Pasteur pipettes 4. Leishman stain and buffer solution pH 7.0 5.CSF diluting fluid 6. Grams stainig reagents 7. Acid fast staining reagents 8.Centrifuge 9. Microscope

Test Principle

Glucose in CSF specimen reacts with ortho-toluidine in hot acidic medium to form a green coloured complex.The intensity of ultimate colour produced is measured by using a photometer at 620nm to 660nm.The measured colour intensity is directly proportional to the concentration of glucose in the specimen.

Normal Values : (fasting) 70-110 mg/dl

Serum/plasma (post prandial) : Up to 130mg/dl.

(2 hrs. after lunch)

Specimen Collection

Fasting sample,post-glucose sample and post prandial samples should be collected for quantitative determination of glucose.

Clinical Significance: Increased levels of glucose can be observed in diabetes mellitus, hyper thyroidism and hyper pituitarism.

Globulin: Presence of globulin in CSF can be detected by a) Pandys test b) Nonne-apelt test

andys Test: Take test tube and to that add 1ml. of pands reagent and to that add 1 drop of CSF.

Result: If bluish white cloud is formed arround CSF drop, increased globulin level is observed in CSF.

Nonne-Apelt Test: Take clean test tube and to it add 1ml saturated ammonium sulphate.To this add 1mof CSF.If white ring is formed between the junction of two liquids,it indicates the presence of globulin in CSF.

Clinical Significance of Pandys Test: This test gives rough ideas about the increased levels of globulin in bacvterial infection.

Clinical Significance of CSF Examination: CSF examination is carried out in the pathological laboratory mainly for the diagnosis of meningitis.CSF examination is required in encephalitis,subarrachnoid haemorrhage,spinal chord tumor and CNS syphylis.

Key Terms

Bodyfluids: The fluids which are present in the various regions of the bodyi.e between different layers and in cavities are known as body fluids

CSF: The fluid which is formed by selective dialysis of plasma by the choroid plexus of the ventricles of the brain is known as CSF

DLC: Differential leucocyte count

Pleural Fluid: The fluid which is present in cavity between the layers surrounding the lungs is called as pleural fluid

Pericardial Fluid: The fluid which is present in double membranous sac which covers the heart is known as pericardial fluid

Peritoneal Fluid: The double serous membrane which covers the organs present in abdominal cavity and pelvic cavity is known as peritoneal fluid

Trauma: Bodily injury:emotional shock
Pancreatitis: Inflamation of the pancreas
Cardial: Related to heart
Choroid plexus: Related to brain
Inflammation: Swollen part of the skin
Intrapleural Malignancy: Cancer of the pleural cavity

Short Answer Type Question

Define CSF
Define pleural fluid
HOW do you collect CSF?

Write down the physical examination of CSF
What is pandys test?
Define pericardial fluid
Write down the normal composition of pleural fluid
How do you collect pericardial fluid?
Define peritoneal fluid
How do you collect peritoneal fluid?

Long Answer Type Questions

Write notes on microscopical examination of CSF
Explain in detail about Pleural fluid
Write notes on peritoneal fluid
Write notes on chemical examination of CSF
Explain about the pericardial fluid.

UNIT 15

Haematology

Structure

15.1 Introduction

The branch dealing with the study of blood and blood components is called as haematology. Haematology encompases the study of blood components and coagulation. Blood may be described as a specialised connective tissue which circulates in a closed system of blood vessels. The circulating blood consists of suspensiion of formed elements such as erythrocytes, leucocytes and platelets in a pale yellow coloured fluid called plasma.

Hematology, also spelled haematology, is the branch of medicine concerned with the study of the cause, prognosis, treatment, and prevention of diseases related to blood. It involves treating diseases that affect the production of blood and its components, such as bloodcells, hemoglobin, blood proteins, bone marrow, platelets, blood vessels, spleen, and the mechanism of coagulation. Such diseases might include hemophilia, blood clots, other bleeding disorders and blood cancers such as leukemia, multiple myeloma, and lymphoma. The laboratory work that goes into the study of blood is frequently performed by a medical technologist or medical laboratory scientist. Many hematologists work as hematologist-oncologists, also providing medical treatment for all types of cancer.

The functions of the blood are as follows:

- Respiration
- Excretion
- Acid-base balance maintenance
- Nutrition
- Regulation of water balance
- Regulation of body temperature
- Transport of harmones, vitamins and salts
- Transport of metabolites
- Defensive action
- Coagulation against haemorrhages

15.2 Collection of Blood

Definition: The way of obtaining blood from veins and capillaries of a human body using disposable syringes is known as collection of blood.

Universal precautions were designed for doctors, nurses, patients, and health care support workers who were required to come into contact with patients or bodily fluids. This included staff and others who might not come into direct contact with patients.

Pathogens fall into two broad categories, bloodborne (carried in the body fluids) and airborne.

Uses

Universal precautions were typically practiced in any environment where workers were exposed to bodily fluids, such as:

- Blood
- Semen
- Vaginal secretions
- Synovial fluid
- Amniotic fluid
- Cerebrospinal fluid
- Pleural fluid
- Peritoneal fluid
- Pericardial fluid
- Bodily fluids that did not require such precautions included:
- Feces
- Nasal secretions Urine
- Vomitus
- Perspiration
- Sputum , Saliva

Methods of Collection

Definition

The various methods which are used to collect the blood from various routes is called as the methods of collection.

The following methods are used to collect the blood

- Venous Method
- Capitallary Method
- Vacutainer Methods

Collection of Venous Blood

Aim: Collection of Venous Blood.

Requirements: Disposable syringe, tourniquet, cotton, spirit.

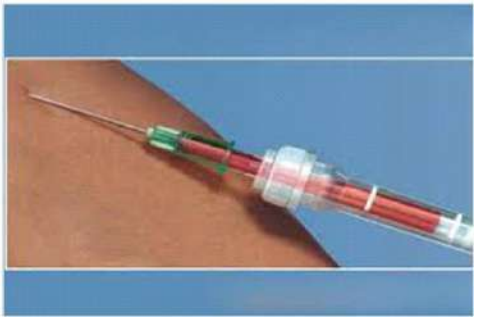


Fig. 15.1 Collection of Venous blood

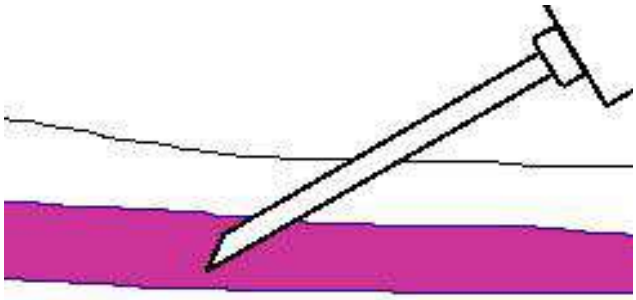
Blood Specimen Collection and Processing

The first step in acquiring a quality lab test result for any patient is the specimen Collection.

Venipuncture Procedure:

1. A phlebotomist must have a professional, courteous, and understanding manner in all contact with all patients.
2. The first step to the collection is to positively identify the patient by two forms of identification; ask the patient to state and spell his/her name and give you his/her birth date. Check these against the requisition (paper or electronic).
3. Check the requisition form for requested tests, other patient information and any special draw requirements. Gather the tubes and supplies that you will need for the draw.
4. Position the patient in a chair, or sitting or lying on a bed.
5. Wash your hands.
6. Select a suitable site for venipuncture, by placing the tourniquet 3 to 4 inches above the selected puncture site on the patient.
7. Do not put the tourniquet on too tightly or leave it on the patient longer than 1 minute.
8. Next, put on non-latex gloves, and palpate for a vein.
9. When a vein is selected, cleanse the area in a circular motion, the area needs to be re-cleansed before the venipuncture is performed.

10. Insert the needle through the skin into the lumen of the vein. The needle should form a 15-30 degree angle with the arm surface.



11. When the last tube is filling, remove the tourniquet.
12. Remove the needle from the patient's arm using a swift backward motion.
13. Apply and hold adequate pressure to avoid formation of a hematoma. After holding pressure for 1-2 minutes, tape a fresh piece of gauze or Band-Aid to the puncture site.
14. Dispose of contaminated materials.

Precautions

1. Void healed burn areas
2. Take steps to avoid haemolys is
3. Always Torniquetor aclothlikeh and key is used to tighten the top portion of the hand, which pools up blood in the collecting site.
4. Always asceptic conditions are followed and disposable syringes and needles are to be used

Advantages

- For separation serum from venous blood(clotted blood as specimen)
- Separated serum from venous blood is used in chemistry testing, serological testing and blood banking (serum as a specimen)
- Whole blood is used in haemotology testing.
- Plasma is used in coagulation studies and in plasma chemistries (plasma as specimen)

Finger Puncture:(Collection of capillary blood)

Definition:The blood which is collected from the capillaries by puncturing the skin is called as the collection of capillary blood)

Collection of Capillary Blood

Aim: Collection of Capillary Blood.

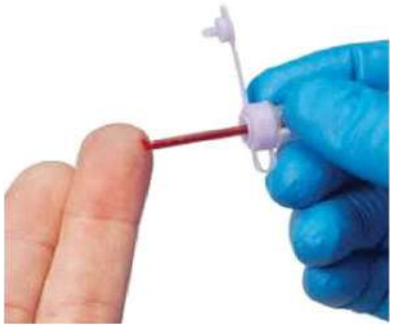
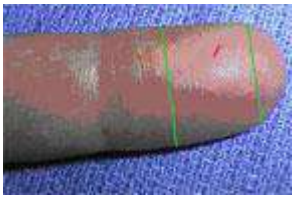


Fig. 15.2 Capillary Blood Collection

Requirements: Disposable needle, spirit, cotton.

Fingerstick Procedure:

1. Follow steps #1 through #5 of the procedure for venipuncture as outlined above.
2. The best locations for fingersticks are the 3rd (middle) and 4th (ring) fingers. Do not use the tip of the finger or the center of the finger. Use the finger where vessels and nerves located.
3. When a site is selected, put on gloves, and cleanse the selected puncture area.
4. Massage the finger toward the selected site prior to the puncture.
5. Using a sterile safety lancet, make a skin puncture just off the center of the finger pad. The puncture should be made perpendicular to the ridges of the fingerprint so that the drop of blood does not run down the ridges.
6. Wipe away the first drop of blood, which tends to contain excess tissue fluid.



7. Collect drops of blood into the collection tube/device by gentle pressure on the finger.
8. Cap, rotate and invert the collection device to mix the blood collected.
9. Have the patient hold a small gauze pad over the puncture site for a few minutes to stop the bleeding.
10. Dispose of contaminated materials/supplies in designated containers.
11. Label all appropriate tubes at the patient bedside.

Precautions

- Blood pipettes used should be clean and dry.
- Clean and dry pipettes ensure filling with ease.
- The site from which capillary blood is collected should be cleansed with cotton soaked in spirit
- After collection of blood from the site, it has to be covered with cotton by pressing it

Advantages

- Collection of capillary blood is very easy when compared to the venous blood collection
- No special technique is needed in collecting the capillary blood
- Majority of haematological tests like, Hb estm, RBC count, WBC count
- DLC, Platelet count are determined
- Less expensive equipment like ,a single disposable needle is needed
- Easy way of collecting the blood

iii) **Vacutainers methods**

Definition: The method of collection of blood by using Vacutainer blood collection tube is called as vacutainer method.

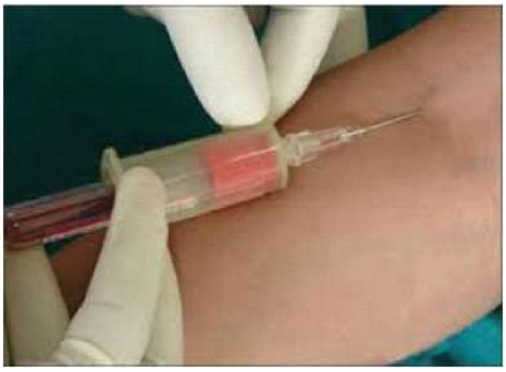


Fig. 15.3 Blood Collection by Vacutainer Method

A **Vacutainer** blood collection tube is a sterile glass or plastic test tube with a colored rubber stopper creating a vacuum seal inside of the tube, facilitating the drawing of a predetermined volume of liquid. Vacutainer tubes are available with a safety-engineered stopper, with a variety of labeling options and draw volumes. The color of the top indicates the additives in the vial.

Vacutainer tubes

Blood Collection byusing Vacutainers

During the bloodcollection process,the rear cannula pushes through the rubber sleeve and puncture the rubber stopper,allowing the vaccum in the tube todrawblood from the vein.

Note

1. All vaccutaner tubes and needles are sterile and intended for single use.
   The needle holders can be reused after sterilisation by autoclaving.
2. The tube stoppers are colour coded so that the person using them find out at glance the type of additive used.

Advantages of Vacutainer System

- It eliminates the preparation of anticoagulated bulbs and tubes
- There is no processing of containers
- It minimises haemolyses in specimens
- It assures accurate blood to additive ratio

POCT

- Point-of-care testing (POCT) is defined as medical testing at or near the site of patient care. POCT includes: blood glucose testing, blood gas and electrolytes analysis, rapid coagulation testing(PT/ INR, Alere), rapid cardiac markers diagnostics (TRIAGE,Alere), drugs of abuse screening, urine strips testing, pregnancy testing, fecal occult blood analysis, food pathogens screening, hemoglobin diagnostics, infectious disease testing and cholesterol screening.
- These tests require only a single drop of whole blood, urine or saliva, and they can be performed and interpreted by any general physician within minutes.

Fig. 15.4 POCT Collection of Blood

POCT has become established worldwide and finds vital roles in public health.

Fig. 15.5 Blood collection at Bed side in case of Children

Fig. 15.6 Blood Collection at Bed side in case of Adults

15.3 Preparation of Anticoagulants

Definition: The chemicals which are used to prevent the coagulation or clooting of blood are called as anticoagulants.

The following are the various types of anticoagulants:

- Double Oxalate
- Sodium Citrate
- Edta
- Heparine

Double Oxalate

Preparation: The solution is prepared with a concentration of two parts of potassium oxalate and three parts of ammonium oxalate by disolving in 0.08 grams of potassium oxalate and 1.2 grams of Ammonium oxalate in 100ml. of distilled water.

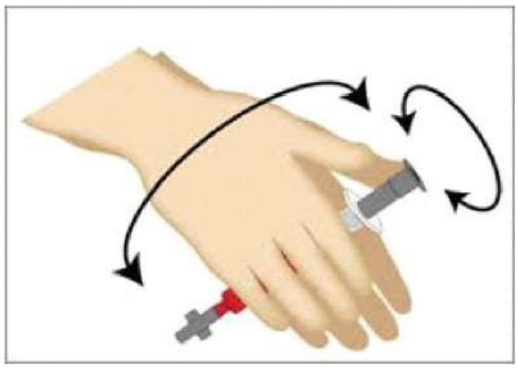


Fig. 15.7 Mixing Anticoagulant with Blood to Prevent Stacking of red blood

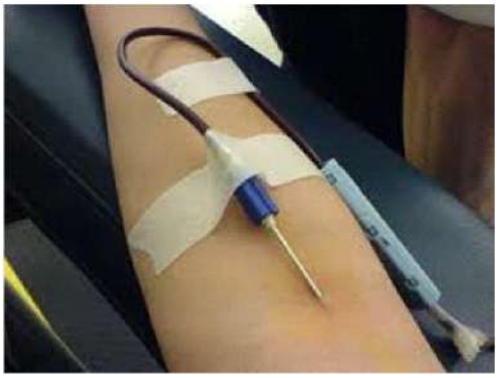


Fig. 15.8 Plastic Bags containing Anticoagulant for Blood Collection

2. **Sodium Citrates 3.8%**

   Preparation: 3.8gm of sodium citrate is dissolved in distilled water and labelled as 3.8% sodium citrate

3. **EDTA(Ethylene diamine tetra acetic Acid):** of EDTA is dissolved in distilled water and solution is prepared.

4. **Heparine**

Preparation

This used in the concentration of 0.1to 0.2mg/ml of blood

15.4 RBC and WBC Count

Introduction

Diagnostics plays prominent role in the field of Medicine. Without proper diagnosis, proper conclusions regarding Medical treatment/ surgery can not be obtained.

The RBC count and WBC count decides about major clinical conditions and status of the patient. Some times in certain cases both the two counts may rise and in certain cases both the two counts may fall down. Fall or rise of both the two counts are having diagnostic importance. So RBC and WBC counts plays an important role in giving information about clinical conditiion of the patient.

RBC Count

A red blood cell (RBC) count is a blood test that tells you how many red blood cells you have.

Red blood cells contain a substance called haemoglobin which transports oxygen around the body. The amount of oxygen that's delivered to your body's tissues will depend on the number of red blood cells you have and how well they work.

A RBC count is usually carried out as part of a full blood cell (FBC) count. A normal RBC count would be:

male–4.7 to 6.1 million cells per micro litre(cells/mcL)

female–4.2 to 5.4 million cells/mcL

The results of an RBC count can be used to help diagnose blood-related conditions, such as irondeficiency anaemia (where there are less red blood cells than normal).

A low RBC count could also indicate a vitamin B6, B12 or folate deficiency. It may also signify internal bleeding, kidney disease or malnutrition (where a person's diet doesn't contain enough nutrients to meet their body's needs).

A high RBC count could be due to a number of health conditions or health-related factors including:

- smoking
- congenital heart disease
- dehydration – for example, from severe diarrhoea low blood oxygen levels (hypoxia)
- pulmonary fibrosis – a lung condition that causes scarring of the lungs

Counting of the number of RBC’s in human blood using haemocytometer is known as RBC Count.

Haemocytometer: The haemocytometer Neubauer counting chamber has a total ruled area of 9sq.mm. It consists of centrally heavy ruled area of 1sq.mm.in size and four other of the same size in each corner. The central area is divided into 25 squares is further subdivided into 16 subsquares. For total RBC counts use 5 squares of the central area. That is 80 ml squares.

Requirements: Haemocytometer, RBC pipette, sterilized needles, microscope, cover glass, cotton, spirit, RBC diluting fluid etc.

RBC pipette: The RBC pipette has red bead. It has two graduations, namely 0.5 and 101.

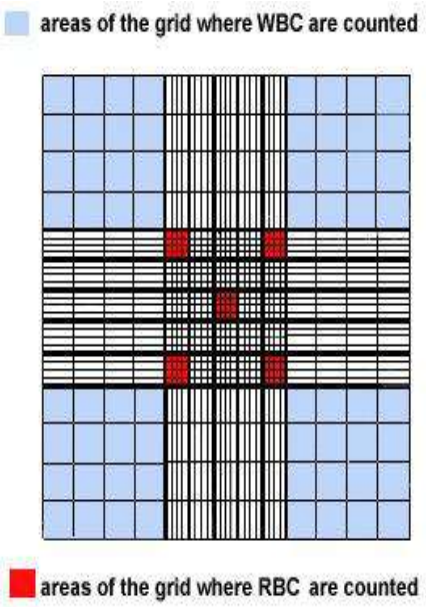


Fig. 15.9 Neubaur Counting Chamber with circles for both RBC count and WBC count

RBC Diluting Fluid: The area two fluids and any one can be prepared and used

(a) FORMAL CITRATE SOLUTION

Trisodium - 3g.
Distilled water - 99ml
Formalin - 1ml.

(b) HAYEM'S FLUID

Sodium Chloride - 0.5g
Sodium Sulphate - 2.5g
Mercuric Chloride - 0.25g.
Distilled Water - 100ml.

Procedure for RBC Count

1. The tip of the index finger is sterilised by rubbing with a cotton soaked in spirit.
2. Make gentle prick with the help of sterilised pin or needlr.
3. The tip of the finger is pressed and blood oozes out.

4. The first drop is wiped out with the help of cotton.
5. Then the blood is aspirated into the RBC pipette exactly upto 0.5 mark.
6. Immediately RBC diluting the fluid is loaded up to the 101 mark.
7. The pipette is rotated between the thumb and forefinger. This will give a dilution of 1:200.
8. Clean the counting chamber and cover glass thoroughly.
9. Place the coverglass in position over the ruled area,using gentle pressure.
10. Mix the suspension thoroughly by rotating the pipitte for about a minute holding it in horizontal position.
11. Fill the chamber by holding the pipette at an angle of 45 degrees
12. Allow two to three minutes for the red corpuscels to settle.
13. Count the number of RBC's in 80 small squares.(4 squares at the four corners and right hand lines, but count the cells touching the upper and left hand lines).

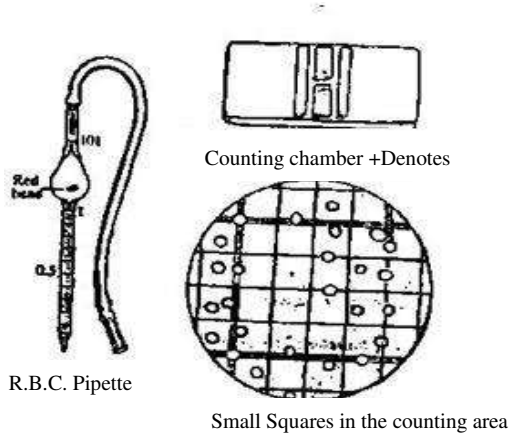


Fig. 15.10 RBC Counting

Trisodium citrate is dissolved in little amount of distilled water and to this 1ml of Formalin is added. Then this is diluted with distilled water and volume is made upto 100ml with distilled water

Calculation

The area of a small square is = 1/400sq.mm.

The depth of the counting chamber is = 1/10mm.

Therfore, the volume of a small square is=1/4000c.mm.

The dilution of the blood is = 1/200

$$\text{Total RBC's} = \frac{423 \times 4000 \times 200}{80 \times 1 \times 1} = 42,30,000 \text{ per c.mm.}$$

Normal: Men 4.5 to 6.5 millions per c.mm. Women 3.9 to 5.6 millions per c.mm.

Clinical Significance

RBC count increases in polycythemia.RBC count decreases in anaemia.

Precautions

1. Care should be taken while puncturing the finger & taking the sample
2. Use sterile needles or lancets.
3. Diluting fluid must be taken accurately up to the mark

This method helps in minimising the pipetting and the dilution errors which are encountered while using the micropipettes. In this method large volumes of blood and diluting fluid are used.

WBC Count:

Counting of the number of WBCs in human blood using haemocytometer is known as WBC Count.

Requirements

Haemocytometer, WBC pipette, sterilised needles, microscope, cover glass,cotton, spirit, WBC diluting fluid etc.

WBC PIPETTE: The WBC pipette has a white bead. It has also two graduations, namely 0.5 and 11 markings.

WBC Diluting Fluid (Truck's Fluid)

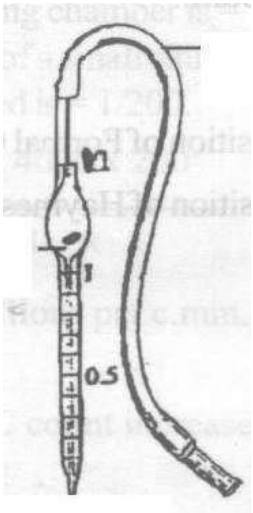
Acetic acid, glass 3ml.

Distilled Water 97ml.

Add Gention voilet to give a pale voilet colour.

Preparation of Diluting Fluid

Add the two lquids. Add gentian voilet to give a pale voilet colour



W.B.C. Pipette

Fig. 15.11

Procedure for White Blood Cell (Leucocyte) Counts

1. Draw the blood upto the 0.5 mark in WBC pipette and dilute upto mark 11 with WBC fluid and fill the counting chamber.
2. Allow 3 minutes for cells to settle.
3. Count the cells in the four corner blocks by using the low power objective
4. In counting the cells, include those cells touching on the inner lines on the right and top, but mot count the cells touching the lines on the left and bottom.

Clinical Significance

WBC count increases in leucocytosis and leukaemia.

WBC count decreases in leucopenia.

Calculation:

No.of WBC's countedin fourcorners=104

Thevolumeof a square =1/10c.mm.

The blood was diluted to =1/20

Therefore numberof cellspercmm $= \frac{104 \times 10 \times 120}{4}$

= 5200 cells per c.mm of undiluted blood

Normal : WBC's per c.mm. of blood 4,000 to 10,000 per c.mm.

Precautions

1. Care should be taken while puncturing the finger & taking the sample
2. Use sterile needles or lancets.
3. Diluting fluid must be taken accurately up to the mark

15.5 Platelet Count

Introduction

The methods used for automated platelet counting are impedance, optical scatter, optical fluorescence, and immunologic flow cytometry. The introduction of the international reference method (IRM) for platelet counting by flow cytometry has improved the precision and accuracy of platelet counting at thrombocytopenic levels and offers a suitable comparator for routine platelet counting methods.

Morphology Platelets . Functions of Platelets. Platelet Diluting Fluid

Procedure of Platelet Counting

Morphology Platelets

Platelets look as round oroval plates with biconvex surfaces when observed microscopically. These are named as thrombocytes platelets are having the 2-4 diameter Platelets contain the granules in the cytoplasm without nucleus. Normal platelets count ie 2,50,000 to 5,00,000 per common of blood. They are the fragments of giant cells called Megakaryocytes produced in the bone marrow. Platelets are non-necleated and irregular in shape. When disintegreted, they release the enzyme thromboplastic and initiate the clotting process, which is very complex one.The blood platelets also help in sealing small vascular openings by forming a platelet plug. Decreased platelet count is seen in thrombocytopenia.

Function Of Platelets

- Initiation of blood clotting by disitegretion and liberation of thromboplatin.
- Speedy repair of capillary endothelial lining.
- Haemostatic mechnism by means of aggulutination and coagulation.
- Hastening the retraction of clot.It is dependent on thrombosthenin.
- Liberating of 5-HT and Histamine to exert vaso constriction which helps in haemostasis.

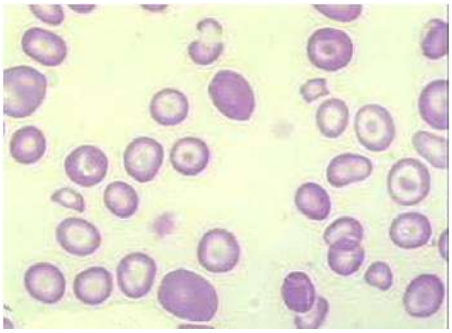


Fig. 15.12 Morphology of Platelets

Platelet Diluting Fluid

Composition

Procaine hydrochloride	3.0gm
Sodium chloride	10.0gm
Distilled water	100ml

Preparation

Above two components are dissolved in few amount of water and volume is made to 100 ml with distilled water

Procedure of Platelet Counting

Requirements

(1) Microscope (2) Improved Neubaur Counting Chamber

(3) RBC Pipette (4) Platelet Diluting Fluid

Procedure

1. Mix the blood specimen carefully.
2. By using RBC pipette draw blood upto 0.5 mark.
3. Wipe excess blood on the outside of the pipette.
4. The diluting drawn upto the mark 101.
5. Mix the contents in the bulb thoroughly.
6. After 5 minutes, discard the first drop, then transfer a small drop on one side of the counting chamber.
7. Place the filled mounted counting chamber under a petridish with a moust filter paper.

8. Let it stay undisturbed for 15 minutes.
9. Place the counting chamber carefully on the stage of the microscope. Under low power magnification focus red cell counting area.
10. Move to view the corner square the red cell area and change to high power objective.
11. Keep the condenser down and reduces the light by adjusting the diaphragm.
12. The platelets will appear like highly refractile particles.Count platelets in all 25 small squares.

Normal Ranges

2,50,000 - 5,00,000 / cumm (Micro Liters)

Clinical Significance

Detrmination of platelts is requested in the investigation of bleeding disorders.Decreased platelets count is often associated with prolonged bleeding and poor clot retraction.Decreased platelet count also occurs in aplastic anaemia, megaloblastic anaemia & acute leukaemia.Increased platelet count is found in polycythemia vera, following spleen ectomy.

15.7 Haemoglobin Estimation

Introduction

HB% in the given sample is estimated by following methods

- Tallquist method
- Sahlis acid haematin method
- Alkali method
- Cyanmethhaemoglobin method

Sahlis Acid Haematin Method

To Determination of HB by Sahlis acid haematin method

Requirements:

- Sahli haemoglobinometer
- 0.1N HCL
- Distilled water
- Pasteur pipettes

The Sahli haemometer method utilizes the conversion of haemoglobin into acid haematin which has a brown colour in solution. The intensity of the colour is related to the amount of

haemoglobin in the blood sample. Water is added to dilute the brown solution until it matches that of a standard. The more haemoglobin, the more water required to obtain a colour match. Haemoglobin values are read at the meniscus of the brown solution.

5.14 Sahlis Acid Haematin Method

Parts-

a. **Sahli'sgraduated hemoglobin tube** (marked in grams percent g% (2-24) and percentage % (10 -140)

b. **Comparator** with a brown glass standard. opaque white glass is present at the back to provide uniform illumination.

c. **Sahli'spipette or hemoglobin pipette** (marked at 20µl or 0.02 ml). No bulb

d. **Stirrer:** Thin glass rod .

INDICATIONS OF HAEMOGLOBIN ESTIMATION

- To determine presence and severity of anemia
- Screening for polycythemia
- To assess response to specific therapy in anemia.
- Estimation of red cell indices
- Selection of blood donors.

PRINCIPLE:

Blood is mixed with an acid solution so that hemoglobin is converted to brown-colored acid hematin. This is then diluted with water till the brown colormatches that of the brown glass standard. The hemoglobin value is read directly from the scale.

Procedure

- The diluent is N/10 Hydrochloric acid (HCL). Add it from the dropping bottle provided to the graduated tube, up to mark 2.
- Measure 0.2 ml (20 µl) of well-mixed blood, with the provided micropipette (Sahli's pipette) and transfer it to the HCL in the tube.
- With the pipette beneath the surface of the acid, gently blow the blood.
- Rinse the pipette by sucking up and blowing out diluent 2-3 times.
- Thoroughly mix blood and acid using a fine glass rod (HCL will react with the haemoglobin and convert it into acid-haematin, which has a brown color).
- Wait up to 3 minutes after adding the blood to allow the color to develop sufficiently to achieve an accurate comparison.
- Add distilled water gradually to the mixture and mix the solution with glass rode.
- Place the tube in the haemoglobinometer and compare it with the standard.
- Continue to add distilled water until the sample firstly appears to be detectably pallor than the standard.

Note the level of the liquid in the tube.

Normal values:	Hb,g/dl
Men	14-18
Women	11.5-16.5
Children(up to 1 yr)	11.0-13.0
Children(10-12yrs)	11.5-14.5
Infants(full term cord)	13.5-19.5

Advantages

- This method is useful for places where a photometer is not available
- Sahlis method is easy to perform and convenient.
- This method is inexpensive.
- It is not very time consuming (maximum takes 15 minutes.

Clinical Significance

Decreased in anemia, hemorrhage, and hemolytic reactions;increased in dehydration, heart and lung disease

A decrease in haemoglobin below normal range is an indication of anaemia. An increase in haemoglobin concentration occurs in haemoconcentration due to loss of body fluid in severe diarrhoea and vomiting.High values are also observed in congenital heart disease and in polycythemia.

CYANMETHHAEMOGLOBIN METHOD

This is the preferred and the most accurate method for determining the hemoglobin concentration. It is the standard method used in most of the centers.

This method is optional for estimation of hemoglobin and this method is recommended by International Committee for Standardization in hemotology. This is because in this method all type of hemoglobin are transformed to cyanmethemoglobin (except sulfhemoglobin), and a firm and trustworthy standard is available.

Principle

When Blood is mixed with a solution of potassium cynide, potassium ferricyanide and Drabkin's solution, the erythrocytes are lysed by producing evenly disturbed hemoglobin solution. Potassium ferricyanide transforms hemoglobin to methemoglobin, and methemoglobin combines with potassium cyanide to produce cyanmethemoglobin. This way all types of hemoglobin present in blood are entirely transformed to a single compound cyanmethemoglobin. When the reaction is entire, absorbance of the solution is deliberate in a spectrophotometer at 540 nanometer. Cyanmethemoglobin has a wide absorbance peak at this wavelength. The absorbance is compared with that of the standard cyanmethemoglobin solution by using a formula to obtain the amount of hemoglobin. The Formed cyanmethemoglobin has a wide absorbance peak at 540nm wave length.

Equipment

- Spectrophotometer or photoelectric colorimeter
- Pipette 5 ml
- Sahli's pipette

Reagent

- Drabkin's Solution
- Cyanmethemoglobin standard solution with known hemoglobin value

Specimen

Blood obtained from skin puncture or EDTA-anticoagulated venous blood.

Procedure

- Take 5 ml of Drabkin's solution in a test tube and add 20 µl of blood. This way, we will get the dilution of 1:25. Now mix the mixture and allow to stand for atleast 5 minutes. This time is adequate for transformation of hemoglobin to cyanmethemoglobin.
- Pour the test sample to a cuvette and read the absorbance of the test sample in a spectrophotometer at 540 nanometer or in a photoelectric colorimeter using a yellow-green filter. Also read the absorbance of the standard solution. Absorbance must be read against Drabkin's solution.
- From the formula given below, the hemoglobin value is derived.

Hemoglobin in gm/dl = [Absorbance of test sample ÷ Absorbance of standard] x concentration of standard x [Dilution factor ÷ 100]

Result

The result is calculated by using the formulae

Hbgm/dl=Absorbanceoftest/AbsorbanceofStandard×conc.of

Standard..

Advantages

- All forms of Hb except Sulphahemoglobin are readily converted to cyanmethemoglobin
- Direct comparison with cyanmethemoglobin standardpossible.
- Stabilityof the diluted sample, readings made at operator's convenience.
- Easy toperform the test.
- Reagents are readily available.
- The standardis stable.

–

Disadvantages

- Increasedabsorbancenotduetohaemoglobinmaybecausedby turbidity due to abnormal plasma proteins, hyperlipaemia, high WBC countor fat droplets.
- Potassium cyanide in the solutions is poisonous, though it is present only in a very low concentration hence the reagents should be handled carefully.

Clinical Significance

Hemoglobin (Hb) has the major function of supplying oxygen tothe tissue cells. Hb estimation is one ofthe commonest screening tests for the diagnosis of Anemia. Decreasedlevelsof hemoglobin concentrations areobserved in all varieties of anemia, resulting from hemorrhage or fromdeficiencyof iron, Vitamin B12 or Folic acid. Increased levels of hemoglobin concentration is observed in polycythemia vera, congenital cyanotic heart disease and in hemoconcentration due to various clinical causes like heat stroke and dehydration

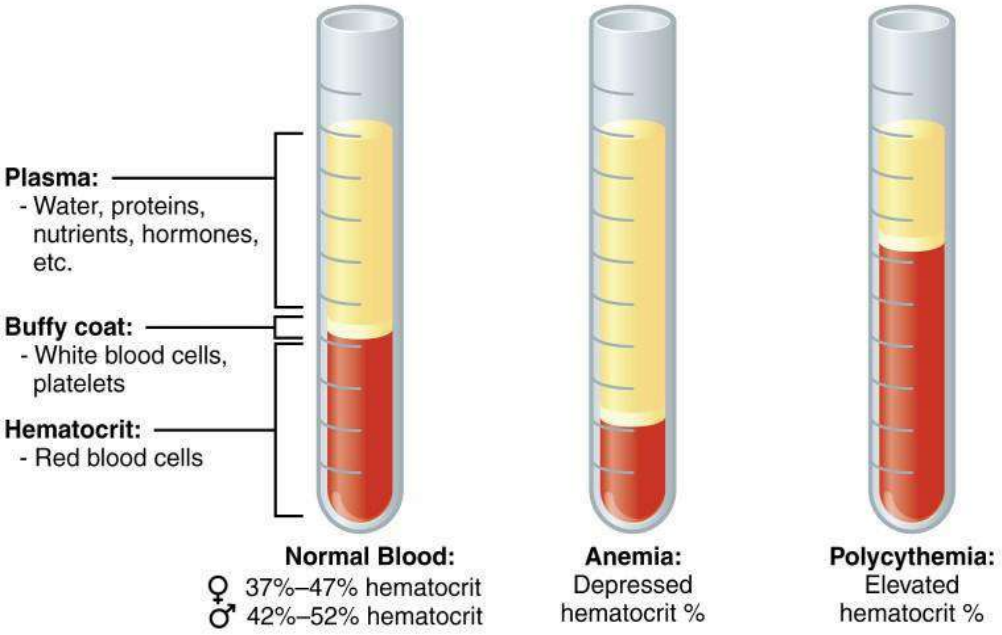
15.8 Estimation of PCV

Introduction

The packed cell volume (PCV) is the measure of the ratio of the volume occupied by the red cells to the volume of whole blood in a sample of capillary, venous, or arterial blood. The ratio is measured after appropriate centrifugation and is expressed as a decimal fraction.

The PCV is an easily obtained measure for detecting anemia or polycythemia and can be useful in estimating changes in hemodilution or hemoconcentration. The PCV is used, together with the red cellcount, in calculating the mean cell volume (MCV) and, together with the hemoglobin content, incalculating the mean corpuscular hemoglobin concentration (MCHC).
Estimation of PCV can be done by Macro method & Micro method:

MACRO METHOD (WINTROBE METHOD) FOR ESTIMATION OF PACKED CELL VOLUME (PCV) OR HEMATOCRIT



Principle

Anticoagulated whole blood is centrifuged in a Wintrobe tube to completely pack the red cells. The volume of packed red cells is read directly from the tube. An advantage with this method is that before performing PCV, test for erythrocyte sedimentation rate can be set up.

Equipment

Wintrobe tube: This tube is about 110 mm in length and has 100 markings, each at theinterval of 1 mm. Internal diameter is 3 mm. It can hold about 3 ml of blood.

Pasteur pipette with a rubber bulb and a sufficient length of capillary to reach the bottom of the Wintrobe tube.

Specimen

Venous blood collected in EDTA (1.5 mg EDTA for 1 ml of blood) or in double oxalate. Test should be performed within 6 hours of collection.

Method

- Mix the anticoagulated blood sample thoroughly.
- Draw the blood sample in a Pasteur pipette and introduce the pipette up to the bottom of the Wintrobe tube. Fill the tube from the bottom exactly up to the 100 mark. During filling, tip of the pipette is raised, but should remain under the rising meniscus to avoid foaming.
- Centrifuge the sample at 2300 g for 30 min (To counterbalance a second Wintrobe tube filled with blood from another patient or water should be placed in the centrifuge).
- Take the reading of the length of the column of red cells.

After centrifugation of anticoagulated whole blood, three zones can be distinguished in the Wintrobe tube from above downwards-plasma, buffy coat layer (a small greyish layer of white cells and platelets, about 1 mm thick), and packed red cells. Normal plasma is straw-colored. It is colorless in iron deficiency anemia, pink in the presence of hemolysis or hemoglobinemia, and yellow if serum bilirubin is raised (jaundice). In hypertriglyceridemia, plasma appears milky. Increased thickness of buffy coat layer occur if white cells or platelets are increased in number (e.g. in leukocytosis, thrombocytosis, or leukemia). Smears can be made from the buffy coat layer for demonstration of lupus erythematosus (LE) cells, malaria parasites, or immature cells

- Plasma colour
- Yellow colour of plasma indicates jaundice.
- Opaque plasma is due tolipaemia.
- Pink colour denotes haemoglobinemia.

Normal Values:

- Men 40-54%
- Women 36-47%
- Atbirth 44-62%

- Oneyear 35%(approx)
- Tenyears 37.5%(approx)

Clinical Significance

Avalue below an individual's normal or below the reference range for the age and sex indicates anaemia and a higher value indicates polycythemia.

Fall of haematocritvaluesare observedin

1. Anaemias 2. Hydraemia(occurrence ofexcessivefluidinbloodin pregnancy)

Increased haematocrit values are observed in

1. Polycythemia vera 2. Dehydration 3.Emphsema 4.Congenital heart disease.

Estimation of PCV(Haematocrit) by Microhaematocrit method

This method is used whenever there is a difficulty in drawing sufficient amount of blood.It is useful particularly in paediatric patients.This is most ideal for skin puncture.

Specimen:1. EDTAor oxalated specimen(use plain capillary tubes)

2. Capillary blood(ysed heparinsed capillaries)

Requirements

- Haematocrit centrifuge
- Haematocrit reader
- Capillary haematocrit tubes
- Soft wax or modelling clay which is used to seal the end of the capillary tube

Procedure

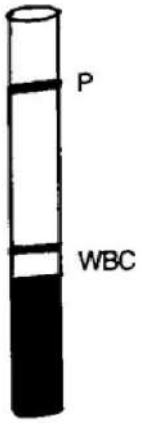
1. In to the capillary tube draw the specimen(anticoagulated blood) appropriately.
2. Fill the capillary tube with anticoagulated blood upto about3/4 length.
3. Seal both ends of the capillary tube wiyh soft wax or modelling clay to a depth of about 1cm.
4. Place the sealed capillary tube in centrifuge cup and place another similar sealed capillary tube at opposite side.
5. Close the centrifuge with cover plate and centrifuge the tubes at high speeds(at 1500RPM) for 5 minutes.
6. Remove the capillary tube.
7. Three layers will be formed after centrifugation.

a) Clear plasma layer will be formed at the top

b) Whitish buffy coat later will be formed at the middle portion

c) Column of red blood cells is formed at the bottom

The bottom of the column of the red blood cells is brought to the same line with horizontal zero line by holding the tube against the haematocrit scale 1.0 mark of haematocrit scale should pass through upper line of plasma(top of the plasma column). Reading on the haematocrit scale corresponing to the upper level of the RBC column has to be noted which is to be taken as the PCV value.



P (plasma) WBC(white blood cells) RBC(red blood cells)

PCV by micro haematocrit method.

After centrifugation the capillary tube will show three layers

- Plasma(P)
- WBC(White blood cells)
- RBC(Red blood cells)

Observations

Haemolysed specimen will yield false low values.Proper mixing of blood and EDTA should be done. Otherwise it gives false values

Advantages

- Only small amount of blood is needed for the test.
- Even capillary blood can be sampled.
- Less time is needed for the test.
- Easier procedure.
- Amount of trapped plasma is much less.Fig. 15.17 Micro method

Clinical Significance

Observe any abnormal findings such as

1. Colour of plasma Yellow colour may indicate Jaundice Reddish colour indicate haemolysis
2. Increased white blood cells will indicate the increased buffy coat layer.

Estimation of Erythrocyte Indices

When a patient has a lower than normal hemoglobin, it is important to determine whether red blood cells are of normal size and if they have a normal concentration of hemoglobin. These measurements, known as erythrocyte or red blood cell indices, provide important information about various types of anemias.

Using haemoglobin concentration,PCV and total RBC count,RBC indices are caluclated.The commonly employed RBC indices are

Mean cell volume(MCV)

Mean cell haemoglobin(MCH)

Mean corpuscular haemoglobin concentration(MCHC) RDW

Colour index

Mean cell volume:

Mean corpuscular volume (MCV) measures the mean or average size of individual red blood cells.

Calculation

MCV=PCV X 10

RBC in millions/cmm

Normal values:82 to 92 cubic microns

Clinical Significance

Increased MCV is observed in Macrocytic anaemia

Decreased MCV is observed in Microcytic anaemia

MCV

MCV values are higher in newborns and infants

Men: 80-98 fl (femoliters)

Women: 96-108 fl

Mean Corpuscular Haemoglobin(MCH)

Mean corpuscular hemoglobin (MCH) measures the amount, or the mass, of hemoglobin present in one RBC. The weight of hemoglobin in an average cell is obtained by dividing the

hemoglobin by the total RBC count. The result is reported by a very small weight called a picogram (pg).

Normal values:

MCH - 17-31 pg (picograms)

27-32 micro micrograms

Clinical Significance

Increased values are observed in Macrocytic anaemia.Decreased values are observed in Hypochromic anaemia.

