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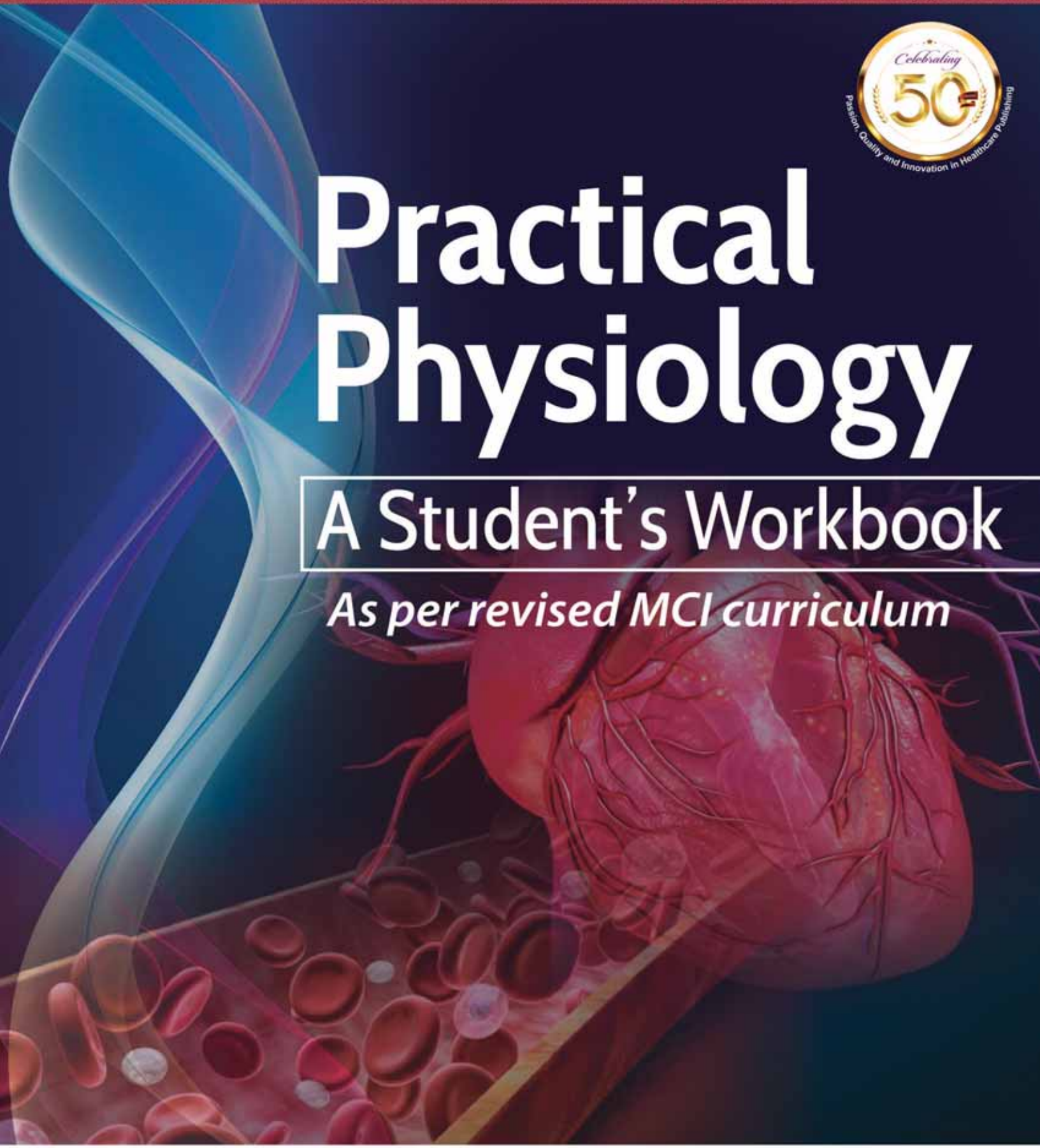

Practical Physiology

A Student's Workbook

As per revised MCI curriculum

VP Varshney
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JAYPEE

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CHAPTER 1.1

The Compound Microscope

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AIM

To study the compound microscope.

THE COMPOUND MICROSCOPE

Antonie van Leeuwenhoek (1632–1723) invented the compound microscope.

The compound microscope magnifies the image of an object that is not visible to the naked eye to an extent where it can be seen clearly. These are of two types:

1. **Monocular microscope:** It has only one eyepiece (Fig. 1.1.1A).
2. **Binocular microscope:** It is a compound bright-field microscope but having two eyepieces instead of one, so that both eyes can be used simultaneously. This prevents eyestrain (Fig. 1.1.1B).



