Discipline Specific Elective Course

(DSE): ZOUELD1: Mammalian Physiology

B.Sc. V semester, Department of Zoology,

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Experiment aim: Preparation of temporary slide of squamous epithelium

Principle: Group of similar type of cells that perform a specific function, is called a tissue. Tissues are organised in a specific proportion and pattern to form different organs. Specific shape and arrangement of cells in tissues helps to identify the particular type and location of tissues. The epithelium or epithelial tissue provides a covering or lining for some parts of the body.

Requirement: Cheek tissue, beakers, glass slides, coverslips, watch glasses, dropping bottle, dropper, required stain, glycerine, NaCl solution (0.9% w/v), needle, forceps, brush, toothpick, water, wash-bottle, dissecting tray, compound microscope.

Procedure

• Rinse your mouth well with water.

• Gently scrap the inside of your cheek with the broad end of a clean toothpick. Discard this material.

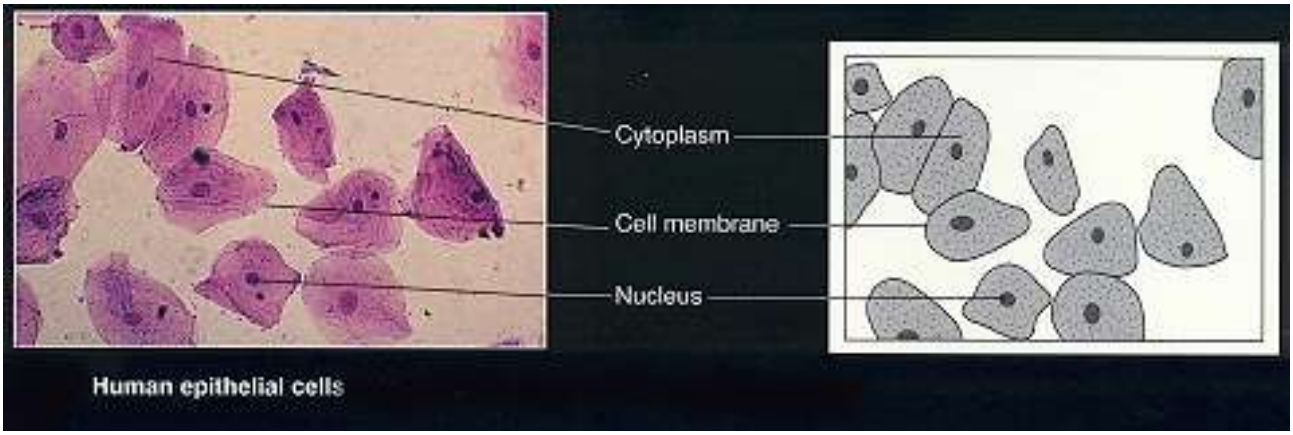
• Scrap again, and spread these cells gently on a clean slide. Add a drop of 0.9% NaCl solution or physiological saline and a drop of methylene blue with the help of a dropper.

• After two minutes, remove the excess stain and saline using the edge of a filter paper. Now, put a drop of glycerine on the cells.

• Place a coverslip over the tissue and gently press it with the back of a pencil to spread the cells.

• Examine the slide under the low power of microscope.

• Draw a labelled diagram of your preparation.



Observation

Record your observations in the tabular form given below:

Features	Observations
1. No. of cells in a focus 2. Shape of cells 3. Nature of cell boundary 4. Nucleus: present/absent- shape- location-	

Discussion:

Epithelial tissue (squamous epithelium) forms the covering tissue of the body. It covers the body surface and lines the body cavities and hollow visceral organs. It may be single or multi-layered. The lower most layer normally rests upon a non-cellular basement membrane. It is protective/sensory/absorptive/and secretory in nature and also helps in exchange and movement of materials inside the body.

Experiment aim: Preparation of temporary slide of striated muscle fibres

Principle: Group of similar type of cells that perform a specific function, is called a tissue. Tissues are organised in a specific proportion and pattern to form different organs. Specific shape and arrangement of cells in tissues helps to identify the particular type and location of tissues.

Requirement: Live material/concerned tissue, beakers, glass slides, coverslips, watch glasses, dropping bottle, dropper, required stain, glycerine, NaCl solution (0.9% w/v), needle, forceps, brush, toothpick, water, wash-bottle, dissecting tray, microscope.

***(*a) Taking out the tissue**

• Place a preserved cockroach in a dissecting tray containing water.

• Cut open the animal to expose its thigh region. (As an alternate preserved sample of striated muscle can be provided).

• Take a small piece of muscle from this region and tease it on a slide with the help of needles to get a few thinnest possible fibres.

• Wash it in water in a petridish, changing the water 2-3 times to remove the preservative, as it may interfere with staining.

(b) Staining and mounting

• Add a few drops of methylene blue to stain the muscle fibres.

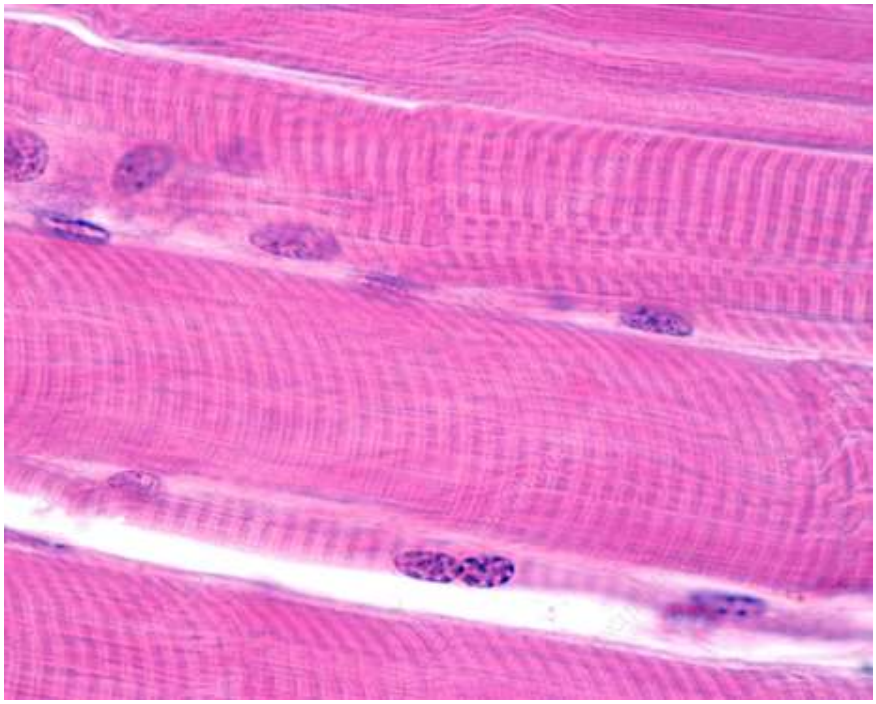
• After staining, put the muscle fibres on a slide and tease it further, if necessary, with needles so that the muscle fibres are well separated.

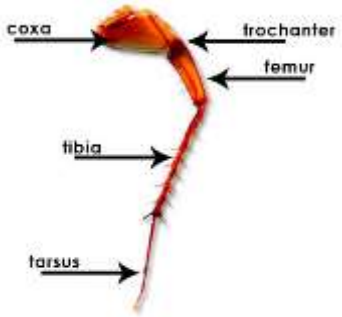
• Blot out the excess of water and stain.

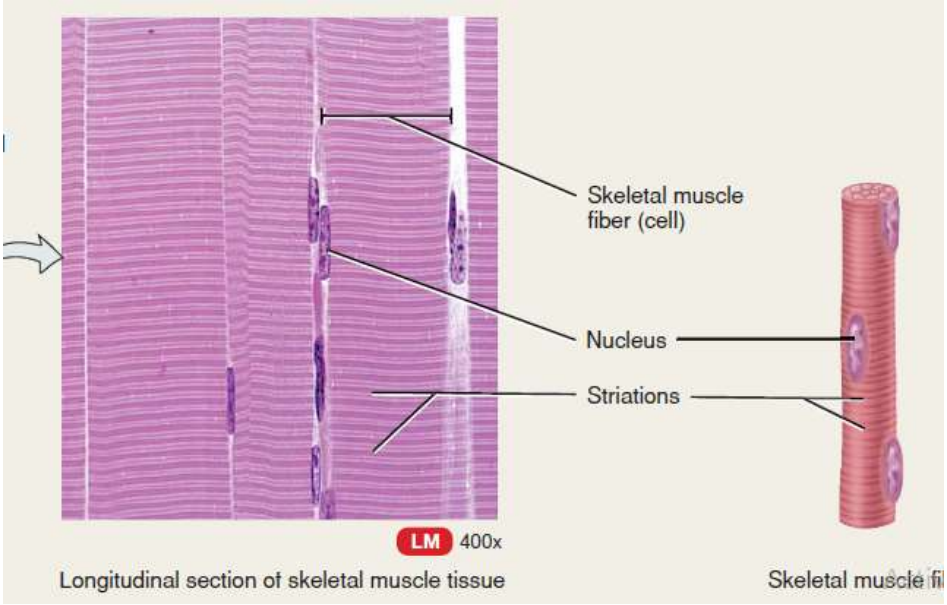
• Add a drop of glycerine on the slide and with the help of a needle gently put the coverslip and avoid the entry of air bubbles.

. Press the coverslip gently with a needle to spread the glycerine and the muscles properly.

• Examine the slide under the microscope.







Observation

• Muscle fibres are elongated, cylindrical and multinucleated (syncytium).

• These fibres are enclosed in a membrane called sarcolemma.

• Several dark and light bands are alternately arranged perpendicularly to the long axis of the fibre. Presence of these bands alternately produce striations, hence these muscles are called striated muscles.

Discussion

Striated muscles constitute the main component of musculature of our body, primarily attached to bones via tendons, and hence are also called skeletal muscles. Their contractions are voluntary in nature and thus are not controlled by autonomic nervous system.