Mean Corpscular Haemoglobin Concentration (MCHC):

The concentration of the average red blood cell containing haemoglobin is called as MCHC.

$$MCHC = \frac{Hb \times 100}{PCV}$$

Normal values: 32-36%

Clinical Significance:

Decreased values are observed in Hypochromic anaemias.Increased values are observed in Spherocytosis.

RDW

The red blood cell distribution width (RDW or RCDW) is a measure of the variation of red blood cell (RBC) width that is reported as part of a standard complete blood count. Usually red blood cells are a standard size of about 6–8 μm. Certain disorders, however, cause a significant variation in cell size. Higher RDW values indicate greater variation in size. Normal reference range in human red blood cells is 11–15%. If anemia is observed.

Calculations

The "width" in RDW is sometimes thought of as "misleading," since it in fact is a measure of deviation of the volume of RBCs, and not directly the diameter. However, "width" refers to the width of the volume curve (distribution width), not the width of the cells.

Thus, it is a reasonably accurate term.

Mathematically the RDW is calculated with the following formula:

$$RDW = (\text{Standard deviation of MCV} \div \text{mean MCV}) \times 100.$$

Clinical Significance

- High RDW
- Iron Deficiency Anemia: high RDW with low MCV
- Folate and vitamin B12 deficiency anemia: High RDW with High MCV
- Recent Hemmorrhage: High RDW with Normal MCV

15.9ESR (ErythrocyticSedimentationRate)Introduction

The ESR is a simple non-specific screening test that indirectlymeasures the presence of inflammation in the body. Changes in redcell shape or numbers m ay alsoaffectthe ESR.Twolayers are formed,the upper plasmalayer andlower o ne of the red bloodcells. The rate atwhich the redcells fallsis known as erythrocytic sedimentation rate.

ESR is greater in women than in men and it is related tothe differencein PCV. During pregnancy ESR graduallyincreases after 3rd month and returns tonormal in about3-4weeks after delivery

METHOS OF ESR DETERMINATION

There are two main methods to determine ESR :

- Wintrobe's method
- Westergren's method

Each method produces slightly different results. Mosely and Bull (1991) concluded that Wintrobe's method is more sensitive when the ESR is low, whereas, when the ESR is high, the Westergren's method is preferably an indication of patient's clinical state.

WINTROBE'S METHOD

This method uses Wintrobe's tube, a narrow glass tube closed at the lower end only. The Wintrobe's tube has a length of 11 cm and internal diameter of 2.5 mm. It contains 0.7-1 ml of blood. The lower 10 cm are in cm and mm. The marking is 0 at the top and 10 at the bottom for ESR. This tube can also be used for PCV. The marking is 10 at the top and 0 at the bottom for PCV.

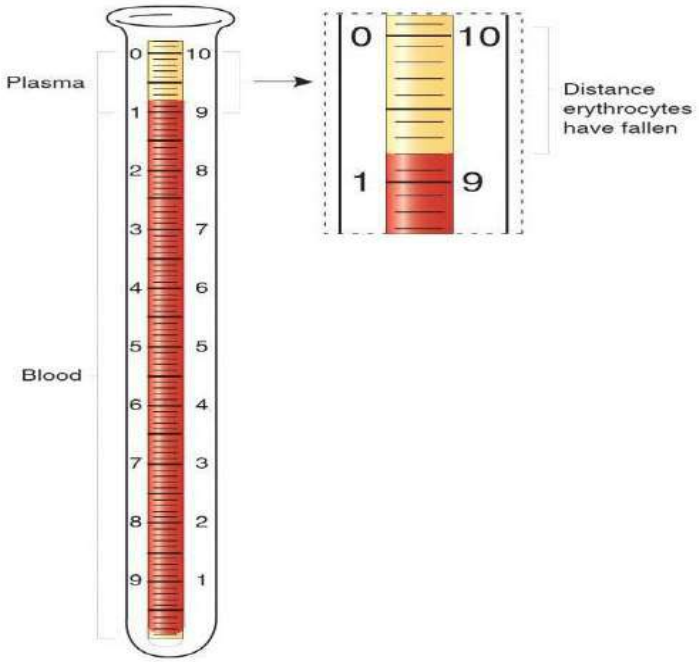
REQUIREMENTS :

- Anticoagulated blood (EDTA, double oxalate)
- Pasteur pipette
- Timer
- Wintrobe's tube Wintrobe's stand

PROCEDURE :

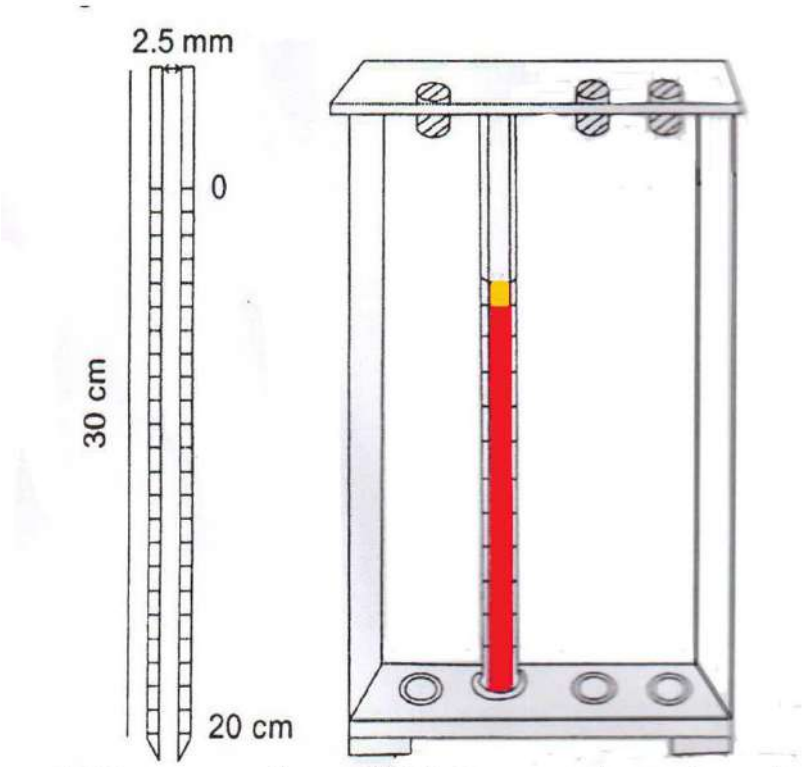
- Mix the anticoagulated blood thoroughly.
- By using Pasteur pipette, fill the Wintrobe's tube upto '0' mark. There should be no bubbles in the blood.
- Place the tube vertically in ESR stand and leave undisturbed for 1 hour.
- At the end of 1 hour, read the result.

NORMAL VALUE: males:0-9mm/hr,females:0-20mm/hr



WESTERGREN'S METHOD

qIt is better method than Wintrobe's method. The reading obtain is magnified as the column is lengtheir. The Westregren tube is open at both ends. It is 30 cm in length and 2.5 mm in diameter. The lower 20 cm are marked with 0 at the top and 200 at the buttom. It contains about 2 ml of blood.



a) Westergren tube b) Westergren tube on the rack

REQUIREMENTS :

Anticoagulated blood (0.4 ml of 3.13% trisodium citrate solution + 1.6 ml blood) Westergren tube

- Westergren stand
- Rubber bulb (sucker)

PROCEDURE :

- Mix the anticoagulated blood thoroughly.
- Draw the blood into the tube upto 0 mark with the help of rubber bulb.
- Wipe out blood from bottom of the tube with cotton.
- Set the tube upright in stand. Make sure the pipette fits snugly to eliminate possible leakage and that the pipette is in vertical position.
- Leave the tube undisturbed for 1 hour.

- At the end of 1 hour, read the result.

NORMAL VALUE :

For males: 0-10 mm/hr

For females: 0-15 mm/hr

Clinical Significance of ESR

The erythrocyte sedimentation rate (ESR) is a non-specific test. It is raised in a wide range of infectious, inflammatory, degenerative, and malignant conditions associated with changes in plasma proteins, particularly increases in fibrinogen, immunoglobulins, and C-reactive protein. The ESR is also affected by many other factors including anaemia, pregnancy, haemoglobinopathies, haemoconcentration and treatment with anti-inflammatory drugs.

Causes of a significantly raised ESR :

All types of anemias except sickle cell anemia

Acute and chronic inflammatory conditions and infections including:

- HIVdisease
- Tuberculosis
- Acuteviralhepatitis
- Arthritis
- Bacterialendocarditis
- Pelvicinflammatorydisease
- Rupturedectopicpregnancy
- Systemic lupus erythematosus

African trypanosomiasis (rises rapidly) Visceral leishmaniasis

Myelomatosis, lymphoma, Hodgkins disease, some tumours Drugs, including oral contraceptives

Causes of Reduced ESR :

- Polycythaemia Poikilocytosis
- Newborn infants Dehydration

- Dengue haemorrhagic feverand other conditions associated with haemoconcentration

Precaution

- Wash the tubes as early as possible, under running tap water.Rinse in deionized water and dry in the incubator between 40-50oC
- Wash the tubes under running tap water by introducing a thick wire in the tube repeatedly to remove the packed cells completely. Afterwards dry the tubes in the incubator.
- In the case of infants and if the blood quantity is insufficient use "Landau" method.

KeyTerms

- **Haematology:** The branch dealing with the study of blood components and coagulation is known as haematology.
- **Blood :** Afluid connective t issue in which blood cells are suspended in fuid plasma
- **Plasma:** The fuid portioned blood
- **Plasma Proteins :** Serum albumim, serum globulin and serum fibrinogen are the plasma proteins
- **Anticoagulants :** Chemicals used to prevent the process of coagulation
- **Coagulation :** The formation of fibrin threads mesh in which blood cells are entangled
- **EDTA :** Ethylene diamine tetra acetic acid
- **ACD :** Acid citrate dextrose
- **E.S.R :** Erythrocyte sedimentation rate
- **Heparine :** Chemical used to prevent coagulation
- **Haemocytometer :** It is an instrument for measuring the number of blood cells
- **Polycythemia Vera:** Condition in which RBC count is high is called as polycythemia vera
- **Anemia:** It is the condition where the levels of RBCS are low.
- **Leucocytosis:** The condition of increased levels of leucocytes is called as leucocytosis
- **Leukaemia :** Abnormal increase in wbc count with immature cells among them
- **Leucopenia:** Decreased levels of wbc count

- **Haemolytic Anaemia :** It is due to excessive or abnormal destruction of red blood cells
- **Haemorrhage :** Escape of blood from a vessel
- **Thrombocytopenia:** Low count of platelets
- **Oedema :** Accumulation of water (fluids) in between body tissues
- **Polyuria:** Secretion of large quantities of urine
- **BT :** Bleeding Time
- **BleedingTime :** The time required for the blood to stop flowing from a deep prickin the finger.
- **ClottingTime :** The interval of time between the appearance of blood and appearance of fibrin threads during the breakage of capillary tubes is known as clotting time.
- **Polynephritis :** Inflammation of the more number of nephrons
- **Pulmonary :** Relating to lungs
- **Erythropoiesis :** Formation of red blood cells
- **Bonemarrow :** Lively material from which blood cells are formed and send to blood circulation
- **Haemolysis :** Breakage of RBC with the release of Haemoglobin
- **Haemolytic Anaemia :** It is due to excessive or abnormal destruction of red blood cells
- **Haematuria :** Excretion of blood in urine
- **Haemoglobin :** The respiratory pigment of the RBC is called as haemoglobin anaemia: the condition where there is reduced levels of RBCS
- **Drabkins Reagent :** Reagent used in cyanmethaemoglobin method of Hb estimation
- **Specific Gravity Method :** One of the methods of Hb estimation
- **Jaundice:** A condition characterized by raised bilirubin level in the blood. Urine appears as yellowish in this condition
- **Lipaemia:** Increased levels of lipoids (especially cholesterol) in the blood.
- **ESR :** The rate at which red cells falls is known as ESR

- **Sedimentation :** Settling of solid components to the bottom of the tube
- **Plasma :** The separated fluid portion of the blood after anticoagulant is added
- **Serum :** The fluid portion formed after formation of clotting

Short Answer Type Questions

- Define coagulation
- What is an anticoagulant?
- Abbreviate EDTA
- What is the sodium citrate?
- Expand ACD
- Where sodium citrate is used?
- How does heparin will act as an anticoagulant?
- For what purposes ammonium and potassium oxalate mixture is used. Write down the composition of wbc diluting fluid.
- Define leucocytosis.
- Write down the normal values of wbc count.
- Define anaemia.
- What is leukaemia?
- Define polycythemia vera.
- Define bleeding time
- What is normal value of platelet count
- Define thrombocytopenia
- Write the compostion of platelet diluting fluid
- What is the importance of platelet count?
- Define Erythropoiesis
- What is anaemia?
- Define haematology
- Write the morphology of reticulocytes
- Expand the term EDTA.
- How do you estimate Hb by specific gravity method?
- What are the normal values of Hb in men and women?

- What are the advantages of specific gravity method?
- What are the disadvantages of specific gravity method?
- What are the advantages of alkaline haematin method?
- What are the disadvantages of alkaline haematin method?
- What are the advantages of Talquist method?
- Write the disadvantages and clinical significance of Talquist method.
- Define the term Haematology
- Define PCV.
- What is the clinicalsignificance of PCV
- Define the Mean cel volume
- Define Mean corpuscular haemaglobin
- Define Mean corpscular haemaglobin consentration
- Define ESR
- Write the methods of ESR determination
- Write the normal values of ESR
- Define roulex formation
- Write the factors affecting the ESR

Long Answer Type Question

- Write notes on RBC count.
- Explain about WBC COUNT.
- Write the morphology and functions of platelets
- Write down the procedure of platelet counting .
- Write notes on reticulocyte counting
- Write notes on advantages of reticulocyte count and morpholgy of reticulocytes.
- How do you estimate haemoglobin by Sahlis acid haematin method?
- Write down the haemoglobin estimation of cyanmethaemoglobin method.
- Explain about the HB estimation by alkaline haematine method
- How do you estimate Hb by specific gravity method?
- Write notes on Hb estimation by Haldanes method.
- Write the advantages and disadvantages of Hb estimation by specific gravity method.
- Write about the importance of haemeglobin in the human body system.
- How do you determine PCV by microhaematocrit method
- How do you determine PCV by macrohaematocrit method
- Write notes on RBC indices
- How do you determine ESR by westergrens method?
- Write the determination of ESR by Wntrobes method

UNIT-16

Disposal of Hospital Waste

Structure

16.1 Introduction

Hospital is a place of Almighty, a place to serve the patient. Hospital waste is a potential health hazard to the health care workers, public and flora and fauna of the area. Hospital acquired infection, transfusion transmitted diseases, rising incidence of Hepatitis B, and HIV, increasing land and water pollution lead to increasing possibility of catching many diseases.

What is hospital waste

Hospital waste refers to all waste generated, discarded and not intended for further use in the hospital.

16.2 Classification of Hospital waste

General waste: Largely composed of domestic or house hold type waste. It is non-hazardous to human beings, e.g. kitchen waste, packaging material, paper, wrappers, plastics.

Pathological waste: Consists of tissue, organ, body part, human foetuses, blood and body fluid. It is hazardous waste.

Fig. 16.1 Hospital waste

Infectious waste: The wastes which contain pathogens in sufficient concentration or quantity that could cause diseases. It is hazardous e.g. culture and stocks of infectious agents from laboratories, waste from surgery, waste originating from infectious patients.

Sharps: Waste materials which could cause the person handling it, a cut or puncture of skin e.g. needles, broken glass, saws, nail, blades, scalpels.

Pharmaceutical waste: This includes pharmaceutical products, drugs, and chemicals that have been returned from wards, have been spilled, are outdated, or contaminated.

Chemical waste: This comprises discarded solid, liquid and gaseous chemicals e.g. cleaning, house keeping, and disinfecting product.

Radioactive waste: It includes solid, liquid, and gaseous waste that is contaminated with radionucleides generated from in-vitro analysis of body tissues and fluid, in-vivo body organ imaging and tumour localization and therapeutic procedures.

Fig. 16.2 Hospital Waste Management Programme

Hospital waste management programme

- Identification of waste types
- Segregation of waste
- Transport & storage of waste
- Proper disposal of waste
- Implementation of contingency plans
- Identify the need for use of personal protective equipment
- Segregation by color coding system
- Three categories
- Infectious waste - Red bags
- Domestic waste - Green Bags
- Sharps - Needle cutters / Puncture proof containers
- Segregation at Source (ward, operation theater, laboratory, labour room, other places)

Transportation

Containers: Puncture proof, leak proof,

Bags: Sturdy, properly tied

Transport trolleys: Designated & Timely

Staff protection: Provided with protective clothing and other items

All infectious waste and sharps containers: Incineration

All Domestic waste: Landfill

All hazardous waste: Chemical treatment before disposal

Implementation of contingency plans

Fig. 16.3 Transportation of Hospital waste

16.3 Disposal of cotton and Swabs

1. Cotton and swabs constitutes the Infectious agents
2. The wastes which contain pathogens in sufficient concentration or quantity that could cause diseases. It is hazardous e.g. culture and stocks of infectious agents from laboratories, waste from surgery, waste originating from infectious patients.

Fig. 16.4 A Portable Incinerator to dispose hospital waste

Fig. 16.5 A Pit to dispose Biodegradable Waste

- Sorting of medical waste in hospital is the first step in handling the Infectious agents
- At th site where it is generated,infectious waste is placed in specially-labled bags and containers for removal by Infectious waste transporters
- The person should wear mask covering the nose and mouth
- The person should wear a cap over the head
- The person should wear foot protectives
- The person should wash his hands with soap and warm water after handling infectious wastes
- The person also should wash all areas of his body with soap and water that he thinks may have come into contact with infectious wastes, even if he is not sure that his body actually touched the infectious wastes
- Keep all sores and cuts covered
- The person should wear an apron or another type of cover to protect his clothes from contact with the waste
- Promptly clean and disinfect soiled, hard-surfaced floors by using a germicidal or bleach solution and mopping up with paper towels
- Red coloured bins/bags should be used for collecting the infectious
- waste.
- The Infectious waste should be disinfected with suitable chemical disinfectants to prevent infection.
- TheInfectious waste should be taken away to a far away place and disposed

- The Infectious waste which is disinfected can also be burried by digging a pit and filled with soil.
- The infectious waste after getting disinfected should be dried and burnt.

16.4 Diposal of Blood and body fluids

- Both solid and pathological waste constitutes the blood and body fluids
- The persons handling these waste should take care while collecting because of transmission of diseases
- These pathological wastes are collected in the concerned coloured bags properly
- The persons handling these wastes should see that,there should be no leakage from the bins or bags.
- The bins or bags should not be overloaded with pathological wastes
- The person handling thein fectious wastes should wear disposable latex hand gloves. Discard the gloves immediately after use.
- The person should wear mask covering the nose and mouth
- The person should wear a cap over the head
- The person should wear foot protectives

- The person should wash his hands with soap and warm water after handling infectious wastes.
- The person should wear an apron or another type of cover to protect his clothes from contact with the waste
- Promptly clean and disinfect soiled, hard-surfaced floors by using a germicidal or bleach solution and mopping up with paper towels.
- These solid and pathological wastes hane to be autoclaved
- Then treated with chemical disinfectants
- And finally burried deep in the soil after digging a pit and closed with filling.

Fig. 16.6 Disposal of blood

16.5Disposal of Syringes and Neddles

1. Waste materials which could cause the person handling it, a cut or puncture of skin e.g. Syringes ,needles, broken glass, saws, nail, blades, scalpels constitute the Sharps.
2. The used disposable needles should be made into pieces by cutting.
3. The used syringes should be destroyed.

Fig. 16.7 Disposal of used syringes in a safety box

Fig. 16.8 Disposal of home generated syrengis

Fig. 16.9 Syringe Needle Clipper

4. After these are taken to a far away place
5. Where these are disinfected and subjected to chemical treatment
6. And finally these mutilated, disinfected, chemically treated sharps are subjected to Incineration.
7. Sometimes liquid wastes like blood after disinfecting with chemicals are discharged into the drainage

16.6 Disposal of Urine

In the postpartum rather than in a clinic, from the d/c Foley bags, as much of the urine as possible was emptied, deflate the balloon, and put the whole shebang into the regular trash.

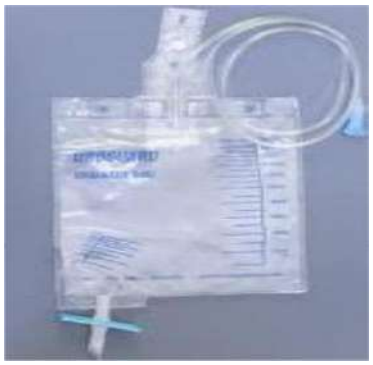


Fig. 16.10 Disposal of Urine in foley bags

The cost of trash disposal is determined by weight, so not emptying the cups/bags would increase the charge for no good reason. The rate for biohazard bags is approximately ten times that of the regular trash, so you don't want anything in there that doesn't have to be.

Emptying the cups into the toilet and then tossing them in the regular trash is the most reasonable method.

Empty the urine out of the cup and place the cups in the red bags. Urine dipsticks go in there, too.

Disposal of Medical Waste

1. Human anotomical are subjected to Incineration and deep burial.
2. Animal waste are also subjected to Incineration and deep burial
3. Microbiology and Biotechnology waste are subjected to Incineration and deep burial

4. Sharps are subjected to the following processes
   i) Incineration ii) Disinfection iii) Chemical treatment iv) Mutilation
5. Medicines and cytotoxic drugs are subjected to
   i) Incineration ii) Destruction and disposal in secured land fill
6. Chemical waste is subjected to chemical treatment and secured landfill
7. Incinerated ash is disposed in Municipal landfil.

Key Terms

Hospital waste : Refers to all waste generated, discarded and not intended for further use in the hospital.

General Waste : Largely composed of domestic or house hold type waste.

Pathological Waste: Consists of tissue, organ, body part, human foetuses, blood and body fluid.

Infectious Waste: The wastes which contain pathogens in sufficient concentration or quantity that could cause diseases.

Sharps : Waste materials which could cause the person handling it, a cut or puncture of skin e.g. needles, broken glass, saws, nail, blades, scalpels.

Pharmaceutical waste: This includes pharmaceutical products, drugs, and chemicals that have been returned from wards, have been spilled, are outdated, or contaminated.

Radioactive waste: It includes solid, liquid, and gaseous waste that is contaminated with radionucleides generated from in-vitro analysis of body tissues and fluid, in-vivo body organ imaging and tumour localization and therapeutic procedures.

Hospital Waste Management Programme : The management programme of hospital waste is called as hospital waste manement preogramme

Chemical Waste: This Comprises discarded solid, liquid and gaseous chemical e.g. cleaning, house keeping, and disinfecting product.

Short Answer Type Questions

- Define hospital waste
- Define general waste
- Define infectious waste
- Define sharps
- Define pharmaceutical waste
- Define chemical waste
- Define Radioactive waste.
- What is hospital waste management programme?.

Long Answer Type Questions

- Write the classification of hospital waste and explain about it in detail
- Explain about the disposal of cotton and swabs
- Write notes on disposal of blood
- Write notes on disposal of syringes, needles and urine.

UNIT-17

Glass Slides, Cover Slips

Structure

17.1 Introduction

History

A microscope slide prepared by Filippo Pacini in 1854, containing reference specimens.

A microscope slide is a thin flat piece of glass, typically 75 by 25 mm (3 by 1 inches) and about 1 mm thick, used to hold objects for examination under a microscope. Microscope slides are often used together with a cover slip or cover glass, a smaller and thinner sheet of glass that is placed over the specimen. Slides are held in place on the microscope's stage by slide clips or slide clamps. Slides are usually made of common glass and their edges are often finely ground or polished. Microscope slides are usually made of glass, such as soda lime glass or borosilicate glass, but specialty plastics are also used. Fused quartz slides are often used when ultraviolet transparency is important, e.g. in fluorescence microscopy.

17.2 Cleaning of glassslides and Cover slips

Load slides and coverslips in the appropriate sized carriers. Fill the Ultrasonic Cleaner with pure water and 1%Micro-90 soap. Submerge the loaded carriers in the cleaner and let sonicate at 60°C for an hour.

Place carriers in a container and rinse under running tap for five minutes. Drain water and repeat the tap waterrinse two more times.

In a similar manner, rinse the loaded carriers under running deionized water for five minutes.

In a similar manner, rinse the loaded carriers by submerging in ultrapure water three times.

Remove carriers from the final rinse and allow excess water to drop for several seconds before placing them in the sealable containers. Fill the carriers with 180 proof ethanol and seal with cover. The glass is now ready for storage.

Before Use

Before using a slide or coverslip, remove it from the ethanol with a pair of flat-ended tweezers and rinse well with a stream of 200 proof ethanol.

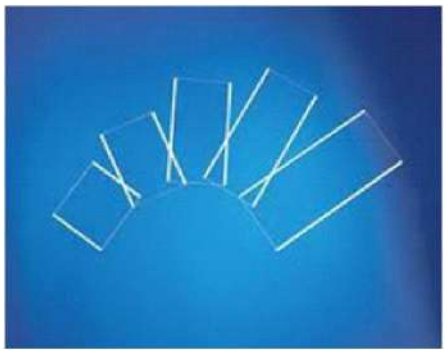
Then thoroughly dry with a clean air gun in a dust free environment. The slide or coverslip will now be ready for use.

Method I.

Squeaky Clean Slides[2]: all done in bath sonicator (Branson 250)

1. Sonicate in Versa Clean and hot water (2%) for 45 minutes
2. Sonicate in just hot water for 30 minutes (to rinse)
3. Sonicate in deionized water for 30 minutes
4. Sonicate in 1 mM EDTA for 30 minutes
5. Sonicate in 70% ethanol for 30 minutes
6. Sonicate in 100% ethanol for 30 minutes
7. Store in ethanol until use

These slides were either flame-dried, air dried, or compressed air dried before use to remove ethanol. The results were not very promising. All the slides had a sort of uniform “fur” covering them with only spots of darkness, indicating a clean area. Plus there were horizontal lines of dirt running across large sections of the slide.



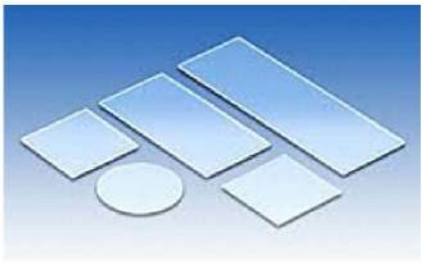


Fig. 8.1 Cover slips for micro array slides Fig. 8.2 Microscope slides and cover slips

Method II

Ultra Clean Slides - Ammonia based glass cleaner and Opti-Clean polymer:

In this method simply used the cleaner with a Kimwipe on the slide. This alone produces poor results under the microscope (darkfield). It does work very well for getting the slide visibly clean (removing the dirt that accumulates on slides over time). After the ammonia-based cleaner is used, it helps to rinse the slides in de-ionized water in a sonicator bath for at least 30 minutes. This removes lint left over from the Kimwipe and other large dust particles.

Key Terms

Glass slides: Used for supporting the specimens to be viewed through microscope

Coverslip : Used to reduce distortion upon viewing and at the same time also protects the specimen from external environment

Wetmount : Used to view microorganisms,stained cross sections or thin slices of tissue

Cleaning : Glass slides after usage washed with cleaning solution is called as cleaning

Ulrasonic Cleaner : Instrument used to clean the used galss slides automatically

Magnification : Enlargement of the image is called as the magnification

Dry mount : Used to observe thin cross sectional segments

Mount : Placement of specimen on the slide and then covering with a cover slip

Maintenance : Protecting the slide right from moumting to finishing of observation of prepared glass slide

Short Anwer Type Questions

Define glass slides and cover slips.

Define wet mount.

Define dry mount.

What is cleaning of glass slides?

Define maitainance of glass slides and cover slips

What are the uses of glass slides and cover slips

What is magnification?

Long Answer Type Questions

Write notes on cleaning of glass slides and cover slips in detail

REFERENCE BOOKS

1. Human Physiology - Dr.Chatterjee . C .C
2. Medical Laboratory Technology - Dr.Ramnik Sood
3. Text Book Of Medical Laboratory Technology - Praful . B.Godkar
4. Notes On Clinical Lab Techniques - Samuel .K.M
5. Text Book Of Pathology - Dr.M.Ramulu & C.Prem Chand
6. Text Book Of Microbiology - Dr.Gyaneshwari
7. Text Book Of Microbiology - Dr.AnanthNarayan
8. Text Book Of BioChemistry - S.Srinivas Rao
9. Text book of Microbiology-Aravind-5th edition
10. Textbook of Microbiology-Principles, procedures & correlations-E.Anne Stiene, Martin
11. Fundamentals of hematology-Richand A Rifkind.

MEDICAL LAB TECHNICIAN

Paper - III

ANOTAMY & PHISIOLOGY

INDEX

UNIT – 1

INTRODUCTION TO HUMAN ANATOMY AND PHYSIOLOGY

HUMAN ANATOMY

Human Anatomy is science that deals with the structure of human body. Study of anatomy helps in understanding the functions of body.

Types

Macroscopic anatomy – It deals with the structure of body with unaided eye

Histology - The study of normal cells and tissues, mainly using microscopes.

Various anatomical positions(Fig 1)

- Superior(Cephalic): Closer to the top of the head. For example, the nose is superior to the chin.
- Inferior (Caudal): Closer to the feet. The chin is inferior to the nose. Caudal is similar to inferior.
- Anterior (Ventral): Closer to the front of the body. For example, the abdominal muscles are anterior to the spine. Ventral is similar to anterior; it means toward the abdomen.
- Posterior (Dorsal): Closer to the back of the body. For example, the spine is posterior to the abdominal muscles.
- Median: At the midline of the body. For example, the nose is a median structure.
- Medial: Closer to the midline of the body. For example, the big toe is medial to the little toe.
- Lateral: Farther away from the middle. For example, the little toe is lateral to the big toe.
- Proximal: Closer to the trunk or closer to the point of origin. For example, the shoulder is proximal to the elbow.
- Distal: Farther from the trunk or from the point of origin. For example, the elbow is distal to the shoulder.

- Superficial: Closer to the surface. The skin is superficial to the muscles.
- Intermediate: In between. The abdominal muscles are intermediate between the skin and the small intestines.
- Deep: Farther from the surface. The abdominal muscles are deep to the skin.

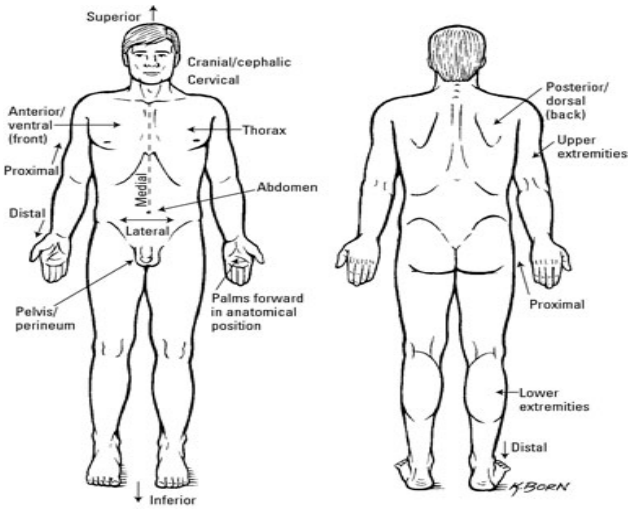


Fig1: Various anatomical positions of the human body

Planes of the human body

- Midsagittal plane: It is a vertical plane that divides the body into left and right halves.
- Sagittal planes: Vertical planes are parallel to the midsagittal plane and divide the body into unequal left and right portions.
- Frontal (coronal) planes: Vertical planes pass through the body at right angles to the midsagittal plane and divide the body into front (anterior) and back (posterior).
- Transverse (horizontal) planes: Horizontal planes pass through the body at right angles to the midsagittal and the frontal planes. They divide the body into superior and inferior portions.

Movements occurring at various joints

Flexion : It is the movement where similar surfaces come nearer to each other reducing the angle between them. eg: Bending of fore arm near elbow.

Extension: It is the movement causing similar surfaces to go apart, whic is opposite to flexion. eg : Straightening of bent fore arm.

Adduction: It is the movement bringing the limb towards mid line.

Rotation: It is the movement around central axis involving 360 degrees.

Medial rotation : Rotation towards medial direction is called medial rotation.

Lateral rotation: Rotation towards lateral direction is called lateral direction.

Circumduction: It is the movement involving flexion, abduction, extension and adduction occuring in sequence.

Important terms that deals the human body

Cell- The tiniest particle of body. It is the basic functional and structural unit of body.

Tissue- A group of cells of similarity in structure, function and origin.

Organ- A group of different kinds of tissues for performing specific functions.

System- The collection of different organs of body to work collectively to conduct some kinds of functions. Eg: Respiratory system, Digestive system, etc.

Organism –It consists of different systems.

HUMAN PHYSIOLOGY

Human physiology is the science of the mechanical, physical and biochemical functions of humans. It is closely related to anatomy as there is an intrinsic link between structure and function of the human body.

The word *physiology* is from the Ancient Greek *phusiología*, "natural philosophy" and it is the study of how organisms perform their vital functions. An example is the study of how a muscle contracts or the force contracting muscles exert on the skeleton. It was introduced by French physician Jean Fernery in 1552. Physiology is better explained by applying the principles of physics, chemistry, biology and anatomy. Anatomy helps in the study of physiology as they are inter related.

Organ systems included in Human anatomy

The following systems make up the human body.

Haemopoieticsystem:Thissystemconsistsofblood.Mainfunctionsof blood are transport of respiratory gases,nutrients,hormones,waste productsetc.

Cardiovascular system: The main organs are heart and blood vessels. The blood vessels transport oxygen, carbon dioxide, nutrients, hormones etc, to and from the cells. The heart acts as a blood pump, pushing blood in the blood vessels to be transported to and from all body tissues.

Digestive system: The organs included are the oral cavity (mouth), oesophagus, stomach, small large intestines, and rectum. The role is to break down food into absorbable molecules and to deliver these to the blood for distribution to body cells.

Endocrine system: The organs included are pituitary, thyroid, parathyroids, adrenals, thymus, pancreas, pineal gland, ovaries (in the female) and testes (in the male). These glands produce and secrete hormones that affect every cell in the body. Metabolism is regulated primarily by these hormones.

Excretory system: The urinary system is mainly involved in excretory function and is composed of the kidneys, ureters, bladder, and urethra. Its main function is to flush wastes from the body in urine, to maintain the body's water and electrolyte balance. Other organs related with excretion are lungs and skin etc.

Integumentary system: It is the external covering of the body (skin).It waterproofs the body and cushions and protects the deeper tissues from injury.

Lymphatic system:It includes lymphatic vessels, lymph nodes, and other lymphoid organs such as the spleen and tonsils. It returns fluid leaked from the blood back to the blood vessels so that blood can be kept continuously circulating through the body. These organs also hold cells involved in immunity.

Muscular system: It is made up of muscles that contract or shorten. The muscles in our body allows for movement and maintain our posture.

Nervous system: It consists of the brain, spinal cord, nerves, and sensory receptors. Central nervous system is concerned with intellectual activity, whereas automatic nervous system is concerned with involuntary functions of body and consists of sympathetic and parasympathetic nerves.

Reproductive system: The male reproductive system includes the testes, scrotum, penis, accessory glands, and the duct system. The female reproductive system includes the ovaries, uterine tubes, uterus, and vagina. The main purpose of these systems is to produce offspring.

Respiratory system : It consists of the nasal passages, pharynx, larynx, trachea, bronchi, and lungs. Its isconcerned with the constant supply of oxygen and to remove carbon dioxide.

Skeletal system: It is made up of bones, cartilages, ligaments and joints. These provide support and protection for body organs.

Special senses: Special senses of body are sight, hearing, taste, smell and touch. Organs related to these functions are eyes, ears, tongue, noseand skin.

Summary

Human Anatomy is the science dealing with the structure of human body, whereas Human Physiology is the study of functions of body. 11 organ systems make up the body like cardiovascular system, skeletal system, muscular system, respiratory system, digestive system, excretory system, endocrine system, reproductive system, nervous system, integumentary system and lymphatic system.

Questions

Anatomy

Essay - Define Anatomy. Describe and various postions of the human body.

Short Answer Questions

1.Mention the types of Anatomy.

2. Write briefly about the variousmovements of the body

3. Name various planes of the body

4. Write the definitions of a) Cell b) Tissue

5. Write the definitions of a) Organ b) system

6.Define Histology

Physiology

Essay- Define Physiology. Describe various organ systems of the body and their functions?

Short Answers

1. What are the organs involved in the excretory system?

2. Write the functions of cardiovascular system

3. Name the organs involved in endocrine system

4. Mention the functions of respiratory system

5. Define the major types of the nervous system.

6. Name the organs involved in lymphatic system.

7. Mention the sensory organs

UNIT – 2

CELL

Definition: Cell is the basic structural, functional and biological unit of all living organisms. It is the smallest unit of the body. Based on the number of cells, organisms can be classified as unicellular (consisting of a single cell eg: bacteria) or multicellular (consisting of many cells eg: plants, mammals).

Types of cells of body: They are two types of cells in body.

1) Somatic cells - Somatic cells are diverse cells which make up somatic structure of body.

2) Gonadal cells- Gonadal cells are gametes which can unite to form new individual.

Structure of cell: Humans contain more than 10 trillion (10^{13}) cells. Most plant and animal cells are visible only under a microscope, with dimensions between 1 and 100 micrometres.

Every cell comprises following parts. 1) Cell membrane2) Cytoplasm 3) Nucleus

1) Cell membrane : It is also called as plamsalemma or plasma membrane. It is a double layered, thin barrier, surrounding the cell to control the entry and exit of certain substances. It cannot be seen by light microscope. It can be seen by electron microscope. It has trilaminar structure of phospholipid bilayer. Outer surface of cell wall contains pinocytotic vesicles. Inner surface is continuous with endoplasmic reticulum (ER).

The functions of cell wall are 1) Transport of materials (main function) 2) Protection of cell 3) Reception of external stimuli 4) Ingestion of nutrients 5) Excretion of waste products of cellular metabolism.

2) Cytoplasm : It is a membrane, which protects the cell by keeping the cell organelles separate from each other. It is the site, where many vital biochemical reactions take place.Cytoplasmic organelles are Endoplasmic reticulum, Golgi apparatus, Mitochondria, Lysosomes, Ribosomes, Centrosomes etc. (Figure 2)

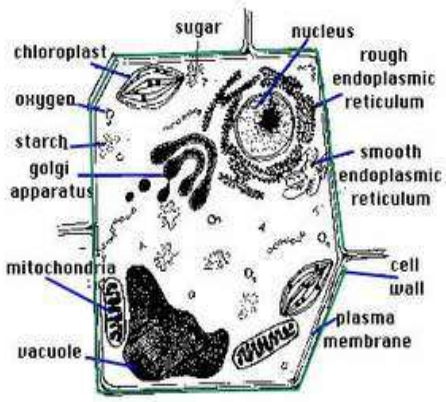


Figure 2 : Structure of cell

Endoplasmic reticulum: Endoplasmic reticulum is a system that continues with infoldings of cell membrane.

It is of two types of endoplasmic reticulum.

1.Smooth ER - It is a network of smooth tubules.

Function: It is concerned with metabolism and synthesis of steroids

2. Rough ER-It consists of ribosomes and isprominent in Adrenal cortex, liver and striated muscle.

Functions are 1) Protein synthesis2) Translation of language of nucleic acids.

Golgi apparatus : It is shaped like network of threads.

Functions: 1) synthesis of various secretions. 2) Storage of enzymes etc. 3) helps in the movement of materials within the cell.

Mitochondria: They are double membrane, rod shaped, filamentous organelles.They vary in size from 0.5 to 5 microns. Inner membrane remains folded to form partitions called cristae mitochondriales. Intramitochondrial space contains fluid called matrix. (Fig 3)

Functions: 1) play a vital role in generating, transforming the energyand supply 95% of cell's energy requirement, hence called power houses2) plays a vital role in various functions of the cell metabolisms including oxidative phosphorylation3) synthesis of RNA and DNA.

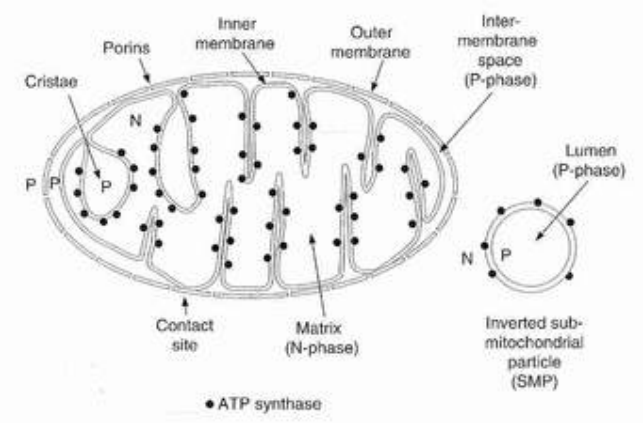


Figure 3: Structure of mitochondria

Lysosomes : They are digestive organs of cells and are also called suicide bags of cells. They contain powerful hydrolytic enzymes. Lysosomes are absent in RBC.

Functions : 1) Digestive organelles of cells and break down of particles taken hydrolytic enzymes.2) Autolysis 3) Phagocytosis 4) Killing of cells 5) Cell division.

Ribosomes : They are scattered throughout cytoplasm singly or as groups. They are ribonucleoprotein in nature.

Functions: Protein synthesis.

Centrosome : Centrosome contains centrioles.

Function : Centrioles control polarisation of spindle fibres. Centriole is closely related to spindle formation during cell division (Mitosis).

Plasmosin : It is constant constituent of cytoplasm. They formtonofibrils in epithelial cells, myofibrils in muscles and neurofibrils in nerves.

They consist of long protein molecules rich in deoxy-ribonucleoprotein.

Vacuoles : They are also cytoplasmic organelles. They are found covered by faton staining with dilute neutral red solution.

Nissl bodies: They are found in nerve cells.

Secretory granules: They store secretory products of cell and are found in- Golgi apparatus and Endoplasmic reticulum.

3) Nucleus : It is the key structure of living cell. Nucleus is covered by nuclear membrane and consists of chromatin and nucleolus. Chromatin is dense chromosomal network. Chromatin contains different genes which determine heredity of cell.Chromosomes are present as individual bodies in interphase as well as in mitosis. Predominant component in chromosome is DNA. Genes are located in chromosome. They are discrete units of transmission of hereditary characters. In female, 2X chromosomes and in males 1X and 1Y chromosomes are present. There is usually single nucleolus or 2-5 nucleoli in a cell.

Differential arrangement of cellular mass: Differentiation of cellular mass is evident when cells arrange in three layers. They are 1) Ectoderm2) Mesoderm 3) Endoderm. Ectoderm gives rise to epithelium of mouth, nose, skin, hair, some glands like sweat, mammary, pituitary, adrenal medulla, brain and nerves. Mesoderm gives rise to connective tissue including blood, bone marrow cells, cortex etc. Endoderm gives rise to epithelium of digestive and respiratory tract, thyroid, parathyroid, thymus and bladder.

Properties of cell :

Properties of cell in unicellular organisms like amoeba are - a) Irritability b) Conductivity

c) Contractility d) Absorption e) Excretion f) Growth and reproduction g) Motility h) Secretion

Questions

Essay Questions

1. Write the structure of cell with diagram.
2. Explain Properties of cell.

Short questions

1. Write the definition of a cell?
2. Mention various properties of the cell.
3. List out cytoplasmic organelles.
5. Explain the types of endoplasmic reticulum and their functions.
6. What are the functions of Golgi apparatus?
8. Draw the diagram of mitochondria.
9. What are Lysosomes ? Write briefly about their functions.
10. What are the elements arise from a) Ectoderm b) Mesoderm C) Endoderm

UNIT – 3

TISSUE

Definition: Tissue is defined as group of cells of similarity in structure,function and genesis.

