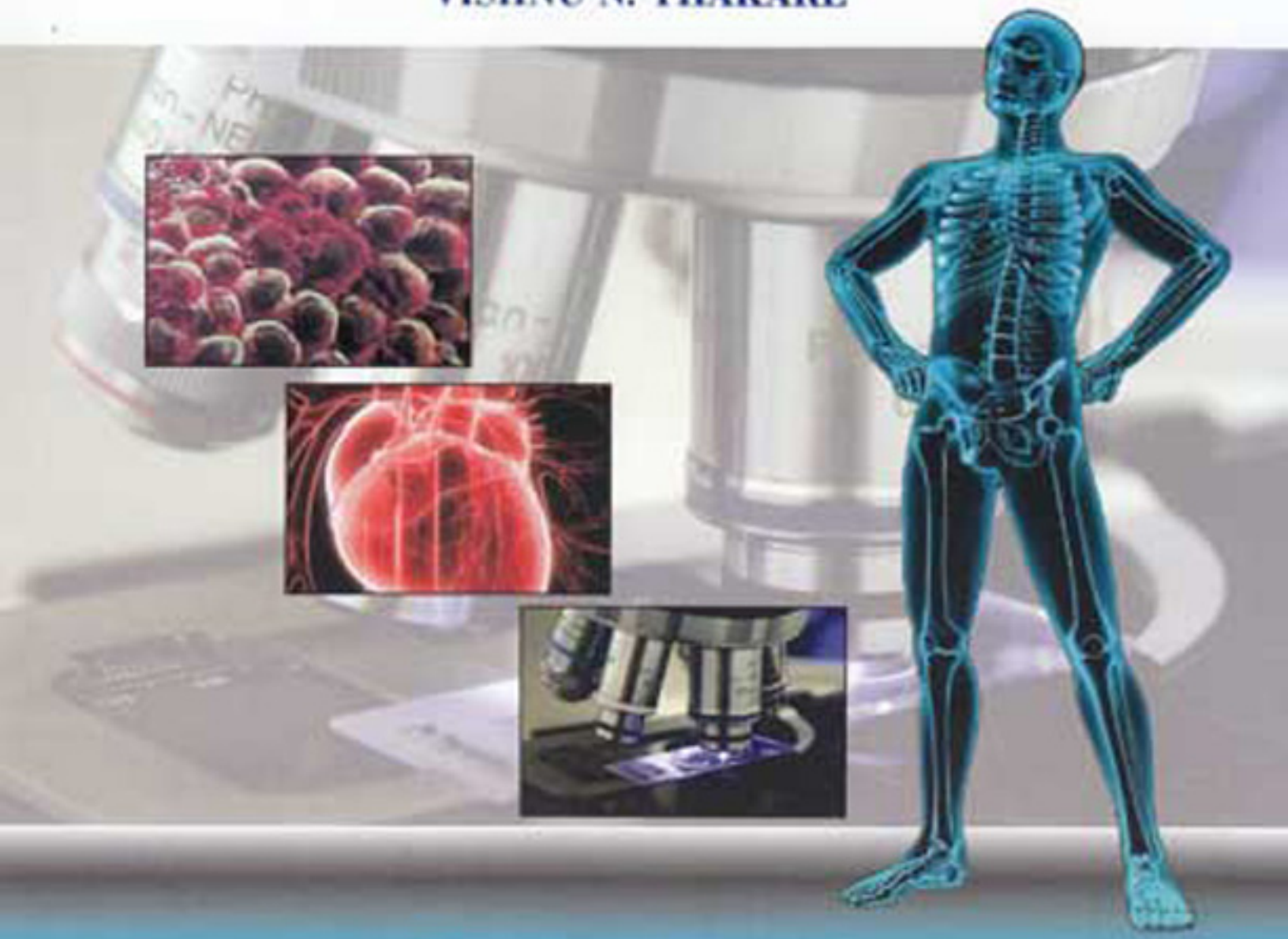
HUMAN ANATOMY AND PHYSIOLOGY

AN EXPERIMENTAL HANDBOOK

VISHNU N. THAKARE



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HUMAN ANATOMY AND PHYSIOLOGY