Experiment aim: Preparation of temporary slide of connective tissue

Principle: Group of similar type of cells that perform a specific function, is called a tissue. Tissues are organised in a specific proportion and pattern to form different organs. Specific shape and arrangement of cells in tissues helps to identify the particular type and location of tissues.

Requirement: Glass slides, coverslips, watch glasses, dropping bottle, dropper, Leishman's stain, NaCl solution (0.9% w/v), needle, forceps, water, wash-bottle, microscope, 70% ethanol or spirit, cotton, glycerine.

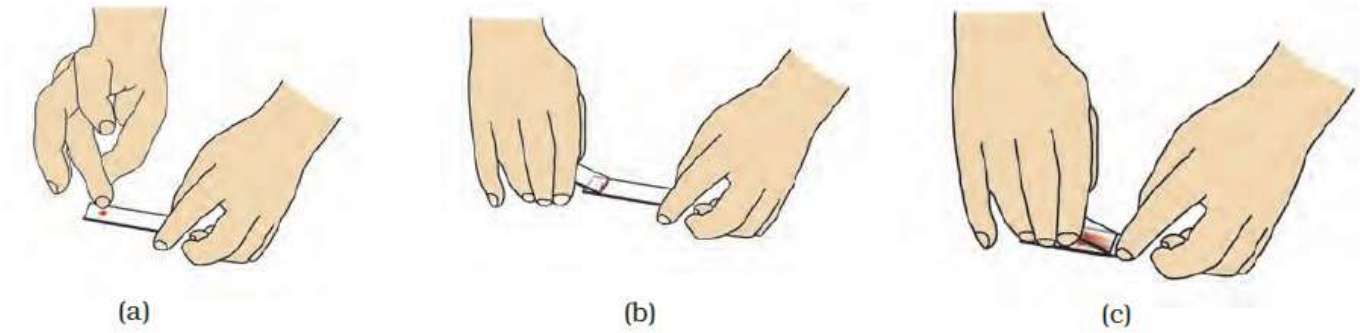
Procedure

• Clean the slides so that it become free from grease, finger prints, etc.

• Clean the tip of your middle finger with rectified spirit and prick with a sterilised needle.

• When a drop of blood appears on the finger tip, wipe it away with cotton dipped in rectified spirit.

• Press the fingertip to get the next drop of blood and touch it with the clean surface of slide (placed on working-table)



• Hold the narrow edge of another slide (2nd slide) at about 45^0 angle to the 1st slide and to the left of the drop of blood

• Pull to the right until the 2nd slide touches the blood. Wait for 2-3 seconds till the blood spreads along the line of contact. Now push the 2nd slide towards the left in a steady but brisk movement.

.Take care to keep the edge pressed uniformly against the surface of the 1st slide. Keep pushing until the other end of the slide is reached. This method spreads the blood thinly (also called a blood film) over the surface of the slide.

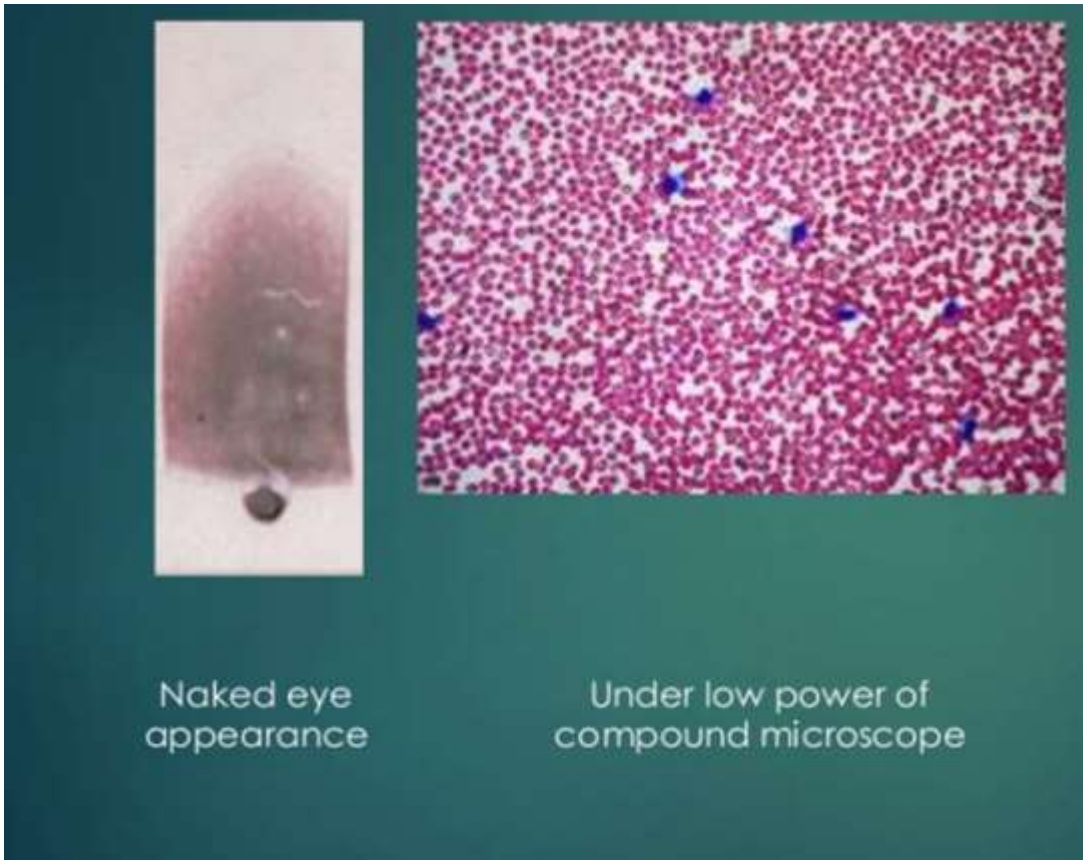
.You may make 3 or 4 such film (smear) preparations. Once the uniform smear is made, air dry the slide for about 10 minutes.

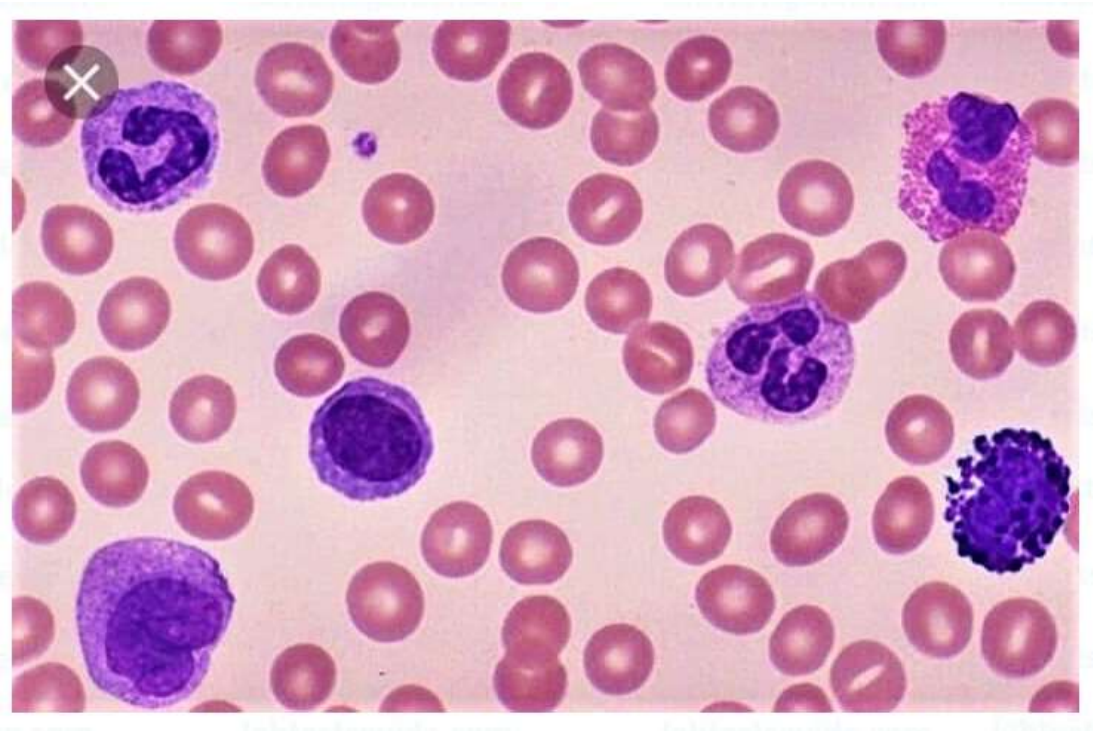
• Cover the region marked with wax pencil with few drops of Leishman's stain. Leave the stain over the smear for 2 to 3 minutes. Now, add an equal amount of distilled water gently with a dropper and leave it for 2 to 4 minutes. Repeat this process till its colour becomes light violet. Air dry the slide thoroughly and mount with a cover slip, using a drop of glycerine.

• Observe the slide under a compound microscope first under low magnification and then at higher magnification.

Observation

The observation under the microscope showed red blood corpuscles (RBC's) of human blood. RBCs (erythrocytes) are biconcave, circular and non-nucleated. Various types of white blood corpuscles (WBC's), the eosinophil's, the basophils and neutrophils were also seen.





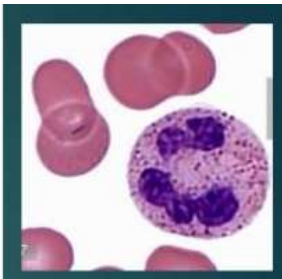
Under high magnification

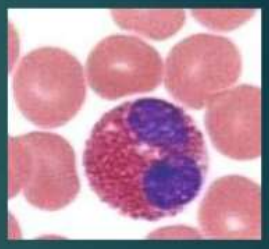
Precaution

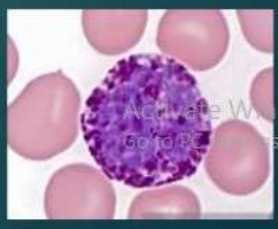
(i) Every student is required to use separate sterile needle (preferably disposable) to avoid any infection.