Classification:

Human body contains following types of tissues.

I) Epithelial tissue II) Connective tissueIII) Muscular tissue IV) Nervous tissue

I. Epithelial tissue:

Epithelial tissues are a diverse group of tissues that include both surface epithelia and solid organs. Surface epithelia cover or line all body surfaces, cavities and tubes and forms epithelial membrane. All epithelia are supported by a basement membrane which separates the epithelium from underlying supporting tissues. Blood vesselsnever cross epithelial basement membranes, so epithelium depend on the diffusion of oxygen and metabolites from adjacent supporting tissues.

Functions of epithelial tissue are -

1) To form protective barrier (protects underlying surfaces) 2) Regulation of the exchange of molecules between compartments (selective diffusion and absorption3) Synthesis and secretion of glandular products 4) Excretion

Classifiacation of epitheilia:

Number of cell layers	**Type of cell**	**Special features**	**Example**
Simple (one layer)	Squamous		Peritoneum, vascular endothelium
	Cuboidal		Collecting tubule of kidney
		Microvilli	Proximal convoluted tubule of kidney
	Columnar	Pseudostratification	Respiratory tract
		Microvilli	Small intestine
		Goblet cells	Small and Large

			intestine
		Surface cilia	Fallopian tube
		Stereocilia	Vas deferens
			Gall bladder
Stratified(Multiple layers)	Squamous	Keratinization	Epidermis of skin
			Oral cavity
	Cuboidal		Exocrine gland ducts
	Transitional		Bladder

1. Simple epithelium: It is defined as surface epithelium consisting of a single layer of cells. Types of simple epithelial tissues are,

a) Squamous or pavement epithelium–It consists of single layer of flat cells.

Functions : - 1) Passage of liquids and gases 2) Protection

b) Cuboidal epithelium–It represents an intermediate form between simple squamous and simple columnar epithelium. It consists of single layer of cuboidal cells of samedimensions and the cells appear as square. Nucleus is round and located in the centre of the cell. Eg: Collecting tubule of kidney, salivary glands, pancreas.etc.

Functions:1) Protection 2) Secretion 3) Excretory etc.

c) Columnar epithelium– It is similar to simple cuboidal epithelium except that the cells are taller and appear columnar in sections perpendicular to the basement membrane.The nucleus is elongated and may be located towards the base; the centre or occasionally at the apex of the cytoplasm; this is known as polarity of the nucleus.

It is found on absorptive surfaces such as in the small intestine, large intestine, alveoli as well as secretory surfaces such as that of the stomach and endocervix, In alimentary canal and nephron, it is brush bordered. Goblet cells are another type of columnar epithelium found in large intestine mainly and secrete mucus.

Functions : 1) Absorption 2) Secretion

Variants: 1.Ciliated columnar and cuboidal epithelium –They have surface cilia. Cilia are much larger than microvilli and are readily visible with the light microscope. Each cell may have upto 300 cilia that beat in a wave – like manner.The waving motion of the cilia propels

fluid and particles over the epithelial surface. It is found in the female reproductive tract(fallopian tube). Ciliary action facilitates transport of the ovum from the ovary towards uterus.

2. Pseudostratified ciliated columnar epithelium (Respiratory epithelium)- It appears as it contains more than one layer of cells. The nuclei are placed at different levels, thus creating illusion of cellular stratification. In fact, this is a true simple epithelium. It is exclusively confined to respiratory tract.

2.Stratified epithelium: It is defined as epithelium consisting of two or more layers of cells.

a) Stratified squamous keratinized epithelium–It has two or more layers and horny due to deposition of keratin.It is found in skin, hairs, nails, horns, enamel of teeth.

Functions: 1) Protection from atmosphere2) Protection from mechanical pressure3) Protection from injury and friction

b) Stratified squamous nonkeratinized epithelium –It has also two or more layers, but keratin is absent. It is found in cornea, mouth, pharynx, oesophagus, anal canal, urethra, vagina and cervix etc.

c) Stratified cuboidal epithelium –It is thin, stratified epithelium that usually consists of only two or three layers of cuboidal cells. It is confined to the large excretory ducts such as the salivary glands.

d) Transitional epithelium (Urothelium)- It is a form of stratified epithelium found only in the urinary tract in mammals. It is so named because it has some features intermediate between stratified squamous and stratified cuboidal epithelium.

Functions: It is highly specialized to accommodate a great degree of stretch and to withstand the toxicity of urine.

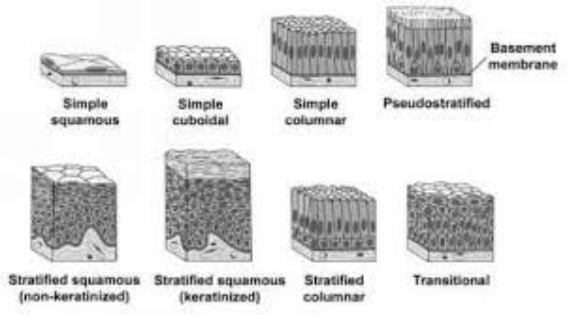


Figure 1: Types of epithelia

II.Connective Tissue: Connective tissue/supporting tissue is the term applied to tissues which provide general structure, mechanical strength, space filling and physical and metabolic support for more specialized tissues. It is also called as mesenchymal tissue. It is developed from mesoderm. Cells will be less and intercellular matrix will be abundant.

Every type of connective tissue has the following components.

1. Matrix fibres - Collagen and elastin
2. Ground substance – Glycoproteins, Glycosaminoglycans
3. Basement membrane –Type IV collagen
4. Support cells -Fibroblasts, Myofibroblasts, Adipocytes, Chondrocytes, Osteoblasts and Osteocytes and neurons.

Types of connective tissue

a) Areolar tissue b) Adipose tissuec) Fibrous tissue d) Elastic tissue e) Bloodf) Hematopoietic tissue g) Cartilaginous tissueh) Osseous tissue i) Jelly like tissue k) Reticuloendothelial tissue

a.Areolar tissue: It is loose connective tissue present in many organs. It is composed of fibres(collagenous and elastic fibres), cells, and ground substance. It acts as a biological packing and wrapping material. It is distributed between muscular, vascular and nervous tissues, subcutaneous, subserous and submucous tissues.

b) Adipose tissue : It is loose connective tissue mostly composed of adipocytes . It is found below skin in mesentery, omentum etc. It is of two types.

1. White adipose tissue- It provides structural fill which gives shape to limbs and forms part of shock absorbing padding and prevents injury to organs.
2. Brown adipose tissue- It is highly metabolically active and helps in the regulation of body temperature and body weight.

c) Fibrous tissue : It is made of white fibres formed by fibroblasts. These fibres are non branching and present in bundles. They are present in tendons and ligaments of limbs. It is made up of collagen.

d) **Elastic tissue** : It is another variety of fibrous tissue. Elastin fibres are eosinophilic, wavy. It is most resistant to many chemicals and is found in areolar tissue throughout body. It is also

present in lung, skin, urinary bladder and blood vessels etc. Elastic fibres in the dermis allow the skin to stretch and recoil, keeping it wrinkle free. In arteries elastin provides the stretch and recoil to smooth and transmit the pulse pressure generated by each heart beat.

e) Blood : Blood is fluid connective tissue of body.

f) Haemotopoietic tissue : There are two types of haematopoietic tissues. They are - a) Myeloid tissue b) Lymphatic tissue

Myeloid tissue **:** Myeloid tissue is blood forming tissue as well as phagocytic. 'Myelos' means marrow. There are two types of bone marrow

a) Red bone marrow ... Active form

b) Yellow bone marrow - Inactive form.

Red bone marrow: Red cells are produced in red bone marrow. In foetal life, most of the bones contain red bone marrow. With advancement of age, it is located only in upper ends of humerus, femur, bones of skull, thorax, vertebrae and pelvic innominate bones.

Yellow bone marrow: It is inactive in adult.

Lymphatic tissue: Lymphatic tissue is two types - It is present in lymph organs - lymph node, spleen, thymus, tonsils.

g) Cartilaginous tissue : It is connective tissue, which is intermediate between fibrous and osseous tissues in firmness and elasticity. Main components are cartilage cells, chondroblasts, inter cellular ground substance called matrix, fibres. It is divided into three types.

a) Hyaline cartilage b) Fibrocartilage c) Elastic cartilage.

a) Hyaline cartilage : It is made of cartilaginous cells and clear homogenous ground substance. Cartilage cells are also called chondrocytes. The small empty spaces in the matrix are called lacunae. Matrix is solid intercellular substance of cartilage or bone. It is distributed in the articular end of bones.

b) Fiibrocartilage: This type of cartilage has great tensile strength with flexibility and rigidity. It can stand with shearing forces. It is found in intervertebral discs, meniscus of knee joints, mandibular joints, pubis symphysis and linings of tendon.

c) Elastic cartilage : Elastic cartilage is histologically similar to hyaline cartilage. It contains elastin fibre networks and collagen type II fibres. The principal protein is elastin. It is

distributed in External ear, Epiglottis and Eustachian tube.These fibres give elastic cartilage great flexibility so that it is able to withstand repeated bending.

Jelly like connective tissue(Wharton's jelly) : It is an embryonic form of areolar tissue. It contains large fibroblasts and mucin. It is found in umbilical cord. It is called as Wharton's jelly here. Vitreous humour of eye ball is composed of this tissue in adult life.

h) Mononuclear phagocytic system (Reticuloendothelial tissue) : It is a part of immune system and consists of phagocytic cells located in reticular connective tissue. The cells are primarily monocytes and macrophages and accumulate in lymph nodes and spleen. Main functions are phagocytosis, antibody formation and formation of new RBC and destruction of old RBC.

i) Osseous tissue : Osseous tissue is specialized connective tissue andis made of bone cells and intercellular ground substance. It acts as structural support and stores minerals .It is seen in skeleton.There are three types of bone cells. They are - Osteoblasts, osteocytes and osteoclasts.

There are 2 types of bone tissues They are 1) woven bone 2) Lamellar bone

1.Woven bone : (Also known as Primary or immature bone) it has irregularly arranged bone matrix seen in bone development.

2.Lamellar bone : (Also known as Secondary or mature bone) It has well organised layers of bone matrix.

There are 2 types of bone tissue based on bony architecture

1.Compact bone : It is dense ,closely packed bony tissue . It is seen in cortical bone of long shafts of bone .

2.Spongy bone : It is also called cancellous or trabecular bone , is lighter than compact bone and is seen in parts of medullary cavity of mature bones. It is present in vertebrae, flat bones, long bones,,

Bone is covered with periosteum. Periosteum has two layers- Outer fibrous layer and inner layer called cambium. Cambium is osteogenic in its functions to produce osteoblasts and osteoclasts. Endosteum is the lining membrane of medullary cavity of all long bones .Bone cavity is the hollow space inside the bone and filled with bone marrow.

Transverse section of bone: T.S. of bone under microscope shows Haversian system consisting of 1) Central haversian canal 2) Lamellae 3) Lacunae 4) Canaliculi.

Central haversian canal contains blood vessels, nerves and lymphatic vessels.

Lamellae are layers of bone deposited in concentric circles around haversian canal.

Lacunae are interlamellar spaces.

Canaliculi are minute canals joining lamellae and communicating with central haversian canal.

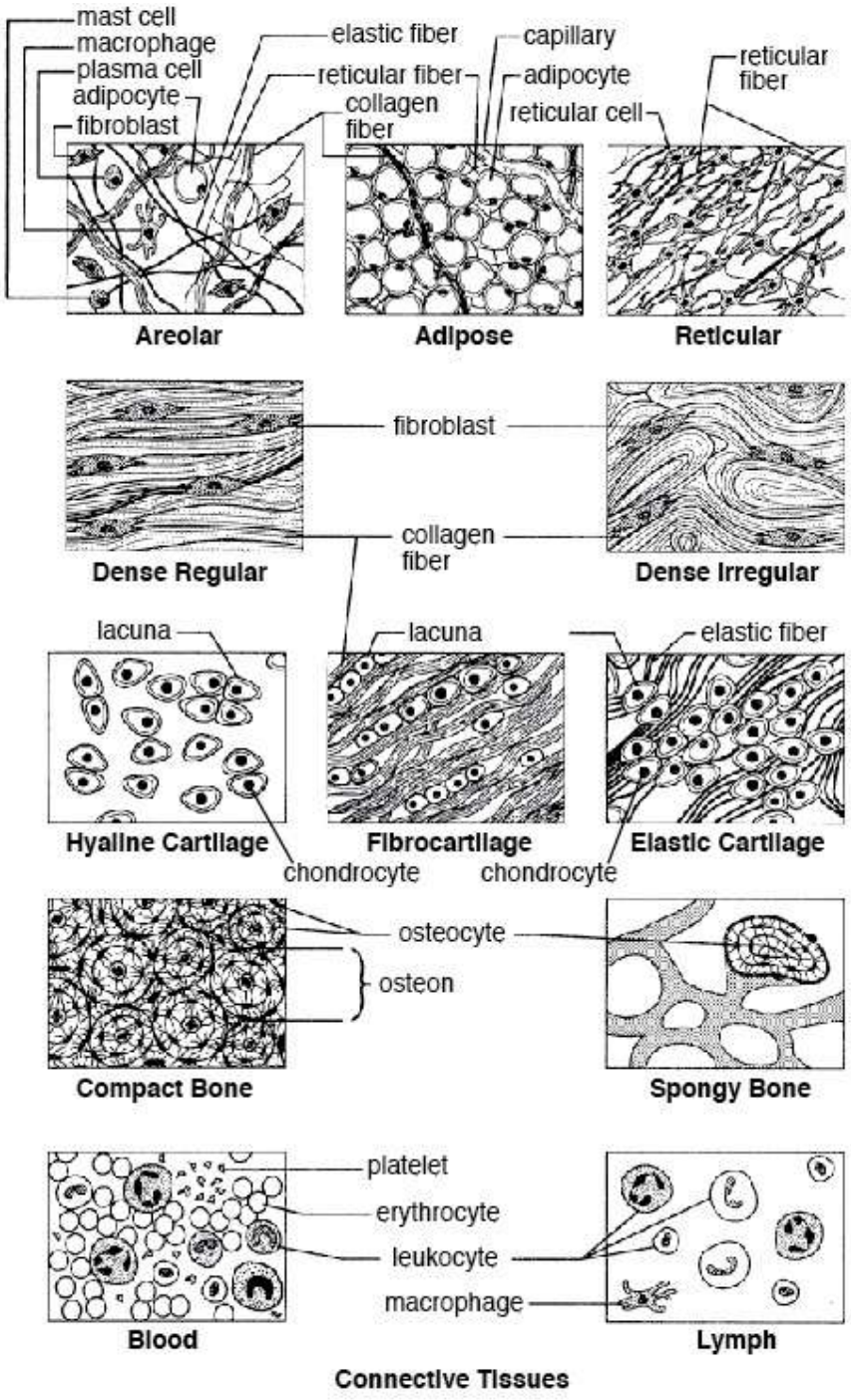


Fig 2: Types of Connective tissues

III) Muscular tissues : These tissues has contractile ability on excitation. It has also property of conductivity. There are different types of muscular tissues.

Types of muscular tissues based on striation: They are two types.

1) Striated muscles : They have cross striations Ex : Skeletal muscle and Cardiac muscle

2) Non striated muscles : They do not have cross striations Ex.Smooth muscle

Types of muscular tissues on the basis of control. They are two types,

1) Voluntary muscles Ex.Skeletal muscle

2) Involuntary muscles Ex : Cardiac and Smoothmuscle

Types of muscular tissues on the basis of distribution. They are of 3 types.

1) Skeletal muscles 2) Cardiac muscles 3) Smooth muscles.

Skeletal muscle : Skeletal muscles are attached to bones.

Epimysium is the outer covering of skeletal muscle.

Perimysium is the sheath of connective tissue surrounding a bundle of muscle fibers.

Smaller bundles into which skeletal muscle is divided are called fasciculi.

Each fasciculus contains muscle fibres. Each fibre is covered by endomysium.

Sarcolemma is the transparent cell wall of muscle fibre.

Cardiac muscle: It is involuntary, striated muscle of heart. It contracts rhythmically and automatically.

Main differences between skeletal and cardiac muscle are seen in the following table 2:

Characteritiscs	**Cardiac Muscle**	**Skeletal Muscle**
Contarctility .	Spontaneous rhythmicity and contractility	Voluntary in action
Muscle'fibers arrangement	Arranged syncytially	individually present
Nucleus	Single oval shaped at centre	Nuclei are seen peripherally

Smooth muscle: They are smooth, involuntary and muscles of viscera.

Visceral muscle fibres are smooth and elongated. They are fusiform with tapering towards periphery.. They contain one oval or rod shaped nucleus at the centre of each cell.

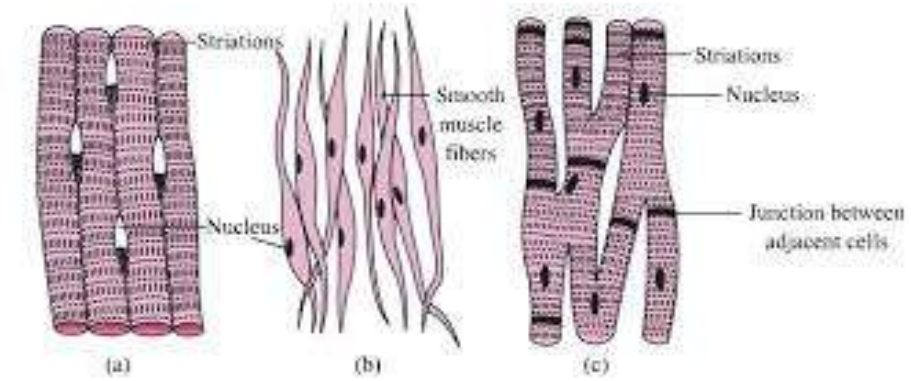


Fig : Types of Muscle tissue

IV. Nervous Tissue:

Nervous tissue is excitable type tissue receiving and transmitting messages. It is composed of neurons. Nervous system is ectodermal in origin. There are three types of matters in nervous tissue. They are -

1) Grey matter - forming nerve cells

2) White matter - forming nerve fibres

3) Neuroglia - holding nerve cells and fibres together and supporting them.

Neuron : Neuron is the basic functional and structural unit of nervous system. Parts of neuron are -

1) Nerve cell body (also called Perikaryon or Neurocyton or Soma)

2) Nerve fibres (also called processes of nerve cells)

Nerve cell body: It is the part of neuron containing cell membrane, neuroplasm and nucleus. Neuroplasm contains neurofibrils, nissl bodies, mitochondria and golgi apparatus. Neurofibrils are fine filaments passing through neuroplasm from dendrites to axon. Nissl bodies are angular granules stained with basic dyes.

Nerve fibres: There are two types of nerve fibres.

a) Dendrites- They are receptive processes (also called dendrites). Dendrites carry impulses from other neurons and carry them towards nerve cell body.

b) Axons- They are discharging processes. It carries impulses away from nerve cell. It consists of three parts - axis cylinder, myelin sheath and neurilemma.

Axis cylinder contains axoplasm, neurofibrils and mitochondria.

Myelin sheath is absent over nerve fibres within grey matter. It is present over nerve fibres after entering white matter. Function of myelin sheath is insulation of nerve fibre.

Neurilemma is the homogeneous nucleated covering over somatic and autonomic nerve fibres outside C.N.S. Myelinated fibres in brain and spinal cord do not have neurilemma.

Peripheral nerves: Fibres of peripheral nerve trunks are divided into bundles. Individual fibres are held together by loose connective tissue called endoneurium. Each bundle is covered by a sheath called perineurium. Epineurium is the tough enclosure of whole nerve trunk.

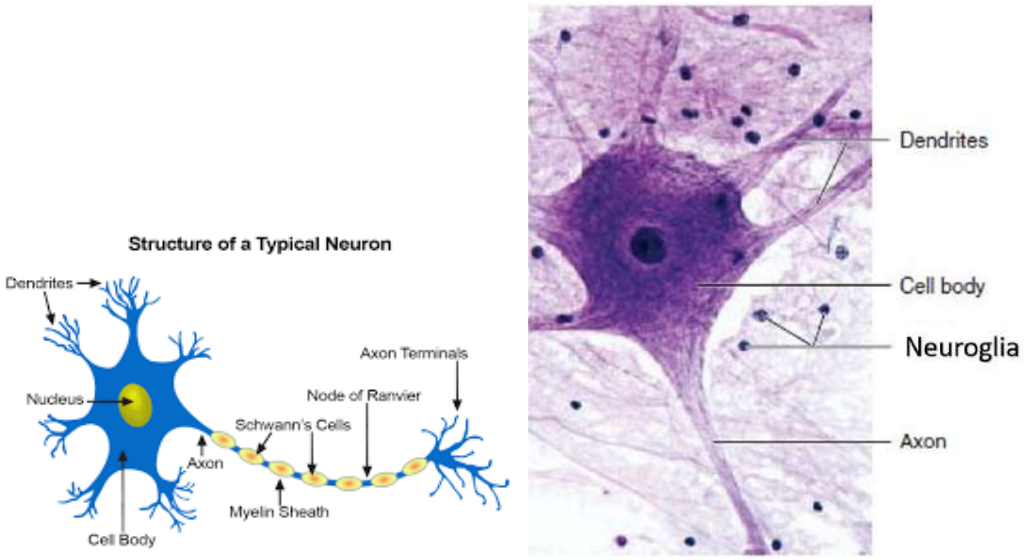


Fig 2: Structure of Neuron

Summary

Tissue is group of cells. Different types of tissues of body are Epithelial tissue, Connective tissue, Muscular tissue and Nervous tissue. Epithelial tissue is two types - simple and compound. Connective tissue is also called mesenchymal tissue. There are several types of connective tissue. Muscular tissue is three types - Skeletal muscle, cardiac muscle, smooth

muscles. Nervous tissue is made of neurons. Each neuron contains nerve cell, dendrites and axon.

Questions

1. Classify the tissues of human body and write about epithelial tissue.
2. What are different types of connective tissue? Explain myeloid tissue and osseous tissue.
3. Add note on Areolar tissue tissue.
4. Add note on nervous tissue. Draw diagram of neuron.

Short Answer Questions

1.Mention major classes of tissues of human body.

2. Define a) Simple epithelium b) Compound epithelium

3. Describe pavement epithelium.

4. Give the distribution of pavement epithelium.

5. Write the description and distribution of cuboidal epithelium.

6. Write about columnar epithelium.

7. Mention the classes of compound epithelium

8. Write the distribution of a) Transitional epitheliumb) Stratified squamous cornified epithelium.

9. Write the functions of Pseudostratified columnar epithelium.

10. What are the types of cells found in areolar tissue?

11. Write about white fibrous tissue.

12. Explain Myeloid tissue.

13. What are different types of cartilaginous tissue?

14. Write about Reticuloendothelial tissue.

15. What are types of bone tissue.

16. Give the T.S. of bone

17. Mention different types of muscular tissues
18. Explain a) Epimysium b) Perimysium c) Endomysium
19. Define a) Sarcolemma b) Sarcoplasm
20. Differentiate between skeletal and cardiac muscular tissues.
21. Write about visceral muscles.
22. What is nervous tissue?
23. Write different types of matters of nervous tissue.
24. What are parts of Neuron?
25. Explain a) Endoneurium b) Perineurium

UNIT – 4

RESPIRATORY SYSTEM

Definition : Respiratory system is defined as the system consisting of parts concerned with inhalation and exhalation. Respiration is defined as the process of gaseous exchange between body tissues and external environment.

Respiratory tumor is divided into 2 parts.

1. Upper respiratory tract extends from upper nares to the vocal cord.

2. Lower respiratory tract extends from vocal cord to the alveoli.

Parts of the respiratory system:

1. Nose

2. Pharynx

3. Larynx

4. Trachea

5. Bronchi

6. Lungs : They have following parts.

a. Bronchioles

b. Alveolar ducts

c. Alveoli

1. Nose: It is the part of respiratory system through which air is inhaled in and exhaled out.

Roof of the nose is formed by ethmoid bone at the base of the skull.Floor of the nose is formed by the hard and soft palates at the roof of the mouth.

External nose is the visible part of nose. It is formed by the two nasal bones and cartilage. It is covered by skin. There are hairs inside.

Nasal cavity is a large cavity divided by a septum. It is lined with ciliated mucous membrane. It is extremely vascular.

Anterior nares are the openings which lead in.

Posterior nares are similar openings at the back and lead into pharynx.

Paranasal sinuses are the cavities in the bones surrounding the nasal cavity, which are lined with mucous membrane and open into nasal cavity.

They are of 4 types. 1. Maxillary sinus 2. Frontal sinus 3. Ethmoidal sinuses 4. Sphenoidal sinus

2.Pharynx : It lies between Nasal cavity and larynx.

Pharynx is divided into three parts. They are.

i) Nasopharynx ii) Oropharynx iii)Laryngopharynx

i)Nasopharynx: It lies between nasal cavity and oropharynx.It is lined with ciliated mucous membrane which is continuous with lining of the nose.

ii) Oropharynx: It extends from soft palate to the level of hyoid bone. The lateral wall contains of lymphoid tissue called tonsils.

iii) Laryngopharynx (Hypopharynx): It is the lowest part of pharynx.

3. Larynx: It lies below pharynx and above trachea. It is continuous with oropharynx. It is composed of several cartilages. They are joined together by ligaments and membranes.

Cartilages of Larynx are-a) Thyroid cartilageb) Cricoid cartilage c) Arytenoid cartilages d) Epiglottis

4) Trachea: It is also called as wind pipe. It is a cylindrical tube of length about 11 cm and begins at the lower end of pharynx. It divides into two bronchi at the level of fifth thoracic vertebra. It is made of sixteen to twenty C-shaped incomplete cartilages. It is lined by ciliated epithelium and contains goblet cells which secrete mucus.

5) Bronchi: Trachea divide into right and left bronchi. Right bronchus leads into right lung and left bronchus leads into left lung. Right bronchus is shorter and wider than left bronchus. Bronchi are made up of complete rings of cartilage.

6) Lungs: Lungs are the principal spongy organs concerned with respiratory process. They are two in number and lie in the thoracic cavity on either side of heart and great vessels. Hilum is a triangular shaped depression on the concave medial surface and is formed by

pulmonary arteries, pulmonary veins, bronchial arteries, bronchial veins, bronchi and lymphatic vessels.

Lungs have apex above and base below. Each lung is divided into lobes by means of fissures. Right lung is divided into three lobes whereas left lung is divided into two lobes. Each lobe is divided into number of lobules. Each lobe contains a) bronchioles b) alveolar ducts c) alveoli.

a) Bronchioles: They are the branches of bronchi and donot have cartilage. They are lined by cuboidal epithelium. They become further smaller to form terminal bronchioles which are lined by a single layer of flattened epithelial cells.

b) Alveolarducts: Terminal bronchioles divide repeatedly to form minute passages called alveolar ducts. They open into alveolar sacs.

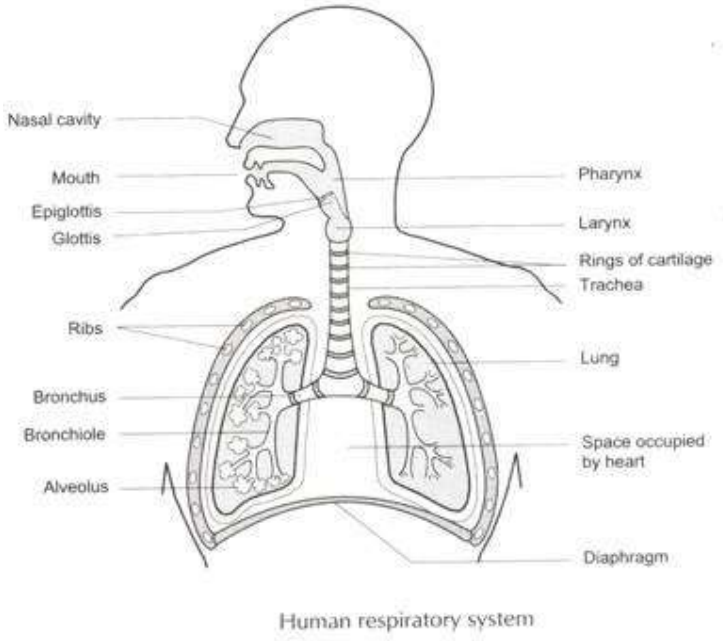
c) Alveoli: Alveolar ducts open into alveoli.They are surrounded by numerous capillaries. Capillary network is the site of exchange of gases between blood and air in the alveoli.

Pleura: It is a serous covering the lungs. It contains two layers, inner visceral layer andouter parietal layer. Pleural fluid lies in the space between these layers.

Respiratory muscles: Intercostal muscles and diaphram are main respiratory muscles. However, during forced respiration sternocleidomastoid, scalenie, mylohyoid, platysma and abdominal muscles also participate.

Intercostal muscles are 11 pairs and are external and internal intercostal muscles. Diaphramis a large dome shaped sheath of muscle. It separates thoracic cavity from abdominal cavity.

Mediastinum: It is a block of tissue in between the two lungs and contains heart, great vessels, trachea, oesophagus, thoracic duct and thymus gland.



Human respiratory system

Fig 1: Parts of sRespratory system

PHYSIOLOGY OF RESPIRATION

Breathing or ventilation

1.External respiration. which is the exchange of gases (oxygen and carbon dioxide) between inhaled air and the blood.

2. Internal respiration, which is the exchange of gases between the blood and tissue fluids.

3.Cellular respiration

In addition to these main processes, the respiratory system serves for:

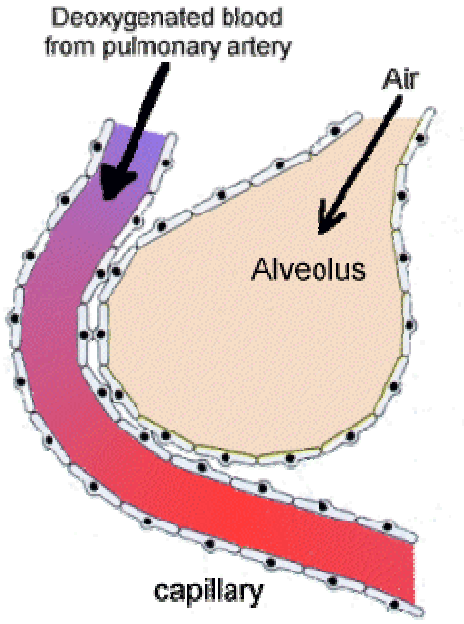
- Regulation of blood pH, which occurs in coordination with the kidneys
- Acts as a defense against microbes
- Control of body temperature due to loss of evaporate during expiration

Mechanics of Breathing

To take a breath in, the external intercostal muscles contract, moving the ribcage up and out. The diaphragm moves down at the same time, creating negative pressure within the thorax. The lungs are held to the thoracic wall by the pleural membranes, and so expand outwards as well. This creates negative pressure within the lungs, and so air rushes in through the upper and lower airways.

Expiration is mainly due to the natural elasticity of the lungs, which tend to collapse if they are not held against the thoracic wall.

Physiology of Gas Exchange



Each branch of the bronchial tree eventually sub-divides to form very narrow terminal bronchioles, which terminate in the **alveoli**. There are many millions of alveloi in each lung, and these are the areas responsible for gaseous exchange, presenting a massive surface area for exchange to occur over.

Each alveolus is very closely associated with a network of capillaries containing deoxygenated blood from the pulmonary artery. The capillary and alveolar walls are very thin, allowing rapid exchange of gases by *passive diffusion along concentration gradients*. CO_2 moves *into* the alveolus as the concentration is much lower in the alveolus than in the blood, and O_2 moves *out of* the alveolus as the continuous flow of blood through the capillaries prevents saturation of the blood wit

Summary

Respiratory system is system consisting of parts related with respiration. Parts of respiratory system are -Nose, pharynx, larynx, trachea, bronchi, bronchioles, alveolar ducts and alveoli.

Alveoli are the ultimate sites of gaseous exchange. Lungs are two in number. Right lung is divided into three lobes. Left lung is divided into two lobes. Each lobe is divided into lobules. Pleura is serous membrane covering lungs. Hilum on each lung is depression through which blood vessels, nerves, lymphatics etc. pass.

Essay Questions

1) Describe in detail about various parts of respiratory system

Short Answer Questions

1) What are the parts of respiratory system, which lead into lungs?

2) Name the parts of respiratory system which lie within lungs.

3) Mention the parts of nose.

4) What are the parts of pharynx?

5) Name the cartilages of larynx.

6) Explain trachea.

7) What are a) Bronchi b) Bronchioles?

8) Write about alveoli.

9) What are the types of respiration?

10) Desribe the mechanics of breathing.

11) Write about the process of brathing.

4. DIGESTIVE SYSTEM AND HEPATO BILIARY SYSTEM

Digestive system consists of gastrointestinal tract(Alimentary canal) and various glands attached. It starts with mouth and ends with anus.The upper gastrointestinal tract consists of the buccalcavity(Mouth), pharynx, esophagus, stomach, and duodenum.The lower gastrointestinal tract includes most of the small intestine, large intestine, rectum and anus.

Various parts of Digestive tract:

1)Mouth

2)Pharynx
4)Stomach

5)Smallintestine

8)Anus

Accesory organs of the digestive tract:

1)Teeth

2)Threepairsofsalivary glands

3)Liverandbiliarysystem

4)Pancreas

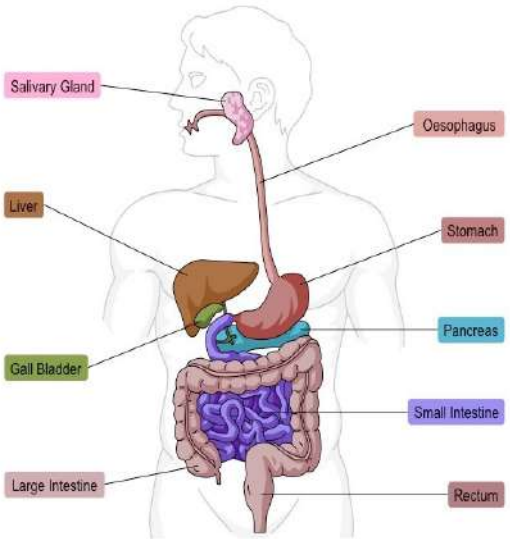


Fig 1: Parts of Digestive system

Mouth: It is the first part of the digestive tract. It opens through upper and lower lips. Roof of the mouth is called palate which is dome shaped . It is divided into hard (front part) and soft palate (back part) . Walls of the mouth are formed by muscles of cheeks. Mouh is lined by mucous membrane. This muscle keeps the mouth closed. Pharyngeal tonsils are on either side at the back of oral cavity. Uvula hangs down from lower border of soft palate.

Tongue: Tongue is at the base of the mouth. It is a musculo-membranous structure. It consists of 1)Stratified and cornified epithelium 2) Voluntary, cross striated muscle fibres and 3)Glands.Epithelium of tongue is modified into papillae and taste buds.

Teeth: Man is provided with two sets of teeth in his life. First set is called as Deciduous teeth or primary teeth. They are 10+10 in number. They eruptthrough the gums during first and second years of life. Second set strarts replacing the first set at about sixth year and process is complete by twenty fifth year.Second set remains upto old age and is called as permanant teeth.

Permanant teeth are 16+16 in number.Four types of teeth are there. They are,

1) Incisor teeth

2) Canine teeth

3) Premolar teeth

4) Molar teeth

Upper teeth and lower teeth are attached to upper Jaw and Lower jaw respectively.

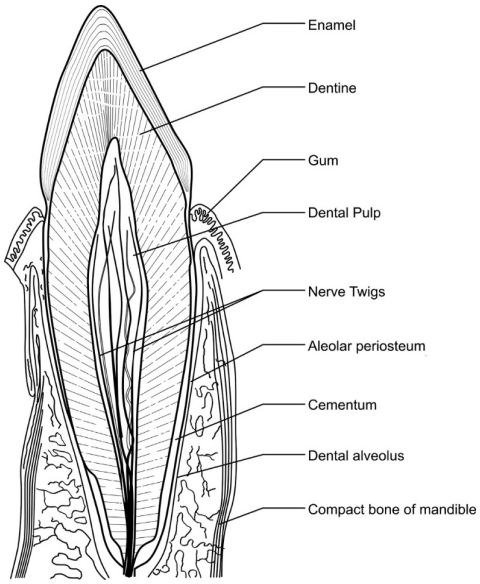


Fig 2: Structure of tooth:

Each tooth consists of three parts.They are 1) Root2) Neck3) Crown

Tooth is composed of three substances. They are 1)Dentine 2)Enamel 3)Cementum

Crowns of Incisor teeth are chisel shaped. Crowns of canine teeth are large and conical.

Crowns of premolar teeth are bicuspid and almost circular. Crowns of Molar teeth are broad and tetra or penta cuspid.

Salivary glands: There are three pairs of salivary glands in the mouth. They are

1)Parotid 2)Submandibular and 3) Sublingual glands.

1) Parotid glands: They are the largest salivary glands and present below the ears. Each gland opens on inner side of cheek opposite to the second upper molar teeth through its duct. Ducts of the parotid glands are called as Stenson's ducts.

2) Submandibular glands:They are also called as Submaxillary glands and are smaller than parotid glands. They lie on each side and lies under the angle of Jaw. Eachsubmandibular gland has a duct called wharton's gland. They open near the midline under the tongue.

3) Sublingual glands:They are the smallest salivary glands. They lie under the tongue. They pour their secretions into the mouth through several openings.

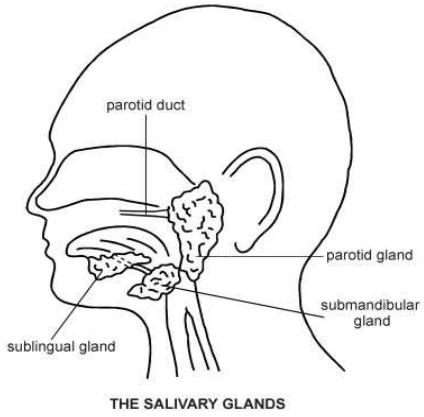


Fig 3: Salivary glands

Pharynx: Pharynx lies between mouth and oesophagus.It serves commonly forboth digestive and respiratory systems. It is divided into 3 parts.

1)Nasopharynx 2)Oropharynx and3)Laryngopharynx.

Oesophagus: It lies in both thoracic and abdominal cavities. Trachea and vertebral column lie in the front and back of oesophagus respectively.Histology of oesophagus shows similar structure as remainder of alimentary canal. It shows the following layers.

1) Mucosa 2)Submucous coat 3) Muscular coat

It is devoid of Serosa. Upper one third of oesophagus consists of smooth muscles. Lower one third contains smooth muscles and middle one third contains both types of muscles.

Stomach: Stomach is the most dilated part of digestive tract and is J shaped. It is situated between the end of the oesophagus and beginning of the small intestine. It lies below the diaphragm in the abdominal cavity.Its major part is to the left of the mid line.It distends when it is filled with food. Average capacity of stomach is 1.5 L in an adult.Stomach has two surfaces,two curvatures, two ends,three parts and two sphincters.They are as follows.

The two surfaces of stomach are-

1) Anterior surface 2) Posterior surface.

Two curvatures are- 1) Lesser curvature 2)Greater curvature.

Three parts of stomach are –

1) Fundus (upper portion) - above the cardiac sphincter

2) Body (middle portion) - between fundus and pylorus.

3) Pylorus (lower portion) - below incisura angularis.Pylorus is subdivided into pyloric antrumand pyloric canal.

Two ends of stomach are-

1) Cardiac end guarded by cardiac sphincter.

2) Pyloric end guarded by pyloric sphincter.

Two sphincters of stomach are-

1) Cardiac sphincter (at the beginning)

2) Pyloric sphincter (at the ending)

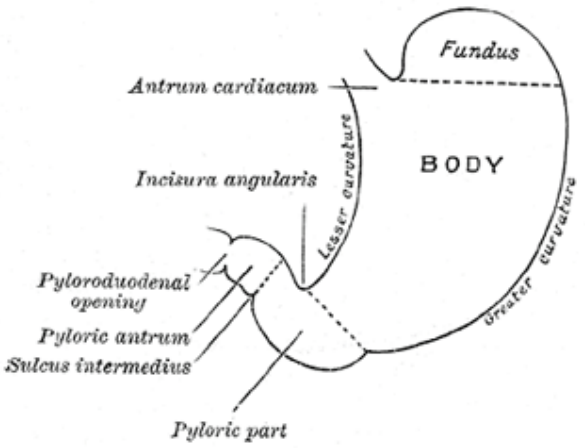


Figure 4: Parts of stomach

Histology of stomach- It has 4 layers

1) Mucousmembrane – innermost layer containing numerous folds (rugae).

2) Submucous layer

3) Muscular coat is made up of three layers consisting of longitudinal, circular and oblique smooth musclefibres.

4) Serous coat, which is the visceral layer of peritoneum.

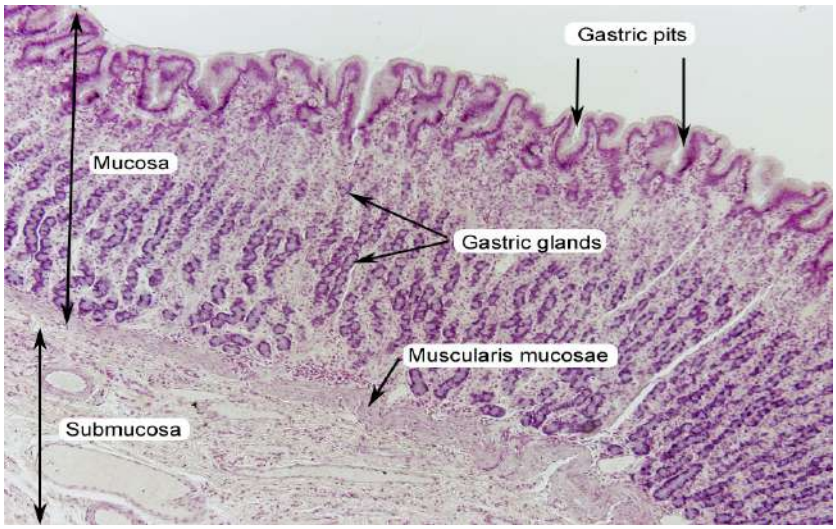


Fig5: Histology of stomach

Folds connected to the stomach are called as omenta. It is divided into,

1) Greater omentum - hangs from lower border of stomach to the front surface of small intestine.

2) Lesser omentum - extends from lower border of liver to the lesser curvature of stomach.

Small intestine:

It is a coiled tubular structure about 6 metres long. It extends from pyloricsphincter to its junction with large intestine at the ileocaecal valve.It is divided into three parts. They are- 1) Duodenum 2) Jejunum 3) Ileum

Histology of small intestine:It has 4 layers.

1) Mucousmembranne(Muscularis interna/Muscularis mucosa) – It is the innermost layer of the small intestine and contains goblet cells and villi. They produce mucus. It contains circular folds. Unlike the rugae of the stomach, they are permenant. They enhance the surface area available for absorption. The intestinal glands secrete intestinal juice.

2) Submucouscoat containing blood vessels, lymph vessels and nerves. It contains Brunner glands in duodenum.

3) Muscular coat with a thin external layer of longitudinal fibres and a thick internal layer of circular fibres.

4) Serosa- It is an outer peritoneal layer

Duodenum: It is the first part of smallintestine and is C shaped. Duct from gall bladder, bile duct and prancreatic duct open into the second part of duodenum through the hepato-panereatic ampulla.

Jejunum: This is the midsection of the small intestine, connecting the duodenum to the ileum. It is about 2.5 m long, and contains the circular folds, and villi that increase its surface area. Products of digestion (sugars, amino acids, and fatty acids) are absorbed into the bloodstream here.