Figs. 1.1.1A and B: (A) Monocular microscope; (B) Binocular microscope.

PARTS OF A COMPOUND MICROSCOPE

The Support System

It acts as a framework to which various functional units are attached (Fig. 1.1.2):

1. **Base:** It is a heavy metallic, U-shaped or horseshoe-shaped base or foot, which supports the microscope on the work table to provide maximum stability.

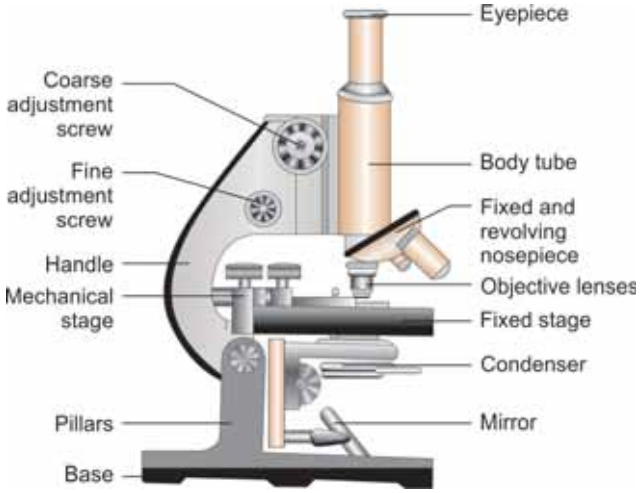

Fig. 1.1.2: Compound microscope.

2. **Pillars:** Two upright pillars project up from the base and are attached to the C-shaped handle. The hinge joint allows the microscope to be tilted at a suitable angle for comfortable viewing.

NOTE
The microscope is never tilted when counting cells in a chamber or when examining a blood film under oil immersion. It can be tilted for viewing histology slides.

3. **Handle (the arm or limb):** The curved handle, which projects up from the hinge joint, supports the focusing and magnifying systems.

Optical (Magnifying) System

It is mounted on the body tube which is in two parts:

1. **External tube:** There is a revolving nosepiece at its lower end in which are fitted three interchangeable objective lenses of different magnifications (Fig. 1.1.3).
   a. Low power objective (LP) (10x)
   b. High power objective (HP) (40x)
   c. Oil immersion objective (OI) (100x)
2. **Inner draw tube:** It has an eyepiece fitted on its top which has a magnification of 10x.

NOTE
"x" is the sign of multiplication. The magnifying power of each objective, as that of the eyepiece, is etched on it.



Fig. 1.1.3: Objective lenses.

NOTE
Parfocal system: The objectives these days are so constructed that when one lens (LP, for example) is in focus, the others are more or less in focus. Thus, switching from one lens to another (e.g. from LP to HP) requires only a little turn of fine adjustment to bring the image into sharp focus. This arrangement of lenses is called "parfocal system".

The Stage

It has three components:

1. **Fixed stage:** It is a square platform on which the slide is placed. There is an aperture in the center through which light enters to reach the object. It is fitted to the limb below the objective lens.
2. **Mechanical stage:** It is a calibrated metal frame fitted on the right edge of the fixed stage. There is a spring-mounted clip to hold the slide or the counting chamber in position. Two screw heads are present that help to move the slide from side to side or forward/backward.
3. **Substage:** It is fitted with a **condenser** and **iris diaphragm.**
   - **Condenser:** It consists of two convex lenses which condense the light rays on the object. It can be raised or lowered with the help of a screw so as to focus the light on the object. Each time the objective is changed, a corresponding adjustment must be performed on the condenser to provide proper light.
   - **The iris diaphragm:** It is fitted immediately below the condenser. It can control the amount of light reaching the object.

NOTE
In a compound microscope proper illumination includes a combination of position of light source, regulation of light intensity, position of condenser and regulation of the size of field of view.

Position of the condenser and the iris diaphragm according to the different objective lenses used is given in Table 1.1.1.

The Illumination System

The illumination system provides uniform, soft and bright illumination of the entire field of view. Two factors are involved in providing such uniform illumination:

1. Position of the condenser.
2. Size of the iris diaphragm.

Table 1.1.1: Important features of compound microscope.

Objective lens	Numerical aperture	Mirror (simple microscope)	Position of condenser	Iris diaphragm
Low power (LP) (10x)	0.25	Concave	Lowest position	Slightly open
High power (HP) (40x)	0.65	Concave	Midway	Half open
Oil Immersion (OI) (100x)	1.25	Plane	Highest position	Fully open

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The illumination system consists of:

1. **Source of light:** In most microscopes, there is a built-in light source. This unit has frosted tungsten lamp to provide uniform white light. Natural light may also be used.
2. **The mirror:** A double-sided mirror, which is plane on one side and concave on the other is located below the condenser.
   - The plane mirror is used with a distant source of light (natural or daylight). The parallel rays of light remain parallel when they enter the iris diaphragm.
   - The concave mirror is employed when the light source is from a bulb. The divergent rays of light are reflected as parallel rays and directed into the iris diaphragm.