(ii) Do not allow the stain to dry. For this, the slide may be covered by a watch glass/petridish.

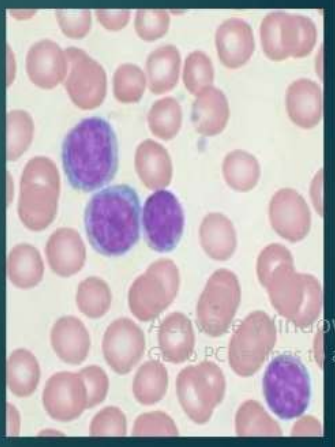
Cell type	Diameter (μm)	Nucleus	Cytoplasm	Cytoplasmic granules
Granulocytes Neutrophils (40–70%)	10–14 (1.5-2X a RBC)	• Blue-violet • 2–6 lobes, connected by chromatin threads Seen clearly through cytoplasm	• Slate-blue in color	• Fine, closely-packed violet pink • Not seen separately • Give ground-glass appearance • Do not cover nucleus
Eosinophils (1–6%)	10–15	• Blue-violet • 2–3 lobes, often bi-lobed, lobes connected by thick or thin chromatin band • Seen clearly through cytoplasm	• Eosinophilic • Light pink-red • Granular	• Large, coarse • Uniform-sized • Brick-red to orange • Seen separately • Do not cover nucleus
Basophils (0–1%)	10–15	• Blue-violet • Irregular shape, may be S-shaped, rarely bilobed • Not clearly seen, because overlaid with granules	• Basophilic • Bluish • Granular	• Large, very coarse • Variable-sized • Deep purple • Seen separately • Completely fill the cell, and cover the nucleus







Cell type	Diameter (μm)	Nucleus	Cytoplasm	Cytoplasmic granules
Agranulocytes				
Monocytes (5–10%)	12–20 (1.5-3 X a RBC)	• Pale blue-violet • Large single • May be indented horse-shoe, or kidney shaped (can appear oval or round, if seen from the side)	• Abundant • 'Frosty' • Slate-blue • Amount may be larger than that of nucleus	• No visible granules
Small Lymphocytes				
(20–40%)	7–9	• Deep blue-violet • Single, large, round, almost fills cell. • Condensed, lumpy chromatin, gives 'ink-spot' appearance	• Hardly visible • Thin crescent of clear, light blue cytoplasm	• No visible granules
Large lymphocytes (5–10½)	10–15	• Deep blue-violet • Single, large, round or oval, almost fills cell • May be central or eccentric	• Large, crescent of clear, light blue cytoplasm • Amount larger than in small lymphocyte	• No visible granules



Leishman stain is a mixture of Methylene blue, and Eosin dye, prepared in Alcohol medium and diluted with buffer or distilled water during staining procedure. Leishman stain is a differential stain that is used to variably stain the various components of the cells and it can be used to study the adherence of pathogenic bacteria to the human cells. It differentially stains the human and bacterial cells and appeared as purple and pink colored bodies respectively. The Leishman stain is one of the best stains for routine blood stain to stain the Peripheral blood smear for the examinations of blood film under the microscope and is satisfactory for malaria and other blood parasites. Giemsa stain gives better results in parasitic studies.

Principle of Leishman staining

Leishman Stain is a neutral stain for blood smears which was devised by the British surgeon W. B. Leishman (1865–1926). It consists of a mixture of eosin (an acidic stain), and Methylene blue (a basic stain) in Methyl alcohol and is usually diluted and buffered during the staining procedure. It stains the different components of blood in a range of shades between red and blue.

It is based on a methanolic mixture of "polychromed" Methylene blue and eosin. The methanolic stock solution is stable and also serves the purpose of directly fixing the smear eliminating a prefixing step.

Leishman stain is commonly used when there is need to examine the Blood smear for the Various blood cells, Differential Leucocyte count, Type of Anemia, Toxic Granules & Platelet count etc. and also used to differentiate nuclear and cytoplasmic morphology of the various cells of the blood like Platelets, RBCs, WBCs as well as for the parasites. This stain is the most dependable stain for Peripheral blood film examination.

The working principle of the Leishman stain is same as described above. As it is a type of Romanowsky stains, it contains both the Acidic and Basic dyes which have the affinity for Basic and Acidic components of the Blood cells respectively. The acidic dye, Eosin, variably stains the Basic components of the cells i.e. the cytoplasm, Granules etc. and the Basic dye, Methylene blue stains the Acidic components, especially the Nucleus of the cell. The stain must be diluted for use with Phosphate buffer to pH 6.8 or 7.2, depending on the specific technique used. The pH 6.8 is preferred when the morphology of blood cells is to be examined and pH 7.2 is good for parasitic studies.

Experiment aim: The main purpose of conducting this experiment is to understand the basic concept of the ABO blood group system and to know our blood group and type.

Principle: The ABO and Rh blood grouping system is based on agglutination reaction. When red blood cells carrying one or both the antigens are exposed to the corresponding antibodies they interact with each other to form visible agglutination or clumping. The ABO blood group antigens are O-linked glycoproteins in which the terminal sugar residues exposed at the cell surface of the red blood cells determine whether the antigen is A or B. Blood group A individuals have A antigens on RBCs and anti-B antibodies in serum. Similarly, blood group B individuals have B antigens on RBCs and anti-A antibodies in serum. Blood group AB individuals have both A and B antigens on RBCs and neither anti-A nor anti-B antibodies in serum. Whereas, blood group O individuals have neither A antigens nor B antigens, but possess both anti-A and anti-B antibodies in serum. The Rh antigens are transmembrane proteins in which the loops exposed on the surface of red blood cells interact with the corresponding antibodies.

Materials Required

- Toothpicks
- Blood sample
- Alcohol Swabs
- Lancet
- Clean glass slide
- Sterile cotton balls
- Biohazard disposal container
- Monoclonal Antibodies (Anti-A, B, and D)

Procedure

 Take a clean glass slide and draw three circles on it.

 Unpack the Monoclonal Antibodies (MAB) kit. In the first circle add Anti-A, to the second circle add Anti-B and to the third circle add Anti-D with the help of a dropper.

 Keep the slide aside safely without disturbing.

 Now wipe the ring finger with the alcohol swabs and rub gently near the fingertip, where the blood sample will be collected.

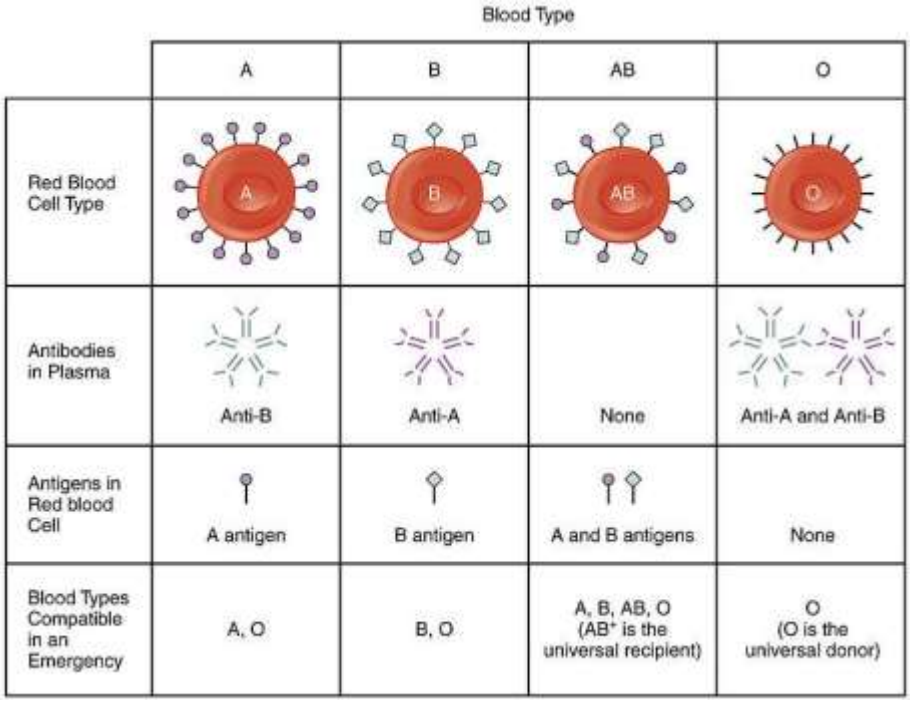
 Prick the ring fingertip with the lancet and wipe off the first drop of the blood.

 As blood starts oozing out, allow it to fall on the three circles of the glass slide by gently pressing the fingertip.

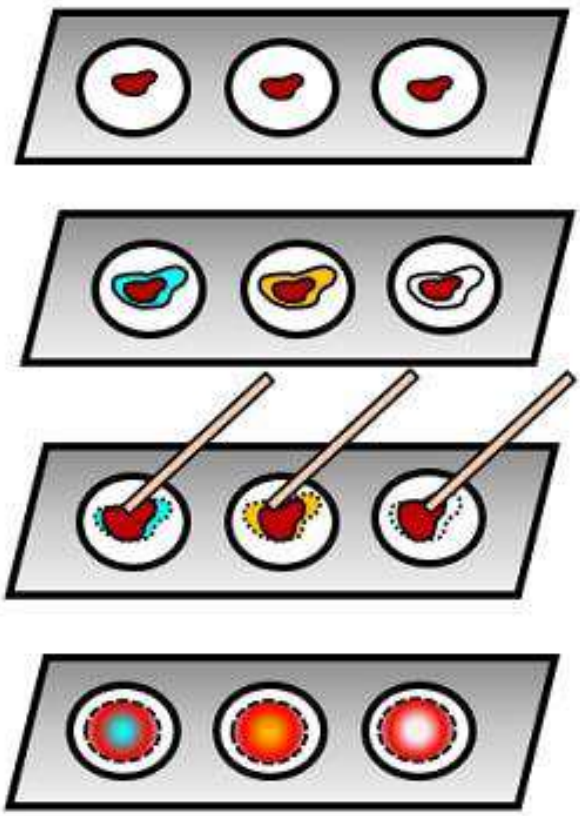
 Apply pressure on the site where it was pricked and to stop blood flow. Use the cotton ball if required.