Ileum:It is the distal three fifth of long and coiled part small intestine. It extends between Jejunum and caecum (beginning of large intestine). There are number of minute lymphoid structures (peyer's patches) in ileum. Ileum has similar structure as Jejunum but more villi. Ileum also contains digestive glands. But they are less than in the jejunum.

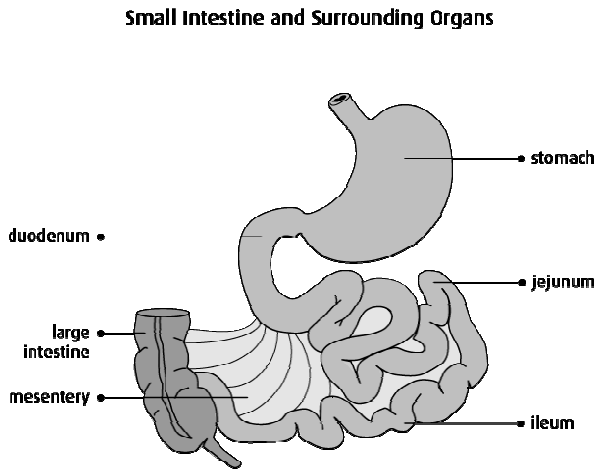


Fig 6 : Small intestine

Large intestine (colon):

Ileum of the small intestine merges into large intestine. Colonmeasures about1.5 metres in length.There is ileocaecalvalve at the junction of ileum and large intestine. Large intestine consists of following parts. They are,

1)Caecum: It is a short rounded sac and lies in the right iliac fossa. It begins at the ileoeacealvalve where ileum and caecum join. It is continuous with ascending colon.

2)Vermiform appendix: It is a vestigial organ and present about an inch from ileocaecal value. Lumen of the appendix communicates with that of caecum. It contains same four layers as intestine but the submucous layer contains lymphoid tissue.

3) Ascending colon: It ascends upwards from caecum and infront of right kidney. It turns to left below the liver. It merges with transverse colon.

4)Transverse colon:It lies transversely below the stomach. It is suspended from its own mesertery from the posterior abdominal wall. It extends to the left and merges with descending colon at the lower surface of spleen.

5)Descending colon: It is situated verticallyon the left side of abdomen. It extends from transverse colon and merges with sigmoid colon.

6)Sigmoid colon: It lies in the pelvis. Hence it is also called as pelvic colon. It is situated at the left. It forms loops. It has a mesentery of its own. It continues below with rectum.

Histology of large intestine:Large intestine has the same structure as small intestine. The difference is, longitudinal muscles are arranged in three bands. Mucous membrane does not contain villi.

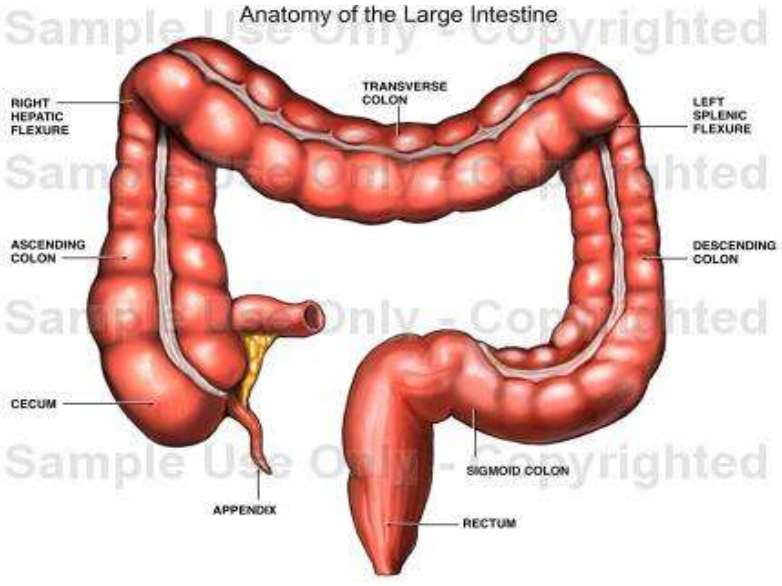


Fig 8: Parts of large intestine

Rectum: It is a straight tube lying in lower posterior part of pelvic region. It is12 cm long extends from sigmoid colon to analcanal. It is situated behind urinary bladder, prostate and seminal vesicles in male and behind uterus and vagina in females. Mucous coat of rectum has longitudinal and transverse folds. Lower portion of rectum is called as rectal ampulla.

Anus:Rectum ends in anus. It is about 1 inch long. It is a small canal guarded by two sphincters. Internal sphincter is involuntary and external sphincter is voluntary.

Mesentery:

Mesentery is the fold of peritoneum which attaches different parts of small intestine to the posterior abdominal will. Blood vessels, nerves and lymphatics enter the intestines through mesentery.

Peritoneum:

Peritoneum is a serous membrane. In males it is a closed sac lining the abdomen. In females, free ends of uterine tubes open into peritoneal cavity. It consists of two layers. They are

1) Parietal layer lining the walls of abdominal cavity.

2) Visceral layer covering the abdominal organs.

Peritonial cavity: It is the space between parietal and visceral layers of perital layers.

Pouch of Douglas: Sac of peritoneum between rectum and uterus is called as pouch of Douglas.

Regions of abdomen: Abdomen is divided into 9 regions.

1) Right hypchondrium
2) Epigastrium
3) Left hypochondrium
4) Right lumbar region
5) Umbilical region
6) Left lumbar region
7) Right iliac fossa.
8) Hypogastrium
9) Left iliac fossa.

Accessory organs of the digestive system: They are teeth, salivary glands, liver, biliary system and pancreas,Teethandsalivaryglandsarecoveredundermouth.

Liver: Liver is the largest organ in the abdomen and is the largest gland in the body. It is situated in the upper right part of abdominal cavity. It lies below the diaphragm under the cover of lower ribs. Falciform ligament divides it into two lobes. They are-

1) Right lobe – It lies over the right colic flexure and right kidney. It is bigger than left lobe.

2) Left lobe - lies over stomach

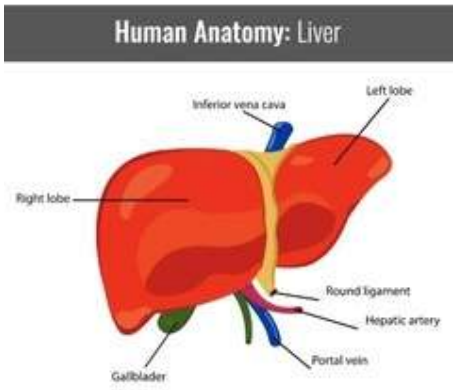


Fig 9: Lobes of liver

Histology of liver:Liver consists of large number of hepatic lobules. They are hexagonal in shape. Each lobule has a small central intra lobular vein, which is a branch of a hepaticvein.Lobules consist of liver cells which are large polygonal cells with abundant eosinophilic cytoplasm. These cells radiate from central vein. Portal triads are present inbetween the lobules. It contains 1) Inter lobular vein 2)hepatic artery 3) bile duct.

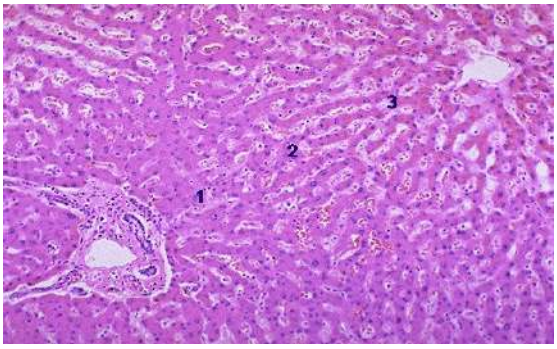


Fig 10: Histology of liver

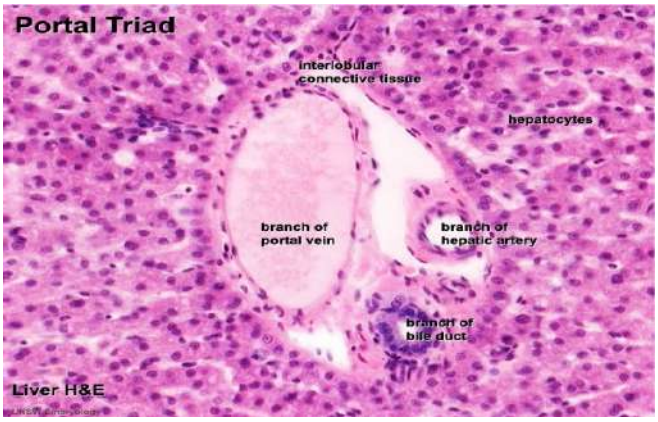


Fig 11: Portal triad

Blood supply of liver:Hepatic artery and portal vein carry blood to liver. Hepatic artery supplies oxygenated blood to liver. It is a branch of coeliac plexus. Portal vein brings blood to liver from stomach, spleen and intestines. It divides into inter lobular veins. They subdivide and finally form central veins.

Biliary system: Biliary system consists of,

1) Common hepaticduct formed by the union of right and left hepatic ducts from liver.

2) Gall bladder

3) Cystic duct from gall bladder

4) Common bile duct formed by union of common hepatic duct and cystic duct.

Gall bladder: It is a pear shaped organ situated at the under surface of right lobe of liver.It has a duct called cystic duct.

It consists of three parts, 1) Fundus 2) Body 3) Neck

Histology of gall bladder: It has three layers.

1) Inner mucous coat.

2) Middle muscular coat

3) Outer serous coat.

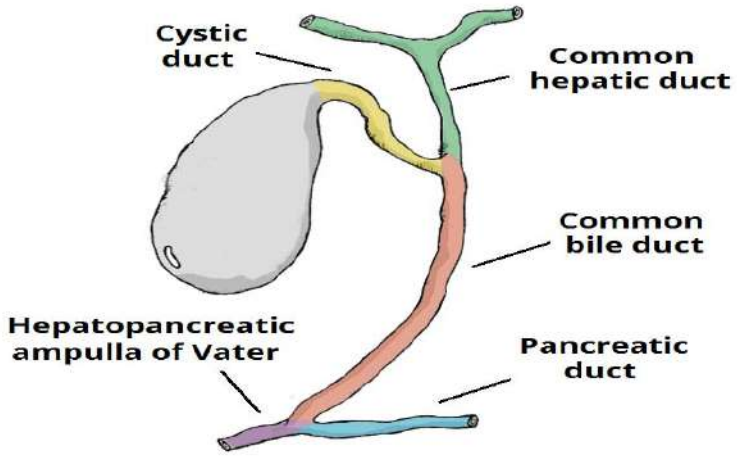


Figure 12: Biliary system

Pancreas: It is a soft greyish pink coloured gland. It is about 12 to 15 cm long. It lies transversely across the posterior abdominal wall behind the stomach.

Pancreas has three parts.1) Head 2) Body and3) Tail.

Head of the pancreas lies within the curve of duodenum. Tail extends as for as the spleen. Body lies between Head and tail. Pancreatic duct lies within the organ. Pancreatic duct joins the bile duct at the head of the pancreas and open together into duodenum at heptopancreatic ampulla.

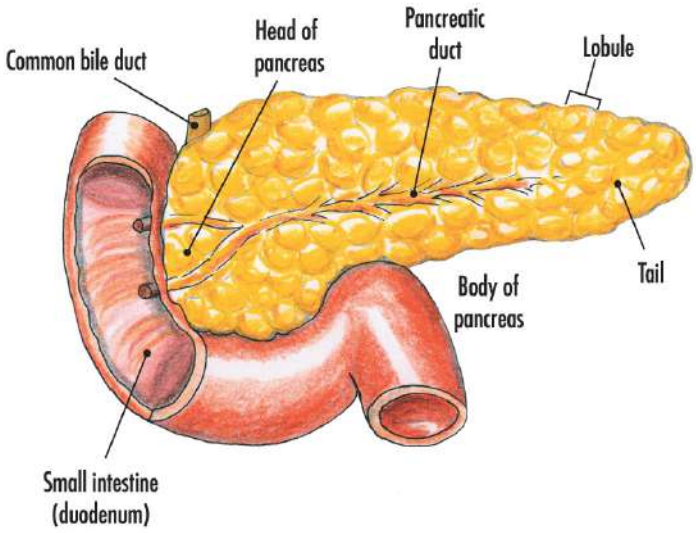


Figure 13: Parts of pancreas

Hitology of pancreas: Pancreas is composed of lobules. Each consists of tiny vessel. All these tiny vessels lead to the main duct and end in number of acini. Acini lined with cells secrete the enzymes trypsinogen, amylase and lipase. In between the acini, aggregates of cells called Islets of langerhans are present. Alpha cells constitute 25 percent of total number of Islets and beta cells constitute 75 percent of the total number of Islets.

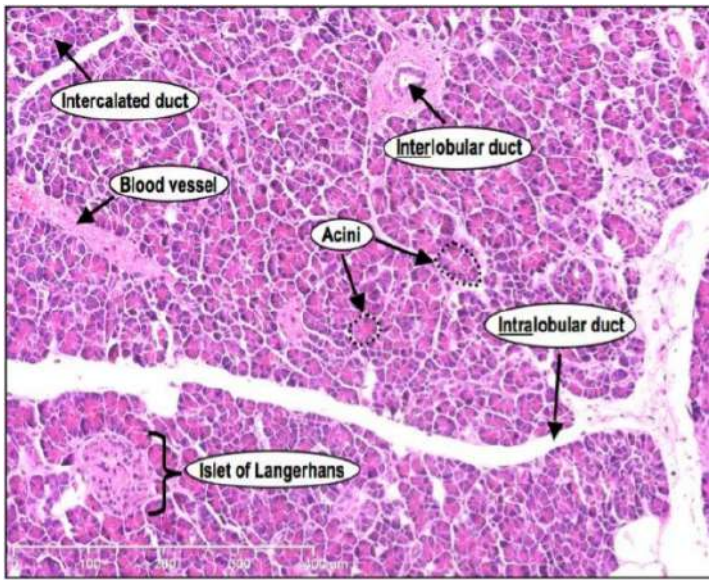


Fig 14: Hostology of pancreas

Summary

Digestive system consists of Gastrointestinal tract and various glands attached.They are mouth, pharynx, oesophagus, stomach, small intestine, large intestine, rectum and anus. These are concerned with functions like ingestion, deglutition, absorption and excretion. Accessory organs are teeth, salivary glands, liver and biliary system, pancreas etc.

Questions

Essay Questions

1) What are the various parts of digestive system? Describe anatomy of teeth

2) Describe the anatomy of pharynx and oesophagus. Draw the diagrams.

3) Write the anatomy of stomach. Draw the diagram and label.

4) What are different parts of small intestine? Desribe in detail the anatomy of each part with diagrams.

5) Write the anatomy of large intestine. Draw the digrams.

6) Desribe in detail about the anatomy and histology of liver.

7) What are the parts of pancreas? Describe the histology ofpancreas.

Short answer questions

1) List the main parts of Digestive system.

2) Mention the names of accessory organs of diagestive system.

3) What are the parts of a tooth?

4) Mention the types of permanant teeth.

5) What are the parts of a tooth?

6) Write the names of the salivary glands.

7) Name the ducts of a)parotid glands b)Submandibular glands.

8) What are different parts of pharynx?

9) What are the Histological layers of oesophagus?

10) Mention various parts of stomach.

11) Name the cuvatures of stomach.

12) List the layers of stomach.

13) Name the parts of small intestine.

14) What are the Histologiacl layers of small intestine?

15) Write about vermiform appendix.

16) What are the lobes of liver?

17) What is a portal triad and write the components of portal traid

18) Name the surfaces of liver.

19) Write the parts of Biliary system?

20) Where is Gall bladder situated? What are its parts?

21) Mention the parts of pancreas.

22) What are islets of langerhans?

UNIT – 5

CARDIO VASCULAR SYSTEM

Cardiovascular system consists of Heart and Vascular system. It is well organised blood transport system of body. Heart is the central pumping organ. Blood vessels constituting vascular system are arteries, arterioles, capillaries, venules and veins.

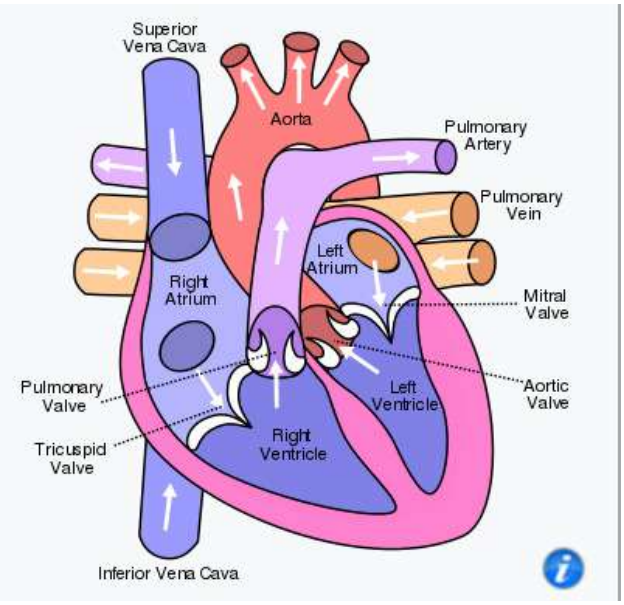
Anatomy of Heart

Heart lies on the left upper part of thoracic cavity and lies between the two lungs.

Chambers of Heart : Heart has four chambers. Two of them are upper chamberscalled atria or auricles. Lower two chambers are called ventricles.

The two atria are separated by interatrial septum.

The two ventricles are separated by interventricular septum.



Atria are filling chambers and ventricles are pumping chambers. Compared to artia, ventricles are thicker since they are pumping chambers. Of the two ventricles, wall of left ventricle is

three times thicker than that of right ventricle since left ventricle pumps oxygenated blood to all parts of body and right ventricle pumps deoxygenated blood to lungs only.

Valves of Heart :

i) Tricuspid valve: Opening between right artium and right ventricle is guarded by tricuspid valve. It prevents back entry of blood into right atrium from right ventricle at the beginning of ventricular systole.

ii) Mitral valve: Opening between left atrium and left ventricle is guarded by the mitral valve. It is also known as the bicuspid valve. It has two flaps that lies between the left atrium and the left ventricle. It prevents back flow of blood into left atrium.

The mitral valve along with the tricuspid valve are known collectively as the atrioventricular valves because they lie between the atria and the ventricles of the heart.

iii) Pulmonary valve: Pulmonary artery is guarded by tricuspid semilunar valve which prevents back flow into right ventricle. It carries deoxygenated blood from the right side of the heart to the lungs.

iv)Aortic valve: Aorta has tricuspid semilunar valve which prevents back flow of blood into left ventricle at the beginning of ventricular diastole.

Chordae tendinae and papillary muscles: Papillary muscles arise from ventricular walls. Chordaetendinae attach apical end of valves and papillary muscles. They prevent over distension of valves during diastole.

Histology of Heart: Heart consists of three layers.

1) Pericardium - outermost layer consisting of a) Visceral pericardium b) Parietal pericardium.

2) Myocardium - Middle layer made of cardiac muscle cells and interstitial cells.

3) Endocardium - Innermost layer.

Pericardium forms bag like structure between visceral and parietal layers containing pericardial fluid.

Blood vessels attached to heart:

1) Superior and inferior vena cava– They carry deoxygenated blood from parts of body to right atrium.

2) Pulmonary artery carries venous blood to lungs from right ventricle.

3) Pulmonary veins carries oxygenated blood from lungs to the left atrium of heart.

4) Aorta carries oxygenated blood to all parts of body from left ventricle of heart.

Blood vessels supplying oxygenated blood to heart: Right and left coronary arteries arising from Aorta supply oxygenated blood to heart.

Blood vessels draining heart: Coronary veins bring deoxygenated blood of heart into coronary sinus, which opens directly into right atrium.

Nerve supply to heart : Sympathetic and vagus nerves supply heart.

Conducting system of heart: System of conducting impulses of cardiac contraction consist of

1) Sinoatrial node (SA node): It is present at the opening of superior venacava into right atrium. It is called pacemaker of heart.

2) Atrioventricular node (AV node) :It is present in the right atrium at the posterior part of inter atrial septum. It is close to the opening of coronary sinus.

3) Bundle ofHis: Main trunk of bundle of His is continuous with AV node. Itpasses through interventricular septum. It is about 20 mm long.

4) Right and left branches of bundle of His :Bundle of His divides into right and left branches. Right branch is longer than left branch. Left branch bifurcates into superior and inferior divisions.

5) Purkinje fibres: They arise from branches of bundles of His. They spread from interventricular septum directly to papillary muscle and ultimately end in sub endocardial network.

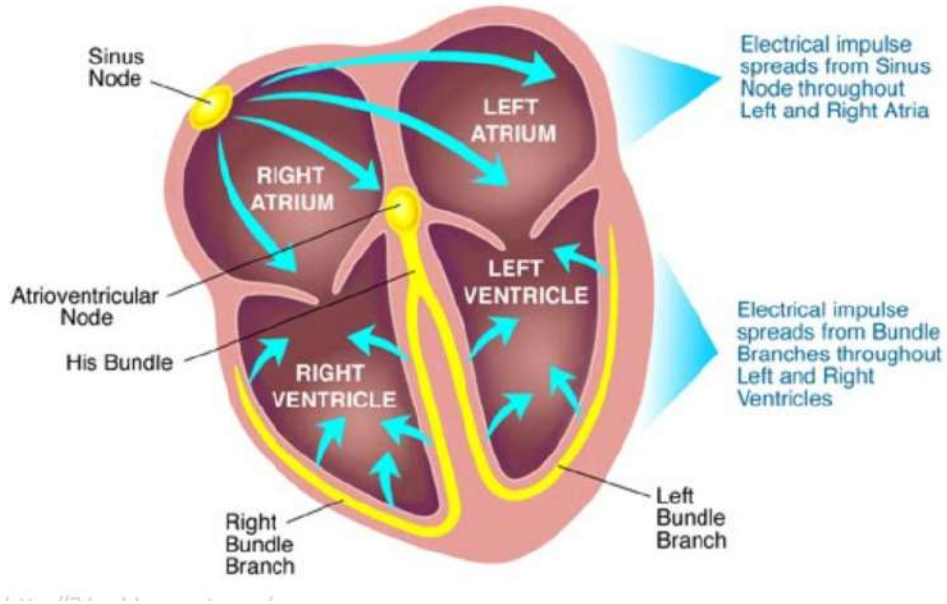


Fig: Nodes of conducting system of heart

BLOOD VESSELS:

Histology of Arteries and Veins:Arteries and veins consist of three layers.

1) Tunica externa - outer layer made of fibrous tissue and elastic tissue. It is also called as tunica adventitia.

2) Tunica media - middle layer of plain muscles and network of elastic fibres.Tunica media in arteries is thicker than in veins.

3) Tunica interna– innermost layer made of endothelial cells and also called tunica intima.

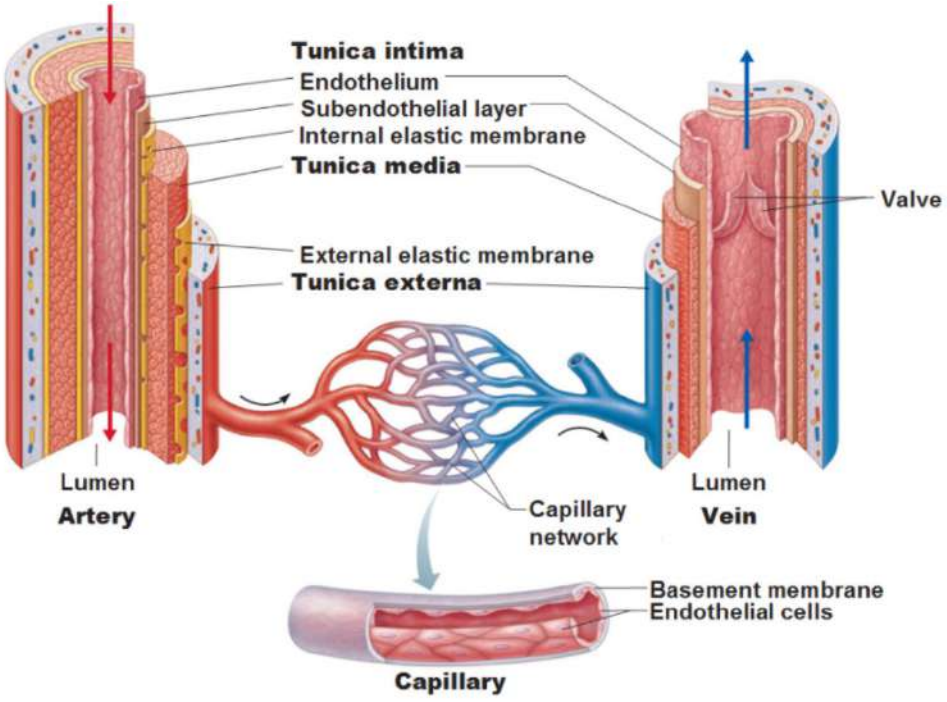


Fig : Layers of blood vessel

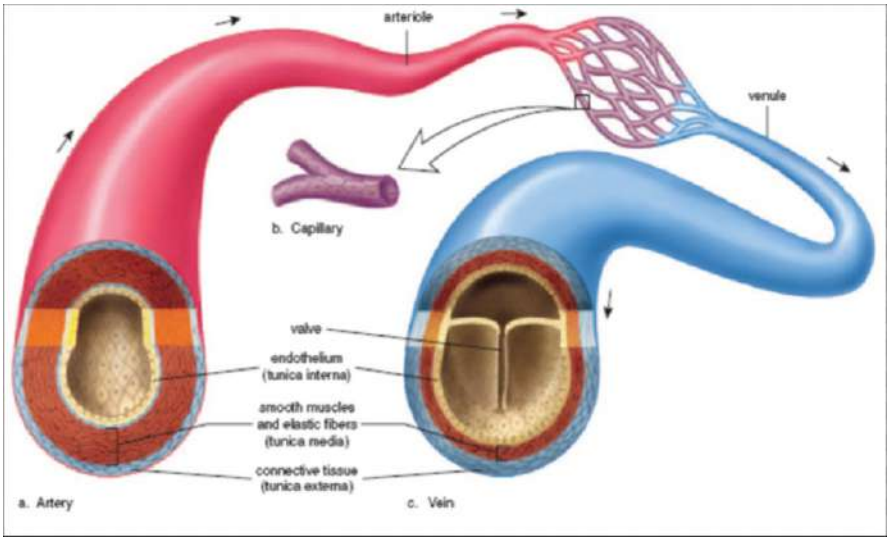


Fig: T.S. of artery and vein

Arteries of the body:

Aorta: It arises from left ventricle of heart is the main artery of body.

It consists of three parts.They are,

1) Ascending aorta:It gives off two branches i) Right coronary artery ii) Left coronary artery. Coronary arteries supply blood to heart.

2) Arch of aorta: It gives off three branches and supplying blood to head, neck and upper limb. The branches of arch of aorta are,

i) Innominate artery –It divides intocommon carotid arteryright subclavian artery

3) Descending aorta–It is divided into,

i) Thoracic aorta –It supplies blood to wall of chest cavity and viscera.

ii) Abdonminal aorta – It supplies wall of abdominal cavity and its viscera. The Branches of abdominal aorta:

i) Coeliac plexus

ii) Mesenteric arteries

iii) Renal arteries

iv) Final branches

i) Coeliac plexus divides into,

a) Hepatic artery - supplies liver

b) Gastric artery - supplies stomach

c) Splenic artery - supplies spleen.

ii) Mesenteric arteries arei) Superior mesenteric arteryii) Inferior mesenteric artery

iii) Renal arteries supply kidney. Final branches are,i) Right common iliac artery ii) Left common iliac artery.

These common iliac arteries divide into,

i) Internal iliac artery - Itsuplies pelvic organs. In females, its branch uterine artery supplies uterus.

ii) External iliac artery**-** Itcontinuousin thigh as femoral artery.Continuing in popliteal fossa as popliteal artery.

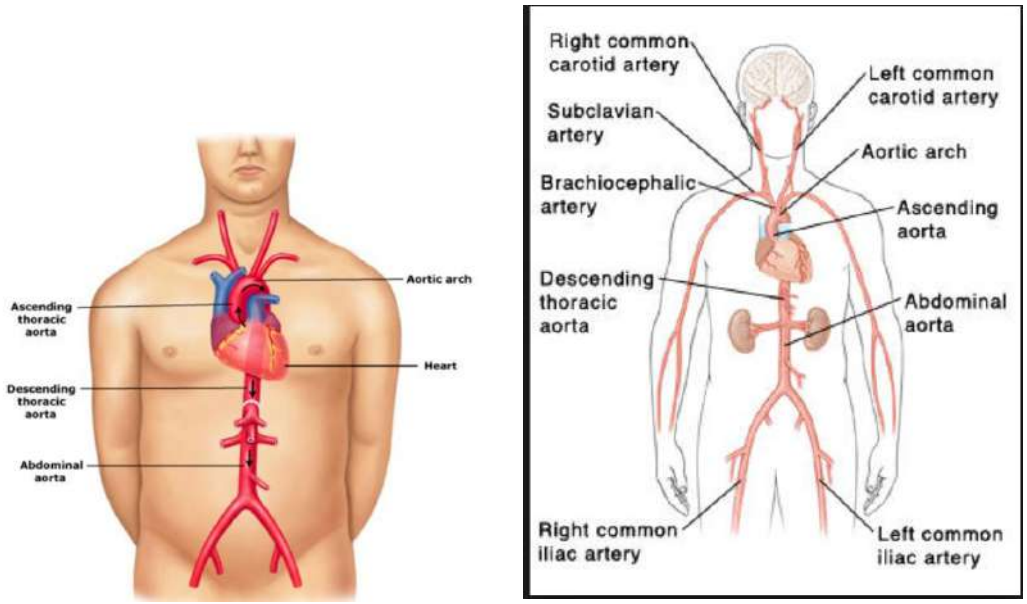


Fig:Arteries of the human body

Veins of the body

All the veins of the body join superior and inferior venacavae and drain the collected blood into right atrium of heart.

Superior venacava: Superior venacava is formed by union of right and left brachiocephalic veins collecting blood from head, neck, upper extremities and some part of thorax.

Inferior venacava: Inferior venacava is formed by union of two common iliac veins collecting blood from lower extremities and abdomen. It extends upwards through abdomen and thorax and opens into right atrium.

Veins of the head, neck and upper limbs:

Internal and external jugular veins drain head and neck.

Subclavian veins collect blood from upper limbs.

Radial veins collect blood from metacarpals. Ulnar vein collects from fingers through palmar arch.

Common iliac vein collects blood from lower extremities and abdomen. In abdominal region, renal veins from kidneys, gonadal veins from testes or ovaries, suprarenal veins from suprarenal glands, hepatic vein from liver, lumbar veins from abdominal wall and internal

iliac veins join inferior venacava. Internal iliac or hypogastric veins drain blood from gluteal muscles, medial side of thigh, urinary bladder, prostate gland, vas-deferens, uterus and vagina.

Types of circulation:

There are mainly two circulatory networks in the body. They are,

1) Systemic circulation

2) Pulmonary circulation

Systemic circulation: Oxygenated blood is circulated to all the parts of body from the left ventricle of heart through aorta. Deoxygenated blood of all parts of body reaches right artium of heart through superior and inferior venacavae. This is the major circulatory network of body and called systemiccirculation or greater circulation.

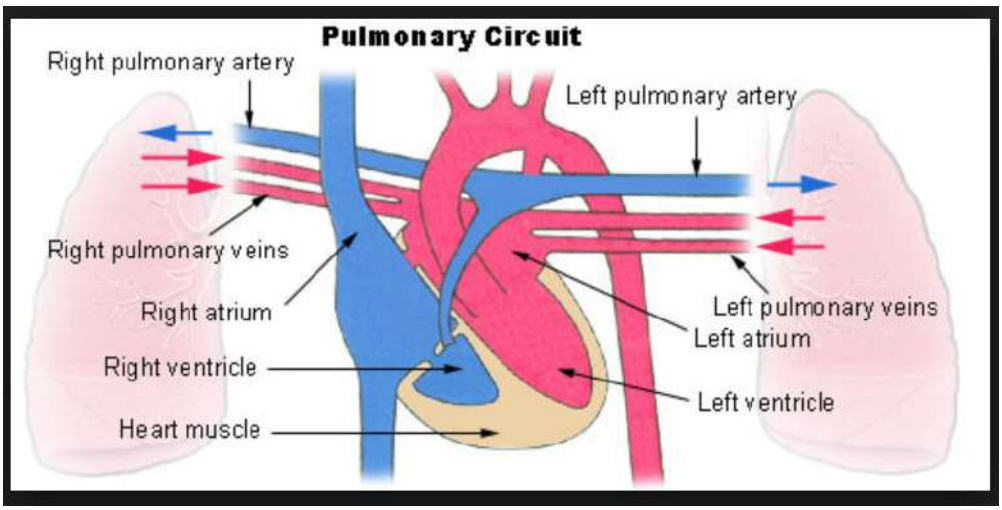


Fig. 5.4Pulmonary circulatory network

Pulmonary circulation : Deoxygenated blood reaching right atrium goes into right ventricle and from here, it reaches lungs through pulmonary artery. After losing CO2 in lungs, it gets oxygenated and reaches left atrium of heart through pulmonary artery.

Coronary circulation : It is the circulatory network, supplying oxygenated blood to heart itself and draining deoxygenated blood from it. Right and left coronary arteries arising from ascending aorta supply oxygenated blood to heart. Coronary veins collecting deoxygenated blood from heart join coronary sinus, which opens into inferior venacava.

Portal circulation : It is the circulatory network through liver. Portal vein and hepatic artery bring blood to liver. Portal vein carries blood into liver through superior mesenteric and splenic veins. Superior mesenteric vein carries blood from mesenteric bed (stomach, small intestine, part of large intestine and pancreas). Splenic vein carries from spleen. Hepatic artery carries oxygenated blood to liver. Capillaries of portal vein join with capillaries of hepatic artery. Hepatic vein carries blood circulated in liver to right atrium of heart through inferior venacava. This circulatory network of liver is called portal circulation.

Summary

Cardio vascular system consists of heart and vascular system. Heart contains four chambers. Upper two chambers are called atria or auricles and lower two chambers are called ventricles. Histology of heart shows pericardium, myocardium and endocardium. Conducting system of heart consists of S.A. node, A.V. node, bundle of His, branches of bundle of His and purkinje fibres. Vascular system consists of arteries, arterioles, capillaries, venules and veins. Arteries and veins contain three layers- tunica externa, tunica media and tunica interna. Aorta is the main artery of the body. Superior and inferior venacavae are the main veins of body. Veins of superior parts of body, upper parts of limbs and some parts of thorax join superior venacava. Veins of abdomen and lower limbs join inferior venacava. Different circulatory networks of body are - systemic circulation, pulmonary circulation, coronary circulation, portal circulation etc.

Questions

Essay Questions

1) Discussthe anatomy of heart. Draw the diagram.

2) Write about the histology of arteries and veins. Discuss arteries of head and neck.

3) Discuss Veins of body.

4)Discuss the types of circulation of body.

Short Answer Questions

1) Name the chambers of Heart.

2) What are the layers of heart?

3) Name the valves of heart.

4) What are the blood vessels that supply heart?

5) Write briefly about the conducting system of heart.

6) Write briefly about S.A. node.

7) Describe A.V. node.

8) What is bundle of His?

9) What are the parts of Aorta?

10) Write the branches of arch of aorta.

11) What are the branches of ascending aorta?

12) Name the divisions of descending aorta.

13) Name the arteries of upper limbs.

14) Mention the branches of abdominal aorta.

15) Write the divisions of coeliac plexus.

16) What are mesenteric arteries?

17) What is plantar arch?

18) What are main veins of body?

19) Name the veins of head and neck.

20) Write the veins of lower limbs.

21) Mention abdominal veins.

22) What is systemic circulation?

23) Write about pulmonary circulation.

24) Explain portal circulation.

25) Write briefly on coronary circulation.

UNIT – 6

LYMPHATIC SYSTEM

The lymphatic system is part of the circulatory system. It is an important part of the immune system, comprising a network of lymphatic vessels that carry a clear fluid called lymph directionally towards the heart

Lymphatic system is a closed system consisting of 1) Lymphatic capillaries2)Lymphatic vessels 3) Lymph nodes and 4) Lymphatic ducts.

1) Lymphatic capillaries:They are fine hair like vessels with porous walls.They arise in the tissue spaces. They unite to form lymphatic vessels. Walls of the capillaries have permeability to substances of greater molecular size than the substances permeable through walls of blood capillaries. Their walls are formed by endothelial cells and supported by fibrous connective tissues.

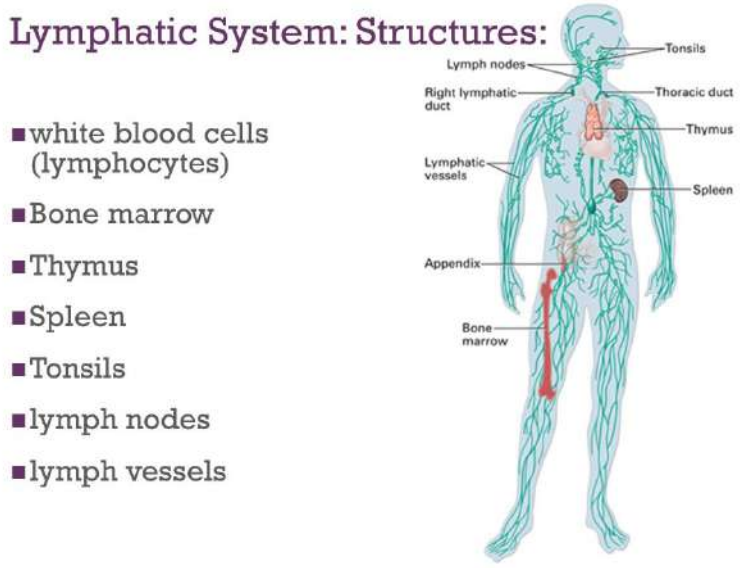


Fig. 6.1 Lymphatic system of human body

2) Lymphatic vessels: Lymphatic capillaries unite to form lymph vessels.

They have one sided valves. They are superficially and deeply located. They are found in skin, muscles and several visceral organs. Lymph vessels pass through lymph nodes. Finally lymph collected from the body pours into right lymphatic duct and left lymphatic duct. Left lymphatic duct is also called as thoracic duct.

3) Lymph nodes: Lymph nodes are small bodies made of lymphatic tissue. They are located both superficially and deeply. Lymphatic vessels bring lymph to lymph nodes.They divide within the node and discharge lymph.Lymphatic vessels entering into the lymph node are called afferent lymph vessels. Lymph vessels leaving the lymph nodes are called as efferent lymph vessels.

Naming of lymph nodes: Lymph nodes are named accordingly as they are located.They are,

a) Cubital and axillary lymph nodes -They are situated in arms.

b) Poplietal and inguinal - Lymph nodes situated in legs are named so.

c) Cervical lymph nodes- Lymph nodes present in the neck.

d) Mediastinal lymph nodes: These are present in Thorax.

e) Abdominal lymph nodes: They are present in abdomen. eg: Mesenteric lymphnodes.

f) Pelvic lymph nodes: Pelvic lymph nodes are present in pelvic organs.

4) Lymph ducts:

i) Right lymphatic duct- Efferent lymph vessels leaving lymph nodes pour lymph into right lymphatic duct and left lymphatic duct (thoracic duct). Right lymphatic duct is comparatively smaller. It is formed by joining of lymphatic vessels from right side of head, thorax and right upper limb at the root of neck.

ii)Thoracic duct – It is left lymphatic duct. It begins at cisternachyli.

iii) Cisternachyli is a small pouch at the back of the abdomen. Lymphatic vessels from lower limbs, abdominal and pelvic organs empty into cysternachyli.

iv) Thoracic duct finally empties into left subclavian vein at its junction with left internal jugular vein. It is provided with unidirectional valves to prevent lymph from flowing in wrong direction.

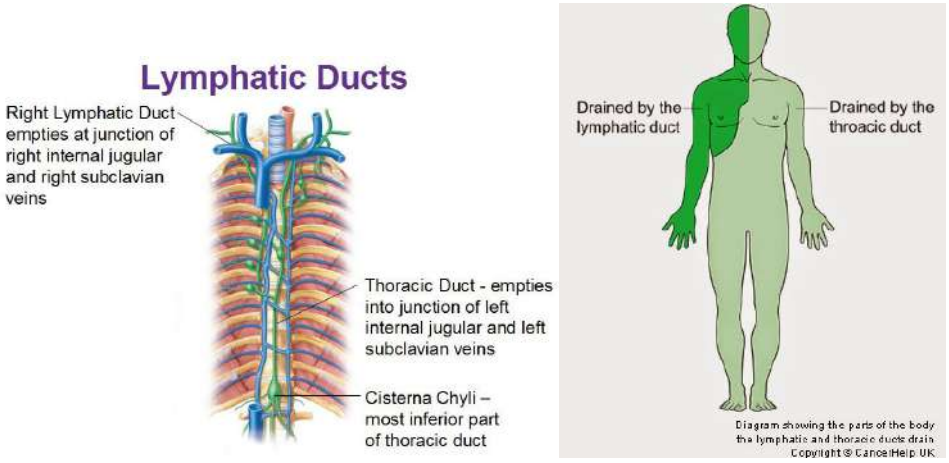


Fig. 6.2 Lymphatic Ducts and Cisterna chyli

Histology of Lymph node: Histology of lymph node shows three parts. They are cortex,medullaandhilum.

1) Cortex: Cortex is the outer part of lymph node. It contains lymphatic nodules peripherally and germinal centres in the inner zone. Germinal centres present in the lymph nodes produce lymphocytes. Lymph sinuses separate lymph nodules from capsule.

ii) Medulla : It is the inner part of lymph node. It is devoid of lymph nodules.It contains reticulo endothelial cells.

iii) Hilum : It is the depression at one side of lymphnode or lymph gland.Through

Hilum, an artery enters and there is exit to a vein and an efferent lymphatic vessel. Afferent lymph vessels enter from all sides but efferent lymph vessel leaves through hilum. Chief efferent vessel leaving lymph node carries filtered and lymphocyte enriched lymph fluid

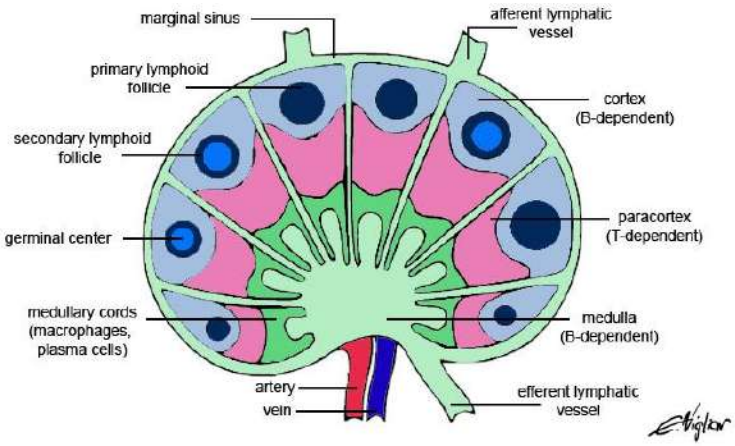


Fig :Normal histology of Lymphnode

Other lymphatic organs: They are spllen, tonsis and thymus

Spleen: Spleen is the largest lymphoid tissue in the body. It is highly vascular organ. It is located in the left hypochondrium beneath the diaphragm. It weighs about 150 g. in adult human being and does not contain afferent lymphatic vessels.It is haemopoieticconnective tissue.