PHYSICAL BASIS OF MICROSCOPY

Resolving Power (Resolution)

- Resolving power is the ability to show closely located structures as separate and distinct from each other.
- Generally, the resolving power of the unaided human eye is said to be between 0.15 mm and 0.25 mm.
- The resolving power of a lens depends on its numerical aperture (NA) as well as the wavelength of light.
- The resolving power of a microscope is expressed in terms of **limit of resolution (LR)**.

Numerical Aperture

The NA of a microscope objective is a measure of its ability to gather light and resolve fine specimen detail at a fixed object distance. The NA of a lens is an index of its power of resolution. As the NA increases, the resolving power of the lens increases.

NOTE

1 nanometer (nm) = 0.001 micrometer (μm) = 0.000001 mm

Magnification

The total magnification of a compound microscope is calculated by multiplying the power of objective with that of eyepiece.

Calculation of Total Magnification

With an eyepiece of 10x, the magnifications with the three objectives will be:

1. **Low power objective (10x)** = 10 × 10 = 100 times.
2. **High power objective (40x)** = 40 × 10 = 400 times.
3. **Oil immersion objective (100x)** = 100 × 10 = 1,000 times.

Image Formation in the Compound Microscope

It is the objective that starts the process of magnification. It forms a **real, inverted and enlarged** image (primary image: A' B') (Fig. 1.1.4) in the upper part of the body tube.

The field lens of the eyepiece collects the divergent rays of light of the primary image and passes these through the eye lens, which therefore the image seen by the eye is—virtual, inverted and highly magnified image. The light rays reaching the observer's eye are divergent and about 25 cm in front of the eye. Figure 1.1.4 shows the ray diagram of a compound microscope.

Working Distance

The working distance is the distance between the objective and the slide under study. This distance decreases with increasing magnification. It is 8–13 mm in LP, 1–3 mm in HP and 0.5–1.5 mm in OI lenses, respectively. Figure 1.1.5 shows the approximate working distances for each lens.

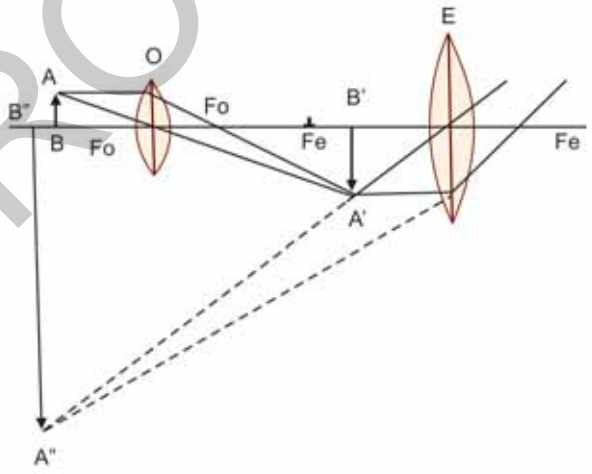

Fig. 1.1.4: The ray diagram of a compound microscope. AB = object; A' B' = real, inverted and magnified image; A" B" = virtual, inverted and magnified image; O = objective lens; E = Eyepiece; Fo = Focus of objective; Fe = Focus of eyepiece.

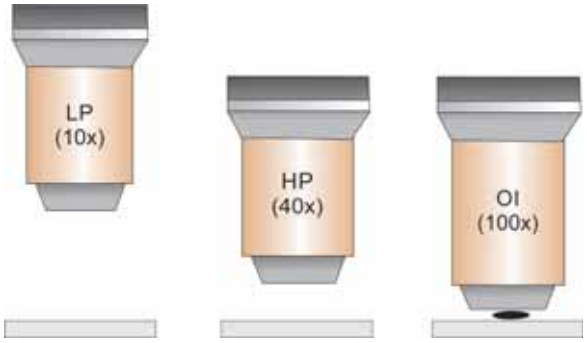

Fig. 1.1.5: Diagram to show the working distances of low power (LP), high power (HP) and oil immersion (OI) lenses.