 Mix the blood sample gently with the help of a toothpick and wait for a minute to observe the result.

Conclusion



Protocol of ABO blood grouping



1.Add three drops of blood in a clean glass slide

2.Add antisera A, B and D sequentially to the 1st, 2nd and 3rd drop of blood

3.Properly mix the antisera with the blood by separate toothpicks

4.Allow to stand for 2-3 minutes and note down the result on the basis of clump formation

Experiment aim: Analysis of Casein from Different Samples of MilkPrinciple: Analysis of different samples of milk to determination amount of casein. Casein is a major constituent in milk and is a mixed phosphorous protein. Casein is present in milk as a caseinated in the form of micells. Micelle have negative charge and adding acid to milk the negative charges are neutralized.

Ca^{2+} + *caseinated* + $2CH_3COOH\ (aq)$ ⟶ *Casein* + $(CH3COO)_2Ca$

Addition of saturated ammonium sulphate solution to the milk casein was precipitated out. Then add 30ml water heat t5he precipitate at 400Cthen add 1% acetic acid drop wise precipitate is obtained and filter the precipitate , dry weigh. The weight of precipitate give the amount of Casein present in a milk. This procedure is repeated for different samples of milk.

Materials: Beaker, Filter Paper, Conical Flask, Glass Rod and Funnel. Saturated Ammonium sulphate, 1% Acetic Acid, Distilled Water and Different Sample of Milk.

PROCEDURE:-

1. Take a clean dry beaker put into it 20cc of cow's milks and 20 ml of saturated ammonium sulphate solution slowly and with stirring. Fat along with casein will precipitate out.
2. Filter the solution and transfer the precipitates in another beaker.
3. Add about 30 ml of water to the precipitate. Only casein dissolves in water forming milky solution leaving fat undissolved.
4. Heat the milky solution to about 40 ˚C and add 1% acetic acid solution drop wise, when casein gets precipitated. Filter the reprecipitate, wash with water and let the precipitate dry.
5. Weigh the dry solid mass in a previously weighed watch glass. Repeat the experiment with other samples of milk.

Observations and Result

1) Volume of milk taken in each case = 20 ml

2) Weight of milk taken = W1g

3) Weight of casein isolated = W2g

4**) Percentage of casein =** $\frac{\text{Weight of casein}}{\text{Weight of milk}} \times 100$

Observation table: Example

Sl. No.	Types of Milk	Volume of Milk	Weight of Milk W1gm.	Weight of Casein W2gm.	% of Casein
1	Arokya Milk	20 ml	14.2	1.19	8.38%
2	Nandini Milk	20 ml	18.2	1.088	5.78%
3.	Cow Milk	20 ml	18.17	0.62	3.1%
4.	Buffalo Milk	20 ml	18.1	0.66	3.3%
5.	Goat Milk	20 ml	17.86	0.56	2.8%
6.	Sheep Milk	20 ml	18.17	0.9	4.9%
7.	Horse Milk	20 ml	17.2	0.93	1.3%

The yield of casein precipitated from the various milk samples of goat milk, cow milk, buffalo, goat, sheep, horse and camel milk contains 7.8gm, 4gm, 6.4gm, 6.5gm, 3gm and 0.626 gm respectively. Similarly, the milk samples availed from the market such as milk like Arokya and Nandini was 1.19 gm and 1.088 gm respectively. This shows that the casein precipitated from the cow milk contains more amount of casein protein than the goat and buffalo milk samples. The lower amount of casein in the buffalo milk is may be due to the more fat content in it.

Conclusion

This study clearly indicated that the amount of casein precipitated from the cow milk was higher than that of the other milk samples. The quantitative analysis of casein precipitated from the various milk samples provide the ample scope to the cottage cheese manufacture.Thus, the cow milk is suitable for the best muscle growth and basic body building achievements. “Different samples of milk contain different percentage of casein.”

Precautions

1) Handle apparatus and chemicals carefully.

2) Add ammonium sulphate solution very slowly.

3) Stir Milk while adding chemicals.

4) Do not disturb milk after adding ammonium sulphate solution and wait same time for fat and casein to precipitate out.

5) Take the amount readings carefully with digital weighing machine only.

E**xperiment aim:** Enumerate the total RBC (erythrocytes) count of your own blood.

Requirements: Micropipette, Haemocytometer, Neubauer's Chamber, Hayem's diluting fluid, microscope, cotton swab, alcohol, coverslip, needle, test tubes.

Principle: The Blood Specimen contains a large number of Red Blood Cells. This is impossible to count under the microscope. The Red Blood cells are counted using a special chamber that is designed to count blood cells within the specimen. This chamber is known as Neubauer's or Hemocytometer.

The blood sample is first diluted (1:200) with RBC diluting fluid (commonly known as the Hayem Fluid), which preserves and fixes the Red blood cells. The Hayem fluid is non-toxic to the Red blood cells. After diluting the specimen the contents are charged on Neubauer's chamber. The cells are then counted in areas that correspond to RBC count.

The composition of Hayem's diluting Fluid

Mercuric Chloride	0.25	grams
Sodium sulphate	2.5	grams
Sodium chloride	0.5	grams
Distilled water	100	ml

The Final pH of the solution (at 25°C) varies from 5.8 – 6.0

RBC dilution fluid is isotonic with blood, so there is no haemolysis. It contains sodium sulphate which discourages clumping of the erythrocytes and the mercuric chloride is a preservative.

Hemocytometer or Neubauer chamber

The Neubauer chamber is a thick crystal slide with the size of a glass slide (30 x 70 mm and 4 mm thickness). In a simple counting chamber, the central area is where the cell counts are performed. The chamber has three parts: (1) the central part, where the counting grid has been set on the glass, and (2) double chambers/two counting areas that can be loaded independently.

Neubauer chamber's counting grid is 3 mm x 3 mm in size. The grid has 9 square subdivisions of width 1mm. In case of blood cell counting, the squares placed at the corners are used for white cell counting. Since their concentration is lower than red blood cells a larger area is required to perform the cell count. The central square is used for platelets and red cells. This

square is split in 25 squares of width 0.2 mm (200 µm). Each one of the 25 central squares is subdivided in 16 small squares. Therefore, the central square is made of 400 small squares.

Glass cover

The glass cover is a squared glass of width 22 mm. The glass cover is placed on the top of the Neubauer chamber, covering the central area. The glass cover leaves room for the cell concentration between the bottom of the chamber and the cover itself. The chamber is designed so that the distance between the bottom of the chamber and the cover is 0.1 mm. It is not uncommon that the glass cover remains slightly lifted when we introduce more liquid than necessary in the chamber. To avoid this, some counting chambers have two special clamps to avoid the cover glass to avoid edge-lift. If the glass cover is lifted, the distance between the chamber and the cover will be higher than 0.1 mm, and the cell counts will not be accurate.

Procedure

1. With a safety bulb draw up to 0.5 marks on RBC's pipette blood and complete to 101 with Hayem's

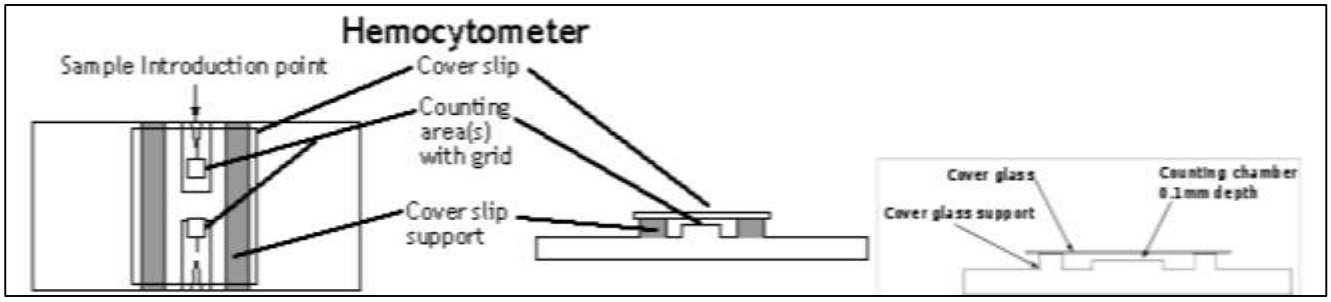
solution.

2. Mix for 2 - 3 minute

3. Charge hemacytometer:

4. Load the counting chamber with diluted blood as follows:

- Discard the first 2-3 drops.
- Place tip of the pipette at edge of the central platform of hemacytometer slide and let a drop of diluted blood run between the hemacytometer slide and cover slip by capillarity.