An Experimental Handbook

For
B. Pharm Students

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Preface

It is with great pleasure that I introduce the book **"Human Anatomy and Physiology – An Experimental Handbook"**. The book allows for the lucid understanding of human anatomy and physiology, which is extremely necessary for the clear understanding of the pathophysiology of various disorders/diseases and effects or actions of drugs. This book is a sincere attempt to explain the basics of experimental human anatomy and physiology in a simple and interesting manner and as per the syllabus prescribed for the first year B. Pharm students by the University of Pune. This book imparts insight into each experiment along with its clinical and pathological significance. Questions that could be asked for viva-voce are also included at the end of each experiment.

All efforts have been made to keep the text error-free and to present the subject in a student friendly and easy to understand manner. However, any suggestions and constructive comments would be highly appreciated and incorporated in the next edition.

Vishnu N. Thakare

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Vishnu N. Thakare

Contents

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EXPERIMENT NO. 1

STUDY OF MICROSCOPE

➢ Aim

To study the microscope

➢ Microscope

Microscope is an optical instrument which uses visible light and a system of lenses to view magnified images of very small objects. This microscope also called light microscope.

➢ Types

(a) Simple microscope – has one set of lens and low magnification.

(b) Compound microscope – has two sets of lenses and objectives with higher magnifications.

(c) Electron microscope – This microscope differs from optical microscope; in that the electrons interact with the sample to generate image instead of light acting as an illuminating source.

In general, experiment involving the study various cells, the compound microscope is used for the study of morphological characteristics of cells. Compound microscope works on the principle of formation of an enlarged image of sample / object in the plane of focus.

➢ Physical terms

(a) Compound microscope : It is an arrangement of objective and eyepiece used to magnify an object to the point where it can be seen with the human eye.

(b) Resolution : The resolution of an optical microscope is defined as the shortest distance between two points on a specimen that can still be distinguished by the observer as separate entities. Consequently it describes how small objects can lie close to each other and can still be recognisable. Resolution with human eye is around 0.25 mm, with light microscope it is around 0.25 μm and with the electron microscope it is 0.5 nm.

(c) Working distance : It is the distance between the objective lens and the specimen. The magnifications increase with decrease in working distance. The ideal working distance is 0.15-1.5 mm for the oil immersion objective, 0.5-4 mm for high power objective and 5-15 mm for low power objective.

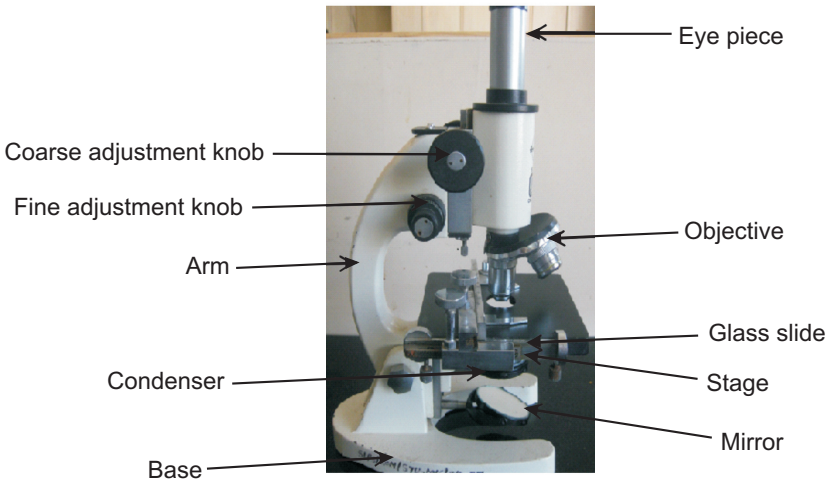

Figure 1.1 : A Compound Microscope

➢ Parts of a Compound Microscope

(1) The support system

(2) The illuminating system

(3) The magnification system

(4) The adjustment system.