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FOCUSING OF AN OBJECT UNDER MICROSCOPE

Focusing Under Low Power (10x)

1. Place the microscope on your worktable in an upright position and raise the body tube 7–8 cm above the stage. Put the slide on the stage and, using the mechanical stage, bring the specimen over the central aperture.
2. Select and adjust the mirror (plane or concave) so that the light shines on the specimen.
3. Under LP before focusing the object, the condenser is brought to the lowest position and the iris diaphragm is slightly opened to cut down excess light.
4. Looking from the side and using the coarse adjustment, bring the body tube down so that the LP lens is about 1 cm above the slide. Now look into the eyepiece and gently raise the tube till the specimen comes into focus. When the image comes into focus, scan the entire field, racking the fine adjustment all the time.

Focusing Under High Power (40x)

1. For focusing under high magnification, simply rotate the nosepiece so that the HP lens clicks into position.
2. The iris diaphragm is partially opened.
3. The condenser has to be slightly raised midway to get maximum clarity in focusing. Use fine adjustment as required.
4. Look from the side and bring the lens down to about 1–2 mm above the slide. Now look into the microscope and raise the tube slowly and gently till the image comes into focus.

Focusing Under Oil Immersion (100x)

1. Place a drop of cedar wood oil on the slide.
2. Rotate the nosepiece so that the oil immersion lens comes above the slide. It will automatically dip into the oil drop.
3. The condenser has to be raised to its highest position and iris diaphragm should be fully opened.
4. Use the fine adjustment for focusing. When you move the slide, the oil will move with it.

NOTE

"Racking the Microscope"

The cells and their constituents are three-dimensional structures and lie at different levels. Therefore, it is important not to keep a fixed focus but **to continuously "rack" the microscope** by using fine adjustment after the specimen has been brought under focus under any magnification.

COMMON DIFFICULTIES ENCOUNTERED BY STUDENTS

1. **The material cannot be focused or the image is very faint:**
   a. The slide may not be near the focus of the objective or there may be no visible material under it. Check this out and start with coarse adjustment once again.
   b. The slide bearing the material may have been placed upside down on the stage. The thickness of the glass slide does not allow the OI lens to reach down to its working distance. Reversing the slide will solve the problem.
2. **There may be a dark shadow in the field:** If the shadow rotates when the eyepiece is rotated, remove it and clean it or there may be an air bubble in the cedar wood oil.
3. **The field of view appears oval instead of round:** This problem arises when the objective has not been properly "clicked" into position.
4. **The illumination of the image is poor:** Check the source of light, angle of the mirror, the position of the condenser and iris diaphragm.
5. **The image does not come into focus even when the objective is in the lowest position and the fine adjustment cannot move down any further:** This happens when the fine adjustment screw reaches the end of its thread (turn) before the image is brought to its focus. To overcome this problem, turn the adjustment screw in the opposite direction for several turns and then use the coarse adjustment screw to regain the focus once again (It is therefore, best to keep the fine adjustment screw near the middle of its turning range).

PRECAUTIONS

1. Students using glasses should put on their glasses while using the microscope.
2. While using one eye with the monocular microscope, do not close the other eye as this will cause lot of strain on that eye.
3. Ensure that all the lenses are clean and free from dust.
4. Check the position of the objective, condenser and diaphragm to ensure optimal illumination.
5. Never lower the optical tube while looking into the microscope.
6. Once a specimen has been focused, continuously "rack" the microscope.
7. Clean all the lenses after using the microscope. Never leave cedar wood oil on the OI lens.
8. Carefully use the fine adjustment screw to obtain the exact focus while using high power and oil immersion lenses.

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9. Use xylene swab to clean the objectives after using the microscope.

OTHER TYPES OF MICROSCOPES

Various types of microscopes have been specially introduced for particular purposes over the past many decades. Some of these are:

1. **Dissection microscope:** It is a binocular microscope used for microdissection under magnification.
2. **Dark-field microscope:** It employs a special condenser that causes light waves to cross on the material under study rather than passing through it. As a result the field of view appears dark (hence called "dark-field" in contrast to "bright-field" microscopy) against which the object appears bright.
3. **Phase-contrast microscope:** In this microscope, a special phase plate is inserted into the condenser, which can retard the speed of some light waves. Since the tissue cells and organisms have different refractive indices, this microscope uses these differences to produce an image with good contrast of light and shade.
4. **Interference-contrast microscope:** A special prism that can split a beam of light is added to the condenser to produce a three-dimensional image.
5. **Polarizing microscope:** It has a polarizer (filter), which is usually placed between the light source and the specimen and an analyzer, which is located between the objective and the eyepiece.
6. **Fluorescence microscope:** A fluorescent dye is used to stain tissues which are then studied under this microscope.
7. **Transmission electron microscope (TEM):** TEM uses a strong beam of electrons instead of light and electromagnetic fields in place of glass lenses.
8. **Scanning electron microscope (SEM):** This microscope, which achieves a resolution of about 30 Å, has been developed for three-dimensional study of surface topography of cells and object.