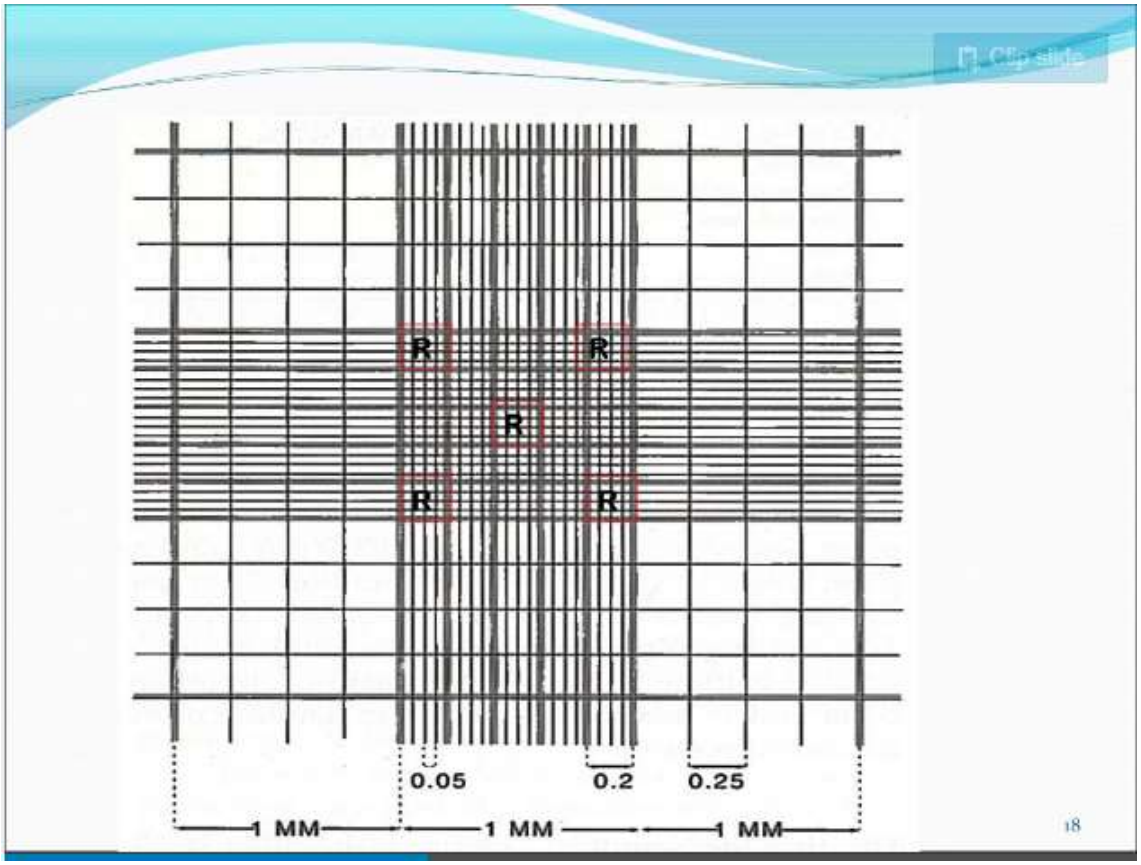


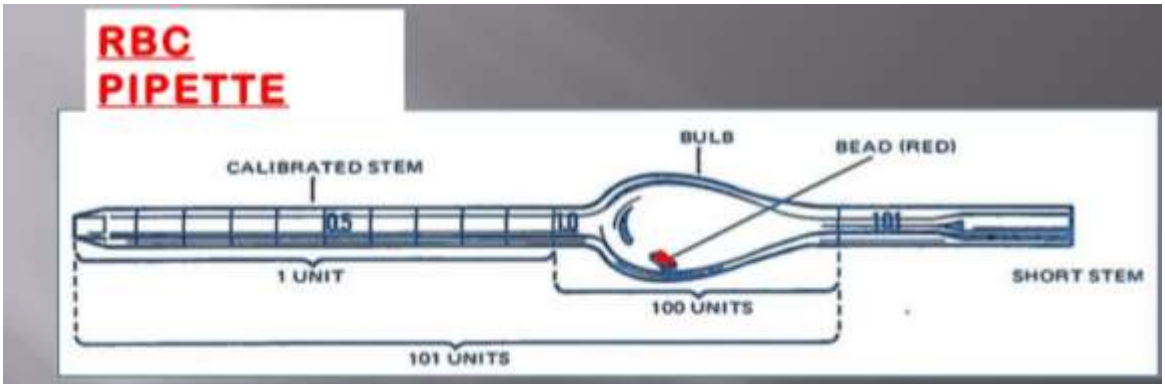
5. Let the hemacytometer to stand on the bench for 3 - 5 minute so the cells are settled down.

6. **An erythrocytes count is performed with a Neubauer hemacytomcter as follows:**

- Using 40 X magnification, count the erythrocytes in the four (4) corner squares and the one in the centre of the large centre square of the counting chamber. Count all cells that touch any of the upper and left lines, do not count any cell that touches a lower or right line.

NOTE: Draw diagram of RBC pipette and counting chamber with proper labelling.





Calculation:

Where (i) Dilution = 1:200 (i.e. 200)

(ii) Area Counted = $\frac{80}{400} = \frac{1}{5}$ Sq. mm.

Since cells are counted in 5 bigger squares and such square is further divided into 16 Small squares. Each small square = $\frac{1}{400}$ sq. mm.

Hence, area of (5 X 16) = 80 such areas = $\frac{80}{400} = \frac{1}{5}$

(iii) Depth of fluid $\frac{1}{10}$ mm.

(iv) Number or red cells counted = N.
Hence, total red blood cells/ cumm

$$M = \frac{N \times 200}{\frac{1}{5} \times \frac{1}{10}} \quad N \times 200 \times 50 = N \times 10{,}000$$

Normal Value:
Male: 4.5 to 6.0 x 10^6 cells/cu.mm (µl)
Female: 4.0 to 44.5 x 10^6 cells/cu.mm (µl)

Results:

Precautions:

- Use new sterilised needle for pricking finger
- Accurately measure the amount of blood specimen and Diluting Fluid to avoid any error in the results.
- Mix the specimen and diluting fluid appropriately by gently rotating in between your palms.
- Carefully charge the Hemocytometer or Neubauer's chamber that it should not be overcharged and do not contain any air bubble in it.

Experiment aim: Enumerate the total W.B.C (Leukocytes) count of your own blood.

Requirements: Micropipette, Haemocytometer, Neubauer's Chamber, W.B.C diluting fluid, microscope, cotton swab, alcohol, coverslip, needle, test tubes.

Principle: WBC diluting fluid is used to perform the WBC (leucocyte) count. By dilution with WBC diluting fluid RBCs are lysed by glacial acetic acid. Gentian violet slightly stains the leukocyte nuclei. The blood specimen is diluted 1:20 (0r 20:380) with the diluting fluid, and the cells are counted under the low power of the microscope using a counting chamber. The number of cells in undiluted blood is reported per cubic mm (µl) of the whole blood.

Composition of WBC Diluting Fluid (100ml)

Glacial acetic acid - 2.00 ml

Gentian Violet (1% w/v) - 1.00 gm

Distilled water- 97.00 ml

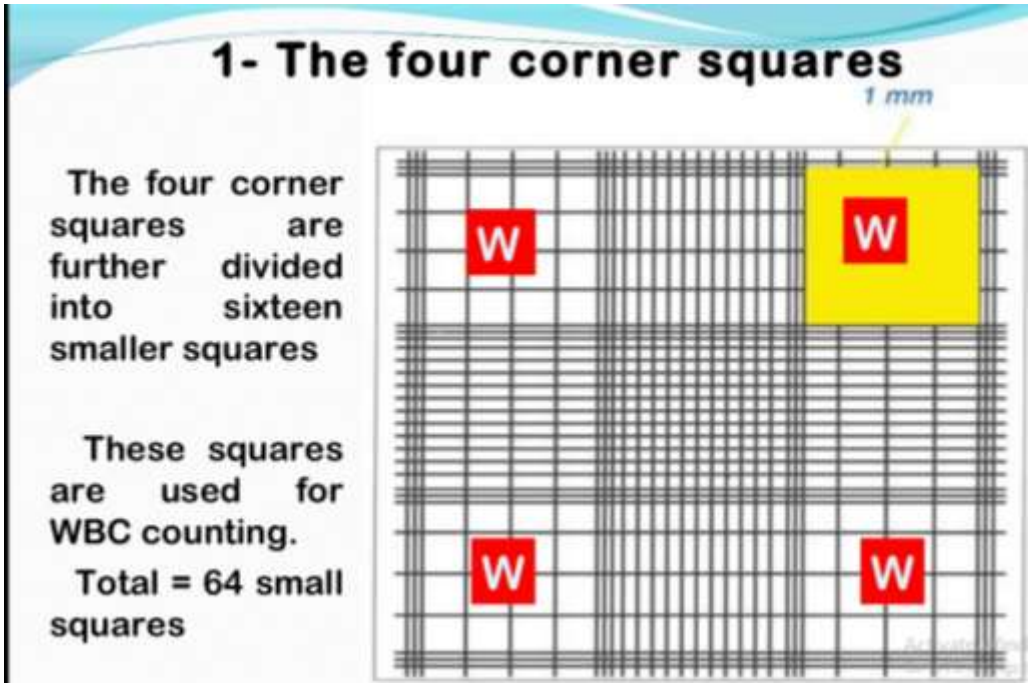
Final pH (at 25°C)- 2.2±0.2

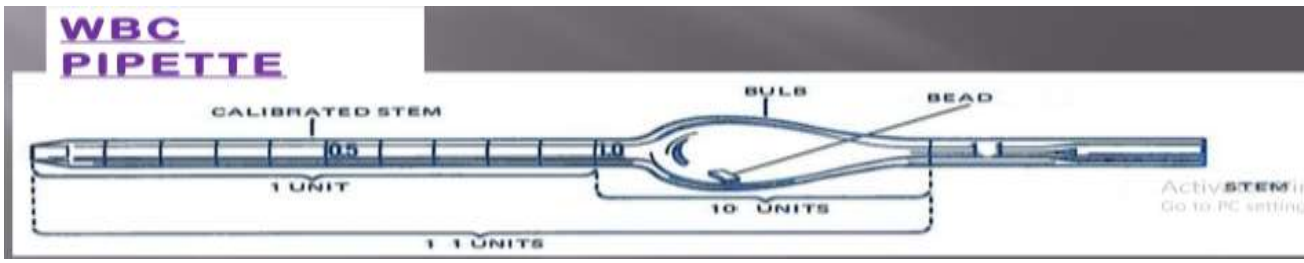
Procedure

- Take 380 µl of WBC diluting fluid in a Clean, Dry and Grease free eppendorf tube.
- Sterilise the tip of your middle finger and puncturing needle with a pad of small cotton wool dipped in 90% alcohol.
- Prick the sterilised finger deeply with the needle so that the blood oozes freely without squeezing.
- Discard the first drop and take 20 µl of blood and add to the eppendorf tube containing WBC diluting fluid with the help of micropipette.
- Mix well for few minutes and Take out the Neubauer's chamber / Hemocytometer from its case and clean it using a swab or gauze piece. Similarly, clean out the cover glass and place it over the grooved area of Hemocytometer.
- Now, take out the micropipette and fill it with the Diluted Specimen, mix the solution well and then discard 1-2 drops from the pipette before charging the chamber.
- Gently press the WBC pipette, so that the next drop of fluid is in hanging position.
- Touch the Tip of the pipette with the hanging drop against the edge of the coverslip making an angle of 45° approximately.