1. **The Support system:** The support system consists of various parts which are described below.

   (a) Base of microscope: It is horse shoe shaped and is the base on which the microscope rests.

   (b) Pillars: Projecting upward from the base, it is joined to the handle of microscope

   (c) Handle (arm) : Handle is curved shaped or 'c' shaped and supports the magnifying and the adjusting system.

   (d) Body tube : It is the tube which houses the lenses and through which light passes to form an image.

   (e) Stage : It is a horizontal platform on which the specimen under observation is placed. Fixed and mechanical stage microscopes are generally used. The specimen is mounted on a slide which is held in place by clips that are present on the stage. The screws enable the movement of the slide sideways or vertically.

Nosepiece : It is the part of the microscope that holds the objective lenses. It is also called as a revolving nosepiece or turret. Nosepiece is attached to the lower end of the body tube and consists of objective lenses of different magnifications attached to it. The

objective of required magnitude can be focused over the specimen by moving the nose piece.

2. The illuminating system : A good illuminating system is the one which provides uniform and bright illumination of the entire field viewed under the microscope.

There are six types of illuminating systems based on which the microscope functions.

(a) Bright field microscope –the source of illumination used is white light, either external sunlight or internal tungsten filament lamp.

(b) Dark field microscope – dark field condenser is used to block scattered light

(c) Fluorescent microscope – UV lamp is used as light source.

The illuminating system of a compound microscope is composed of a light source, a condenser, and a diaphragm.

Mirror is fixed at the base and is used to reflect light from an external light source up through the bottom of the stage. As the rays of light are reflected by mirror through the condenser on the object, the object on the stage appears clear.

Condenser – the rays of light reflected by the mirror pass through the condenser located in between the mirror and stage of the microscope, then fall onto the object under examination and thus help in resolving the image. The position of the condenser needs to be adjusted with each object used in order to alter the light and to improve the resolving power of the microscope.

3. Magnification system: As the name indicates, it directly implies magnifying the image of the object under observation and comprises of the eyepiece and objectives.

(a) Eye piece: The eyepiece is a lens that fits into the top of the body. It magnifies the image formed by the objectives; generally 5x and 10x eyepieces are used. Monocular microscope uses one eyepiece; where as binocular microscope has provision for fitting 2 eyepieces. The magnification formed by the eyepiece multiplied by objective magnification gives the total magnification of the object being viewed.

Objectives : Generally these objectives are screwed into the resolving nosepiece in a compound microscope. The nosepiece is a pivot that ensures quick changes of objectives.

(1) 10X - low power objective- magnifies the image ten times greater.

(2) 40 or 45X - high power objective- magnifies images 40 or 45 times . It is used for a broad view of blood films or histological sections prior to their examination under oil-immersion objectives.

(3) Oil-immersion objective (100X) - This requires immersion oil viz, cedar wood oil. Oil is employed to increase the numerical aperture (is a ratio of the diameter of lens to its focal length) and the resolving power of the objectives. Light passes through glass at same speed as it travels through the immersion oil. Hence, the ray of light that passes through oil undergoes minimum diffraction when it passes through glass. As a result the resulting image is much clearer and sharper.

4. Adjusting system: Two adjustment systems are used

(a) Coarse adjustment-Two coarse adjustment screws are employed for coarse adjustment. These screws are mounted at the top of the handle by a double side micrometer mechanism, one on each side.

(b) Fine adjustment-Two fine adjustment screws are mounted on the handle below the coarse adjustment screws by double side micrometer mechanism, one on each side.

EXERCISE

(a) Give the principle of microscope.

(b) Enlist the various parts of a compound microscope.

(c) What is resolution? Give its significance.

(d) State the importance of condenser and iris diaphragm

(e) Give the role of illuminating system in a microscope.

(f) Give the significance of oil used in oil immersion objectives.