Objective Structured Practical Examination

Aim: To focus a given slide of blood film under LP/HP/OI lens.
Procedural steps: See text above.
Checklist:

1. Raise the body tube, put the slide on the stage and use mechanical stage to bring the object over the central aperture.
2. Choose the light source and correctly bring the different objective lenses into position.
3. Adjust the position of the condenser and iris diaphragm for:
   a. LP b. HP c. OI
4. Look from the side while lowering the body tube.
5. Adjust the light and use coarse and fine adjustment screws.
6. Rack the microscope constantly.

QUESTIONS

Q.1. Why is the microscope called a compound microscope? What type of image is produced by it?

Q.2. When will we use a plane mirror or a concave mirror in a light microscope?

Q.3. What is the total magnification of a compound microscope and how will you calculate it?

Q.4. What is meant by the term numerical aperture? What is its significance?

Q.5. How will you identify oil immersion objective lens? Why is cedar wood oil used with this lens and not with others?

There is a thin layer of air between this objective and the glass slide when the lens is in focus. When light passes from a denser medium (glass of the slide) into a rarer medium (the thin layer of air), they are refracted away from the normal. As a result, when light rays emerge from the slide, many of them are refracted away from the aperture of the objective and very few enter it and a faint image results. Cedar wood oil, which has the same refractive index as that of glass, i.e. 1.55 (air = 1.00; water = 1.33), removes this layer of air so that the glass of the slide and the objective lens become a continuous column (thus avoiding refraction) and allow enough light to enter the objective. **Other mediums that can be used are glycerin and paraffin, their refractive indexes being 1.35–1.40. However, cedar wood oil, though costly, gives best results.**

Q.6. Will you see any image with the oil immersion lens without the cedar wood oil?

Q.7. Why does the oil immersion lens have pinhole-sized aperture?

The aperture being very small, it allows only the central cone of light to pass through and form the image. Had the diameter been large, excessive refraction would have caused spherical and chromatic aberrations, thus making the image indistinct.

Q.8. Why should the position of the condenser be low with the LP lens and highest with oil immersion lens?

Since the aperture of the LP lens is wide, a high condenser would allow too much light to enter the microscope and cause glare. The position of the condenser with the oil immersion lens has to be highest to allow enough light to enter it through its pin-hole aperture.

Q.9. What are the factors affecting illumination when using a microscope and why?

The clarity of an image depends on an optimal (ideal) amount of light available. The illumination (the process of providing light) can be altered by raising or lowering the condenser and opening or closing the diaphragm. A proper combination of the two has to be selected under different conditions. In general, we require less illumination when viewing a clear, unstained object and greater illumination when viewing a stained preparation.

Q.10. What is meant by racking the microscope and what is its importance?

Since the cells and their components are three-dimensional entities and situated at different levels, the focus has to be constantly changed to see all these structures.

Q.11. What are the other types of microscopes?

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STUDY NOTES

Teacher's Signature

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Date .....

CHAPTER 1.2

Collection of Blood Sample

AIM

Collection of blood sample.

INTRODUCTION

Two types of blood samples are used. When small quantity of blood is required for investigations, capillary blood is used. When the large quantity of blood is required venous blood is used.

ASEPSIS

The term asepsis refers to the condition of being free from septic or infectious material—bacteria, viruses, etc. Puncturing the skin always poses the danger of infection. In order to achieve asepsis, the following aspects need to be kept in mind.

Sterilization of Equipment

All the instruments to be used for collecting blood—syringes, needles, lancets and cotton and gauze swabs—should preferably be sterilized in an autoclave. Disposable sterile needles and lancets are now in common use.

Cleaning/Sterilization of Skin

At least 2–3 sterile cotton/gauze swabs soaked in 70% alcohol, methylated spirit or ether should be used to clean and scrub the area.

NOTE

After cleaning the skin, allow the alcohol to dry by evaporation (do not blow on it), because sterilization with alcohol is effective only after it has dried.

Prevention of Contamination

Any material used for skin puncture or the operator's hands may cause contamination. Therefore, once the site has been cleaned and dried, it should not be touched again.

COLLECTION OF BLOOD SAMPLE

Capillary Blood

The skin and other tissues are richly supplied with capillaries, so when a drop or a few drops of blood are required, as for estimation of Hb, cell counts, bleeding time (BT) and clotting time (CT), blood films, microchemical tests, etc., blood from a skin puncture (skin-prick) with a lancet or needle is adequate. Capillary blood is also called **"peripheral blood"** as it comes out of the peripheral vessels (capillaries) in contrast to venous blood (Table 1.2.1).