- Allow a small amount of fluid from the pipette to fill into the chamber which occurs by the Capillary action. Do not overcharge the chamber and there should be no air bubble in the Chamber.
- Allow the corpuscles to settle down for 5min. WBCs are recognised under low magnification by their refractile appearance and by slight colour given to them by diluting fluid.
- The counting is performed in the four corners of 1 square millimetre.

  NOTE: Draw diagram of WBC pipette and counting chamber with proper labelling.





Calculation:

Results:

Precautions:

- Use new sterilised needle for pricking finger
- Accurately measure the amount of blood specimen and Diluting Fluid to avoid any error in the results.
- Mix the specimen and diluting fluid appropriately by gently rotating in between your palms.
- Carefully charge the Hemocytometer or Neubauer's chamber that it should not be overcharged and do not contain any air bubble in it.

Experiment aim: Enumerate the Differential Leukocyte count of your own blood.

Requirements: Cotton, Spirit, needle, Glass slide, Distilled water, Leishman's stain, Light microscope.

Principle: A differential white blood cell count is performed to determine the percentage of each of the various types of white blood cells present in a blood sample. The test is useful because the relative proportions of white blood cells may change in particular diseases.

Procedure

Preparing the blood smear Sterilize the finger tip of the subject with a cotton swab dipped in 70% alcohol and is dried.

Take a bold prick on the fingertip to have free flow of flood.

Collect drops of blood on the end side of a glass slide. Spread the blood drop with another glass slide by placing it at an angle of 45 degree and Hold the spreader firmly and move it on the previous slide to the other end in straight line and equal pressure. Allow the glass slide to dry after formation of the smear.

Staining the slide

Keep the smeared glass slide on a flat surface with the smeared surface facing upwards.

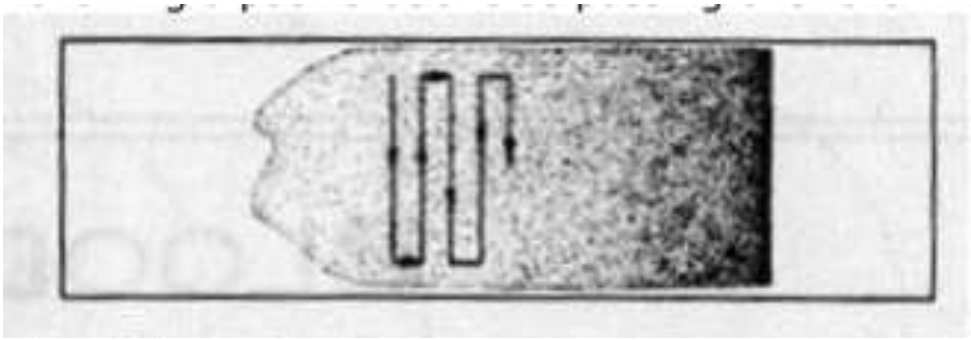
Pour drops of Leishman's stain on the glass slide to cover the smear or film.

Keep it undisturbed for 2-5 minutes.

Pour drops of distilled water on the slide and leave it for 10 minutes. Keep the slide aside for some time to dry.

Observation of the glass slide and counting of cells

1. Focus on the cells at one end of a prepared blood slide, using high-power magnification or an oil immersion objective.
2. Move the blood slide back and forth slowly following a path that avoids passing over the same cells twice (See Figure).



3. Each time you encounter a white blood cell, identify its type and record it in table (use a hash mark for each cell).
4. Continue searching for and identifying white blood cells until you have recorded a total of one hundred cells in the data chart. Because percent means 'parts of one hundred," for each type of white blood cell, the total number observed is equal to its percentage in the blood sample.
5. Record your percentages in the boxes below.

Result:

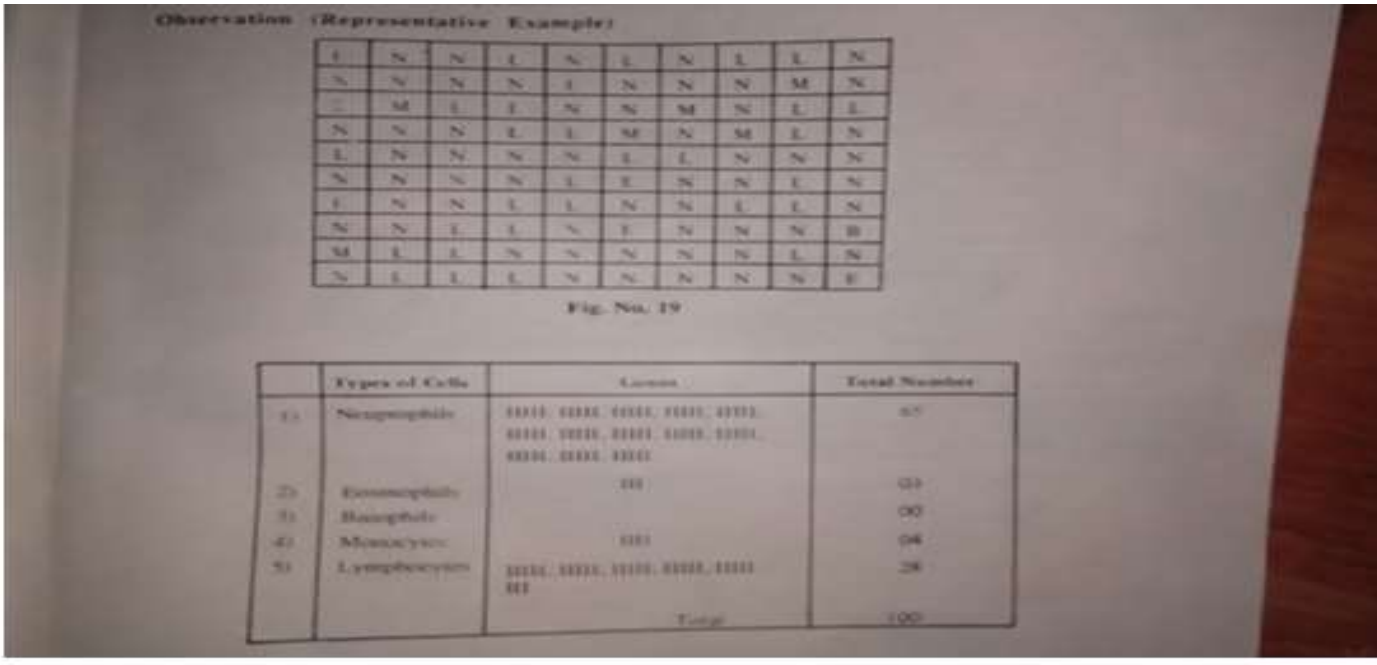

Observation (Representative Example)

Fig. No. 19

	Types of Cells	Counts	Total Number
1)	Neutrophils	𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸	67
2)	Eosinophils	III	03
3)	Basophils		00
4)	Monocytes	IIII	04
5)	Lymphocytes	𝍸 𝍸 𝍸 𝍸 𝍸 III	26
		Total	100

Figure: Showing an example of observation and recording table

Conclusion:

Differential count of given sample of blood is as follows:

Cells	Percentage X/100
Neutrophils	
Basophil	
Eosinophil	
Lymphocyte	
Monocytes	

Precautions:

- Use new sterilised needle for pricking finger
- Make thin smear of blood and avoid overlapping of the smear
- Do not over stain the slide

Activity 1: Demonstration of Knee-jerk reflex.

Introduction: Reflexes are rapid, predictable responses to stimuli. The pathway along which the electrical signals travel is called a reflex arc.

There are five parts to a reflex arc: 1. The receptor detects a stimulus. 2. The sensory (afferent) neuron sends an electrical signal to the CNS. 3. The integration center consists of one or more synapses in the CNS, and processes the information. 4. The motor (efferent) neuron sends an electrical signal from the CNS to the effector. 5. The effector, which may be muscle tissue or a gland, responds appropriately. A monosynaptic reflex has only one synapse. An example is the patellar or knee-jerk reflex, which we will demonstrate today. Most reflexes, however, are polysynaptic, involving more than one synapse. The more synapses involved, the longer the reflex takes. A spinal reflex needs only the spinal cord to function, while other more complex reflexes require brain participation. Somatic reflexes involve skeletal muscle stimulation by the somatic division of the nervous system. Autonomic reflexes are dealt with through the autonomic division and activate smooth muscle, cardiac muscle or glands. Reflex testing is an important diagnostic tool for assessing the general health of the nervous system. Distorted, exaggerated or absent reflexes may indicate pathology. If the spinal cord is damaged, reflex tests can help pinpoint the level of damage.