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EXPERIMENT NO. 2

DETERMINATION OF HAEMOGLOBIN CONTENT AND OXYGEN CARRYING CAPACITY OF BLOOD SAMPLE

➢ Aim

To determine the haemoglobin (Hb) content and oxygen carrying capacity of one's own blood sample.

➢ Apparatus

Sahli's Hellige haemoglobinometer (Figure 2.1), stirrer, micropipette (200 cubic millimeter), disposable needle (24 gauge), Pipette-having single mark 0.02 ml (20 cu nm) without any bulb, 0.1N HCL, 70% alcohol or spirit, cotton, distilled water.

Sahli's haemoglobinometer consists of comparator, tube, pipette and stirrer.

Comparator-the haemoglobinometer at the center point is provided with opening which holds the haemoglobin tube. Two non fading standard brown tubes are provided on both sides of the central haemoglobin glass tube for colour matching.

Tube- Gram% markers on one side up to 30 and % mark (20-140) on another side for easy reading.

➢ Principle

Reaction of blood with hydrochloric acid (HCl) causes the formation of hematin acid by hydrolysis of haemoglobin. Acid hematin is reddish brown in colour. This is then diluted with distilled water until its colour matches exactly with that of the permanent standard of the comparator block. Matching of sample with standard tubes gives exact concentration of haemoglobin of sampled blood.

➢ Theory

Haemoglobin (Hb) is a protein comprising of heme and globin present in RBCs, which carries oxygen and carbon dioxide. Heme portion is involved in transportation of oxygen from lungs to the tissue. Oxyhaemoglobin is the combination of oxygen (4) with one molecule of haemoglobin.

1 gram of Hb carries 1.34ml of oxygen. Haemoglobin also acts as a buffer by maintaining blood pH.

- **Normal Range**

Adult male : 14-18 gm% of blood

Adult female : 12-16 gm% of blood

In newborn : 16-22 gm% of blood

Infants : 12-40 gm% of blood

- **Procedure**

1. Take Sahli's haemoglobinometer and pipette and make sure that it is dry.
2. Fill the haemoglobinometer up to its lowest mark 10% (2 gram %) by adding 0.1N HCL with the help of a dropper.
3. Sterilise the finger tip with spirit or 70% alcohol and prick the finger tip with a sterile needle to allow free flow of blood.
4. Allow a large drop of blood to form on the finger tip, dip the tip of pipette on to the blood drop and suck up to 0.02 ml mark taking care to avoid the formation of an air bubble.
5. Transfer immediately 0.02 ml blood into the haemoglobinometer (containing 0.1N HCL) by blowing pipette.
6. Leave the solution in the tube haemoglobinometer for about 10min.
7. After 10 min, dilute the solution with distilled water, drop by drop and mix it with a stirrer. Keep adding water until colour of solution in the tube matches with standard of the comparator (while matching the two colours, take care to hold the stirrer above the level of the solution).
8. Note the reading when the colour of solution matches to the standard and express the haemoglobin concentration as gram%.

- **Precaution**

Following precautions to be taken while performing this experiment.

1. Blood should be immediately transferred from pipette into haemoglobinometer tube to prevent clotting of blood in the tube.

2. 10 min should be given after addition of blood into haemoglobinometer tube, for complete conversion of haemoglobin to acid hematin.

Result : The haemoglobin content of the blood sample was found to be …… gm%.