In adults and older children, capillary blood is generally obtained from a skin puncture made on the tip of the middle or ring finger or on the lobe of the ear. In infants and young children in whom the fingers are too small for a prick, the medial or lateral side of the pad of the big toe or heel is used. The site for skin-prick should be clean and free from edema, infection, skin disease, callus or circulatory defects.

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Table 1.2.1: Differences between venous and capillary blood.

Venous blood	*Capillary blood*
It is obtained from a superficial vein by venepuncture	It is obtained from a skin puncture, usually over a finger, ear lobe/or the heel of a foot
A clean venepuncture provides blood without any contamination with tissue fluid	Blood from a skin prick comes from punctured capillaries and from smallest arterioles and venules
There is less risk of contamination since sterile syringe and needle are used	There is greater risk of contamination and transmission of disease as one may be careless about sterilization since skin prick is considered a harmless procedure
Cell counts, Hb and PCV values are generally higher	These values are likely to be on the lower side since some tissue fluid is bound to dilute the blood even when it is free-flowing
Venous blood is preferable when normal blood standards are to be established or when two samples from the same person are to be compared at different times	Capillary blood is not suitable for these purposes

(PCV: packed cell volume)

Apparatus

1. **Blood lancet/Pricking needle:** Disposable, sterile, one-time use blood lancets are commercially available and should be preferred.
2. Sterile gauze/cotton moist with 70% alcohol/methylated spirit.
3. Glass slides, pipettes, etc. according to requirements.

Procedure

1. All aseptic precautions must be taken. Clean the finger with the spirit swab.
2. Allow the alcohol to dry by evaporation for the following reasons:
   - Sterilization with alcohol/spirit is effective only after it has dried by evaporation.
   - The thin film of alcohol can cause the blood drop to spread sideways along with alcohol so that it will not form a satisfactory round drop.
     - The alcohol may cause hemolysis of blood.
3. Prick the finger sharply and quickly to a depth of 3–4 mm. The blood should start to flow slowly, spontaneously and freely (without any squeezing)—if a good prick has been given.

IMPORTANT

Do not squeeze or press the finger as the tissue fluid squeezed out will dilute the blood and give false low values. The forearm or the hand may be squeezed or milked toward the fingers to facilitate blood flow. If all efforts fail, a fresh prick may be required.

4 Wipe away the first drops of blood with dry, sterile gauze as it may be contaminated with tissue fluid.
5. Allow a fresh drop of blood of sufficiently large size (about 3–4 mm diameter) to well up from the wound and make a blood smear or fill a pipette as the case may be.
6. Clean the area of the prick with a fresh swab and ask the subject to keep the swab pressed on the wound with his/her thumb till the bleeding stops, which occurs in a minute or so.

Precautions

1. The selected site should be clean, free from infection, edema or skin disease.
2. The site should be cleaned with sterile gauze and alcohol.
3. The lancet/needle should be sterile.
4. The puncture should be deep enough to give free-flowing blood but not so deep that it takes inordinately long time for the bleeding to stop.
5. Do not press or squeeze the finger to increase the blood flow.

Earlobe Prick

With the use of a sterile needle or corner edge of a sterile blade give a 2-mm deep prick (The skin here is usually thinner than at the fingertip). Wipe away the first drop and allow a new one to form.

Pricking the Heel

In infants and young children, blood can be collected from the cleaned medial or lateral areas of the heel.

NOTE

The central plantar and the posterior curvature areas of the heel should be avoided as the prick may cause injury to the underlying tarsal bones which lie near the surface.

Venous Blood

When larger amount of blood are needed as for complete hematological and biochemical investigations, venous blood is obtained with a syringe and needle by puncturing a superficial vein.

NOTE

Venous blood is always preferred for clinical tests.

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Apparatus

Keep the following equipment ready before venepuncture:

1. Disposable gloves.
2. Sterile, disposable, 10 mL syringe with side nozzle.
3. 10 mL test tubes/vials with or without anticoagulant.
4. Sterile gauze pieces moist with 70% alcohol/methylated spirit.
5. Tourniquet: A 2–3 cm wide elastic bandage with Velcro strips to keep it securely in place can be used. An inflated BP cuff can also be used.