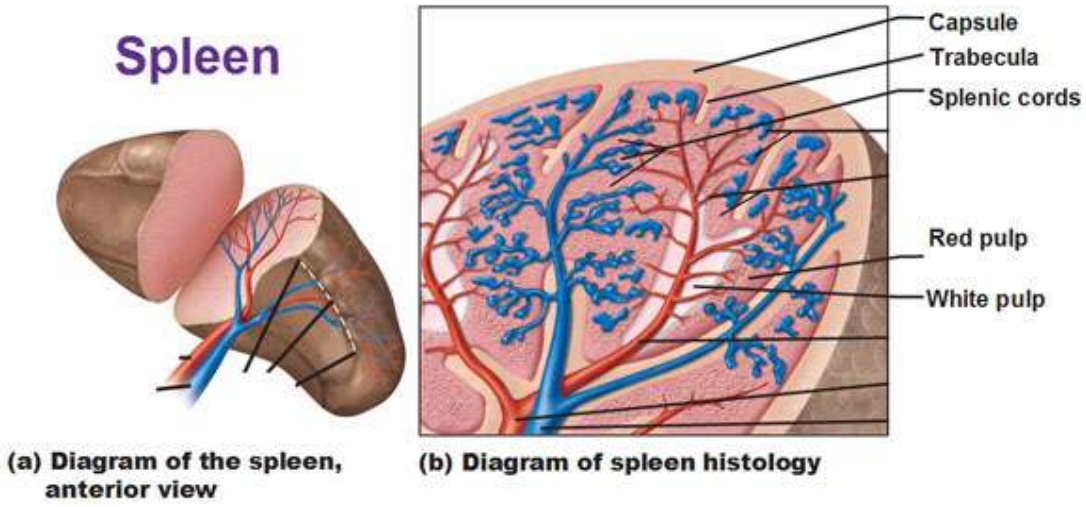


Fig: T.S. of Spleen

Histology of spleen:Histology of spleen shows,

1) Capsules 2) Trabeculae3) Hilum 4) White pulp5) Red pulp 6) Reticular mesh work

7) Blood vessels.

Splenic pulp : Splenic pulp is the parenchymal tissue within the capsule. It is two types 1) White pulp 2) Red pulp.

White pulp : It contains mostly lymphocytes.

Red Pulp : It containssinusoids the blood filled areas.It is concerned with disposing of worn out RBCs and blood borne pathogens.

Splenic sinuses: Splenic sinuses are long vascular channels.

Splenic cords: They are continuous partitions in between splenic sinuses.

3. Tonsils : Tonsils are well-defined organs of accumulated lymphoid tissue inthe mucous membrane at the root of tongue. Tonsils are present at the surrounding of pharynx, where nasal and oral passages unite. Tonsils do not possess afferent lymphatic vessels.

Tonsils can be divided into three groups.

1) Palantine tonsils -covered by stratified squamous epithelium

2) Lingual tonsil- situated at the root of tongue.

3) Pharyngeal tonsils - one on each side in the median posterior wall of nasopharynx.

4.Thymus: Thymus is partly endocrine gland and partly lymphoid structure. It is present in anterior and superior mediastinum of thorax. It extends from pericardium up into neck. It consists of two lobes.

Histology of thymus shows -

1) Capsule 2) Cortex 3) Medulla

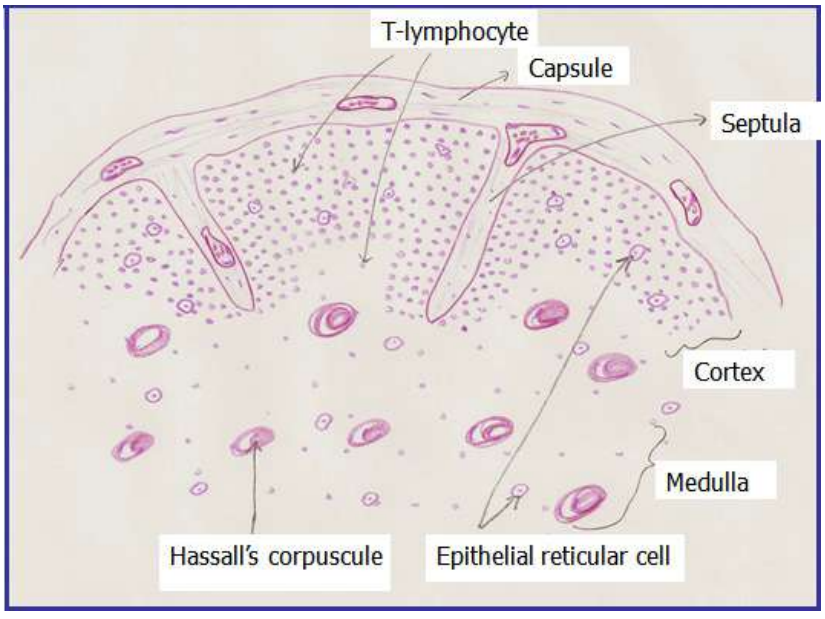


Fig : Histology of Thymus

SUMMARY

Lymphatic system is a closed system consisting of lymphatic capillaries, lymphatic vessels, lymph nodes and lymph ducts. Spleen, tonsils and thymus are also lymphatic tissues. Spleen is haemopoietic organ. Splenic pulp is parenchymal tissue within the capsule of spleen.

Essay questions

1) Write the anatomy of lymphatic system.
2) Write in detail about the anatomy and histology of spleen.

Short Answer Questions

1) Define lymphatic system.

2) Write briefly about lymphatic capillaries.

3) Name various types of lymphnodes.

4) What are a) Cubital and axillary lymph nodes b) Mediastinal lymph nodes.

5) Write about histology of lymph node.

6) Name the lymph ducts.

7) What is splenic pulp?

8) What are tonsils?

9) Write breifle about Thymus.

UNIT – 7

BONES AND JOINTS

Bones and Joints form the skeletal system of body. There are about 206 bones in human body.

Functions of skeletal system

1) Gives support and protection to soft tissues and vital organs.

2) Gives attachment to muscles and assists in body movements.

3) Formation of blood cells in the red bone marrow.

4) Storage of mineral salts like calcium and phosphorous.

Composition of bone :

Bone is structurally a complex organ and has 30% water and 70% Bone Matrix.

Structure of bone tissue : Refer to Cell and Tissues.

Functions of bone marrow :

Bone marrow performs functions of

1) Formation of blood cells(Haemopoeisis).

2) Destruction of old RBC with the help of Reticuloendothelial cells(haemolysis)

3) Protection of body against infections by microbes with the help of Reticuloendothelial cells (Defence mechanism)

Ossification : Ossification is the process of bone formation. Development of bones takes place from spindle shaped cells called osteoblasts.There are two types of ossification.

1) Intra membranous ossification.

2) Intra cartilaginous ossification.

1) Intra membranous ossification : It is the type of ossification in which, dense connective tissue is replaced by deposits of calcium and forms bone. Eg: Bones of skull are formed by this process.

2) Intra cartilaginous ossification: It is the type of ossification in which, cartilages are replaced by bone is called as intracartilaginous ossification. Most of the bones of the body are formed by this process.

Types of Bones: Bones are mainly three types.

1) Long bones.2)Short bones 3) Flat bones 4) Irregular bones 5) Seasmoid bones.

1) Long bones: Long bones are found in limbs. It has two ends called as epiphyses.These two ends are connected by shaft, which is called as diaphysis. The outer membrane covering the bone is called periosteum. It is followed by layer of compact bone. There is central medullary canal.It contains yellow bone marrow.Extremities consist of mass of spongy bone, which contains red bone marrow. Yellow bone marrow contains fat and blood cells but is not rich in blood supply or red blood cells. Arteries enter the bone through nutrient foramen

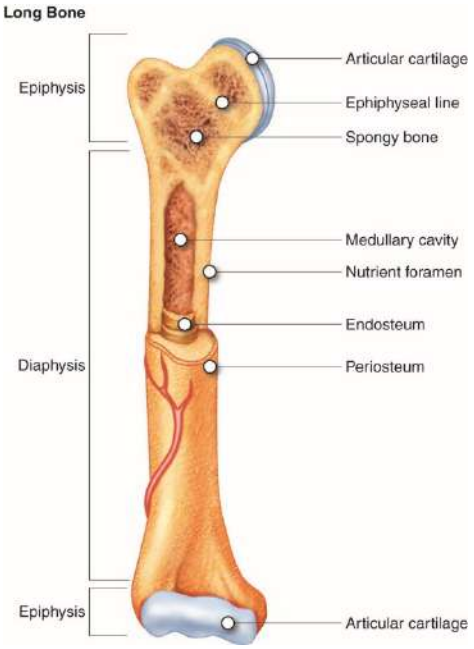


Fig. 7.1 Long bone

2)Short bones : They do not have shaft. They contain spongy substancecovered by shell of compact bone.eg: small bones of wrist and ankle.

3) Flat bones : They contain two layers of compact bone with spongy substance.

4) Irregular bones : Bones which do not fall into any category are irregular bones. ex: vertebrae and bones of face.

5) Seasmoid bones : They are small bones and develop in tendons of muscles. eg: Patella of knee joint.

Classification of bones:

Total 206 bones forming the human skeleton can be divided into

1) Bones of Axial skeleton:

i) Bones of skull: Bones of cranium and face.

ii) Bones of trunk:Sternum,Ribs,Vertebral column.

2)Bones of appendicular skeleton:

i)Bones of upper limbs

ii)Bones of lower limbs and pelvic girdle

Bones of Axial Skeleton:

I) Bones of skull

i) Bones of Cranium : Cranium is called as brain box. It is a large, hollow bony case. It is formed by fusion of various bones with zigzag edges. Cranium is formed by 8 bones.

1Frontal bone

2Parietal bone

2 Temporal bones

1 Occipital bone

1 Sphenoid bone

1 Ethmoid bone

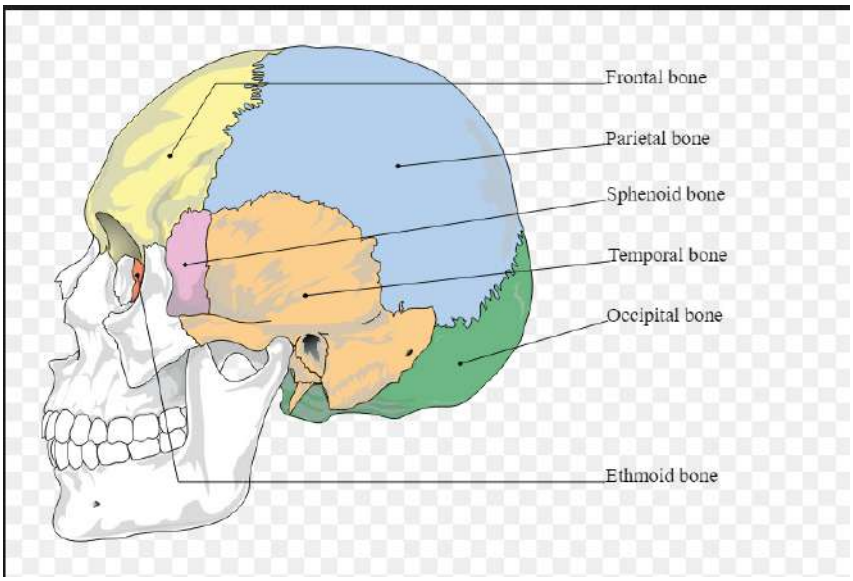


Fig 7.2 – Cranial Bones

Cranial Sutures : Immovable joints of bones of skull are called sutures. There are 3 sutures in cranium.

1. Coronal suture : It is the immovable joint between frontal bone and parietal bones
2. Sagital suture : It is the immovable joint between the two parietal bones.
3. Lambdoid suture : It is the immovable joint between occipital bone and parietal bones.

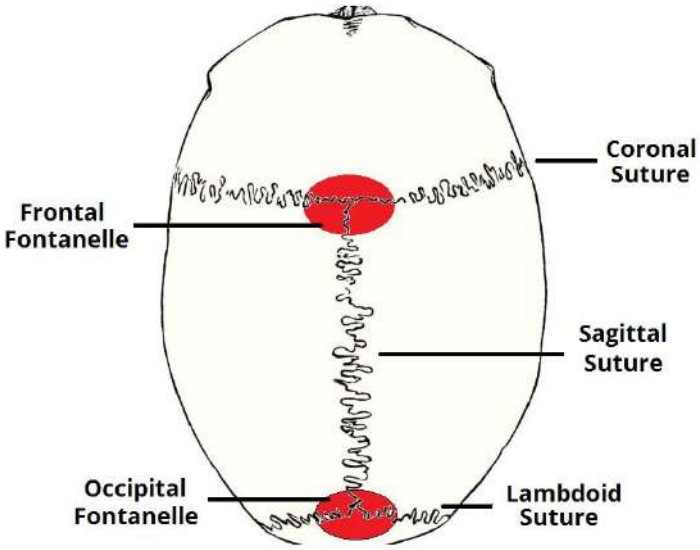


Fig 7.3 Cranial sutures .

ii) Bones of Face : Bones making the face are 14 in number.

i) Nasal bone

ii) Lacrimal bone

iii) Ethmoid bone

iv) Maxilla - 2 (upper jaw)

v) Mandible-1 (lower jaw)

vi) Mastoid process

vii) Zygomatic bones -2 (cheek bones)

viii) Palate bones -2 (roof of mouth cavity and hard palate)

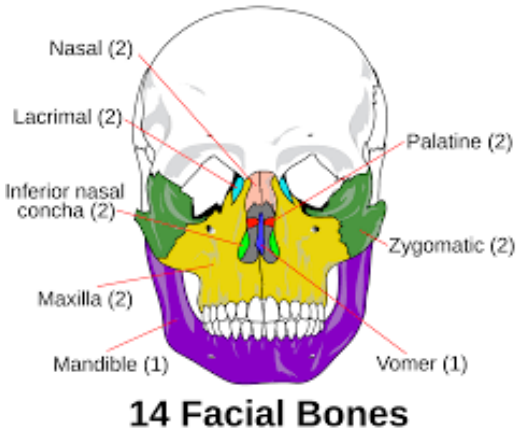


Fig 7.4 Facial bones

Sinuses : Sinuses are the cavities in the bones of skull and communicating with nose.

1) Frontal sinuses

2) Maxillary sinuses

3) Ethmoidal and

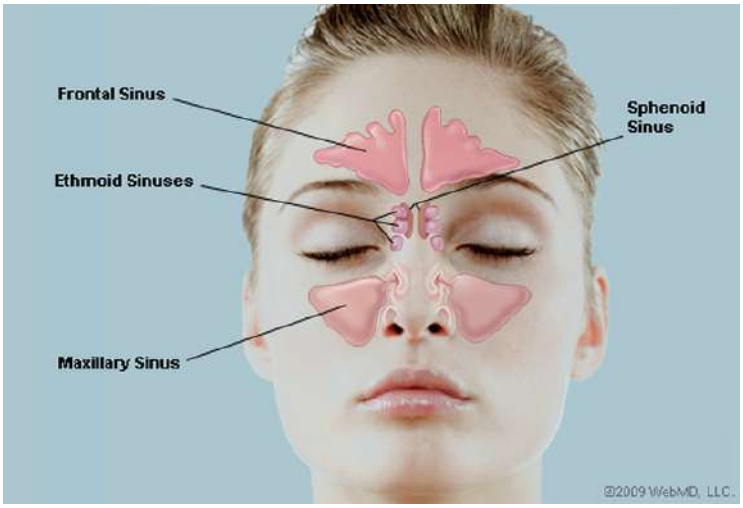
4) sphenoidal sinuses.

Functions of sinuses:

1) Lightening of bones of face and cranium.

2) Giving resonance to voice

Fig 7.5 Sinuses



Cranial fossae:

Base of the skull is divided into three fossae.

1) Anterior cranial fossa: It is is formed by horizontal plates of frontal bone..

2) Middle cranial fossa: It is formed by sphenoid bone and petrous portion of temporal bones

3) Posterior cranial fossa.: It is formed by occipital bone

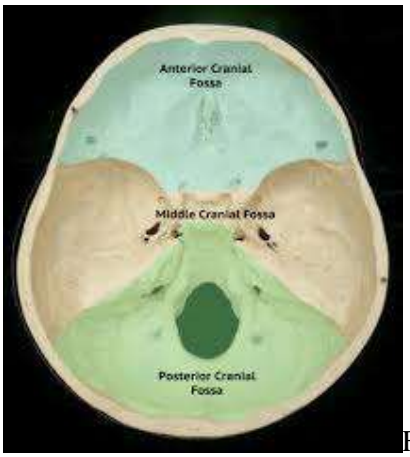


Fig. 7.6 Cranial fossa

II) Bones of trunk:

i) Sternum : It is a long flat bone. Itruns down the front of thorax. It is divided into three parts.

a) Manubrium sterni b) Body c) Xiphoid process.

ii) Ribs : They are 12 pairs of arched bones attached on back side to thoracic vertebrae.On the basis of attachment to sternum, they are classifiedas,

1) True ribs -attached to the sternum directly. First seven pairs are true ribs.

2) False ribs - attached to the sternum throughcostal cartilages.Remaining five pairs are false ribs. Of these, last two pairs are known as floating ribs.

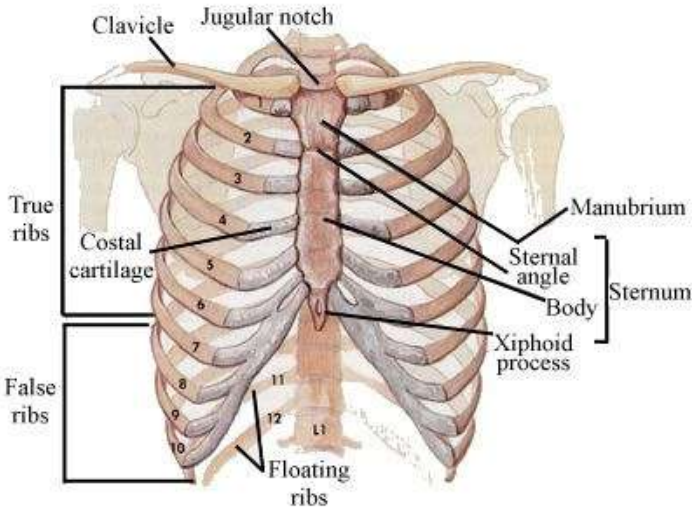


Fig 7.7 Rib Cage

iii) Vertebral column : Vertebral column made of a number of irregular bones called vertebrae. There are 33 vertebrae which are connected to one another.They are capable of limited movement. Main functions of vertebral column are,

1) Supporting spinal cord.

2) Protecting spinal cord.

3) Cushioning when jumping and landing on feet

Ligaments holding the vertebrae are,

1) Anterior and posterior ligaments

2) Ligamenta flava

3) Supraspinous ligaments

4) Intervertebral discs

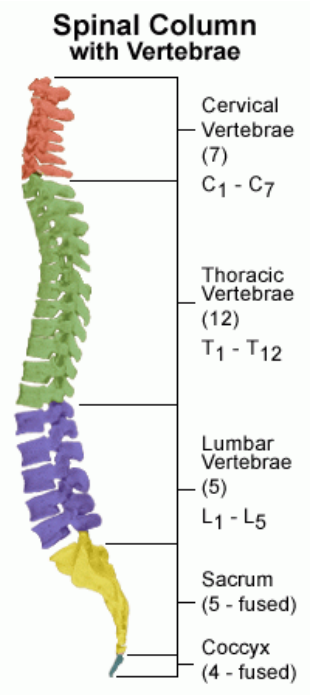


Fig. 7.8 Vertebral column

Structure of typical vertebra : Except atlas and axis, remaining vertebrae have common features. Each vertebra consists of the following parts.

a) Body - cylindrical in shape and lying to the front.

b) Vertebral arch (also called neural arch) - posterior part. It encloses vertebral foramen.

c) Vertebral foramen - Spinal cord passes through this foramen.

d) Spinous process - directed backwards and downwards.

e) Two transverse processes - projecting laterally for attachment of muscles and ligaments

f) 4 articular processes -They meet corresponding processes of adjoining vertebrae.

g) Laminae - wide parts of arch carrying spinous process.

h) Intervertebral discs - They are discs of fibrocartilage for connecting one vertebra to another. Each disc has outer ring of fibrous cartilage and inner core callednucleus pulposus.

Vertebrae are divided into five groups.

1) Cervical vertebrae- 7 in number forming the neck region.They are the smallest. First cervical vertebra iscalled atlas and the 2nd vertebra is called Axis

2) Thoracic vertebrae - 12 in number forming Thorax

3) Lumbar vertebrae - 5 in number forming lumbar region.

4) Sacral vertebrae - 5 in number forming sacrum.

5) Coccygeal vertebrae - 5 in number forming coccyx

III) Appendicular skeleton:

i) Bones of upper limb :

Upper limb consists of shoulder, upper arm, fore arm, wrist and fingers.

a) Bones of shoulder girdle - Scapula, clavicle on each side

b) Bones of upper arm-Humerus on each side.

c) Bones of fore arm -Radius and ulna on each side.

d) Bones of wrist : - 8 carpal bones arranged in two rows.

e)Bones of palm - metacarpals on each side.

f) Bones of fingers - phalanges

Functions of upper limb are locomotion, posture, giving stability to trunk.

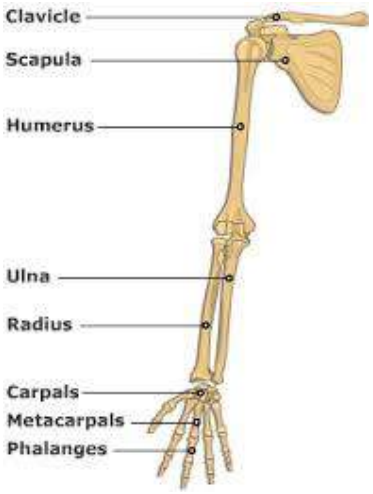


Fig 7.9 upper limb

ii) Bones of lower limbs and pelvic girdle :

Bones of pelvic girdle : Pelvic girdle forms link between trunk and lower limbs.

Differences between male and female pelvis :

Female pelvis is shorter , wider , shallower than male pelvis.

Pubic arch forms obtuse angle in females whereas it forms acute angle in males.

Bones of the lower limb:

i) Femur (thigh bone) : It is the longest and the strongest bone of the body

ii) Patella (knee cap)

iii)Tibia : It is inner bone of leg and is stronger than fibula

iv) Fibula : It is outer bone.It does not participate in weight bearing.

v) Tarsal bones(ankle bones)

vi) Metatarsal bones

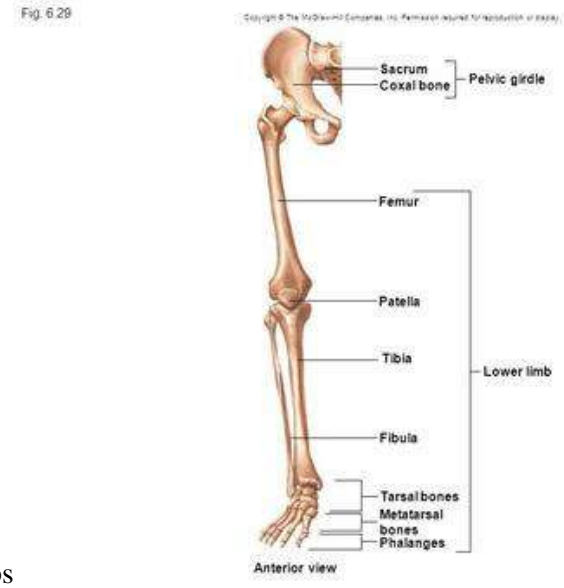


fig 7.10 lower limbs

JOINTS

Definition : Joint or articulation is a junction between two or more bones. Joint is completely surrounded by a fibrous capsule lined with synovial membrane.eg: Elbow joint

Classification of joints : Joints are classified depending on the degree of movement allowed. There are three types of joints.

1) Fibrous joints (Immovable)

2) Cartilaginous joints (Slightly movable)

3) Synovial joints (Freely movable).: It is the joint between the bones where bones are free.

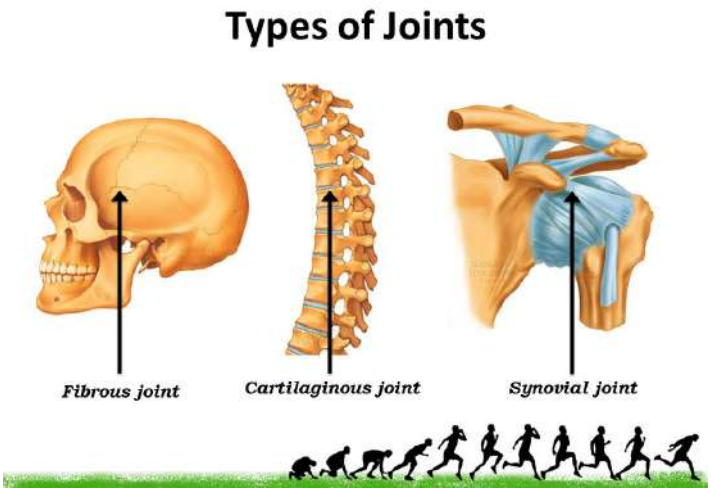


Fig 7.11 Types of joints

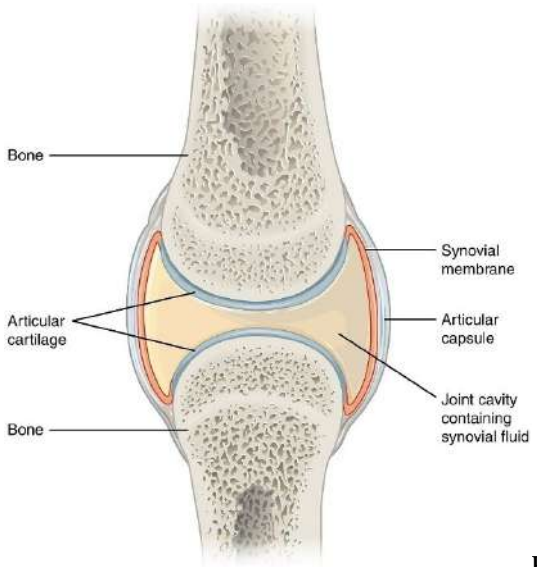


Fig 7.12 synovial jointS

Questions

Essay questions:

1) Classify bones of human skeleton and write about bones of cranium.

2) Classify bones. Write in detail about the bones of trunk.

3) Write note on bones of upper limb, lower limb and bones of wrist and hand.

4) Classify joints. What are different synovial joints?

Short Answer Questions

1) What are the functions of skeletal system?

2) Define ossification. What are the types?

3) Write the classification of bones?

4) Name the bones of skull.

5) Write the list of bones of the wrist.

6) Write the bones of upper limb.

7) Write the bones of lower limb.

8) Write the composition of bone.

9) What are sutures. What are the types?

12) What are fontanelles? Mention the types.

13) Mention the sinuses of skull.

14) What are the functions of sinuses.

15) Mention the bones of the face.

17) Name the bones of upper limb.

20) Mention bones of thorax.

21) What are different types of vertebrae?

22) Mention the number of 1) Thoracic vertebrae 2) Lumbar vertebrae

23) Write the names of pelvic girdle.

24) What are the features of pubis bone?

25) Write the parts of pelvis.

26) What are the differences between male and female pelvis.

27) Mention the bones of lower limb.

30) What are the bones of foot?

35) Mention the types of joints.

36) Write the characteristics of synovial joints.

UNIT – 8

NERVOUS SYSTEM

Nervous system controls and integrates the functions of human body. It consists of neurons, its fibres, dendrites and axons.

Nervous tissue: It is composed of 1) Neurons 2) Neuroglia

1) Neuron:

i) It is the structural and functional unit of nervous system.

ii) It is made of nerve cell body, dendrites and axons.

ii) Nerve cell body consists of large nucleus, neurofibrils and Nissl granules, which are present in the neuroplasm.

iii) Dendrites receive impulses and transmit them to the nerve cell body.

iv) Axons carry impulses away from the nerve cell body.

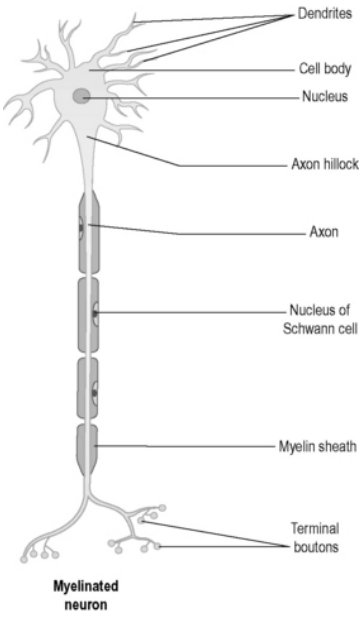


Fig 8.1 Neuron

Types of neurons: On the basis of number of processes, they are classified into,

a) Apolar neurons – Neurons having no processes (seen in fetal life)

b) Unipolar neurons – Neurons having only one process axon (seen in fetal life)

c) Bipolar neurons – Neurons having one axon at one pole and dendrite at the other pole. (Eg: Retina)

d) Pseudo unipolar neurons – Neurons, which are typically bipolar at first and spindle shaped, but later processes converge to meet at one side of the cell body. Eg: They are found in all spinal ganglia and ganglia of cranial nerves except 8th cranial nerve.

e) Multipolar neurons – Neurons having most varied form. (Eg: Cerabral cortex)

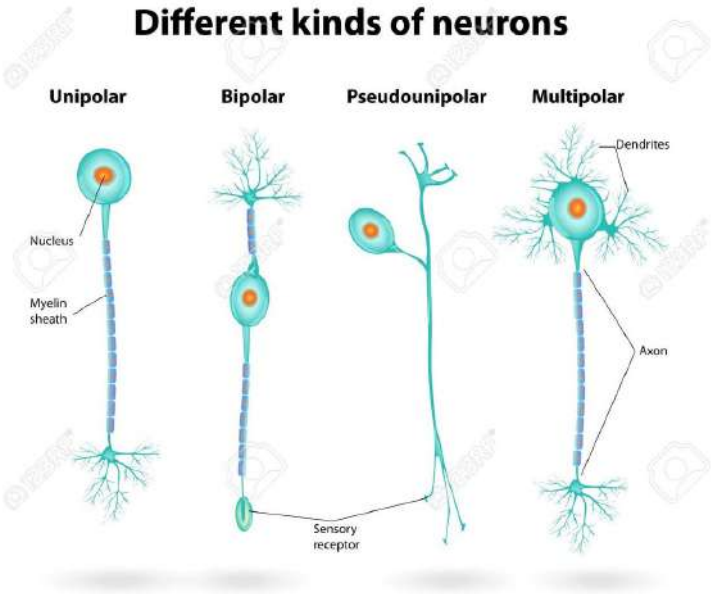


Fig 8.2 Different kinds of Neurons

Types of nerve fibres: Histologically there are two types of nerve fibres.

I) Myelinated nerve fibres - Axon is covered by myelin sheath except at the nodes of Ranvier.

2) Non myelinated nerve fibres - Axons are not covered by myelin sheath.

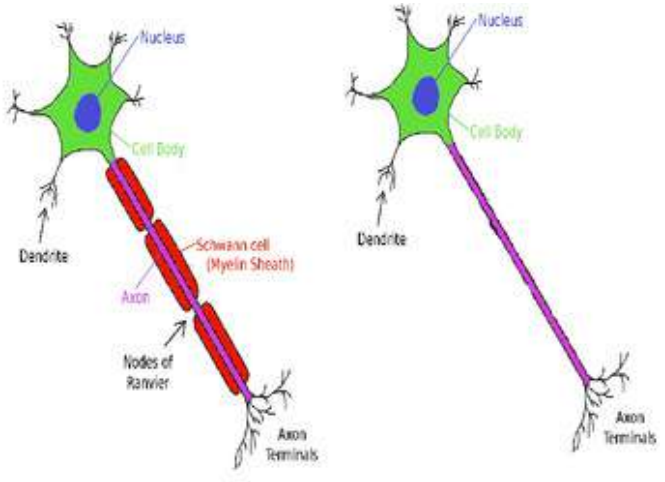


Fig 8.3 Myelinated and non myelinated neuron

2) Neuroglia: Neuroglia is a special type of interstitial tissue present both in grey and white matter. There are three types of neuroglia.

1) Astrocytes 2) Oligodendroglia 3) Microglia

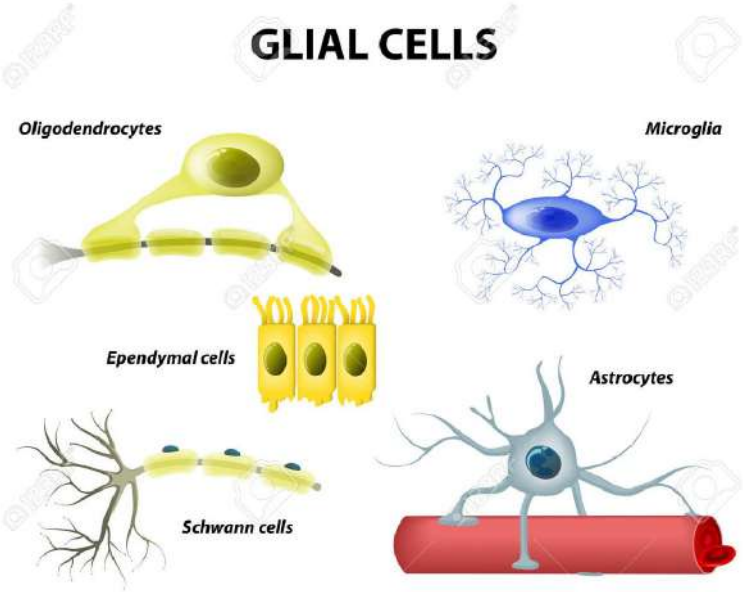


Fig : 8.4 Neuroglial cells

Synapse: Synapse is the junction where one neuron ends and another neuron begins. There are 3 types of synapses.

1) Axosomatic synapse : Presynaptic terminal of the axon ends in the cell body of neuron.

2) Axodendritic synapse : Presynaptic fibres of any axon end in the dendrites of postsynaptic cell.

3) Axo-axonic synapse. : Presynaptic fibres of any axon ends in the axon of the postsynaptic cells.

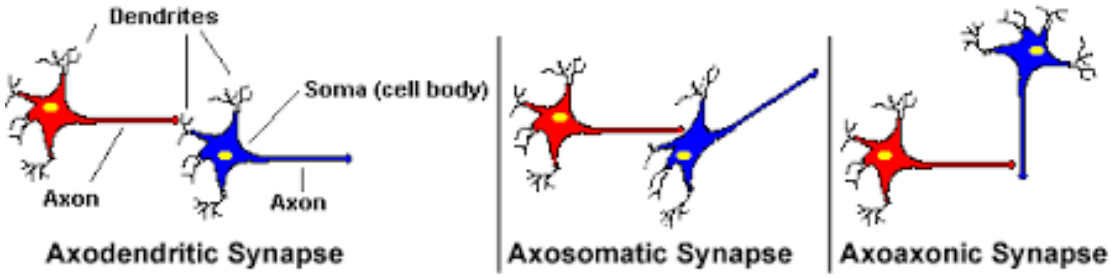


Fig 8.5 Types of synapse

Neuromuscular junction: It is the junction where motor nerve ends into muscle. It is contact between a motor neuron and a muscle fibre causing muscle contraction.

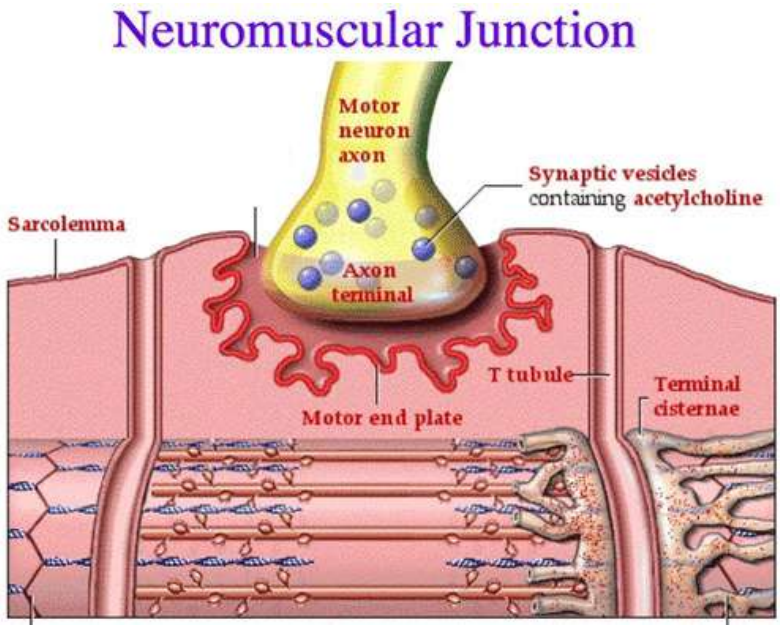


Fig 8.6 Neuromuscular junction

Functionally, the nerve endings are two types. 1) Motor nerve endings 2) Sensory endings (sensory receptors).

Receptors: Sensory receptors are a specialised structure that can be stimulated by environmental changes as well as by changes within the body. It can be defined as the terminal afferent endings that undergo depolarisation in response to specific type of physical stimuli.

Reflex arc: Reflex arc is complete pathway for a reflex action. It comprises of three parts.

1) Afferent limb- It consists of receptor and afferent nerve fibre.

2) Centre- It consists of nerve cells

3) Efferent limb- It consists of a) efferent or motor nerve fibre and its endings b) effector organ i,e, muscle.

4) Synapse – This is link between two neurones.

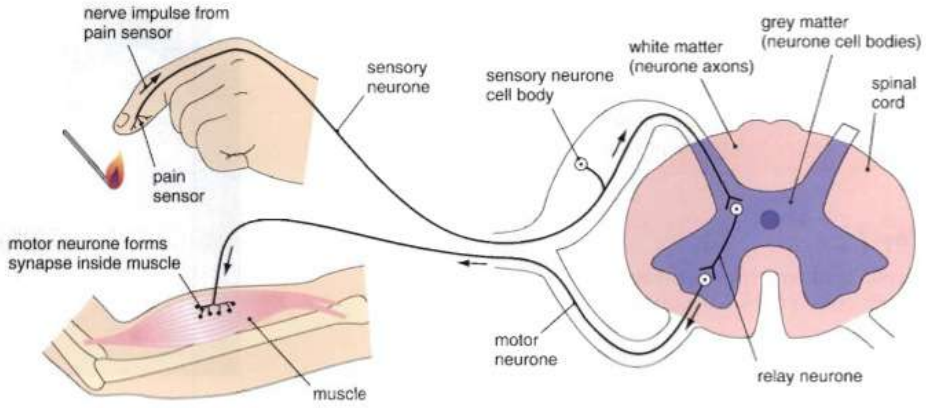


Fig 8.7 Reflex ARC

Brain: It consists of Cerebrum , Cerebellum and Brain stem.

Cerebrum: The cerebrum is the largest part of the brain and is composed of right and left hemispheres. It performs higher functions like interpreting touch, vision and hearing as well as speech, reasoning, emotions, learning, and fine control of movement. They are connected by corpus callosum which is broad band of commissural fibres. Gray matter of Cerebrum is called cortex.

Each hemisphere has five main lobes.

1) Frontal lobe → mainly consists of speech centre.

2) Parietal lobe → higher appreciation of sensation

3) Occipital lobe → consists mainly of visual cortex

4) Temporal lobe → consists of auditory cortex

5) Limbic area.

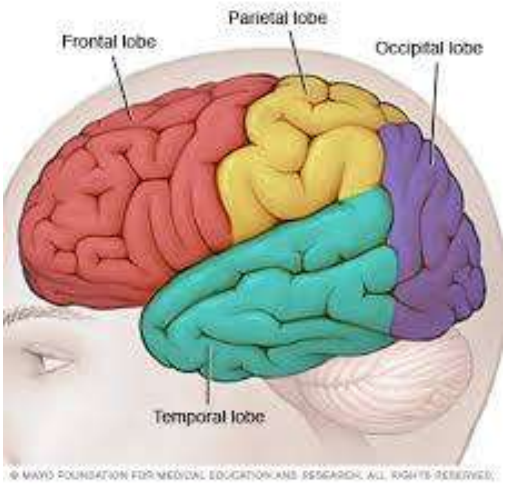


Fig 8. 8: Lobes of cerebrum

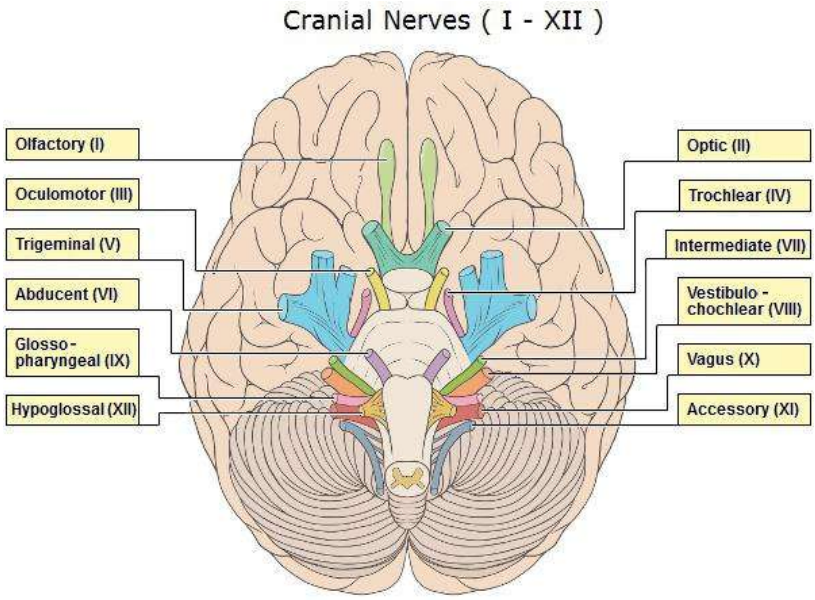


Fig 8.9 Cranial Nerves

Cerebellum: The **cerebellum** is located under the cerebrum. Its function is to coordinate muscle movements, maintain posture, and balance.

Brain stem: It includes,

1) Midbrain: It is connection between fore brain and hindbrain.

2) Medulla oblongata: It is also called spinal bulb. It is continuation of cervical part of spinal cord and is conically expanded. It extends from foramen magnum to the caudal border of pons.

3) Cerebellum: Cerebellum is the largest part of hind brain. It lies behind pons and medulla oblongata.

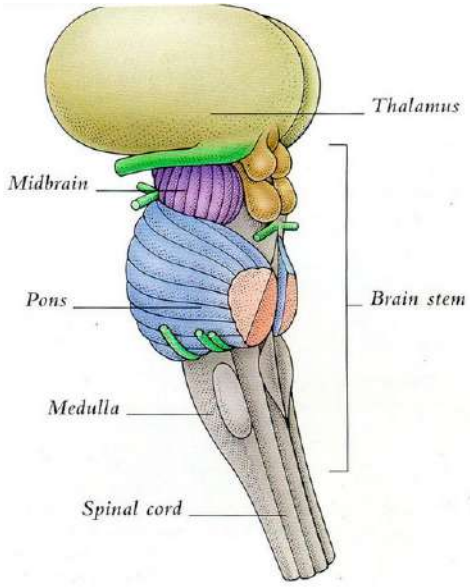


Fig 8.10 –Brain stem

Deep structures

Hypothalamus - It is located in the floor of the third ventricle and is the master control of the autonomic system. It plays a role in controlling behaviors such as hunger, thirst, sleep, and sexual response. It also regulates body temperature, blood pressure, emotions, and secretion of hormones.

Pituitary gland – It lies in a small pocket of bone at the skull base called the sella turcica. The pituitary gland is connected to the hypothalamus of the brain by the pituitary stalk. Known as the "master gland," it controls other endocrine glands in the body. It secretes hormones that control sexual development, promote bone and muscle growth, respond to stress, and fight disease.