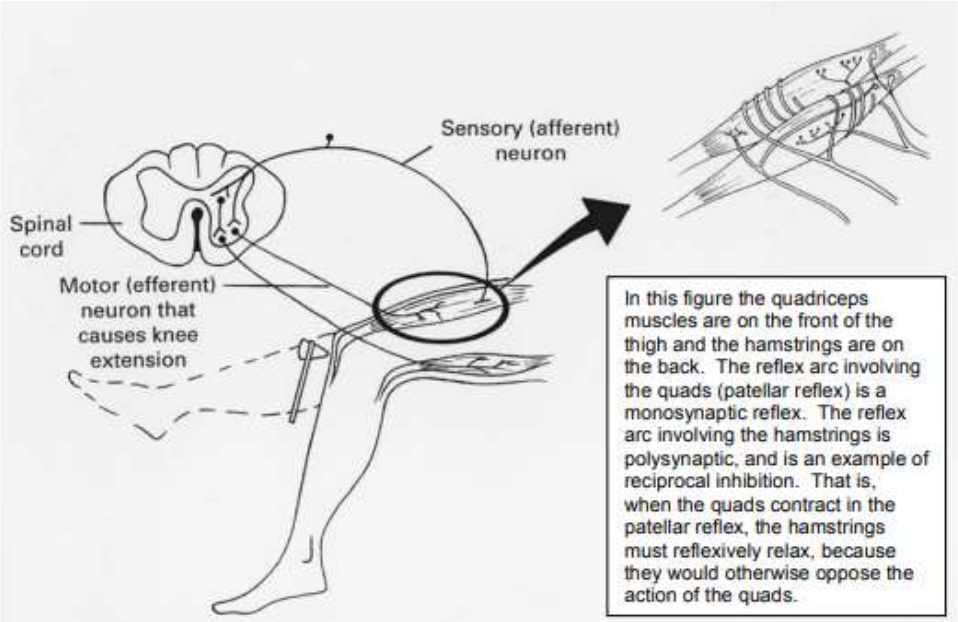
Activity 1: The Patellar Reflex The patellar (or knee-jerk) reflex is called a stretch reflex because it is initiated by tapping a tendon, which stretches the muscle, stimulating the muscle spindle (the proprioceptor inside the muscle) and causing reflex contraction of the quadriceps muscles. Stretch reflexes generally act to maintain posture, balance and locomotion. While this reflex is occurring, the antagonistic muscle group, in this case the hamstrings, reflexively relaxes to prevent interference with the patellar reflex. The brain will also receive information and the subject will be consciously aware of what is happening, although this is not necessary for the reflex to operate. Stretch reflexes tend to be absent or hypoactive with peripheral nerve damage or ventral horn disease, and hyperactive in corticospinal tract lesions. They are absent with deep sedation or coma.

Procedure:

1. The subject should sit on the lab bench with legs hanging freely. Tap the patellar ligament (see figure above). This assesses the L2-L4 level of the spinal cord. Test both sides. This will represent the baseline response.

2. Have the subject add several numbers together as you test again. This tests the effect of mental distraction. Is the response greater than or less than the baseline?

Observation: Write your observation



Experiment aim: To prepare gastrocnemius muscle nerve.

Procedure: While making muscle nerve preparation, following rules must be observed

(1) Never use a knife when experimenting on a frog, but cut with scissors only.

(2) Do not touch the nerves with instruments.

(3) Always keep the tissues, especially the nerve, moist with 0.6% NaCI solution.

(4) Always pith the frog for above preparation.

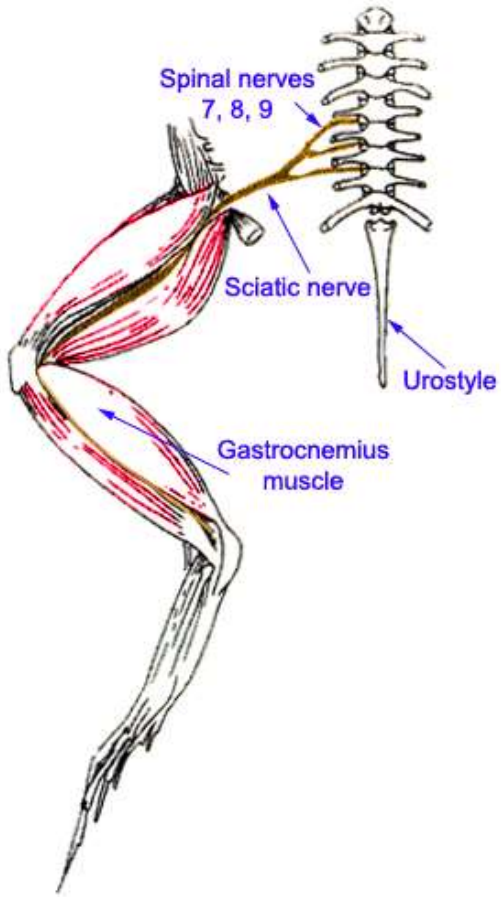
Pithing: For pithing, take a living frog and catch it firmly in the left hand. Make a bold incision into the skin transversely along outside cutting the atlanto-occipital ligament and pass the pithing needle into the skull above the exposed spinal cord to destroy the central nervous system by moving the needle to and fro, forwards and backwards. Take out the needle, pass it downwards through vertebral canal performing side to side movement of the pithing needle, so as to destroy the spinal cord. Complete destruction of the spinal cord is ensured by the absence of cutaneous reflex of the hind limbs.

Preparation of muscle nerve: (1) Cut the pithed frog across about 1 cm anterior to the sacro-iliac joints, remove any viscera remaining in the body cavity. Cut the skin between the legs, catch the skin at the transverse cut with the finger and thumb of one hand and the end of the spinal column with the other and pull the skin downwards towards the toes. It will be peeled off easily and cleanly. Leave the second leg covered with skin.

(2) Lay the legs on their ventral surface, grasp the urostyle with forceps and remove it by cutting away the muscle on both sides, and finally cut it from the end of the spinal column. Tum the preparation to the dorsal side and observe the sciatic nerve lying against back of the abdominal cavity. Snip through the sacro-iliac joint on the side of the skinned leg, dissect the end of the spinal column longitudinally, by means of one strong steady cut with scissors, carefully avoiding injury to nerves. By holding the piece of vertebrae so isolated, the dissection of the

nerve can be carried out without pinching it with forceps. Lay the preparation on the ventral surface and pin it fmnly to the dissecting board.

(3) Now holding the piece of the bone with forceps, dissect the nerve down towards the thigh, separate the muscular masses on the dorsal side of the thigh, carefully keeping in mind for the sciatic nerve and artery lying side by side. Continue the dissection upto the knee, do not let nerve lie on the skin of the frog, moisten with normal saline. Tie a ligature round the Achilles' tendon, cut this on distal side, snip through connective tissue attaching the gastrocnemius muscle to the leg. Cut through the leg bone just below the knee, and the whole thing just above the knee, carefully leaving gastrocnemius muscle and the sciatic nerve intact. Don't dissect the nerve at the knee.



(4) The nerve muscle preparation is now complete which consists of vertebrae with origins of Sciatic nerve, knee joint, gastrocnemius, muscle, tendon and thread. Fasten it on to the myograph board with a pin through the knee joint, attach the thread to the short arm of the level, so that preparation is ready for experimentation.

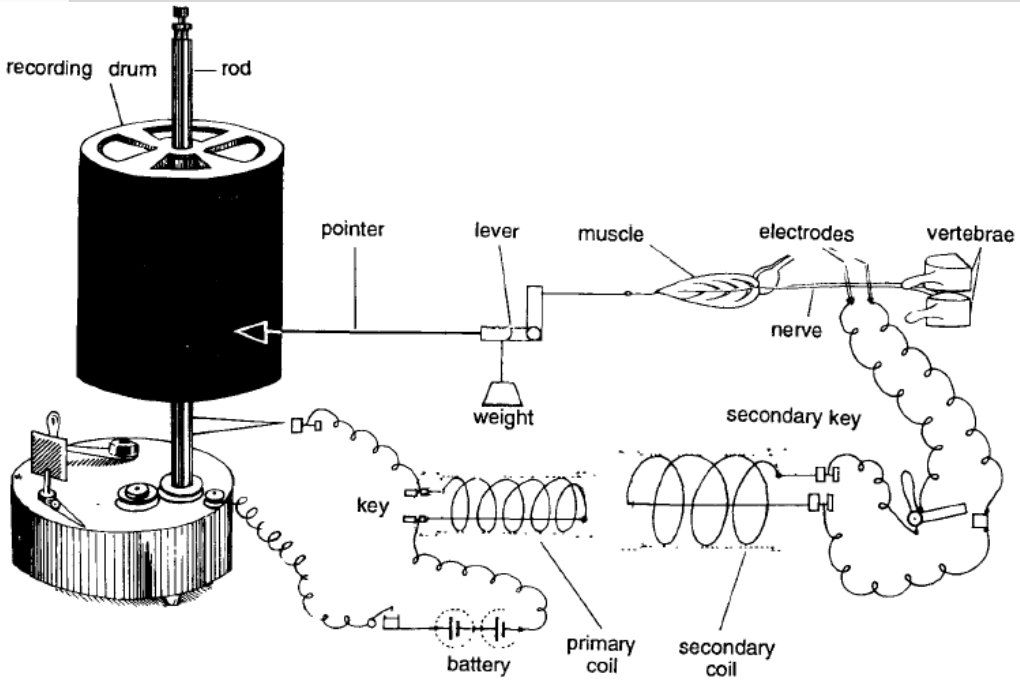


Fig. 4. Connection, recording drum and nerve muscle for simple muscle contraction.

***Experiment aim*:** To demonstrate or draw a simple muscle curve (simple twitch).

Requirements: Dissecting board, adjustable stand, crank myograph, pointer, recording drum or kymograph, primary key, induction coil, short circuiting key, electrodes, connecting wires, pithing needle, scalpel, scissors and hand gloves.

Connections: It consists of primary circuit with drum in circuit. In the secondary circuit, the two terminals of secondary coil are connected to the two side terminals of the short circuiting key. Current from the other two terminals of the short circuiting key is led to the electrodes which are used to stimulate the muscle through its nerve. Speed of the drum is kept fast.

Procedure:

(1) After making all the necessary connections, adjust the base of the recording drum in the horizontal position by levelling the screws. Wrap the smoked paper around the recording drum and making a baseline on the recording drum.

(2) Place the gastrocnemius sciatic preparation on the myograph, connect the tip of the muscle with the vertical

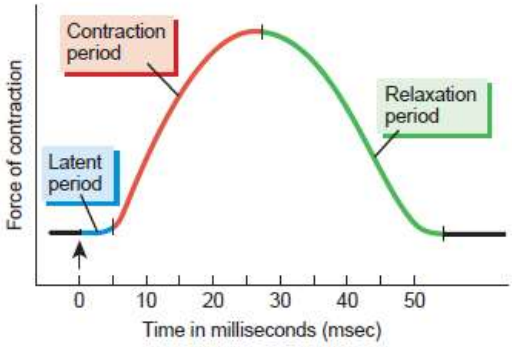
limb of the myograph lever and insert a pin below the knee joint. Other end of the sciatic nerve is laid on the electrodes. Adjust the pointer on the drum to draw the curve.