Procedure

1. Compress the upper arm to make the veins prominent. The antecubital vein is embedded in subcutaneous fat and is usually sufficiently large to take a wide-bore needle. It also runs straight for about 3 cm and is usually palpable—even in obese subjects.
2. Apply the tourniquet about 2–3 cm above the elbow to obstruct the venous return. The subject may open and close the fist to increase the venous return and make the veins engorged (filled) with blood.
3. Clean the skin over the selected vein with gauze and alcohol and allow it to dry.
4. Puncture the skin and push in the needle under the skin with a firm and smooth thrust, at an angle of 15–20° to the skin.
5. Slightly pull the plunger back with your thumb and little finger to produce a little negative pressure in the syringe. Advance the needle gently along the vein and puncture it from the side, a few milliliters ahead of the skin puncture. This prevents counterpuncture of the opposite wall of the vein and formation of a hematoma (local leakage of blood).
6. As the vein is punctured, all resistance will suddenly cease and blood will start to enter the syringe. Do not withdraw blood faster than the punctured veins is filling; as too much pressure applied to the plunger is likely to cause mechanical injury and hemolysis of red cells. The subject may open and close the fist to enhance venous return.
7. When enough blood has been collected, release the tourniquet and press a fresh swab over the skin puncture. Withdraw the needle gently but keep the swab in position. Ask the subject to flex the arm and keep it so as to maintain pressure on the puncture site till the bleeding stops.

   Expel the blood gently into the container; do not apply force as it may cause mechanical injury to red cells. Gently shake or swirl the container between your palms so that the anticoagulant (if used) mixes well with the blood without frothing.

IMPORTANT

The entire process of withdrawing blood should be completed within 2 minutes of applying the tourniquet because stagnation of blood in the vein is likely to increase the cell counts.

Precautions

1. All aseptic precautions must be observed and disposable gloves, syringe and needles must be used.
2. The tourniquet (or the BP cuff) must be removed before taking the needle out of the vein to avoid formation of hematoma.
3. The blood from the syringe should be transferred to the container without delay to prevent clotting.
4. Ask the subject to keep the swab in position till the bleeding from the puncture site stops.

Arterial Blood

When arterial blood is needed for special tests, such as blood pH, gas levels, etc. an artery, such as radial or femoral is punctured with a syringe and needle. This, however, is not a routine procedure.

For a sample of whole blood or plasma: The blood is transferred to a container containing a suitable anticoagulant. This is to prevent clotting of blood.

COMMONLY USED ANTICOAGULANTS

Anticoagulants are substances employed to delay, suppress or prevent clotting of blood.

Anticoagulants for In Vitro Use

Ethylenediamine Tetraacetic Acid (EDTA)

Both the potassium and sodium salts of EDTA are strong anticoagulants. The dry (anhydrous) dipotassium salt of EDTA, being more readily soluble than the sodium salt, is the anticoagulant of choice. The tripotassium salt of EDTA causes some shrinkage of RBCs that results in 2–3% decrease in packed cell volume.

Mode of action: EDTA prevents clotting by removing ionic calcium (which is an essential clotting factor) from the blood sample by **chelation**. The platelets appear clear and are neither aggregated nor destroyed.

Preparation: EDTA is used in a concentration of 1 mg/mL of blood. 0.2 mL of 2.5% solution of the salt placed in a container and dried in gentle heat in an oven is sufficient for 5 mL of blood. This provides 1 mg of EDTA/mL of blood

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(a number of containers can be prepared from the stock solution at a time).

NOTE

Excess of EDTA (more than 2 mg/mL blood) affects all blood cells. Red cells shrink, thus reducing packed cell volume (PCV), while WBCs show degenerative changes. Platelets break up into large enough fragments to be counted as normal platelets. Care should, therefore, be taken to use correct amount of EDTA and blood should be thoroughly mixed with the anticoagulant.

Uses: Except for coagulation studies EDTA is used for most hematological tests.

Trisodium Citrate

Trisodium citrate is the anticoagulant of choice in blood tests for disorders of coagulation.

Mode of action: Acts as a chelating agent (inactivates calcium ions).

Preparation: 3.8% solution is prepared in distilled water and then sterilized.

Uses: Citrate and blood in the ratio of 1:9, is used for coagulation studies and for ESR test by the Westergren's method in the ratio of 1:4. Along with other components, sodium citrate is used for storing donated blood in blood banks (*Refer* Chapter 1.9), since it can be safely given intravenously. Oxalates are toxic and cannot be given intravenously.

Double Oxalate Mixture

This is a mixture of ammonium oxalate and potassium oxalate in the ratio of 3:2. It is thus called double oxalate. It is an effective anticoagulant.

Mode of action: Oxalates prevent clotting by forming insoluble calcium salts, thus removing ionic calcium.