Pineal gland – It is located behind the third ventricle. It helps regulate the body's internal clock and circadian rhythms by secreting melatonin. It has some role in sexual development.

Thalamus – It serves as a relay station for almost all information that comes and goes to the cortex (Fig. 5). It plays a role in pain sensation, attention, alertness and memory.

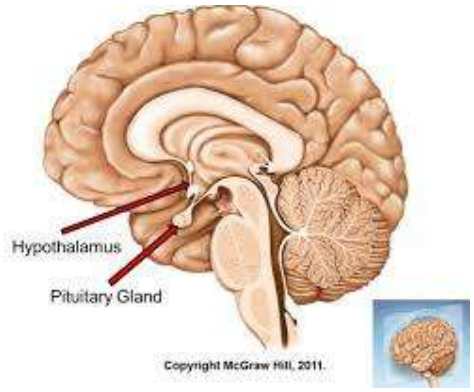


Fig 8.11 Hypothalamus

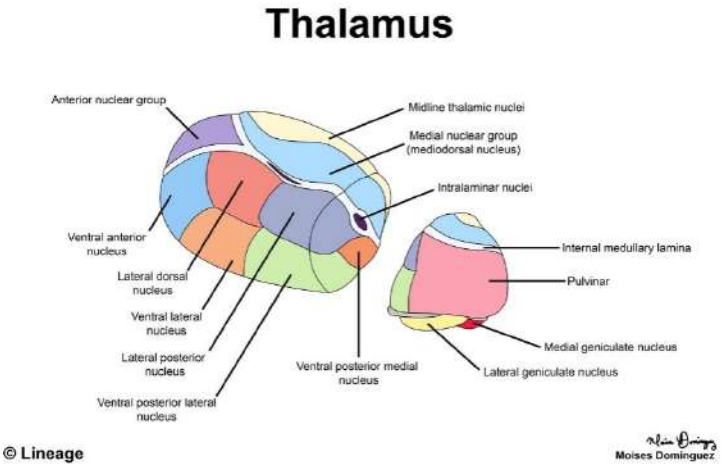


Fig. 8.12 Principal thalamic nuclei

Meninges:

The brain and spinal cord are surrounded by three coverings beneath the bones.

These layers from within outward are

1. Piamater: (closely covers the brain and spinal cord.)

2. Arachnoid mater: It is delicate layer lying between dura mater and pia mater. In between arachnoid mater and piamater there is a space called subarchnoid space. Cerebrospinal fluid fills up this space.

3. Dura mater: It is the outer most, tough fibrous membrane.

There is a space between dura mater and arachnoid mater called subdural space.

All the meninges give protection to the brain and the spinal cord.

Cerebral Ventricles: These are the cavities in the brain where CSF flows and bathes the whole brain. There are four ventricles

i) Two lateral ventricles - They present in the cerebrum

ii) Third ventricle – It lies in between two halves of the thalamus

III) Fourth ventricle - It lies in front of cerebellum and behind the medulla oblongata and pons.

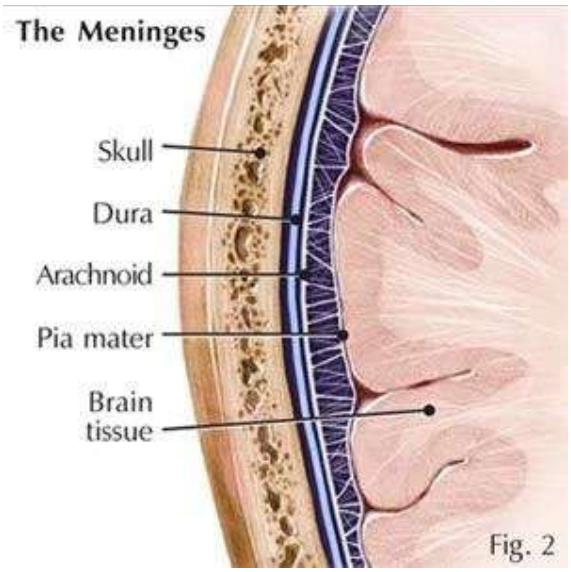


Fig 8.13 Meninges

Cranial nerves : They are twelve pairs.

Table 1: Cranial nerves

Number	Name	Function
I	Olfactory	Smell
0II	Optic	Sight
III	Oculomotor	Moves eyes, pupil
IV	Trocheal	Moves eyes
V	Trigeminal	Face sensation

VI	Abducens	Moves face
VII	Facial	Moves face
VIII	Vestibulocochlear	Salivation, hearing , balance
IX	Glassopharyngeal	Taste, Swallow
X	Vagus	Heart rate, digestion
XI	Accessory	Moves head
XII	Hypoglossal	Moves tongue

Classification of cranial nerves based on function

Sensory nerves :

I Olfactory - smell

II Optic - vision

VIII Vestibulochochlear - for hearing and balance

Motor :

III Oculomotor - eye movements

IV Trochlear - innervates Superior Oblique muscle depresses and rotates eye

VI Abducens - abducts the eye by innervating lateral rectus muscle.

XII Accessory - controls Sternocleidomastoid and Trapezius muscle.

Mixed – Both sensory and Motor

V Trigeminal - Muscles of Mastication

VII Facial - helps in facial expression and taste

IX Glossopharyngeal – Taste

X Vagus – Parasympathetic action

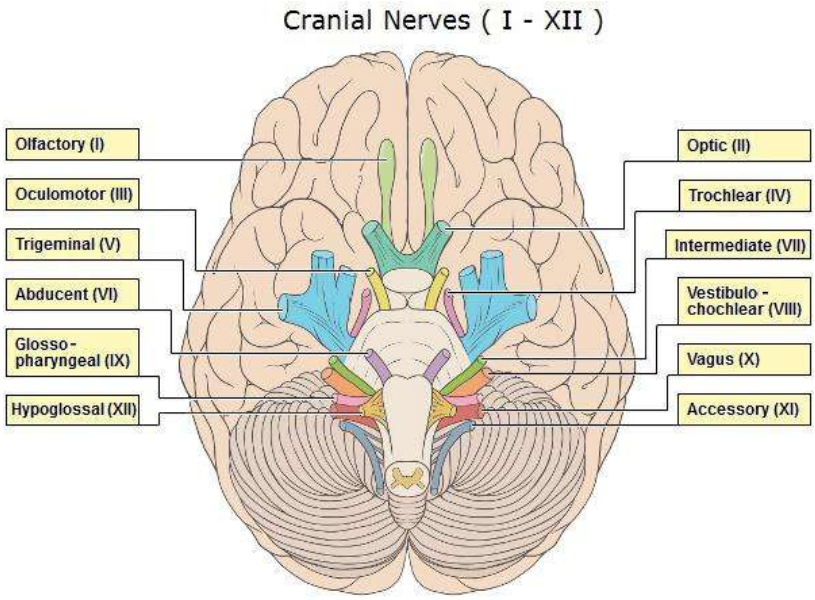


Fig 8.14 – Cranial Nerves

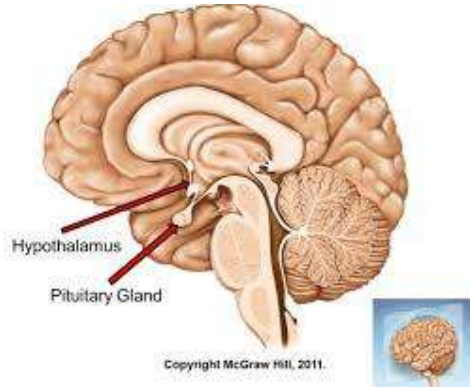


Fig 8.15 Hypothalamus

Thalamus: It is a large collection of nerve cells.

It is useful for relaying of sensory signals and motor signals.

It is helpful in regulation of consciousness, sleep and alertness.

It is located at the top of mid brain.

Basal ganglia

i) Corpus striatum (caudate nucleus and putamen)

ii) Globus pallidus

iii) Substantia nigra (contains melanin)

iv) Subthalamic nucleus

Internal capsule: It is a 'V' shaped band of fibres. It is bounded medially by thalamus and caudate nucleus. Reticular formation which play role in wakefulness.

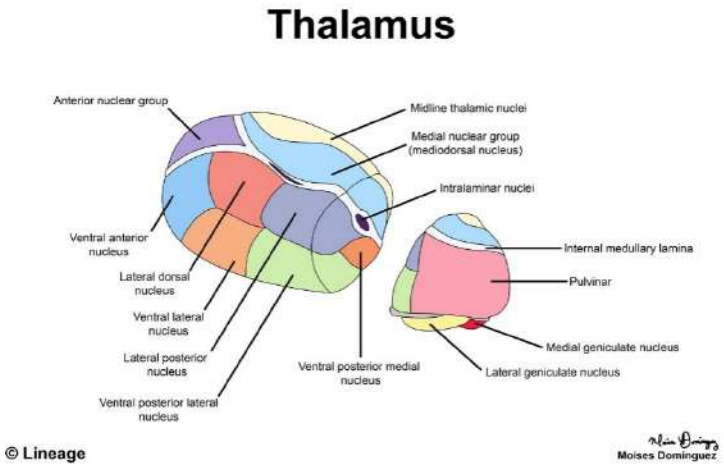


Fig. 8.16 Principal thalamic nuclei

Brain stem: It includes,

1) Midbrain: Midbrain is connection between fore brain and hindbrain.

2) Medulla oblongata: It is also called spinal bulb. It is continuation of cervical part of spinal cord and is conically expanded. It extends from foramen magnum to the caudal border of pons.

3) Cerebellum: Cerebellum is the largest part of hind brain. It lies behind pons and medulla oblongata.

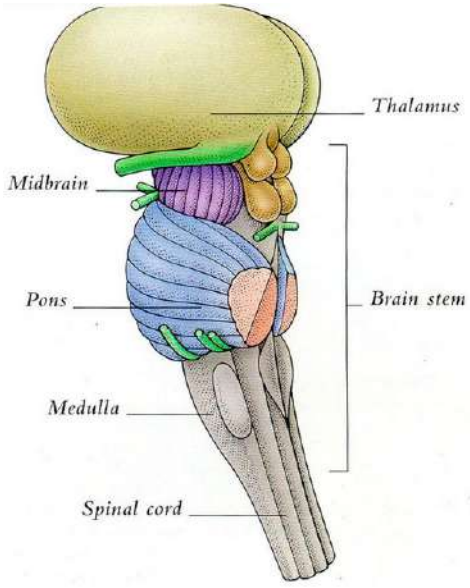


Fig 8.17 –Brain stem

Spinal cord:

It is a long cylindrical flattened nervous cord.

It is located in the vertebral column.

It extends from level of foramen magnum above to the level of the disc between 1st and 2nd lumbar vertebrae below.

Lower end of spinal cord consists of a bunch of nerves called as cauda equina.

The terminal nerve is thin and long. It is called filum terminale.

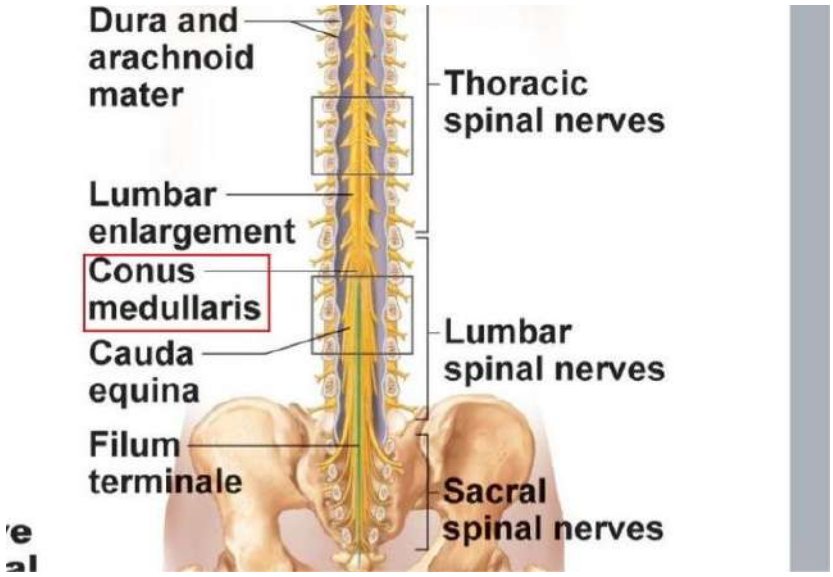


Fig 8.18 Spinal cord

T.S. of spinal cord:

T.S. of Spinal cord shows 1) Central canal 2) Gray matter 3) White matter

1) Central Canal: Central canal is in the middle of the spinal cord. It is lined by cubical ciliated epithelium. Cerebrospinal fluid (C.S.F) circulates through this canal.

2) Gray matter: It is divided into 2 parts

i) Gray matter in front of the central canal is known as anterior (ventral) gray commissure.

ii) Gray matter behind the central canal is known as posterior (dorsal) gray commissure.

It is chiefly composed of three elements a) nerve cells b) neuroglia c) nerve fibres.

3) White matter: It surrounds the gray matter and consists of myelinated and unmyelinated fibres , predominantly myelinated fibres.

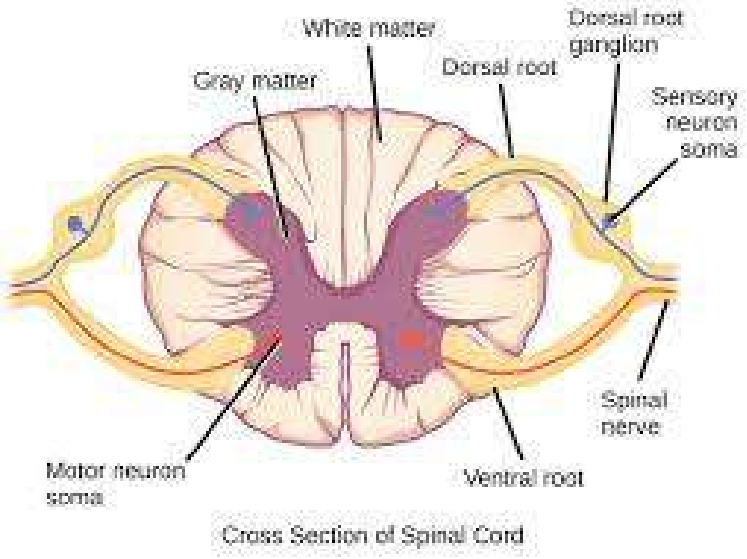


Fig 8.19 T.S of Spinal cord

Spinal nerves: There are 31 pairs spinal nerves. They are 7 cervical, 12 thoracic, 5 lumbar, 5 sacral and 2-3 coccygeal.

i) The first cervical nerve originates from the medulla oblongata and leaves the spinal canal between the occipital bone and atlas.

ii) The eighth spinal nerve emerges from the vertebral column below the seventh cervical vertebra.

iii) All the other spinal nerves emerge from the spinal cord below the vertebra whose number it bears. The coccygeal nerves pass from the lower extremity of the spinal canal.

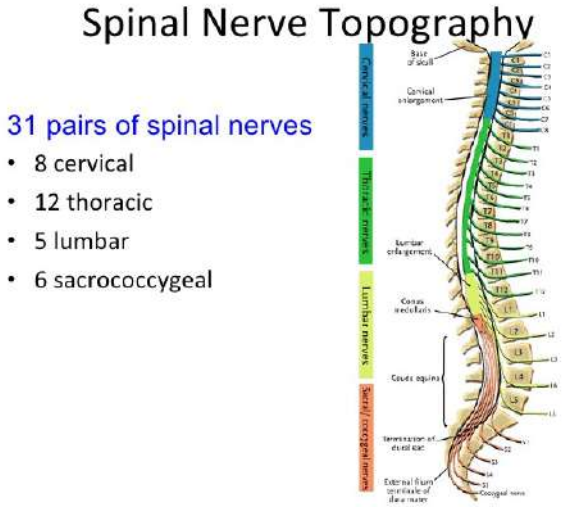


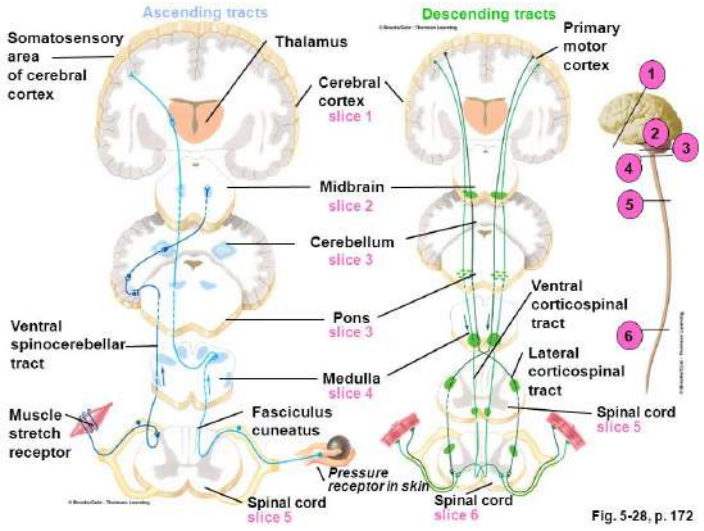
Fig 8.20 Spinal Nerves

Tracts: Fibres carrying different sensations enter the spinal cord through the posterior roots. These are divided into 3 classes.

1) Ascending tracts: Sensory tracts (carry impulses from sensory organs to Brain)

2) Descending tracts: Motor tracts (carry information from brain to voluntary organs which does the action).

3) Intersegmental fibres: Both ascending and descending.



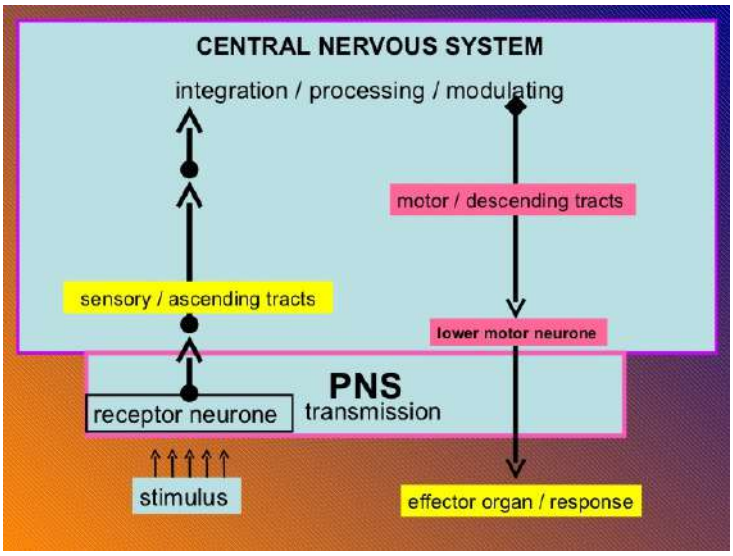


Fig 8.21 Tracts of Brain

Summary

Nervous system consists of neurons, its fibres, dendrites and axons. Nervous tissue is made of neurons and neuroglia. Neuron is the structural and functional unit of nervous system. Synapse is the junction where one neuron ends and another neuron begins. At neuromuscular junction, motor nerve fibre ends into muscle fibre. Reflex arc is complete path way for a reflex action. Cerebellum is the largest part of hind brain. Thalamus is located at the top of mid brain. Cerebrum consists of two symmetrical hemispheres. Histologically spinal cord consists of central canal, Gray matter and white matter. Spinal nerves are 31 pairs. They are 7 cervical, 12 thoracic, 5 lumbar, 5 sacral and 2-3 coccygeal. Brainstem includes mid brain, medulla oblongata and pons. Brain and spinal cord are covered by piamater, arachnoid matter and duramater. Cranial nerves are 12 pairs in number. Autonomic nervous system is divided into craniosacral and thoracolumbar systems. Diencephalon contains thalamus and hypothalamus.

Questions

Essay Questions

1) Write the anatomy of brainstem.

2) What are cranial nerves and their distribution?

3) Write in detail about the anatomy of Spinal cord.

Short Answer Questions

1) What is nervous tissue?

2) Define neuron and mention its types.

3) Write the definition and types of synapse.

4) Neuromuscular junction?

5) Receptors.

6) What are parts of reflex arc?

8) Mention types of spinal tracts.

9) What are the parts of brainstem?

10) Write about medulla oblongata.

11) Name the nuclear masses of thalamus.

12) Basal ganglia.

13) Write the lobes of each cerebral hemisphere.

14) Name the coverings of brain and spinal cord.

15) What are organs of distribution of a) Olfactory b) Facial nerves

16) Name the divisions of ANS.

17) Write the parts of Diencephalon.

18) Where is speech centre located?

UNIT - 9

ENDOCRINE SYSTEM

Endocrine system consists of endocrine glands of body.There are two types of glands in body.

I) Exocrine glands II) Endocrine glands

I) Exocrine glands : They are glands of the body with ducts.eg: mammary glands, sweat glands, lacrimal glands, salivary glands.

II) Endocrine glands: Endocrine glands are ductless glands which pour their secretions directly into blood circulation from where these secretions (hormones) reach their site of action.

I) Endocrine glands :

1) Pituitary gland (Master gland)

2) Thyroid gland

3) Parathyroid gland

4) Adrenal glands

5) Pancreas

6) Testes

7) Ovaries

8) Placenta (during pregnancy)

Thymus and pineal body are glands with probable endocrine function. Stomach, small intestine and kidneys also have endocrine activity.

Hypothalamus

Hypothalamus is a complex neurohormonal regulatory part. Diencephalon contains thalamus and hypothalamus. Hypothalamus forms lower part of lateral ventricle. It forms anterior wall of third ventricle. It is situated at the interpedencular space below the thalamus. It forms complex nuclei and fibres.

1) Pituitary gland

It is called the master gland of the body. It is reddish gray coloured and small oval shaped structure. It is located at the base of the brain in the sellaturcica of sphenoid bone. Average weight is 0.5 to 0.6 g. In females it weighs from 0.6-0.7 g. Its dimensions are 10 mm (anterio posteriorly), 6 mm (dorsoventrally) and 13 mm (laterally).

Anatomically, it has two lobes

i) Anterior lobe of pituitary gland

ii) Posterior lobe of pituitary gland

Histology of pituitary gland: It shows 6 parts.

1) Pars distalis (pars anterior) 2) Pars tuberalis3) Pars intermedia4) Pars nervosa (pars posterior or processusinfundibulis or lobusnervosus)5) Median eminence of tubercinerium. 6) Infundibulum or pituitary stalk.

Anterior lobe consists of pars distalis and pars tuberalis. Posterior lobe consists of pars intermedia and pars nervosa. Adenohypophysis consists of pars distalis, pars tuberalis and pars intermedia. Neurohypophysis consists of pars nervosa (lobusnervosus) and infundibulum (pituitary stalk or neural stalk).

Pars distalis contains acidophils, basophils and chromophobes. Pars intermedia contains basophilic polygonal or prismatic cells. Pars tuberalis contains mainly cuboidal columnar cells.

Blood Supply

Blood supply of anterior lobe : Anterior lobe of pituitary gland is supplied blood by several hypophyseal arteries. These originate from internal carotid artery and circle of willis.

Blood supply to posterior lobe : Neural lobe is supplied blood by inferior hypophyseal arteries. Vessels form capillary network while ending in pars nervosa.

Nerve supply : Few fibres from hypothalamohypophyseal tract or carotid plexus or from greater superficial petrosal nerves have control over this gland. Probably, they may be vasomotor nerves.

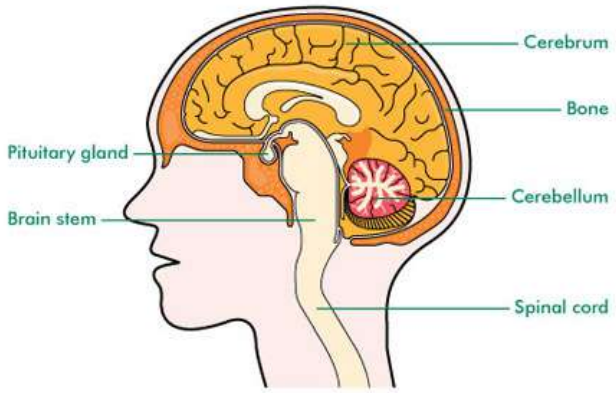


Figure :9.1 Pitutitary gland

Thyroid

This gland is situated at the root of the throat. It has two fairly lateral lobes, which are symmetrical. Each measures 5 x 2 x 2 cm3 approximately. These lobes are present one on either side of trachea. They are connected by as isthmus. Thyroid gland moves upwards during swallowing. Weight in adults is between 20-25 g. It is highly vascular gland.

Histology: It shows follicles lined by single layer of granular cuboidal cells. Bases of cells are in contact with fine basement membrane. Follicles are surrounded by highly vascular stroma. Electron microscopy reveals two types of cells, principal cells and para follicular cells.

Blood supply : Superior and inferior thyroid arteries supply thyroid gland. Internal jugular vein and innominate vein drain the gland.

Lymphatic drainage : Lateral lymph nodes of neck commonly drain lymph. Anterior mediastinal lymph nodes drain to some extent.

Nerve supply: Sympathetic fibres derive from superior, middle and inferior cervical ganglia. Parasympathetic fibres derive from superior and inferior recurrent laryngeal branches of vagus.

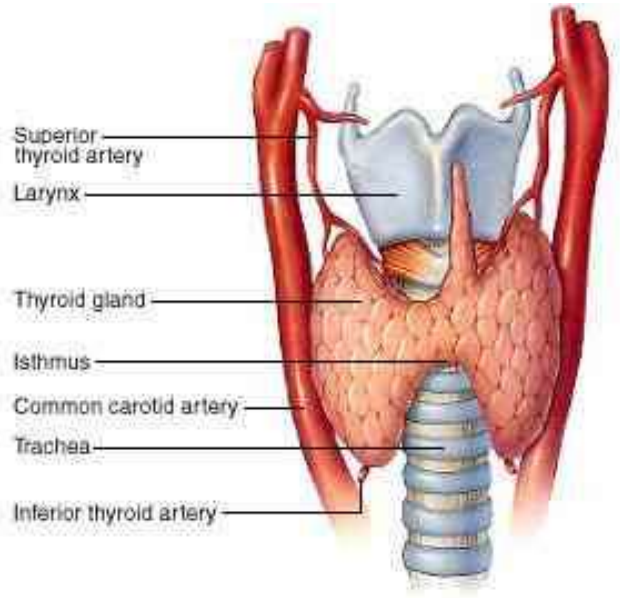


Fig. 9.2 Location and gross structure of thyroid gland

Parathyroid

It consists of four oval bodies embedded in posterior surface of thyroid. Each body measures 6 x 3 x 2 mm3. Each of the two pairs are present vertically behind each of the two lobes of thyroid. Total weight is about 140 mg. Gland is highly vascular.

Blood supply: Superior and inferior thyroid arteries supply blood.

Nerve supply:It is same as for thyroid.

Histology: There are two types of cells in parathyroid.

1) Chief cells or principal cells 2) Oxyphil cells or eosinophil cells.

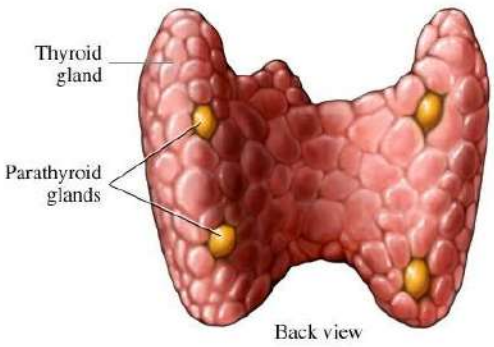


Fig. 9.3 Parathyroid glands

Adrenal glands

Adrenal glands are two in number. They are also called suprarenal glands as two glands are located on upper pole of each kidney. Right suprarenal gland is smaller than left. Dimensions of each gland are 50 x 30-40 x 10 mm3 . Average weight of each is 5-9 g. in adults.

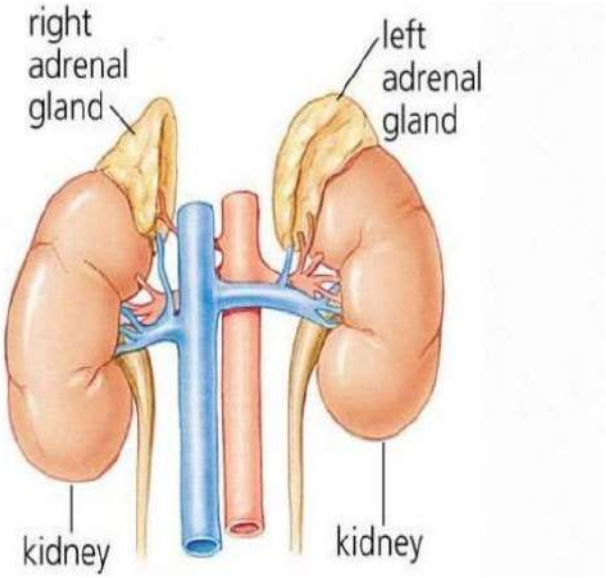


Fig. 9.4 Section of adrenal glands

Histology : There are two parts in adrenal gland.

1) Adrenal cortex - outer part consisting of,

a) Zona glomerulosa (outer) b) Zona fasciculata (middle) c) Zona reticularis (inner)

2) Adrenal medulla - Inner part consisting of irregular masses of polyhedral granular cells.

Pancreas

Human pancreas is large gland which has both exocrine and endocrine functions. It lies transversely across posterior abdominal wall behind the stomach at the level of 1st and 2nd lumbar vertebrae. It contains both exocrine cells and endocrine cells. Endocrine cells (Islets of Langerhans) are distributed all over the gland. These islets are not connected with duct system of the gland. Endocrine part constitutes about 1-2% of the gland.

There are mainly three distinct types of islet cells in human pancreas, α – cells, β – cells, δ - cells of islets of Langerhans.

Blood supply: The pancreas is supplied arterially by the pancreaticoduodenal arteries

- the superior mesenteric artery feeds the inferior pancreaticoduodenal arteries
- the gastroduodenal artery feeds the superior pancreaticoduodenal artery

Nerve supply: The pancreas receives neural innervation from the vagus (cranial X). This is part of the autonomic parasympathetic supply. The role of the vagus is to stimulate secretion of the pancreatic digestive juices.

Histology:

Darker-staining cells form clusters called acini, which are arranged in lobes separated by a thin fibrous barrier. The secretory cells of each acinus surround a small intercalated duct.The intercalated ducts drain into larger ducts within the lobule, and finally interlobular ducts. The ducts are lined by a single layer of columnar epithelium. With increasing diameter, several layers of columnar cells may be seen.

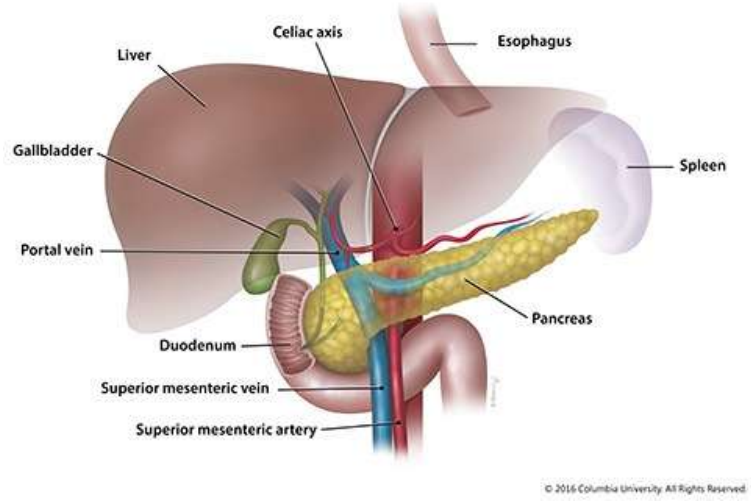


Figure :9.5 Pancreas

Testes

Testes are the male reproductive organs concerned with spermatogenesis. For anatomy refer to Anatomy of Reproductive system.

Ovaries

Ovaries Refer to Anatomy of Reproductive system.

Placenta

Placenta Refer to Anatomy of Reproductive system.

Thymus

It is both endocrine and lymphoid structure, located in the anterior and superior mediastina of thorax behind sternum. It extends from pericardium upwards upto the lower border of thyroid. There are two lobes which are fused and asymmetrical. Right lobe is bigger than left lobe. Each lobe consists of numerous lobules. Follicles of lobules have diameter of 1 mm each.

Histology: It has 3 parts

a) Capsule –It is dense connective tissue, rich in macrophages, mast cells, granulocytes and fat cells etc.

b) Cortex - which is similar to lymph tissue of ordinary lymph nodes,but deficit of primary follicles.

c) Medulla - broad, branched band of thymic tissue.

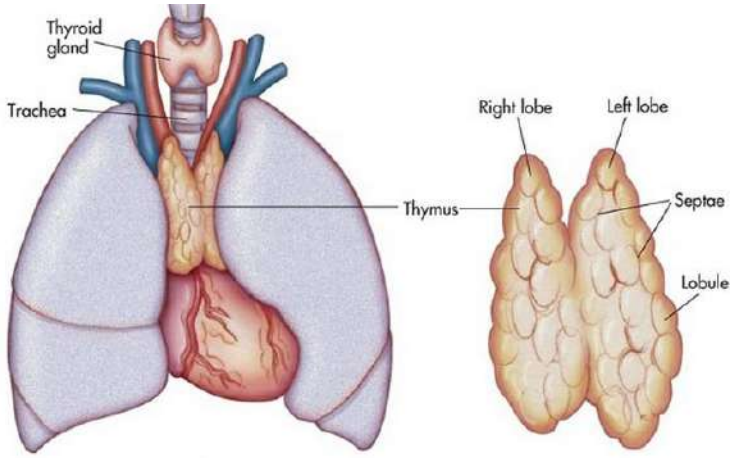


Fig. 9.6 Thymus gland

Pineal body:

Pineal body is also called epiphysis cerebri. It is flat, cone shaped and grey colored. It's length is about 5-8 mm and breadth is about 3-5 mm. Pineal body is attached to the roof of third ventricle by means of a short hollow stalk. Its histology shows two major types of cells, which are neutral in origin. They are1) Parenchymal or chief cells. 2) Interstitial or supportive cells.

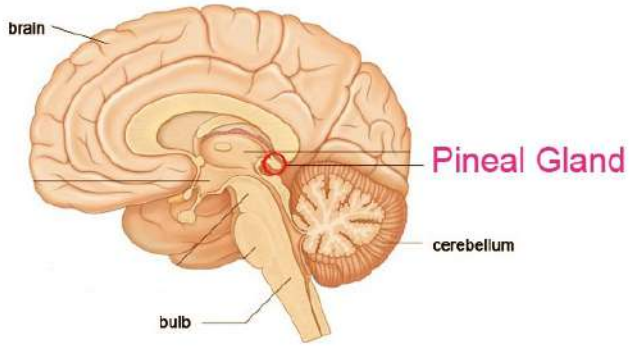


Fig. 9.7 Pineal gland

Gastro intestinal tract:

Certain localised area of GIT acts as endocrine to secrete gastro intestinal hormones. Cells responsible for endocrine activity in GIT are not known. Gastrin I and II are produced in modified epithelial cells of glandular mucosa of pyloric part of stomach. Mucosa of upper part of small intestine secretes cholecystokinin - pancreozymin hormone. Duodenal muscosa produces secretin. Wall of stomach and small intestine produce gut glucagon hormone. Human gastric muscosa contains gastrone. Mucosa of upper small intestine produces villikinin. Intestinal lumen secretes enterocrinin.

Kidneys:

Juxtaglomerular cells produce renin. Erythropoietin is largely produced by kidneys. Prostaglandins are also produced by kidneys.

Summary

Endocrines system consists of endocrine glands of body. Endocrine glands are ductless glands. Endocrine glands of human body are pituitary, thyroid, parathyroid, andrenals, pancreas, testes, ovaries and placenta (during pregnancy). Pituitary has two lobes - anterior and posterior lobes. It is located at the base of brain in sellaturica of sphenoid bone. Thyroid is located at the root of the throat. It has two lobes. Parathyroid consists of four oval bodies. Each of the two pairs are present vertically behind each of the two lobes of thyroid. Adrenal glands are present on upper pole of each kidney. Pancreas lies transversely across posterior abdominal wall behind the stomach at the level of 1st and 2nd lumbar vertebrae. Testes, ovaries and placenta have endocrine activity of producing sex hormones.

Thymus is located in the anterior and posterior mediastina of thorax behind sternum. Pineal body is attached to the roof of third ventricle.

Questions

Essay Questions

1) What are different endocrine glands of body? Write the anatomy of pituitary.

2) Discuss anatomy and histology of thyroid with diagrams.

3) Disribe the anatomy and histoly of pancreas with diagrams.

Short Answer Questions

1)What are the major types of endocrine gland.

2) What are exocrine glands.

2)Write any four endocrine glands of body.

3) Write the anatomical location of pituitary gland

4) What are the lobes of pituitary?

5)Mention the parts of adenohypophysis and neurohypophysis.

6)Where is thyroid located? Write the dimensions.

7)Write the location and dimensions of parathyroid.

8)What are the types cells in parathyroid?

9)Mention the parts in adrenal gland.

10)What are different islet cells of pancreas?

11)Mention the endocrines secreting female sex hormones.

12)Where is thymus situated?

13)Write the histological parts of thymus.

14)Write the major types of cells of pineal body.

UNIT – 10

REPRODUCTIVE SYSTEM

Reproduction is the process of producing same type of offsprings.

There are two sexes.

1. Males - masculine characters are dominant and feminine features are rudimentary.
2. Females - feminine characters dominant and masculine features are rudimentary.

Balance of male and female sex hormorones is essential for physical and mental get up of male or female.

Puberty : Puberty is onset of reproductive life. Usually, onset of puberty is between 12 and 16 years. Reproductive capacity stops in old age.

Menarche : It is the first appearance of menstruation in females.

Menopause : It is cessation of menstruation in females. It is usually between 45th and 55th years of age.

Differentiation of sex: Male spermatogonia and female oogonia contain 23 pairs of chromosomes each as in somatic cells. These are divided into,

1. Autosomes - 22 pairs do not play any role in sex determination.

2.Sex chromosomes - Last pair plays important role in sex determination. In females, there are 2 X chromosomes i.e. XX which are of homoglogous type. In males there are one X and one Y and hence called heterologous type.

In the process of fertilisation,zygote is formed by union of sperm and ovum. If X chromosome of male unites with ovum (X), two sex chromosomes will be identical and resulting offspring is female (XX). If Y sex chromosome of male unites with ovum (X), two sex chromosomes will be different and resulting offspring is male (XY).

Testes

Functions of testes: 1) Spermatogenesis takes place in seminiferous tubules.There are 2 stages.

a)Spermatocytogenesis- It is the first stage in the formation ofspermatozoa. Spermatogonia divide into spermatocytes and then into spermatids.

b) Spermiogenesis - Spermatids are converted iinto spermatozoa.

2) Secretion of testosterone by interstitial cells of Leydig -They occupy less than 10% of volume of testes. They secrete a hormone called testosterone. At puberty, pituitary interstitial cell stimulating hormone (ICSH) rises and stimulates the development of interstitial cells of Leydig.

Semen : It is suspension of spermatozoa in the fluid secreted by epiddiymis, prostate, seminal vesicle, cowper's glands (bulbo-urethral glands). Volume of semen at each emission is about 3-4 ml. Reaction ranges from 7.2 to 8.9 (alkaline). Average pH is 7.8. Normal sperm count is 40-300 millions/ ml. of semen. Count of spermatozoa less than 20 millions/ml. of semen can generally cause infertility.

Sperm count is same as WBC count using WBC pipette. Diluting fluid is different. Motility of spermatozoa is determined by counting at least 500 spermatozoa and taking average.

Ovaries

Functions of ovaries :

1) Formation of mature ova: Each ovary contains germinal epithelium, the outermost covering of ovaries. It sends down genital cords. They cut off from surface and break into small islands of cells. Of these cells, one enlarges and gets differentiated from neighbouring cells. It is called primary oocyte. Rest of the cells surround this primary oocyte and form primordial follicles. From the onset of puberty, under the influence of Follicle stimulating hormone (FSH), only one follicle matures, discharging one ovum at each menstrual cycle. During the whole sexual life of female, only 400-500 mature and ovulate. Others degenrate.

2) Secretion of hormones- Four hormones are secreted. a) Oestrogen b) Progesteronec) Androgen d) Relaxin.

With these four hormones, ovary controls whole reproductive life of female. It is responsible for 1)Puberty changes 2) Pregnancy and associated changes 3) Parturition.

Ovulation : The process of rupture causing release of ovum is called ovulation. It occurs between 13th to 17th day after first day of menstruation in human female.Maturation of follicle takes place in 10-14 days. It increases in size gradualy and migrates to the surface.

Surface layer of ovary undergoes necrosis. Enlarged follicle protrudes from the surface of ovary. Follicle ruptures due to increasing pressure. Ovum is discharged near the end of follopian tube. Ciliary movement of tubular epithelium carries the ovum released near the mouth of follopian tube along the tube. Approximately, in 72 hours after ovulation, ovum arrives at uterus. Ovum will not remain functionally active after few days.

Ovarian cycle : Adult ovary undergoes recurring cyclic process of ovulation and menstruation. It occupies about 28 days. The cycle begins on the first day of menstruation.

Ovarian cycle consists of two phases.

1) Follicular phase 2) Luteal phase

Ovulation occurs normally between 13th to 17th days of cycle, separating the two phases. Menstruation takes place 14 days after ovulation, if fertilisation does not take place.

Ejaculation : It is the act of sudden ejection of semen out of urethra at the time of orgasm. It is a sympathetic activity. At the time of ejaculation, sympathetic impulses prevent micturition by causing - a) Relaxation of detrussor muscle& b) Constriction of internal sphincter. This act also prevents reflux of semen into bladder.

Fertilisation : Fertilisation is the process of penetration of ovum by spermatozoa recently deposited in the genital tract. Fertilisation usually occurs at ampullaryisthmic junction of fallopian tube. Once fertilisation of ovum takes place, further penetration by other sperms is prevented. Blastocyst is formed after fertilisation. Blastocyst is the developing embryo, which moves down the uterine tube into uterus.

Implantation: Implantation is attachment of blastocyst to uterus.It usually occurs between seventh and ninth days ofafter ovulation.Implantation occurs by erosion of epithelial cells of uterine mucosa and penetration of blastocyst.

Menstruation: It is the process of cyclical discharge of unfertilised ovum along with blood, mucus, strips of endometrium and leukocytes. After discharge, clotting takes place due to rapid formation of fibrin.

In each ovarian cycle, endometrium proliferates to prepare suitable bed to receive and implant blastocyst. This proliferated mucosa is converted into placenta in case of conception.If conception does not take place, this hypertrophied mucosa breaks down and discharged along with unfertilised ovum, blood and leucocytes.

Endometrial changes during menstrual cycle are divided into

1)Resting phase or follicular phase of healing of endometrium.(1st-5thday)

2)Proliferative phase of maturation of graafian follicle.(6th-14th day until ovulation)

3)Premenstrual or luteal phase of growth of corpus luteum.(15th-28th day)

4)Destructive or menstrual phase of degeneration of corpus luteum.(starting on 28th day and lasting upto 4-6 days)

Pregnancy : Conception occurs if ovum is fertilised. At the end of pregnancy, parturition takes place. It is normally 280 days of gestation period in human females (10 menstrual cycles).