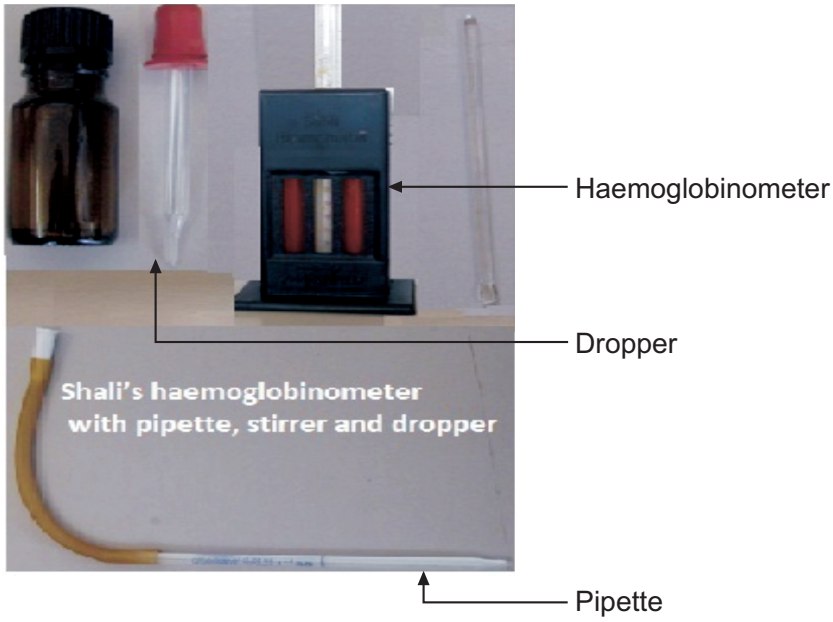


Figure 2.1 : SAHLI'S Haemoglobinometer

➢ Calculation

Observed reading of haemoglobin ------ gm%.

Percentage of haemoglobin

14.5 gms Hb = 100%.

Observed Hb value = ------ gm%.

= 100 × observed value / 14.5

= Y %

➢ Oxygen carrying capacity of haemoglobin

100% Hb = 18.5 cc of oxygen

Z = Y% × 18.5 / 100 Zcc of blood.

➢ Other various methods of haemoglobin content estimation

1. Dare's method
2. Harden's method
3. Wintrobe's method
4. Halden's method
5. Tallquist's method
6. Gasometric method
7. Spectrophotometric oxyhaemoglobin method - A cyanmethaemoglobin method
8. Specific gravity method

➢ Significance

Physiological

RBC contains Hb that forms nearly about 90% of dry weight of the cell.

RBC is called so, due to presence of red coloured haemoglobin. Reduced supply of haemoglobin to cell/ tissues leads to hypoxic condition, since haemoglobin carries oxygen to various cell/ tissues.

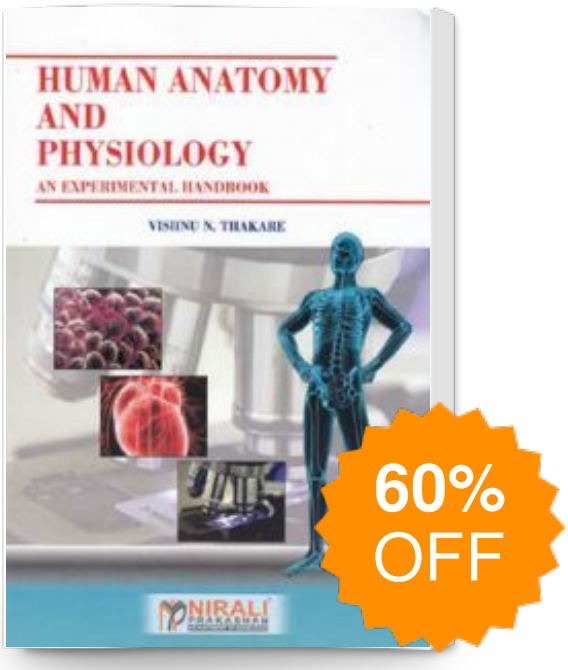
Clinical

Determination of haemoglobin content in blood will give an idea about whether the person is suffering from anaemia (a condition in which the oxygen carrying capacity of blood is reduced) causing weakness, muscle cramp or not. A person is said to be anemic if the Hb content falls below the normal range. Symptoms of anemic person are weakness muscle cramp, nausia, abdominal pain etc.

Hb content increases in the following conditions.

- High altitude
- Excessive sweating
- New born/ infants
- Diarrhoea

Human Anatomy And Physiology



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