Preparation: A large number of containers can be prepared at a time by placing 0.2 mL of oxalate mixture (3.0 g of ammonium oxalate and 2.0 g of potassium oxalate in 100 mL of distilled water) in each container and drying in gentle heat in an oven. This amount is sufficient for 8–10 mL of blood. Too much oxalate is hypertonic and damages all blood cells, while too little will not prevent clotting.

Uses: Though each oxalate by itself can prevent clotting, a mixture is used. Sodium oxalate should not be used since it causes crenation of red cells. Ammonium salt should not be used in urea and nonprotein nitrogen tests. It is used in the tests where the cell volume should remain unaffected, e.g. erythrocyte sedimentation rate (ESR), PCV.

Sodium Fluoride

A mixture of 10 mg of sodium fluoride and 1 mg thymol is an anticoagulant as well as a preservative when a blood sample has to be stored for a few days. Since fluoride inhibits glycolytic enzymes (thus preventing loss of glucose), it is employed when plasma glucose is to be estimated.

Heparin

Heparin, a highly charged mixture of sulfated polysaccharides and related to chondroitin, has a molecular weight ranging from 15,000 to 18,000 and is a naturally occurring powerful anticoagulant. It is normally secreted by mast cells that are present in many tissues, especially immediately outside many of the capillaries in the body. Both mast cells and basophils release heparin directly into blood. Heparin is also a cofactor for the lipoprotein lipase—the clearing factor.

Commercial heparin is extracted from many different tissues and is available in almost pure form (it was first extracted from the liver—hence the name "heparin"). Low molecular weight fragments (5,000 units) have been produced from unfractionated heparin and are being used clinically since they have a longer half-life and produce more predictable results.

Mode of action and uses: Heparin by itself has no anticoagulant activity. However, when it combines with **antithrombin III,** the ability of the latter to remove thrombin (as soon as it is formed) increases hundreds of times. The complex of these two substances removes many other activated clotting factors—IX, X, XI and XII.

Preparation: Theoretically, heparin is an ideal anticoagulant since no foreign substance is introduced into the blood. The required amount of stock solution is taken in a number of containers and dried at low heat. At a concentration of 10–20 IU/mL blood, it does not change red cell size and their osmotic fragility. It is, however, inferior to EDTA for general use. It should not be used for leukocyte counts, as these cells tend to clump. It also imparts a blue tinge to the background of blood films.

Uses: It is used for estimation of blood gases, pH assay and osmotic fragility. Clinically, it is used to prevent intravascular clotting of blood.

Acid-citrate-dextrose and Citrate-phosphate-dextrose-adenine

Acid-citrate-dextrose (ACD) and citrate-phosphate-dextrose-adenine (CPD-A) are the anticoagulants of choice for storing donated blood in blood banks (*Refer* Chapter 1.9). The CPD-A mixture is preferred as it preserves 2, 3-diphosphoglycerate (2, 3-DPG) better.

Anticoagulants for In Vivo Use

The two in vivo anticoagulants are heparin and coumarins. Patients at increased risk of forming blood clots in their

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blood vessels, e.g. leg veins during prolonged confinement to bed or during long flights, are sometimes put on these drugs (e.g. warfarin) to prevent thromboembolism. Their bleeding time, clotting time and prothrombin time (PT) are checked from time to time to adjust the dosage of the drug.

Dicoumarol and Warfarin

The coumarin derivatives are vitamin K antagonists and thus inhibit the action of this vitamin that is essential as a cofactor for the synthesis of six glutamic acid-containing proteins—namely, factors II (prothrombin), VII, IX and X, protein C and protein S. The action of this anticoagulant is, however, slower than that of heparin.

Heparin

It is particularly used during open-heart surgery in which the blood has to be passed through a heart-lung machine; or the dialysis machine during hemodialysis in kidney failure and then back into the patient.

IMPORTANT

Anticoagulant therapy should not be confused with thrombolytic agents employed for dissolving blood clots (*Refer* Chapter 1.10).

QUESTIONS

Q.1. What measures are taken to prevent infection during venepuncture and skin-pricking?
Q.2. What are the sources and main differences between venous blood and capillary blood? Why is capillary blood called peripheral blood?
Q.3. What is the difference between plasma and serum? How will you get a sample of each?
Q.4. What precautions will you observe to prevent hemolysis of venous blood sample?
Q.5. What are anticoagulants? What is meant by *in vivo* and *in vitro* anticoagulants?
Q.6. Why are the thumb and little finger not pricked?
Q.7. What are the sites for collecting capillary blood in infants?
Q.8. Why should the pricked finger not be squeezed? What is meant by free-flowing blood and why should it be preferred over squeezed blood?

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STUDY NOTES

Teacher's Signature

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ISBN 978-93-89188-32-5