Physiological changes during pregnancy are Hypertrophy and thus enlargement of uterus, Development of placenta, Enlargement of birth canal and relaxation of pelvic ligaments, Proliferation and development of breasts, Formation and growth of corpus luteum, Cessation of ovulation, Raise of blood volume,blood cholesterol,plasma fibrinogen,plasma globulin etc., Lowered plasma albumin,plasma iron levels, Increased erythrocyte sedimentation rate, Increased cardiac output, Increased vital capacity,tidal volume and pulmonary ventilation, Nausea&vomiting in early months,hypochlorohydria and hypotonicity of colon often seen. Other changes are Increased glomerular filtration and some times glycosuria,Excretion of oestrogen, pregnanediol and placental gonadotrophin, Enlarged thyroid gland and increased thyroid hormones secretion, Enlarged adrenal cortex (Zona fasciculata) and increased secretion of cortisol, Enlarged parathyroid glands and increased secretion of parathormone,Lowered renal threshold of glucose causing glycosuria, Positive nitrogen balance and retention of more nitrogen in the body, Increased water retention in the later months in omniotic fluid, placenta, foetus, breast, uterus, blood and other tissues,Increased retention of sodium, Stimulated synthesis of hormone binding proteins by liver and Increased formation of renin substrate by liver.

Placenta: It is the functional connection between embryo and uterus. It is necessary in mammals as the foetus is developed in uterus. It is developed from uterus.

Functionsof Placenta -

1) Supply of nutrients from maternal blood to foetus.

2) Excretion of foetal metabolites by diffusion into maternal blood.

3) Passage of O2 from maternal blood to foetal blood and CO2 from foetal blood to maternal blood etc.

Parturition: Parturition is the process of child birth at the end of gestation. It occurs at about 280th day after last menstrual period. Periodic contraction of smooth muscles of uterus and skeletal muscles of abdomen aid parturition. Oestrogen stimulates uterine contraction.

Stages of Labour: Duration of labour varies from 12-18 hours.

1) First stage or stage of dilatation of rupture of amnion and expulsion of amniotic fluid.

2) Secondary stage or stage of descent of child through vagina and expulsion.

3) Third stage of expulsion of membranes of foetus.

Involution : It is the process of rapid decrease of size of uterus to normal. It occurs by gradual autolysis or self digestion within 6-8 weeks.

Multiple Births: Giving birth to more than one child at once is called multiple births.Giving birthto two children at a time is called twin birth.

Twins can be divided into two types - 1) Monozygotic twins 2) Dizygotic twins.

Monozygotic twins : One zygote is formed by penetration of single sperm into single ovum. Zygotic material divides into two halves and gives rise to two separate embryos. In this type, twins of same sex, same blood group and tissues, same antigenic potencies are born.

Dizygotic twins:In this case, two ova are discharged at a time and they are fertilised by two sperms. Two zygotes are formed. In such case, twins are not identical and called dizytotic twins.

Triplets, quadruplets or quintuplets may be born from any of above process or combination.

Methods of controlled reproduction

Populatin explosion is a major problem in the today world. It is necesary to follow contraceptive measures to bring the population to a balance. In a country like India, people have to be mass educated regarding population control.

1) Temporary methods

a) Natural methods b) Methods using barriers c) IUCD d) Oral contraceptives e) Spermicidal jellies, sponges, tampoons, powders, f) Implant g) Douching

a) Natural methods : These methods include1) Coitus interruptus 2) Rhythm method

Coitus interruptus: In this method, penis is withdrawn from vagina just before discharge of semen. It can cause psychic disturbance. It may fail, when it is not possible to have control.

Rhythm : Intercourse should be avoided in unsafe period of menstrual cycle. This natural method is not fool proof.

In this method , In a regular menstrual cycle of 28 days,

-1-5 are days of menstruation.-6-9 days of are safe.-10-18 days are not safe.-19-28 days are safe.

b) Methods using barriers : Barriers are condom, membrane sheath and diaphragm.

c) Use of intrauterine contraceptive device: If a foreign body is placed in uterus, implantation of blastocyst becomes difficult. Presence of IUCD in uterus interferes with chain of events in the implantation of blastocyst in uterine wall.Some of the complications are execessive bleeding, pain, expulsion of IUCD, infection and uterine perforation. Failure rate is about 5%.

d) Oral contraceptives: There are three regimes of oral contraceptives.They are1)Classical pills 2) Sequential pills 3)Luteal supplementation pills.

e) Spermicidal jellies, sponges, tampoons and powders.

f) Implants : Progestin capsules are implanted in body. Action lasts for months or years. Progestasert is such an implant.It can be taken out when pregnancy is needed.

g) Douching : It is washing vagina with irritating fluid like salt solution, lactic acid solution, lemon juice or alum solution after coitus. Tap water can also be used for douching.

Termination of pregnancy : It is also a method of contraception when conception has already occured.It should be conducted in accordance with Medical termination of pregnancy act enacted in 1971.

2) Permanant methods : Vasectomy for males, tubectomy for females and laproscopic methods for women.

In vasectomy, vasdeferens is cut on both sides and ligated. In tubectomy, fallopian tubes are ligated. In laproscopic method, fallopian tubes are viewed using laproscope and ligated or lumen is sealed.It is usually done after 5th day of menstruation. A small incision is made on abdomen for this operation near umbilicus. This procedure requires 10 to 15 minutes.

Sex hormones

I) Male sexual hormones:1) Testosterone 2) Androsterone and 3) Dehydroepiandrosterone (DHEA).Testosterone is primarily produced by Leydig cells of testes in testes in response to FSH and LH released by anterior lobe of pituitary gland. Testosterone and other androgens in turn inhibit the release of FSH and LH by negative feedback mechanism.

Levels of testesterone in plasma of men are 5 to 100 times greater than the levels in plasma of women. About 4-12 mg. is produced in young males per day where as 0.5 - 2.9 mg. is produced in young females per day. Testesterone is converted by androgen responsive tissues in the body to highly active metabolite - dihydrotestosterone. It is believed to be the active form of the hormone.

Physiological actions of male sex hormones :

They are three main functional components of male sexual hormones.

1) Androgenic functions :

In foetus:Testosterone and dihydro testosterone cause masculinising effects in male foetus. They cause development of prostate, penis and related sexual tissues.

At puberty: Secretion of testosterone by testes increases greatly at puberty. At this stage androgens are responsible for growth of male sex organs, increase in the activity of these organs, secondary sexual characters.

Growth of male sex organs:Penis,testes,seminal vesicles,prostate and epididymis develop in size by the activity of male sex hormones.

Increase in the activity of these organs:Spermatogenesis by testes begins. Secretions by epididymis, prostate, seminal vesicles and Cowper's glands start. Secretion of semen starts. Nocturnal emissions occur.

Secondary sexual characters:Secondary sexual characters develop.They are 1) growth of moustache and beard, pubic hair and hair in other parts of body likechest, axillae etc. 2) Change in voice to male type

2) Anabolic functions: There are increase in protein anabolic activity,increase in muscle mass and rapid growth of long bones etc.

3) Psychological aspect :Androgens play important role in development of male psychology and behaviour. Emotional get up of male type is developed. These hormones are responsible for libido which provides back ground for sexual functions.

Female sex hormones: Oestrogens, progesterone,relaxin, androgens.

1) Oestrogens : It is the most important member of this group. It is produced by graafian follicle of ovaries.It is excreted as such or as metabolites.

2)Progesterone : It is secreted by corpus luteum of ovaries and placenta. Pregnanediol is the metabolic product of progesterone and it is biologically inactive. It is also found in adrenal cortex.

Functions of oestrogens and progesterone:

At puberty:These hormones are responsible for,

1) Growth of female sex organs: Oestrogen stimulates development and maintains morphological and functional state of female sex organs- vagina, uterus, fallopian tubes. Oestrogen is responsible for enlargement of vagina to adult size.

2) Secondary sexual characters: Mainly oestrogens and to a lesser extent, progesterone are responsible for devolopement of secondary sexual characters in women at puberty. They are - devolopement of breast,growth of pubic hair and underarm hair,pigmentation of nipples and genital tissues.

Development of breast at puberty is caused by both oestrogens and progesterone.Oestrogens cause proliferation of ductile system of breast. Progesterone stimulates development of alveolar system. Oestrogens contribute to the shape of breast and function.Important role of oestrogens is attribution of feminity.

In menstrual cycle: Progesterone causes premenstrual changes in endometrium after proliferative changes have been made by oestrogens. Oestrogen has direct stimulatory effect on follicle growth.

In Pregnancy : Oestrogen promotes motility of fallopian tube. This plays prominant role in sperm transport.Most important activity of progesterone during pregnancy is depressed contractility of uterus. In the third trimster of pregnancy, progesterone secretion is decreased and oestrogen secretion is increased and thus uterus becomes excitable in preparation for parturition.

Progesterone has important role to play in pregnancy.Functions of progesterone in pregnancy are1) Embedding of ovum, 2) Formation of placenta, 3) Inhibition of uterine excitability before third trimster, 4) Development of breasts in synergism with oestrogens, 5) Inhibition of menstrual cycle and ovulation and 6) Enlargement of birth canal due to growth of vagina and relaxation of pelvic ligaments in synergism with oestrogens.

3) Relaxin : It is the hormone present in pregnant mammalian ovary, placenta and uterus. Its level reaches maximum at the terminal stages of pregnancy. It is useful in affecting parturition.

4)Androgens: Small amounts of testosterone present in females are responsible for development of libido.

Oestrogens and progesterone also have various other actions of which, effect of oestrogen increasing the skeletal growth, lowering of plasma cholesterol level are some.

Summary

Reproduction is the process of producing same type of offsprings. Functions of testes are 1) Spermatogenesis 2) Secretion of testosterone. Semen is suspension of spermatozoa in the fluid secreted by epididymis, prostate, seminal vesicles and Cowper's glands. Functions of ovaries are 1) formation of mature ova 2) secretion of hormones. Fertilisation is penetration of ovum by spermatozoa. Menstrual cycle consists of i) Menstrual phase ii) Follicular phase iii) Luteal phase. Pregnancy ends with child birth. Methods of family planning are i) Temporary methods ii) Permanant methods. Male sex hormones are i) Testosterone ii)Androsterone iii)Dehydro epiandrosterone.Female sex hormones are - oestrogens, progesterone and relaxin. Small quantities of androgens are also present in women and are responsible for libido.

Essay Questions

1)Write the functions of testes in detail.

2)Explain the functions of ovaries.

3)Write about pregnancy and physiological changes during pregnancy.

4)Discuss the methods of controlled reproduction.

5)What are the male sex hormones? Write note on them.

6)List out female sex hormones. Add a note on them.

Short Answer Questions

1)What is puberty?

2)Define a) Menarch b) Menopause

3)Mention the functions of testes.

4)What is spermatogenesis ?

5) What is spermiogenesis?

6)What is semen?

7)Mention the functions of interstitial cells of Leydig.

8)Mention the functions of ovaries.

9)What is ovarian cycle? Mention the phases.

10)Define a) Fertilisation b) Implantation

11) What are the types of twins Explain a) Monozygotic twins b) Dizygotic twins.

12)What is menstruation? Mention the phases of menstrual cycle.

13)Define parturition and write the stages of labour.

14)What are the temporary& natural methods of controlled reproduction?

15)Mention the permanant methods of controlled reproduction

16)Mention male sexual hormones.

17)Mention the main functional components of male sexual hormones.

18)Write the secondary sexual characters in a) Males b) Females

19)What are the androgenic functions of male sex homones at puberty?

20)What are the anabolic functions of androgens?

Stress

Definition: A state of mental or emotional strain or tension resulting from adverse or demanding circumstances.

The other similar terms are strain, pressure, tension, nervous tension, worry, anxiety, nervousness. It is your body's response to certain situations. It is subjective. Stress can affect your physical and mental health, and your behavior.

Types of stress

1. Acute stress- It is the most common type of stress.

2.Episodic acute stress- When acute stress happens frequently, it is called episodic stress.

3.Chronic stress- If acute stress isn't resolved and begins to increase or lasts for long periods of time, it becomes chronic stress.

1. Acute stress - Acute stress is the most common type of stress. It's your body's immediate reaction to a new challenge, event, or demand, and it triggers your fight-or-flight response. As the pressures of a near-miss automobile accident, an argument with a family member, or a costly mistake at work sink in, your body turns on this biological response.

Severe acute stress such as stress suffered as the victim of a crime or life-threatening situation can lead to mental health problems, such as post-traumatic stress disorder or acute stress disorder.

2. Episodic acute stress -When acute stress happens frequently, it's called episodic acute stress. People who always seem to be having a crisis tend to have episodic acute stress. They are often short-tempered, irritable, and anxious. People who are "worry warts" or pessimistic or who tend to see the negative side of everything also tend to have episodic acute stress.

Negative health effects are persistent in people with episodic acute stress. It may be hard for people with this type of stress to change their lifestyle, as they accept stress as a part of life.

3. Chronic stress - If acute stress isn't resolved and begins to increase or lasts for long periods of time, it becomes chronic stress. This stress is constant and doesn't go away. It can stem from such things as poverty, a dysfunctional family, an unhappy marriage and a bad job

Chronic stress can be detrimental to your health, as it can contribute to several serious diseases or health risks, such as heart disease, cancer, lung disease, accidents, cirrhosis of the liver and suicide.

Managing stress(Regulation of stress)

Stress affects each person differently. Some people may get headaches or stomachaches, while others may lose sleep or get depressed or angry. People under constant stress may also get sick a lot. Managing stress is important to staying healthy.

It is impossible to completely get rid of stress. The goal of stress management is to identify your stressors, which are the things that cause you the most problems or demand the most of your energy. In doing so, we can overcome the negative stress those things induce.

The Centers for Disease Control and Prevention recommend the following to help cope with stress:

- take care of ourself, by eating healthy, exercising, and getting plenty of sleep
- find support by talking to other people to get our problems off
- connect socially, as it's easy to isolate ourself after a stressful event
- take a break from whatever is causing stress
- avoid drugs and alcohol, which may seem to help with stress in the short term, but can actually cause more problems in the long term

UNIT – 11

EXCRETORY SYSTEM

Excretory system consists of organs concerned with excretion of waste products formed in the cellular metabolism of body.

Excretory organs:

Kidneys(2)

Liver

Lungs(2)

Digestive tract

Salivary glands

Urinary system: Urinary system consists of,

Kidneys(2)

Ureters (2)

Urinary bladder

Urinogental tract / Urethra

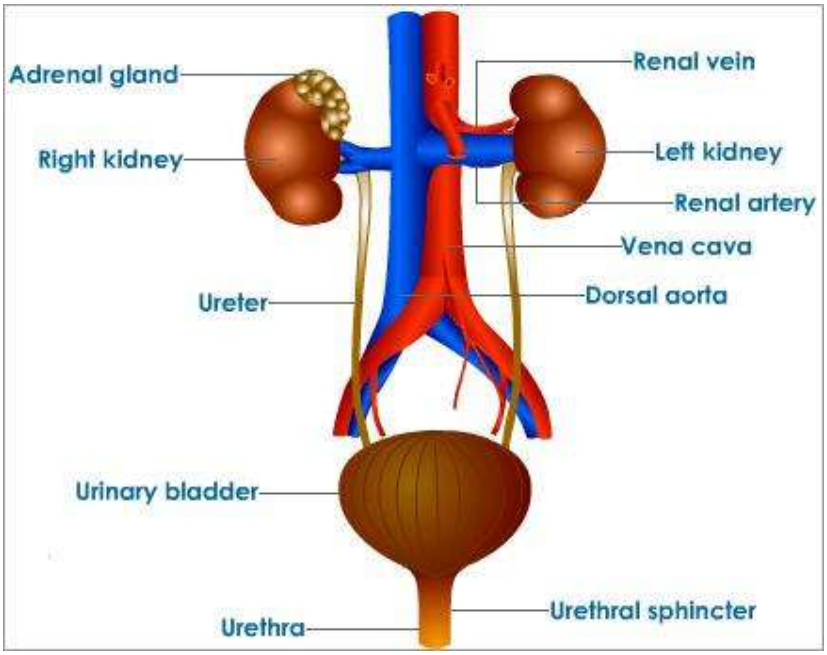


Fig.11.1 Urinarysystem

Kidneys: Kidneys are the main organs of urinary system.They are two bean shaped organs lying on the posterior wall of upper abdomen, one on each side of vertebral column.Right kidney is located slightly lower than left kidney.Each kidney measures about 11 x 5 x 3 cm and weighs 150g approximately.They are embedded by fat called perirenal fat.Each kidney is convex on its outer border and concave in the centre of its inner border (Hilus).At this point,blood vessels,nerves and ureter enter and leave kidney.On each kidney, an adrenal gland is present.

Structure of Kidney: Kidney is surrounded by a fibrous capsule. It can be stripped off easily. Portion inside this fibrous capsule can be divided into,

1. Outer cortex - Cortex is the outer reddish brown coloured portion. Medulla is the inner lighter area.
2. Inner medulla - Medulla is subdivided into 10 to 15 conical areas called renal pyramids. Pyramids have their broad base towards cortex and apex projecting into lumen of minor calyx. Columns of Bertin are the projections of cortex. They form the boundaries of the pyramids.

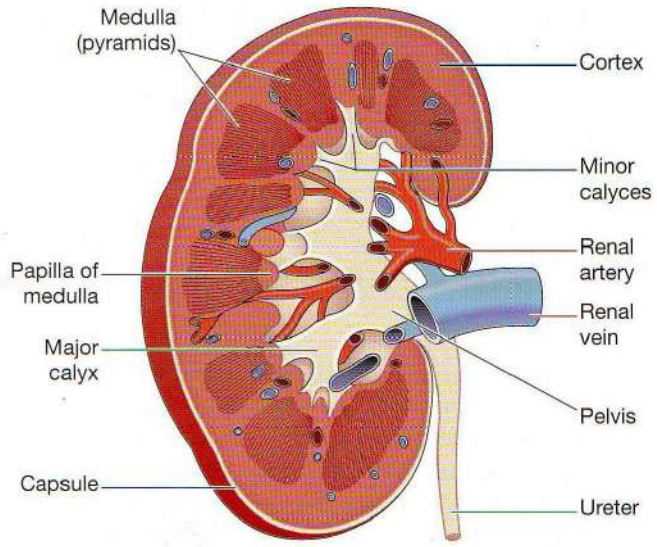


Fig.11.2 L.S. of Kidney

Histology of kidney:

Uriniferous tubules consist of two portions 1) Nephron 2) Collecting tubule.

1) Nephrons : Eachkidney consists of number of minute units called nephrons. They are basic structural and functional units of kidney. They are about one million nephrons in each kidney. Nephrons drain into pelvis of ureter and then into urinary bladder.

Parts of Nephron :

1. Malphigian body: It is also called as renal capsule. It lies in cortex of kidney. It consists of two parts.

A) Glomerulus is tuft of about 6-8 renal capillaries invaginating into the end of tubule. It has two poles 1) Vascular pole - where bloods vessels are attached 2) Tubular pole - Where renal tubule begins. Afferent arteriole brings blood to glomerular tuft. It is short and wide. This capillary tuft reunites and forms efferent arteriole. It is long and narrow. This arrangement builds up a pressure gradient of 70 mmHg and facilitates filtration.

B) Bowman's capsule is the dilated end of nephron. It is invaginated by glomerular tuft. It is made of two layers called parietal and visceral layers. It gradually continues with tubule.

2) Renal tubule: Renal tubule begins at the tubular pole of glomerulus. Renal tubule is about 3 cm long and 20-60 microns wide. Short constricted part of tubule just below the glomerulus is neck.Parts of the renal tubule after neck are, proximal convoluted tubule, loop of Henle and distal convoluted tubule.

A) Proximal convoluted tubule :It measures about 14 mm. It is lined by cubical cells arranged in single layers. Free borders of the cells are brush bordered. This portion of nephron lies in cortex of kidney.

B) Loop of Henle: It is U-shaped loop. It is anatomically divided into1) Descending limb of loop of Henle2) Thin walled ascending limb of loop of Henle 3) Thick walled ascending limb of loop of Henle.Variable length of loop of Henle lies in medulla. It is made of epithelial cells with variable shape in different portions of loop.

C) Distal convoluted tubule: Average length is about 4.9 mm. It is lined by cubical epithelium.

D) Collecting tubule: It is non-secretory portion of uriniferous tubule. It is collecting system. It is about 20mm long. It is lined by pale cuboidal cells. Several collecting tubules from

nephrons join to form duct of Bellini. Nephrons ultimately drain into pelvis of ureter. From here urine collects into urinary bladder. Urine is passed out into exterior through urethra.

Renal Circulation:

There are two circulations in kidney.1) Greater circulation 2) Lesser circulation

Greater circulation carries 85% of blood and lesser circulation carries 15% of blood. Renal arteries enter into kidneys through respective hilus. On or before entering the hilus, renal artery on each side divides into anterior and posterior divisions. They divide to form segmental arteries and drain into lobar branches one for each pyramid.

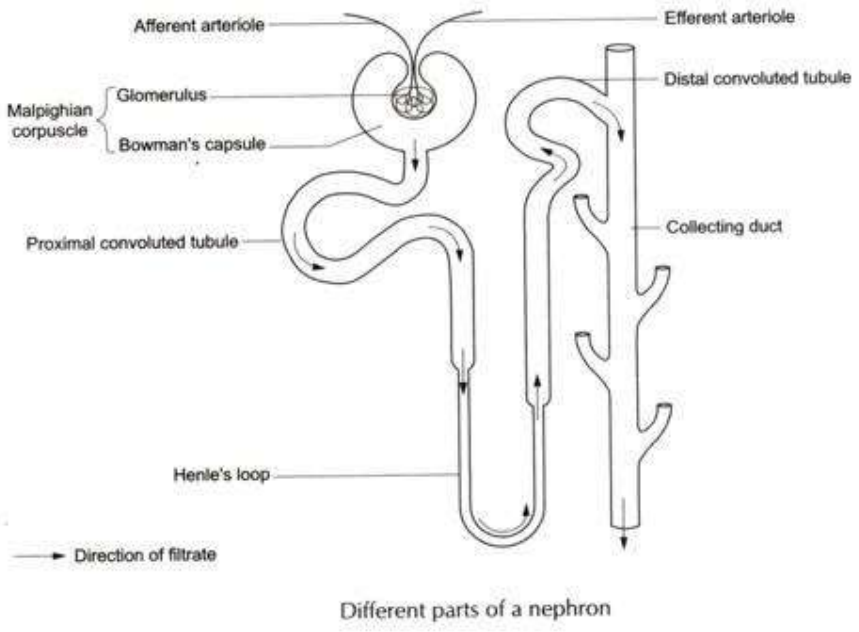


Fig.11.3 Parts of nephron

Ureters: Ureters transport urine formed in kidneys to urinary bladder.

Urinary bladder: From urinary bladder,urine is passed to the exterior through urethra.

Urethra: In males it also for passage of semen.Hence it is also called urinogenital tract in males. In females, it is independent.

UNIT – 12

BLOOD

Blood is defined as specialised fluid connective tissue of body containing blood cells suspended in plasma.

Functions of blood :

1)Transport of oxygen from lungs to tissues and carbondioxide from tissues to lungs.

2)Transport of end products of digestion absorbed from intestines to cells for utilisation.

3)Carriage of essential chemicals like hormones,vitamins and other substances to the sites of their activities.

4)Transport of waste products of cellular metabolism to the excretory organs.

5)Maintenance of acid base equilibrium.

6)Maintenance of water balance.

7)Maintenance of ionic balance.

8)Regulation of body temperature.

9)Regulation of blood pressure

10)Guarding against haemorrhage by its property of coagulation.

11)Defence mechanism by means of phagocytosis by white cells and development of antibodies.

12)Maintenance of osmotic pressure due to presence of albumin (plasma protein) etc.

Physical properties:

1) Colour : Red

2) Reaction:Slightly alkaline

3) pH:7.36-7.45(average 7.4)

4) Specific gravity : 1.048-1.066

5) Consistency :Viscous

6) Bleeding time: 2-5minutes(3.25 minutes on average) by Duke's method.

7) Clotting time:6-17 minutes

8) Prothrombin time : 11-16 secs.

9) E.S.R:0-6.5 mm/hour

Wintrobe'smethod : Average being 9.6mm/hour

Westergrenmethod :0-5mm/hour in men and 0-7mm/hour in females

10.Plasma volume:52-55%

11. Packed cell volume:45-48% { 45%in males&40% in females } .

12. R.B.C.count:Nomal average count in

Adult males -5millions/mm3

Females - 4.5millions/mm3

Infants-6-7millions/mm3 -value

Foetus -7-8millions/mm3

13. Haemoglobin:14-18g %

Males 11.5-16.5g %

Females 13.5-19.5g%

Infants 11-13g%

Children upto 1year 11.5-14.5g%

Children of 10-12years

14.Total count of white cells:

4000-1000/mm3 in adults,

1000-25000 at birth,

6000-18000 for age group of 1-3years,

6000-15000 for 4-7age group

4.5-13.5 thousand for 8-12 years age group.

Platelet count:2.5-4.5L/mm3

Composition of blood: Blood is composed of two parts.

1)Plasma - It is aqueous solution of various organic and inorganic constituents and also acting as suspending medium for blood cells.It constitutes about 55% of whole blood. It contains91-92% water and 8-9% solid components.Solid components of plasma are,

i)Inorganic constituents: They constitute 0.9%.They are sodium, potassium, calcium, magnesium, phosphorous, iron and copper etc.

ii) Organic constituents of plasma:They are Proteins, Nonproteinous nitrogen(NPN) substances, Carbohydrates, Lipids, Pigments.

2)Cells - Different types of blood cells having diverse functions are suspended in plasma.

They constitute about 45% of whole blood. There are three types of blood cells.

a) Red blood corpuscles (RBC) or erythrocytes.

b) White blood corpuscles (WBC) or leukocytes.

c) Platelets or thrombocytes.

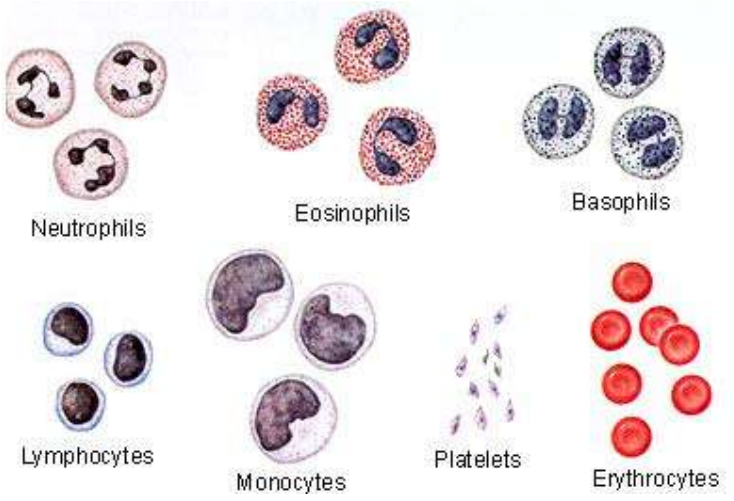


Fig 2.1 Types of blood cells.

Blood gases: They are oxygen and carbon dioxide.

Forms of Blood:

Forms of blood used as specimens in diagnostic testing procedures are,

a) Whole blood

b) Plasma

c) Serum: It is supernatant fluid collected after retraction of clotted blood

d)Cells

Plasma- It is supernatant fluid collected from blood after sedimentation of cells. It contains fibrinogen and prothrombin whereas serum does not contain fibrinogen and prothrombin.

Plasma proteins and their functions:

Plasma proteins are

1) Albumin -It is responsible for exerting 80% of total Osmotic Pressure exerted by plasma proteins.

2) Globulin - They are responsible for viscosity and blood pressure of blood and they are attributed to their higher molecular weight and asymmetry. They also play important role in body's defence mechanism(Immunoglobulins).

3) Prothrombin- It plays role in blood clotting.

4) Fibrinogen - It plays role in blood clotting. It is converted to fibrin during clotting. It is also concerned with erythrocyte sedimentation rate.

Increased fibrinogen levels raise sedimentation rate of blood cells by accelerating rouleaux formation.

Other functions of plasma proteins :

1) Acting as reservoir.

2) Helping in CO_2 carriage by formation of carbamino proteins.

3) Transport of hormones, enzymes, clotting factors, iron, copper etc.

4) Protein binding of certain drugs helps their transport.

Red blood corpuscles (RBC):

Structure of RBC : Mature RBC of human being is circular biconcave disc shaped and not containing nucleus.It appears like a dumbell on viewing from side. It appears light brownish under microscope. Hemoglobin is present inside of RBC.

Size: 7.2 μ diameter and similar to the size of an RBC.

Composition of RBC:

Water : It is the major component constituting about 60-70 %,.

Solids :They constitute about 30%- 40%. They are haemoglobin,protein, phospholipid ,cholesterol,cholesterides etc.

Erythropoiesis: Erythropoiesis is the process of formation of RBC.

There are two theories of origin of RBC.

They are 1) Theory of intravascular origin 2) Theory of extravascular origin.

1) According to extravascular theory, erythropoiesis begins with haemocytoblast.

2) As per intravascular theory, erythropoiesis beings with endothelial cells.

In foetal life, sites of synthesis of RBC are liver and spleen. After birth, erythropoiesis takes place in all bones. As age advances, most of the bones except upper end of humerus and femur stop erythropoiesis.Flat bones like ribs, vertebrae, skull, sternum, pelvis etc. continue to produce blood cells.

Stages of development into normal erythrocyte as per extravascular theory are as follows Heamocytoblast ------->Proerthyroblast ------> Early normoblast -----> Intermediate normoblast --->Latenormoblast -----> reticulocyte -----> Normal erythrocyte.

Stages of development into normal erythrocyte as per intravascular theory are Endothelial cell ----->Megaloblast -----> Early erythroblast ----->Late erythroblast ---->Normoblast ----> Reticulocyte ----->Normal erythrocyte.

Requirements for erythropoiesis: 1) Vitamin B12 and Folic acid are essential.

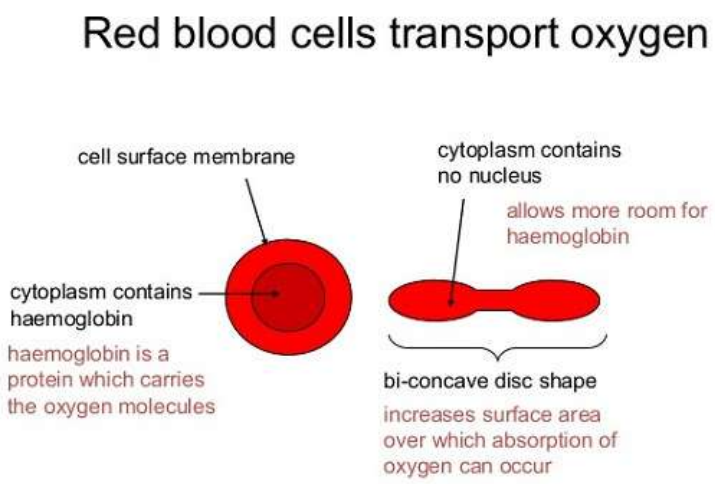


Fig. 2.2 Diagram of RBC

Life span of R.B.C:120days

Fate of R.B.C :

i) At the end of life span,erythrocytes disintegrate.

ii) RES swallows and digests the fragments.

iii) Haemoglobin is broken down into haem and globin.

iv) Globin breaks into amino acids.

v) Haem breaks into iron and non iron residue of haemoglobin i.e. protoporphyrin.

vi) Iron is stored as ferritin and haemosidrin.

vii) They are used up in haemoglobin synthesis.

viii) Green coloured biliverdin is formed from protoporphyrin and reduced to yellow coloured bilirubin or haemoglobin is formed first and oxidised to biliverdin.

Method of counting RBC:

Instruments required: Improved Neubauer's haemocytometer,diluting fluid,cover slip and microscope are required for counting RBC.Haemocytometer consists of special glass slide(counting chamber),RBC pipette and WBC pipette.

ii) Blood is sucked upto a mark on the stem of RBC pipette and diluting fluid is sucked upto mark above the central bulb.One drop of diluted blood is loaded into the counting chamber under cover slip,focussed under microscope

iii). Red cells in the 5small squares(four corners and central) of central counting area are counted.Number of cells per cu.mm can be got by multiplying the number of cells counted with dilution factor and dividing this figure with the volume of total small squares counted.

Functions of RBC :

1) RBCs involve in transport of respiratory gases - O2 and CO2 and thus help in respiration.

2)Buffering action of Hb of RBC helps in acid-base equilibrium.

3)Ionic balance is maintained by special permeability of cell membrane.

4)Maintenance of viscosity

5)Haemolysis gives raise to various pigments - bilirubin, biliveridin etc.

Haemoglobin : Haemoglobin is the red pigment of blood. It is respiratory pigment of blood. It has two parts.

1) Haem (4%) -Metalloporphyrin containing iron in ferrous form

2) Globin (96%) - Protein

Varieties of haemoglobin: There are two varieties of haemoglobin in man.

1) HbF (Foetal haemoglobin)

2) HbA (Adult haemoglobin) Foetal haemoglobin has greater affinity for oxygen. It releases CO2 more readily.

Factors favouring synthesis of haemoglobin:

1) Proteins of high biological value

2)Metals like iron, copper, manganese and cobalt

3)Hormones - Thyroxine

4)Vitamins - B12, Vitamin-C,folic acidriboflavin,nicotinic acid, pantothenic acid and pyridoxine

Estmationion of Haemoglobin :

1)Sahli'sacid hematin method

2)Cyanmethoaemoglobin method

3) Gasometric method

4) Chemical method

5)Tallqvist method

6) Specific gravity method

Functions of haemoglobin:

1)Transport of respiratory gases - O2 and CO2

2)Maintenance of pH

3)Maintenance of Ion balance

4)Formation of pigments of bile, urine and stool

Disorders related to RBC and Haemoglobin:

Anisocytosis : Variation in size of RBC is called anisocytosis.Macrocytes are red larger than normal size are called macrocytes .Microcytes are red cells smaller than normal size are called microcytes.

Poikilocytosis : It is condition in which there is deviation in shape from normal.Eg: Ovalocytes, tear drop cells, target cells and pencil cells.

Polycythemia : It is clinical condition in which erythrocyte count is above normal. In this condition there is also raised Hb content above normal and elevated haematocrit.

Anaemia : It is reduction in RBC count or Hb content or both below normal. There are different types of anaemia.

1.Anaemia caused due to blood loss:

a) Acute post haemorrhagic severe blood loss and causes iron deficiency anemia.

2. Anaemia caused by haemolysis (Haemolytic anaemia): RBC formation is abnormal making them fragile. Different types of haemolytic anaemia are,

i) Sicklecell anaemia - Red cells are sickle shaped.

ii) Hereditory spherocytosis - Red cells are small and spherical.

iii) Thalasaemia- RBCs contain HbF and thus fragile.

In haemolytic anaemia, there may be jaundice along with anaemia due to increased formation of bile pigments from lysing red cells.

3) Aplastic anaemia – It is due to failure of bone marrow itself, exposure of X-rays or γ rays, cancer of bone marrow etc. It is of normochromic, normocytic type.

4. Nutritional anaemia-

a) Pernicious anaemia - It is macrocytic anaemia and is due to Vit B12, and folic acid deficiency.It is also called megaloblastic anaemia as maturating factors converting megaloblasts (proerythroblasts) into erythrocytes are deficient.

b) Iron deficiency anaemia – It is microcytic anaemia and is due to inadequate intake of hypochromic iron or enhanced requirement of iron.

Microcytic Anemia (<76 fL)	Macrocytic Anemia (>98fL)	Normocytic Anemia (76-98 fL)
1) Iron Deficiency anemia	Vit. B12 & folate deficiency	Vit B2 & B6
2) Thalassemia	Alcoholism	Hemolytic Anemia
3) Sideroblastic Anemia	Acute Blood Loss	Post Hemorrhagic Anemia
4) Lead Poisoning Anemia	Liver disease	Sickle Cell Anemia
5) Chronic Disease Anemia	Aplastic Anemia	Anemia in Pregnancy

Fig 2.3 Causes of Anemia

Some important indices of RBC and haemoglobin:

Mean corpuscular volume(MCV)

Mean corpuscular haemoglobin(MCH)

Mean corpuscular haemoglobin concentration(MCHC) etc.

White blood Corpuscles (WBC):

They are also called leucocytes.They are nucleated and bigger than RBC. Lifespan of WBC is shorter than RBC.Origin of WBC is purely extravascular.

Classification of leucocytes:

1) Granulocytes -Granules are present in their cytoplasm. They are again three types.

i)Neutrophils or polymorphs - Nucleus is multilobed. Granules take neutral dye.

ii)Eosinophils - Nucleus is two or three lobed. Granules take eosin (acidic dye).

iii) Basophils - Nucleus is lobed.Granules take basic dye.

2)Agranulocytes- Granules are absent in the cytoplasm.They are again two types.

i) Lymphocytes a) Small lymphocytes b) Large lymphocytes

ii) Monocytes

Normal differential leucotye count-

1.Neutrophils-60-70% (3,000-6,000/mm3)

2.Eosinophils-1-4% (150-400/mm3)

3.Basophils- 0-1% (0-100/mm3)

4.Lymphocytes - 25-30% (1500-2700/mm3)

5.Monocytes-5-10% (350-800mm3)

Origin of Leucocytes: In early embryo, all blood cells originate from single primitive reticuloendothelial cell. In postnatal life, their origin is extravascular.

Granulocytes are derived exclusively from red bone marrow.

Lymphocytes and monocytes originate from spleen, lymphglands and bone marrow.

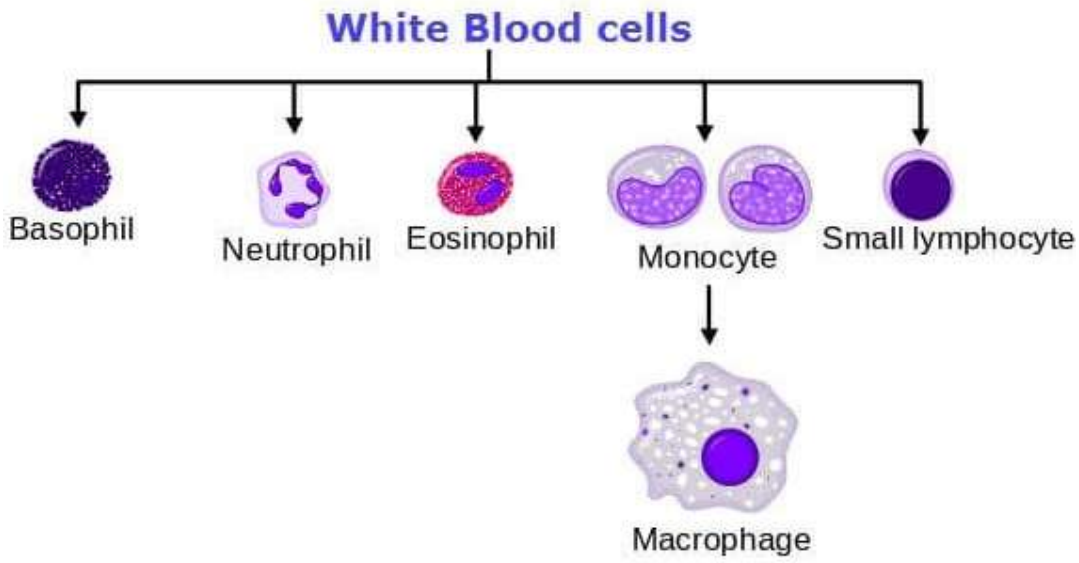


Fig. 2.4 Different types of Leukocytes

Life span of WBC:

Neutrophils 2-4 days

Eosinophils8-11 days

Basophils 12-15 days

Lymphocytes 2-3 days

Fate of WBC:

1.Granulocytes fragment in blood and subjected to monocytesphagocytic action by reticulo-endothelial cells.

2.Lymphocytes pass through intestinal and other mucosa or subjected to phagocytic action of reticulo endothelial cells

Functions of Leucocytes :

1)Phagocytosis: Engulfment of bacteria and foreign particles and their digestion by neutrophils is phagocytosis.

2)Manufacture of β and γ globulins : Important role is played by lymphocytes in body defence mechanism by manufacture of β and γ globulins.

3)Process of repair:Conversion of lymphocytes into fibroblasts in the area of inflammation helps in the process of repair.

4)Nutrition,growth and repair of tissues:Leucocytes influence nutrition, growth and repair of tissues.

5)**Prevention of intravascular clotting:**Secretion of heparin by basophils prevents intravascular clotting.

Disorders related to WBC count:

Leucopenia: Decrease in WBC count below 4,000/mm3

Leucocytosis:Raise of WBC count above 11,000/mm3

Agranulocytosis:It is great fall of circulating granulocytes. It may be due to harmful effects of certain drugs.

Leukaemia: It is malignant disease of one or more varieties of WBC.

Thrombocytes(Platelets)

i) Platelets are non-nucleated round or oval biconvex discs.

ii) Their size varies from 2-5 μ.

iii) Platelets arrange in clumps as well as discretely.

Origin of Thrombocytes: Megakaryocytes introduce pseudopodia through walls of sinusoids. They are broken in a way so that, unit membrane envelopes individual fragment. These fragments with unit membranes are washed away into blood stream. These are called as platelets.

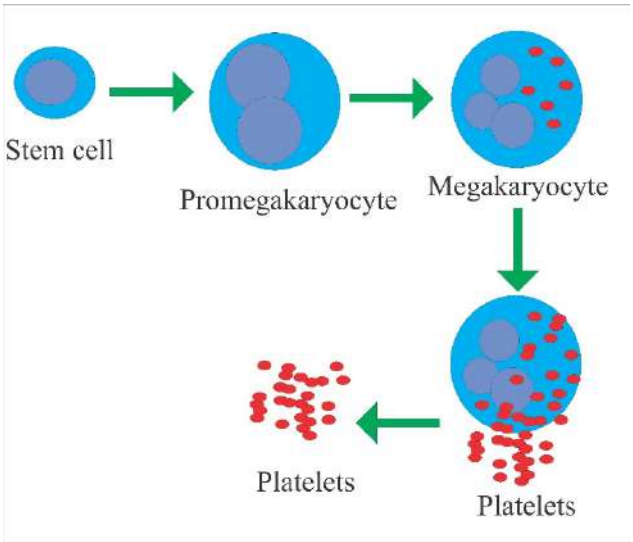


Fig. 2.5 platelet formation

Life span of thrombocytes:5-9 days

Fate of thrombocytes: They are destroyed in spleen and reticuloendothelial cells.

Functions of platelets:

1)Initiation of blood clotting by disintegration and liberation of thromboplastin.

2)Speedy repair of capillary endothelial lining.

3)Haemostatic mechanism by means of agglutination and coagulation.

4)Hastening the retraction of clot. It is dependent on thrombosthenin.

5)Liberation of 5-HT and Histamine to exert vasoconstriction which helps in haemostasis.

Thrombocytopenic purpura :

It is due to diminution of platelets in blood.There is haemorrhage beneath skin and mucous membrane.Coagulation time is normal. Bleeding time gets prolonged. Clot retraction does not take place.

Blood clotting (coagulation) and clotting factors: Coagulation of blood is important for stopping further bleeding during injuries. Clotted blood plugs the bleeding point of blood vessels thus acting as haemostat.

Mechanism of blood clotting: When bleeding starts, blood comes into contact with rough surface. Platelets disintegrate and thromboplastin is released. Damaged tissues in the area of

injury also release certain amount of thromboplastin.This thromboplastin converts prothrombin into thrombin with the help of calcium ions. Thrombin interacts with fibrinogen to form fibrin (clot).

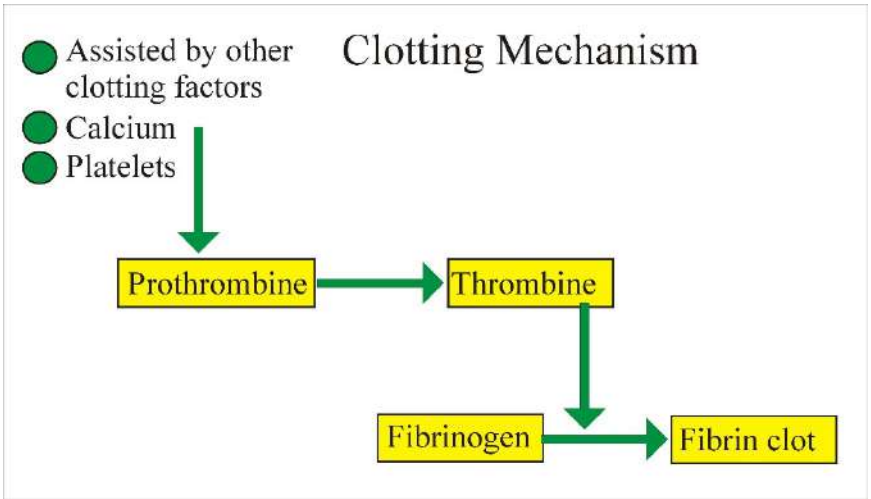


Fig 2.6 Clot Formation

Cellular elements of blood :

1) Erythrocytes (Red blood corpuscles)

2) Leukocytes (White blood corpuscles)

3) Thrombocytes (platelets)

Clotting Factors : Clotting mechanism is a complex process and several factors are involved in this process.

1)Factor–I Fibrinogen

2)Factor–II Prothrombin

3)Factor–IIITissue thromboplastin

4)Factor–IV Calcium

5)Factor–V Proaccelerin or acclerator globulin

6)Factor–VI Accelerin

7)Factor–VII Cothromboplastin

8)Factor-VIII Antihaemophilic globulin (AHG)

9)Factor-IX Christmas factor

10)Factor-X Stuart factor

11)Factor-XI Plasma thromboplastin antecedent (PTA)

12)Factor-XII Hageman factor.

13)Factor-XIII

Complete thromboplastin or thrombokinase.

Fibronogen (F-I) :It is the plasma protein converted to fibrin during clotting.

Prothrombin (F-II): It is converted into thrombin during clotting. In normal plasma, prothrombin is single compound of calcium. Prothrombin activity is measured by prothrombin time.Prothrombin is manufactured in liver. Vitamin 'K' is essential for prothromb in synthesis.

Tissue thromboplastin (F-III):Prothrombin is converted to thrombin with help ofF-III and Ca++ ion. It is also called extrinsic thromboplastin or platelet factor. It is derived from two sources.

1) Extrinsic (in tissues)

2) Intrinsic (in plasma) Calcium (F-IV) : Calcium is essential in blood clotting.

It is essential for

1) Thromboplastin formation

2) Conversion of prothrombin to thrombin.

Proaccelerin (F-IV) : Is is also called as accelerator globulin or Ac globulin or Ac G or thrombogene. It is necessary for complete conversion of prothrombin into thrombin by extrinsic or intrinsic thromboplastin.

Accelerin (F-VI) : It is hypothetical acctivation product of factor-V.

Cothrombolpastin (F-VII): It is also called stable factor or proconvertin or auto prothrombin-I. It accelerates tissue thromboplastin synthesis. It is not used up during clotting. It is converted to convertin during clotting

Antihaemophilic globulin (F-VIII) : It is also called antihaemophilic factor (AHF) or platelet cofactor-I. It helps in intrinsic thromboplastin formation and intrinsic prothrombin conversion. Absence of this factor causes haemoplilia (breeders disease). Haemophilia occurs in males. It is transmitted as sex linked recessive trait.

Christmas factor (F-IX) : It is also called plasma thromboplastin component (PTC) or platelet co factor-II. It is neccssary for intrinsic thromboplastin synthesis.It's absence causes haemophilia in males, which is transmittedas sex linked recessive trait. This factor is precipitated by 50% ammonium sulphate.

Stuart factor (F-X) : It has many properties similar to factor VII. Its absence leads to mild haemorrhagic diathesis. It is stable at R.T. It is destroyed at 560C. Plasma thromboplastin antecedent

(PTA) (F-XI) : It is activated by Hageman factor. It leads to formation of thrombin. Its deficiency causes mild bleeding tendencies of haempphiloid D type. It is transmitted as sex linked dominant to both sexes.

Hageman factor (F-XII) : It is also called surface factor. It activates enzyme kallikerin to produce plasma kinins. This results in increase of vascular permeabiliy. I also causes dilation of blood vessels.

Loki-Lorand factor F-XIII) : It is also called Fibrin stabilising factor. Its active form converts soft fibrin to hard fibrin clot with the help of Ca++. People having its congenital malformation will suffer from poor wound healing.

There are two related path ways involved in clotting.

a) Extrinsic system.

b) Intrinsic system.

Extrinsic system is initiated by damaged tissue and it is faster (1-2 minutes).

Intrinsic system is initiated by blood it self. It takes about 4-8 minutes.

Thrombus : Thrombus is a clot formed inside blood vessels. Intravascular thrombosis occurs in coronary and cerebral thrombosis.

Fibrinolysis : It is the process of breakdown of clotted blood when it is not kept sterile. It is brought about by proteolytic enzyme called plasmin. It is also called fibrinolysin.

Factors inhibiting clotting of blood:

1) Lowering of temperature.

2) Avoiding contact with rough surface

3) Removing calcium ions.

4) Precipitating fibrinogen

5) Addition of anti coagulants.

Factors accelerating clotting of blood :

1) Raising temparature (causing warmth)

2) Contact with rough surface

3) Addition of thrombin

4) Addition of thromboplastin.

5) Injection of Vitamin-K.

6) Adding CaCl2.

7) Adrenaline injection

Blood grouping: Karl Land steiner discovered fundamental principles of blood grouping in 1900.Blood grouping is important to avoid mismatching of blood groups as mismatching would cause hazards to recipient. Death also may occur in mismatched blood transfusions.

It is also needed in

i) Paternity testing,

ii)Forensic medicine,

iii) Blood diseases,

iv) Experimental purposes etc.

Blood transfusion is intravenous administration of blood to compensate blood loss in haemorrhage or otherwise.Blood transfusion corrects shock and vascular collapse.

Agglutinogens: Erythrocytes contain antigens on the surface of their cell membrane. They are chemically lipoproteins.Surface antigens of red cells are also called agglutinogens. Types of agglutinogens:They are two types - A and B

Agglutinins:Agglutinins are antibodies present in plasma or serum.

Types of agglutinins:They are two types α and β.

Haemagglutinationreactions : Reactions between agglutinogens present on cells surfaces of erythrocytes and agglutinins are called haemagglutination reactions.

These antigen - antibody reactions are made use of to detect the type of antigen.

Human blood group systems:There are nearly 300 blood group systems discovered so far.

Some of them are -1) ABO system 2) Rh system 3) MN system 4)P system Major systems are

1) ABO system of blood grouping 2) Rh system of blood grouping ABO system of blood grouping : It was discovered by Karl Land steiner in 1900. According to this system, there are four blood groups. i)A- group - containing agglutinogen 'A' on cell surfaces of erythrocytes and β agglutinin in plasma.

ii)B - group - agglutinogen B, agglutinin α.

iii) AB- group -agglutinogens 'A' and 'B', no agglutinins

iv)O- group - No agglutinogens, α and β agglutinins.

Universal donor: As O group blood can be donated to all groups, it is called universal donor. Universal recipient : As 'AB' group can receive blood from all, it is called universal recipient. Determination of blood group in ABO:Blood group can be determined using known anti A and anti B reagents. To two drops of blood taken on a slide, anti'A' is added to one drop and anti 'B' to another drop. Group is determined by aglutination in the drops.

1)A group :Agglutination takes place only in the drop of blood to which anti A is added.

2)B group: Agglutination takes place only in the drop of blood to which anti B is added.

3)AB group:Agglutination takes place in both the drops.

4)O group:Agglutination does not take place in both the drops.

Distribution pattern of blood groups in indian population.

A - 27%B - 31%AB - 8%O - 34%

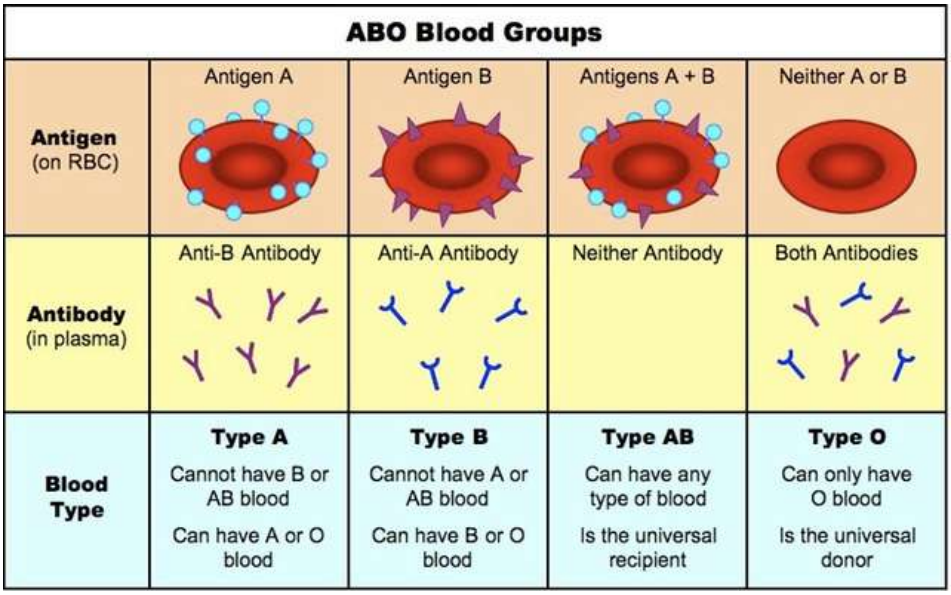


Fig 2.7 ABO blood grouping

Rh - system of blood grouping : It was discovered by Landsteiner and Wiener in 1940. Their study reveals that agglutinogen of Rhesus monkey is present in 85% of white people and 95% or even more in Indians and Ceylonese.

Rh - Agglutinogens: There are six Rh agglutinogens.They are C, c, D, d, E, e.

D and d are most common.Rh positive people contain 'D' antigen or Dd and Rh negative people contain 'd' antigen.

Rh antibodies:All the six Rh agglutinogens stimulate antibody formation,'D' antigen stimulates anti-D antibody.

Rh group determination: When anti 'D' is added to drop of blood, taken on a clean and dry side, agglutination indicates Rh +ve group and non agglutination indicates Rh -ve group.

Clinical significance of Rh grouping in blood transfusion: When Rh +ve blood (containing 'D' antigen) is given to Rh-ve recipient for the first time, anti 'Rh' antibodies develop in recipient's serum but Rh incompatibility reaction does not take place.If same

patient receives same blood for second time,incompatability reaction can take place between 'D' antigens of donor's blood and anti 'D' antibodies developed in patient's blood. Thus agglutination and haemolysis can take place in recipient's blood.

Significance of Rh factor in pregnancy : If mother is Rh -ve and Father is Rh+ve, there is strong possibility of foetus inheriting Rh+ve factor. Rh+ve cells of feotus produce anti 'Rh' antibodies in mother's blood. In the subsequent pregnancies, problem araises if the baby is again Rh +ve.

This is due to passing of anti Rh antibodies from mother's blood through plaenta into foetus, causing haemagglutination reaction. If the antibody titre is very high, foetus may die and gets expelled before end of normal gestation period. If the antibody titre is not high enough to cause death of foetus, child will be born alive and develops haemolytic jaundice. This condition is called erythroblastosis foetalis. If the case is servere, child may die if complete replacement of blood is not undertaken after birth.

Risks of blood transfusion: There is risk of transmission of Hepatitis and AIDS,if proper screening is not done prior to transfusion

Screening of blood:Donor's blood should be screened for malarial parasites,microfilariae,VDRL,HIV,hepatitis virus etc.before transfusion.

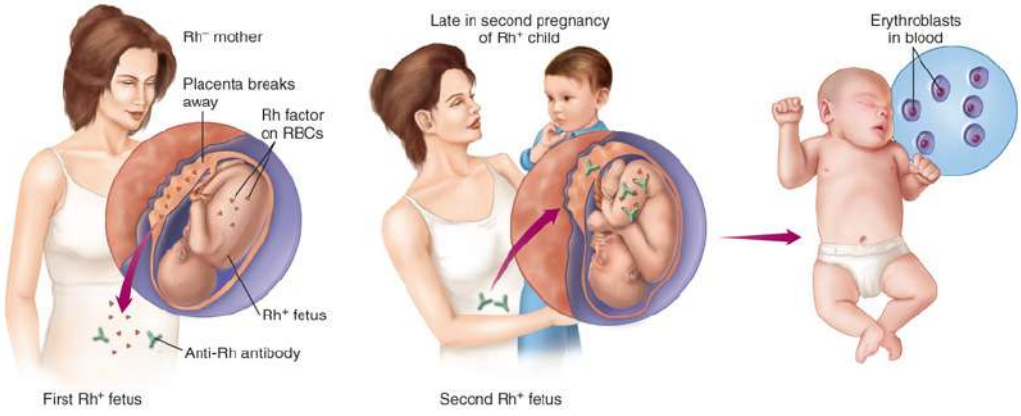


Fig 2.8 Rh incompatability

Summary

Blood is specialised fluid connective system of body. Main function of blood is transport of respiratory gases. Blood is composed of 55% plasma and 45% cells. Plasma proteins are Albumin, globulin,prothrombin and fibrinogen. Blood cells are RBC, WBC and platelets. Blood has the property of clotting when exposed to rough surface. Various clotting factors are responsible for blood clotting and they are I, II, III, IV, V, VI, VII, VIII, VIII, IX, X, XI, XII and XIII. Anticoagulants prevent blood clotting. Blood grouping is needed to present mismatching and thus hazards of incompatible blood transfusion. Different blood grouping systems are ABO, Rh, MN and P. Different goups in ABO systems are 'A', 'B', 'AB' and 'O'. According to Rh grouping Rh +ve and Rh -ve groups are there. Rh in compatibility is significant in blood transfusion and pregnancy.

Essay Questions

1)Write the functions, properties and composition of blood.

2)Discuss plasma proteins and their functions.

3)Write about coagulation, its mechanism and clotting factors.

4)Describe the structure of RBC. Write its composition, erhtyropoiesis and functions of RBC.

5)Write about Haemoglobin in detail.

6)Explain the disorders of RBC and haemoglobin.

7)What are different types of WBC? Write different aspects related to WBC.

8)Write about platelets in detail. 9)Explain blood grouping.

Short Answer Questions

1)Define blood.

2)Mention main functions of blood.

3)What are the parts of blood?

4)Write the forms of blood used as specimens in diagnosis.

5)List the protein and non-protein constituents of plasma.

6)Mention the plasma proteins.

7)Write different globlulin fractions.

8)What is the mechanism of blood clotting?

9)What are the sysnonyms of factor - IV, Factor-VIII, Factor-I and Factor XIII.

10)Write about antihaemophilic globulin.

11)Define a) Syneresis b) Thrombus

12)What are the factors inhibiting blood coagulation?

13)Mention the factors acclerating coagulation of blood?

14)What is the composition of RBC?

15)What are the stages of development of RBC according to extravascular theory?

16)Mention the functions of RBC.

17)Write the parts of haemoglobin.

18)Mention the varieties of haemoglobin.

19)What are the factors favouring synthesis of haemoglobin?

20)Mention the normal values of haemoglobin.

21)List various methods of haemoglobin determination.

22)Write functions of haemoglobin. 23)Define a) Polycythaemia b) Anaemia

24)What are different types of anaemia caused by haemolysis?

25)Write the fate of a) RBC b) WBC.

26)What is nontropical sprue? Write about size and colour of RBC in this condition.

27)Mention different types of leucocytes.

28)Write the values in total count of WBC.

29)Give any four functions of WBC.

30)Write lifespans of a) RBC b) WBC.

31)Explain structure of platelets by light microscope.

32)What are the properties of thrombocytes?

33)Give the a) lifespan &b)fate of thrombocytes.

34)What are the functions of thrombocytes?

35)Discuss thrombocytopenic purpura.

36)Mention few blood group systems.

37)What are different agglutionogens and agglutinins according to ABO system?

38)Mention different blood groups according to ABO and Rh systems.

39)How do you determine blood group by ABO system?

40)What are the antigens of Rh grouping?

41)Write the clinical significance of Rh grouping in blood transfusion.

42)What is the significance of 'Rh' factor in pregnancy?

UNIT – 13

SENSE ORGANS

The sense organsare eyes, ears, tongue, skin, and nose. Of these five organs, first four are organs of special senses. Skin is the organ of general sensations.

Theyhelp in protection of the body. The human sense organs contain receptors that relay information through sensory neurons to the appropriate places within the nervous system.

Eye: Eye is the organ of special sense of vision. It consists of i) Eyeball 2) Accessory structures.

i) Eye ball: It is almost spherical in shape. It is situated in anterior part of orbital cavity. It contains three coats and light transmitting structures.

Layers of eye ball: Layers of eye ball are,

1)Outer fibrous coat- It contains,

a) Sclera - posterior opaque part forms 5/6th of outer fibrous coat.

b) Cornea -anterior transparent forms 1/6th of outer fibrous coat.

2)Middle vascular coat - It contains,

a) Choriod is highly vascular.It forms 5/6th of middle coat. It is dark.

b) Ciliary bodyis in between choroid and iris.

c) Iris- is anterior continuation of ciliary body and is a pigmented membrane. Its central opening is called pupil. It is controlled by circular and radial muscles. Circular muscles are pupillary constrictors and radial muscles are pupillary dilators.

3)Inner nervous coat –It is also called as retina and is the innermost nervous coat of eye ball. It contains special structures called,

a) Rods -Rods are for critical vision and cones for dim vision. Each retina contains 120 million rods.

b)Cones - They are for reception of light. Each retina contains 6 million cones.

Optic disc: It is the point where optic nerve leaves eye ball. This point doesnot contain retina and thus it is photo insensitive. It is also called blind spot.

Macula: It is a small area of retina. It is also called yellow spot situated opposite to the centre of pupil lateral to the entrance of optic nerve. It is for focussing near vision.

Conjuctiva: It is the thin stratified mucous membrane covering the exposed part of eye ball

Light transmitting structures of Eye ball:

1. Aqueous humour-It is the fluid present in anterior chamber of eye.

2. Lens – It is behind iris and pupil. It is trhe organ of refraction of light onto retina.

3. Vitreous humour –It is jelly like fluid in between lens and retina. It is responsible for maintenance of shape of eye.

Accesory structures of eye:

i)Eyebrows: They are arches of thick skin present over the eyes and containing thick hairs.

ii) Eyelids: They are musculocutaneous layer like structures infront of eye. Upper eyelids are bigger than lower eyelids. Eye lashes are hairs projecting from eyelids.

iii) Lachrymal apparatus:It consists of,

a) Lacrimal gland situated in the lateral end of upper eyelid.

b) Lacrimal duct through which tears come out.

c) Lacrimal sac

d) Nasolacrimal duct through which tears flow into nasal cavity.

Extrinsic muscles of eye: There are six muscles moving the eyeball. They are,

I. Four straight muscles
   1) Superior rectus- upward movement of eye.
   2) Inferior rectus- downwards movement of eye.
   3) Medial rectus- inwards movement of eye.
   4) Lateral rectus- outwards movement of eye.

II. Two oblique muscles.

5) Inferior oblique- upward and outward movement of eye.

6) Superior oblique- Downward and out ward movement of eye.

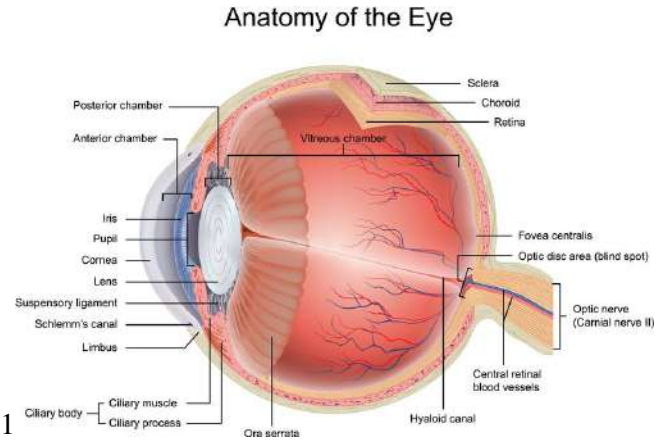


Fig 13.1

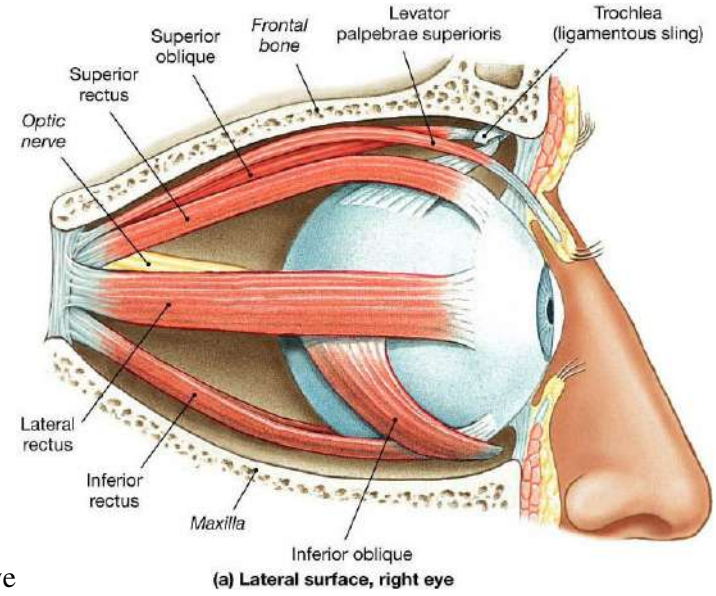


Fig 13.2Muscles of Eye

.

Ear: Ear is the organ of special sense of hearing. It is also responsible for equilibrium. It is divided into three parts.

Parts of ear:

1) External ear : It lies outside the skull. It contains two parts.

i) Pinna –It is funnelshaped organ made of fibroelastic cartilage. It is the organ of collection of sound waves.

ii) External auditory meatus –It is small channel of about 3cm length.It is lined with skin and wax creating glands. Itsinner part is closed by a thin membrane called tympanic membrane or ear drum.This canal is the organ of conveyance of vibrations of sound to the tympanic membrane.

2) Middle ear: It lies inside the skull. It is a small cavity in the temporal bone,internal to tympanicmembrane. It contains air.It contains,

i. Fenestra ovalis (oval window) and fenestrarotundum (round window).

ii. Eustachian tube - It communicates with nasopharynx.It helpsin equalisation of pressure on both sides of tympanic membrane.

iii. Auditus –It is a channel connecting middle ear posteriorly with mastoid antrum of temporal bone.

iv. Auditory ossicles - Malleus, incus and stapes arranged across middle ear. These are minute bones of middle ear and are bound by ligaments.They vibrate as a single unit when sound waves impinge on tympanic membrane.

3. Internal ear: It contains,

i. Bony labyrinth –It is present in petrous portion of temporal bone.

ii. Membranous labyrinth –It lies with the bony labyrinth.

Fluids of Internal ear : Perilymph is the fluid of bony labyrinth. Endolymph is the fluid of membranous labyrinth.

Structures of bony labyrinth : Bony labyrinth contains vestibule, cochlea and 3 semicircular canals. Vestibule is present between vestibule and semicircular canals.Cochlea is the organ of hearing and semicircular canals for equilibrium.

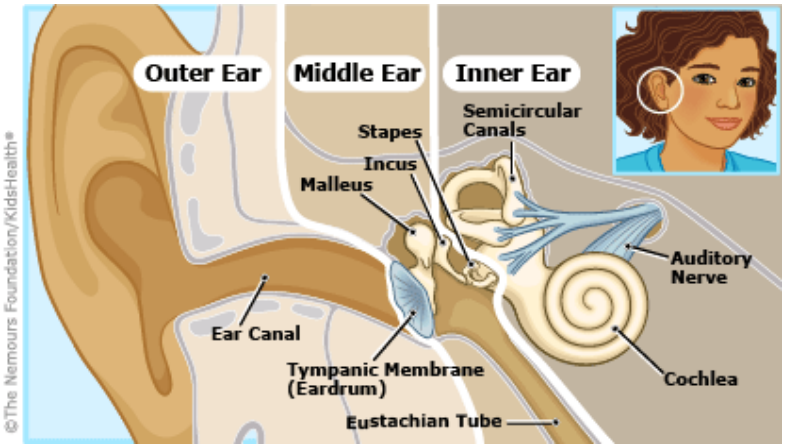


Fig. 13.3 Parts of ear

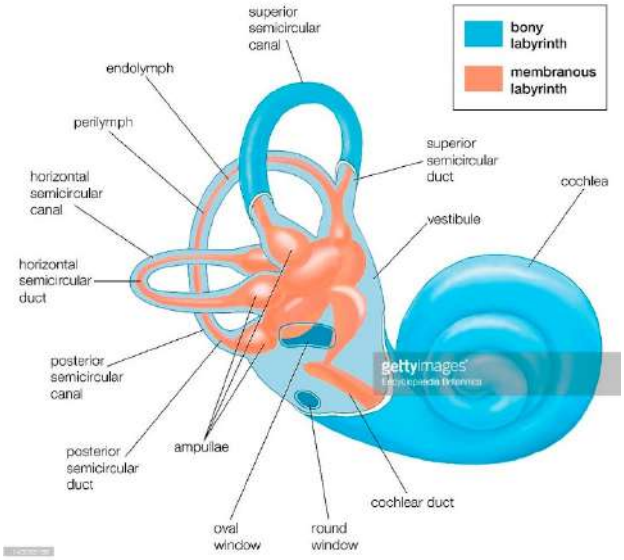


Fig. 13.4 Semi-circular canals

3. Tongue

Tongue is the organ containing taste buds.Taste buds are receptors of special sensation of taste.Epithelium of tongue is modified into papillae and taste buds.Taste buds are located on the sides of papillae.There are four types of taste buds based on sensation of taste - bitter, sour, salt and sweet.

Branch of facial nerve innervates anterior 2/3rds oftongue.Glossopharyngeal nerve innervates posterior 1/3rd of tongue.

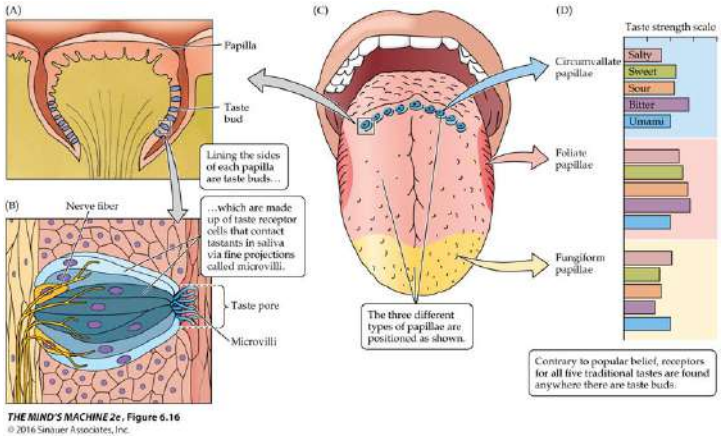
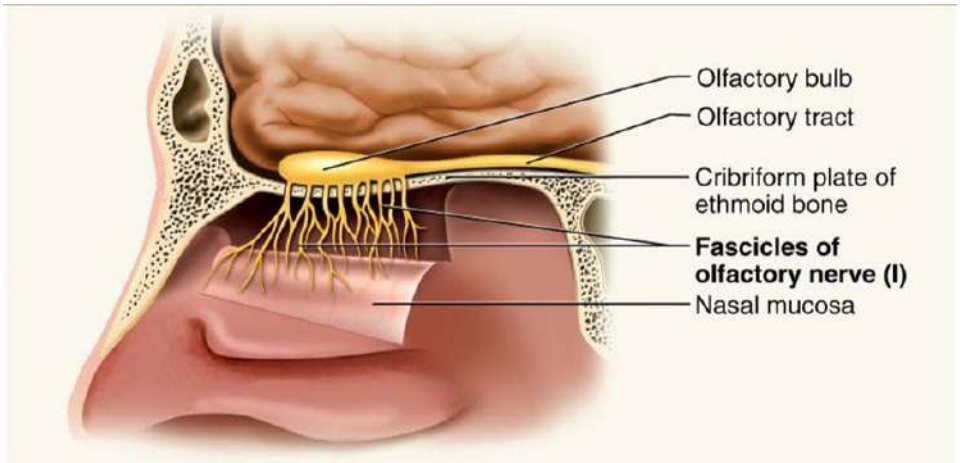


Fig. 13.5 Taste bud

4. Nose (olfactory receptors)



13.5 olfactory bulb

Olfactory receptors are specialised bipolar nerve cells present in the olfactory area of mucous membrane of upper part of nasal cavity. They are about 10-20millions in man.They receive sensation of smell. Ends of olfactory receptors join to form olfactory nerve (1stcranial nerve). Olfactory nerve passes through root of nose and ends in olfactory bulb. Olfactory bulb is connected to olfactory centre in the cerebrum through olfactory tract.

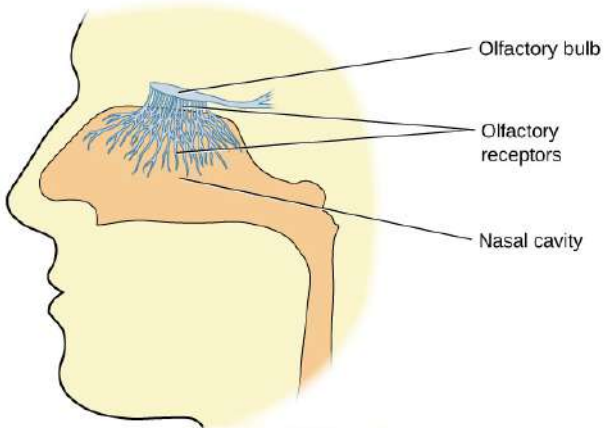


Fig. 13.6 Olfactory receptor

5. Skin

Itconsists of two layers. They are i) Superfial Epidermis ii) Deep Dermis

i) Epidermis: It is made of stratified epithelium. It has following layers,

a) Stratum corneum – It contains scale like cells. They have keratin protein and these cells are constantly replaced.

b) Stratum lucidum – It is a glistening layer.

c) Stratum granulosum – It is made of spindle shaped cells. They have granules in their cytoplasm.

d) Stratum germinatum – It is made of cuboidal cells. Multiplication of skin cells takes place in this layer.

e) Stratum basalis - Itcontains melanophorecells containing melanin pigment.

ii) Dermis: Dermis is deep layer of the skin. Itcontains arterial and venous capillaries, sensory nerve endings, sweat glands and sebaceous glands, roots of hairs, erector pili muscles (Hair straightening muscles).

Various sensory nerve endings of skin are encapsulated in connective tissue and are responsible for receiving various sensations. They are as follows.

1.Meissner's corpuscles – Mechano receptors for touch or light pressure

2.Pacinian Corpuscles – Mechanoreceptors for deep pressure

3.Krause's end bulbs - Thermoceptors for temparature

4.Some naked nerve fibres - Nociceptors for pain, itch, excess pressure etc

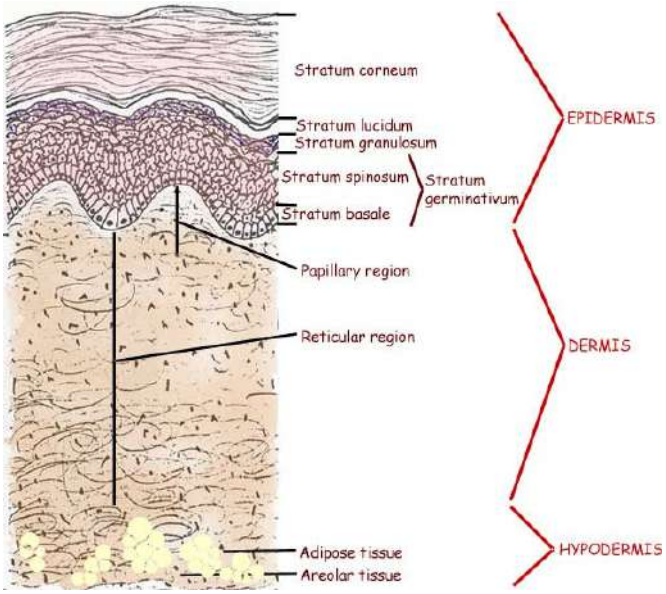


Fig 13.7 skin

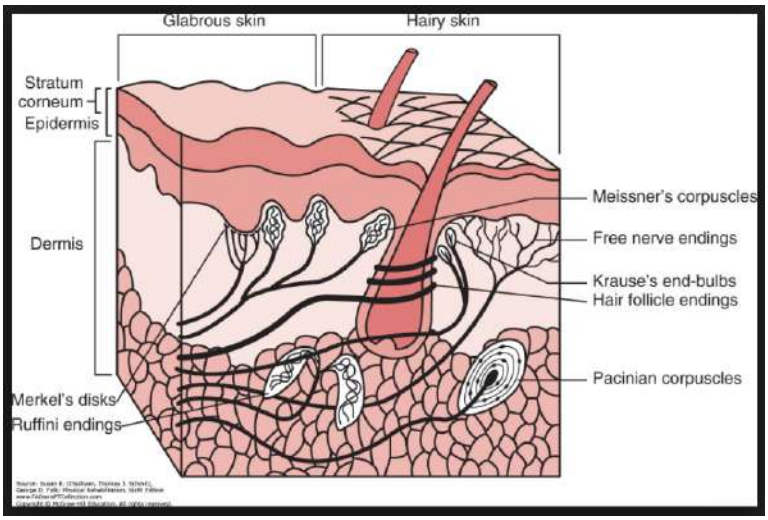


Fig. 13.6 Superficial receptors of skin

Summary

Eye,ear,tongue and nose are special sense organs. Of these five organs, first four are organs of special senses. Skin is the organ of general sensations..

Eye consists of Eye ball and accessory structures. Ear consists of external ear,middle ear and internal ear.Tongue consists of taste buds surrounded by stratified squamous epithelium and taste buds. Olfactory receptors of nose are present in the mucous membrane of upper part of nasal cavity.Skin consists of epidermis and dermis. Epidermis contains stratum corneum,stratum lucidum,stratum granulosum,stratum germinatum and stratum basalis. Several receptors of skin are mechanoceptors, thermoceptors, nosiceptors and naked nerve endings etc.

Questions

Essay questions

1. Write the structure of Eye, draw the diagram and label.

2.Discuss the Anatomy of ear with diagram.

3.Write in detail about the Anatomy of Tongue and draw diagrm .

4.Describe the layers of skin. Draw diagram.

Short answer questions

1.Mention organs of special senses ?

2.What are the layers of Eyeball ?

3.List the accessory structures of Eye ?

4. Write briefly about choroid, ciliary body and iris ?

5.Explain (a) Blindspot (b) Macula

6.What are the light transmitting structures of eyeball ?

7.Name the parts of lachrymal apparatus.

8.Mention the three main parts of ear.

9.What are the parts of external ear ?

10.What are the parts of middle ear.

11.Write the main parts of internal ear.

12.Write the auditory ossicles (bones of middle ear).

13.Mention types of taste buds.

14.Define (a) Taste buds (b) Olfactory receptors

15.What are layers of epidermis ?

16.What are components of dermis ?

17.Name different sensory receptors of skin ?

MEDICAL LAB TECHNICIAN

I YEAR

PART B – VOCATIONAL SUBJECTS

PAPER – I BIO CHEMISTRY – I

BLUE PRINT

PERIODS/WEEK **PERIODS/YEAR: 110**

TIME SCHEDULE, WEIGHTAGE & BLUE PRINT

S.No	NAME OF THE UNIT	No. Of Periods	Weightage in marks	Short answer questions	Essay/ Problem questions
		Theory			
01	Metabolism	10	8	1	1
02	Instrumental methods of Biochemical Analysis	10	6	-	1
03	Separation Techniques	10	8	1	1
04	Nutrition	10	4	-	2
05	Immune Assays	10	4	-	2
06	Distribution of Water & Electro lights	10	8	1	1
07	Liver Function Tests	10	8	1	1
08	Kidneys Function Tests	15	6	-	1
09	Gastric & Pancreatic Function Tests	5	8	1	1
10	Thyroid Function Tests	10	4	2	-
11	Clinical Enzymology	5	6	-	1
12	Body Fluids	5	8	1	1
13	Animation & Quality Control of Biochemistry Labs. Usages of Computer in Labs	5	6	-	1
14	Interpretation of common Clinical Biochemistry investigation	15	8	1	1
	Total	135	68	10	

Note: The question paper contains two sections.

Section - A contains 10 short Questions carries 2 marks each.

Section - B contains 8 Long Questions carries 6 marks each.

The Student has to answer all questions in Section A & any 5 questions in Section B.

MEDICAL LAB TECHNICIAN

I YEAR

PART B – VOCATIONAL SUBJECTS

PAPER – II MICROBIOLOGY& PATHOLOGY

BLUE PRINT

PERIODS/WEEK **PERIODS/YEAR: 110**

TIME SCHEDULE, WEIGHTAGE & BLUE PRINT

.No.	Name of the unit	No. Of Periods	Wight age In marks	Short answer questions	Essay / Problem questions
	MICROBIOLOGY				
I	**Historical introduction to Microbiology**	4	2	1	
II	**Microscopy**	05	6		1
III.	**Sterilization and disinfection–classification**	06	6		1
IV.	**Cleaning, drying &Sterilization of Glass ware**	5	2	1	
V.	**Morphology and classification of Bacteria**	10	6		1
VI.	**Methods of Collection of Clinical Specimen**	**5**	**2**	**1**	
VII.	**Processing of clinical specimen collected**	**10**	**8**	**1**	**1**
VIII.	**Composition and preparation of staining reagents and different methods of**	**10**	**6**		**1**
IX	**Culture Media–Classification of Media, Composition, preparation**	**10**	**2**	**1**	

	PATHOLOGY				
I	**Urine Analysis:**	10	6		1
II	**Preparation of reagents, procedures, principles**	5	2	1	
III	**Sputum Analysis**	10	2	1	
IV	**Semen Analysis**	5	2	1	
V.	**Body fluids–Collection**	10	2	1	
VI.	**Hematology**	20	8		1
VII.	**Disposal of hospital waste**	5	6		1
VIII.	**Glass slides, cover slips– Cleaning&**	5	2	1	

Note: The question paper contains two sections.

Section - A contains 10 short Questions carries 2 marks each.

Section - B contains 8 Long Questions carries 6 marks each.

The Student has to answer all questions in Section A & any 5 questions in Section B.

MEDICAL LAB TECHNICIAN

I YEAR

PART B – VOCATIONAL SUBJECTS

PAPER – III ANATOMY AND PHYSIOLOGY

BLUE PRINT

PERIODS/WEEK **PERIODS/YEAR: 110**

TIME SCHEDULE, WEIGHTAGE & BLUE PRINT

S.No.	NAMEOFTHEUNIT	No. Of Periods	Weight age in marks	Short answer	Essay/ Problem
1.	**Introduction to Human Anatomy and Physiology,**	12	2	1	
2.	**Cell–Definition and properties**	6	2	1	-
3	**Tissue–Classification**	6	2	1	-
4	**Respiratory system**	6	6		1
5.	**Digestive system**	6	8	1	1
6	**Structure of heart**	5	6		1
7	**Lymphatic system.**	5	2	1	
8	**Bones &Joints.**	5	6	-	1
	Nervous system–CNS	5	4	2	-
10	**Excretory system–Urinary system& Physiology of execration**	5	6	-	1
11	**Endocrine system**	5	6		1
12.	**Reproductive system-**	5	6		1
13	**Sense organs-Eye ,ear,**	5	2	1	
14	**Blood**	5	8	1	1
15	**Stress, types and its regulation methods**	5	2	1	
	Total	135	68	10	8

MODEL QUESTION PAPER

MEDICAL LAB TECHNICIAN

I YEAR

PAPER – I BIOCHEMISTRY – I

Time: 3 Hrs **Max.Marks : 50**

SECTION - A

Note: (i) Answer all the Questions
(ii) Each Questioncarries2marks 10 x 2 = 20

1. Give the normal values of Blood sugars.
2. Define solution.
3. Mention the different methods of Blood collection.
4. What are the different types of Urine specimens? Give examples of urinary preservatives.
5. Explain the terms a) Solute b) Slvent.
6. What are the hygroscopic substances? Give examples.
7. Expand GTT and give the normal values of serum uric acid.
8. Write the source of Vitamin-A .And write the diseases caused by its deficiency.
9. Write the names of water soluble vitamins.
10. What is diabetes

SECTION - B

Note: (i) Answer Any five Questions
(ii) Each Questioncarries6marks 5 x 6 = 30

11. Give the classification of Lipids and write the biological importance.
12. Write the determination of Blood glucose using GOD-POD method.
13. Give an oteon different types of Glassware used in Bio-chemistry lab. Write the applications.
14. Describe the prevention, safety and first-Aid in lab accidents.
15. Write the principle and construction of Electrical Centrifuge. And give the applications.
16. Define Colorimetry. Describe the construction, operation and uses of colorimeter.
17. What is Phlebotomy? Describe the collection of venous blood.
18. Write the principle, construction and applications of Spectrophotometer.

MODEL QUESTION PAPER

MEDICAL LAB TECHNICIAN

I YEAR

PAPER – II MICROBIOLOGY & PATHOLOGY

Time: 3 Hrs **Max.Marks : 50**

SECTION - A

Note: (i) Answer all the Questions
(ii) Each Questioncarries2marks 10 x 2 = 20

1. Mention the names of different body fluids.
2. Define Anuria, olaguria and poly urea.
3. Write the names of Bile salts and Bile Pigments.
4. Name acid fast bacteria
5. Write about a) Glycosurea b) Hematuria.
6. What is Liquification time of Semen?
7. Define Sterilization and disinfection
8. Name reagents used in Gramstaining
9. Write the principle of Compound Micro-scope
10. Write the contributions of Antonyvan Leeuwenhoek & Louis Pasteur to Microbiology

SECTION - B

Note: (i) Answer Any five Questions
(ii) Each Questioncarries6marks 5 x 6 = 30

11. Explain about hanging drop preparation
12. Describe the Estimation of ESR. Give the normal values of and write the clinical importance.
13. Write the Qualitative determination of Urine sugar. And write the clinical importance.
14. How do you count WBC. Write its clinical significance
15. Describe the construction & operation of compound Microscope and write the applications.
16. What are the different methods of Sterilization? Describe the construction and operation of Autoclave
17. Write a note on Media for Blood cultures and Anaerobic media. Write the composition & preparation of Zeil Nelsons stain.
18. Estimation of Hemoglobin by Sahli's method.

MODEL QUESTION PAPER

MEDICAL LAB TECHNICIAN

I YEAR

PAPER – III ANATOMY & PHISIOLOGY

Time: 3 Hrs **Max.Marks : 50**

SECTION - A

Note: (i) Answer all the Questions
(ii) Each Questioncarries2marks 10 x 2 = 20

1. Define Anatomy & Physiology
2. Mention the varieties of tissues in our body
3. Write the functions of saliva
4. Define cell name any two cell organelles
5. What is alveoli
6. What is Neuron? Write the parts of neuron
7. What are the endocrine Glands
8. Write the composition of urine
9. Mention the parts of female reproductive system
10. Name the hormons of thyroid gland

SECTION - B

Note: (i) Answer Any five Questions
(ii) Each Questioncarries6marks 5 x 6 = 30

11. Draw the Neat and labeled diagram of heart and explain coronary circulation
12. Write the classification of Bones with examples and mention the functions of Bones
13. Explain about Physiology of Respiration
14. What is stress? Explain types of stress and regulation tips
15. Draw the labeled structure of stomach and explain the functions of liver
16. Write the composition of blood and functions of blood
17. Explain the structure of uterus with neat label diagram
18. Draw neat diagram of urinary system with labeling, explain the formation of urine