(3) Apply the current on the nerve.

Observations: When the electrical stimulus is applied to the sciatic nerve, the gastrocnemius muscle contracts and a curve is drawn on the smoked paper. This curve shows moment of stimulus, latent period, phase of contraction and phase of relaxation.

Figure 10.14 Myogram of a twitch contraction. The arrow indicates the time at which the stimulus occurred.

A myogram is a record of a muscle contraction.



Precautions:

(i) Switches should be used very carefully.

(ii) Connections should be tight.

(iii) Place of recording drum must be in the horizontal position.

Experiment aim: To measure Blood Pressure by using Sphygmomanometer

Requirement: Sphygmomanometer (A sphygmomanometer consists of an inflatable bag inside a covering called a cuff, an inflating bulb, a manometer from which blood pressure can be read, and a valve that is used for deflation.), Stethoscope, Chair, Table or other surface to support arm

Principle: Blood pressure is the force exerted by blood against the walls of arteries and veins. It is created by the pumping action of the heart. Blood pressure is measured in millimetres of mercury (mm Hg) and is expressed by two numbers—120/80, for example. The higher number is systolic blood pressure, the maximum pressure that occurs when the heart contracts. The lower number is diastolic blood pressure, the pressure when the heart is relaxed between contractions.

Procedure:

1. The subject should sit comfortably, with the arm slightly flexed, palm up, and the forearm supported at heart level on a table or other smooth surface. If such a surface isn't available, you will need to support the subject's forearm while you take the measurements.

2. Place the deflated cuff on the subject's upper arm, with the lower edge of the cuff about 1 inch above the inner elbow crease. The inflatable bag should rest on the brachial artery, which is on the inner part of the upper arm. The inflatable bag should encircle at least 80% of the arm; if it does not, use a larger sphygmomanometer.

3. Apply the stethoscope lightly to the arm, just at the inner elbow crease. Make sure the stethoscope doesn't touch the cuff or any of the tubing from the sphygmomanometer.

4. While watching the manometer and listening for pulse sounds through the stethoscope, inflate the bag about 30 mm Hg above the point at which pulse sounds disappear. (Inflating the bag closes off the blood flow in the brachial artery, causing the pulse sounds to stop.)

5. Slowly deflate the bag at a rate of about 3 mm Hg per second (or per heartbeat). As you release the pressure, pulse sounds will become audible, go through several changes in clarity and intensity, and then disappear again. You must listen carefully to the pulse sounds while you watch the readings on the manometer.

6. Systolic pressure is the point at which pulse sounds first become audible. You should hear faint but clear tapping sounds.

7. Diastolic pressure is the point at which the pulse sounds disappear.

8. Wait 1–2 minutes and then repeat the test. Record both results and indicate which arm was used for the measurements. For more accurate results, readings should be taken by several different people or on several different occasions.

Result:

Systolic pressure: reading 1 and 2

Diastolic pressure: reading 1 and 2

(Note: Average the results of the two sets of measurements. Refer to the table below for the rating.)

Average pressure:_____/_____ (systolic) (diastolic)

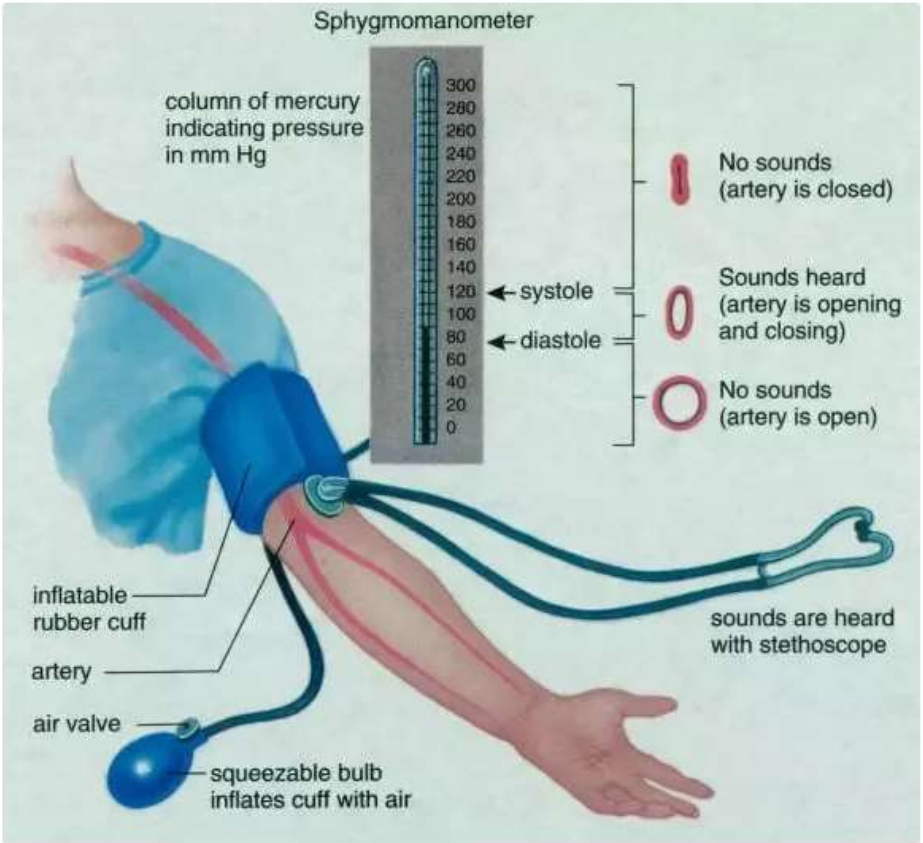
Classification: __high/low/normal

Category	**Systolic (mm Hg)**		**Diastolic (mm Hg)**
Normal	below 120	and	Below 80
Prehypertension	120–139	or	80–89
Hypertension			
Stage 1	140–159	or	90–99
Stage 2	160 and above	or	100 and above

- When systolic and diastolic pressure fall into different categories, the higher category should be used to classify blood pressure status.
- The risk of death from **stroke** begins to rise when blood pressure is above 115/75.
- Based on the average of two or more readings taken at different times. In persons older than 50 years, systolic blood pressure greater than 140 is a much more significant CVD risk factor than diastolic blood pressure.

Precaution:

- For the person being tested: Wear clothing that allows your upper arm to be bare.
- Avoid heavy exercise or eating prior to the test.
- Don’t smoke or ingest caffeine for at least 30–60 minutes before being tested.
- Sit quietly for several minutes before the test begins.



Experiment aim: Determination of rate of oxygen consumption in cockroach using a respirometer

Introduction: Aerobic organisms require a continuous supply of oxygen for the conversion of nutrients into energy. Carbod, dioxide and water are the end products of the respiratory matabolism. In effect the utilisation of oxygen and release of CO, are the two major components of respiration in living organisms. In this experiment you will attempt to quantify the rate of oxygen consymed by an insect using a simple respirometer. Aerial respiration is studied by manometric techniques. Warburg's manometer is the instrument of choice for such studies. But this instrument is quite expensive and you may construct a simple device of your own to make measurements of oxygen consumption in small organisms.

Objectives: At the end of this exercise, you should be able to: construct simple devices for measuring certain of biological activities. measure the rate of respiration in small organisms using simple respirometers

MATERIALS REQUIRED

- 4 oz. bottles - 2
- One holed rubber stopper - 2
- 2ml. graduated pipette - 2
- Filter paper bits
- Small pieces of wire gauze (about 1" square)
- 15% KOH solution
- Cockroaches.,

PROCEDURE

1. Fit the 4 oz. bottle with one holed rubber stopper (Fig. 18.1).
2. Insert a 2ml. graduated pipette through the hole in such a way that only 115th to 114th of the pipette is inside the bottle and rest is projecting out.
3. Place at the bottom of the bottle filter paper bits soaked in 15% KOH solution. A piece of wire gauze may be used to wrap around the filter paper bits so that when the insect is introduced in the bottle it does not come in contact with the alkali.
4. Weigh the cockroach in a balance. Introduce it intb the respirometer and close tightly.

5. Prepare a control respirometer (a thermobarometer) without the insect. (This means you will prepare a respirometer similar to the experimental one except that you will not introduce the insect into it.)

6. Immerse, both the experimental and control respirometers for equilibration in a tray containing water. While doing so, let the open end of the pipette of each respirometer be outside the water. This procedure equilibrates the temperature between air and water, and takes about fifteen minutes. At the end of the equilibration period submerge the open tip of the respirometers into the water.

Precaution: When the respirometer is immersed, water should not enter rapidly into the graduated pipette. This would suggest leakage in the system. Y u should have an airtight device for the success of the experiment. If necessary you may seal the mouth of the bottle with molten wax. Insufficient equilibration may also ause the rapid entry of water into the graduated pipette. In that case, equilibrate the resirometer for a longer period

OBSERVATIONS AND RESULTS

Observe the slow entry of water into the graduated tube even as the insect consumed oxygen and the released carbon dioxide is absorbed by KOH. In this experimental set up water in the tray serves as the manometric fluid, and you would observe the water meniscus in the pipette moves continuously. Record the volume of water that has entered into the pipette after one hour. This volume represents the volume of oxygen consumed by the insect in millilitres or cubic centimeters in one hour. The respirometer is very sensitive to changes in temperature and pressure. This is the reason why you have to set a control thermobarometer. Any volume changes occuriug due to changes in temperature and pressure will be recorded by this control device. Note volume changes in the control respirometer and this would represent the correction factor. The correction factor has to be added to or subtracted from the experimental value.

Volume of O_2 consumed by the insect = volume recorded in the experimental respirometer ± volume recorded in the control respirometer

The rate of oxygen consumed per gram weight of the body of the insect per unit time

$$= \frac{\text{Weight of oxygen consumed in one hour}}{\text{Weight of the insect}}$$

= .....ml of oxygen/gram weight of cockroach/hr at the given temperature

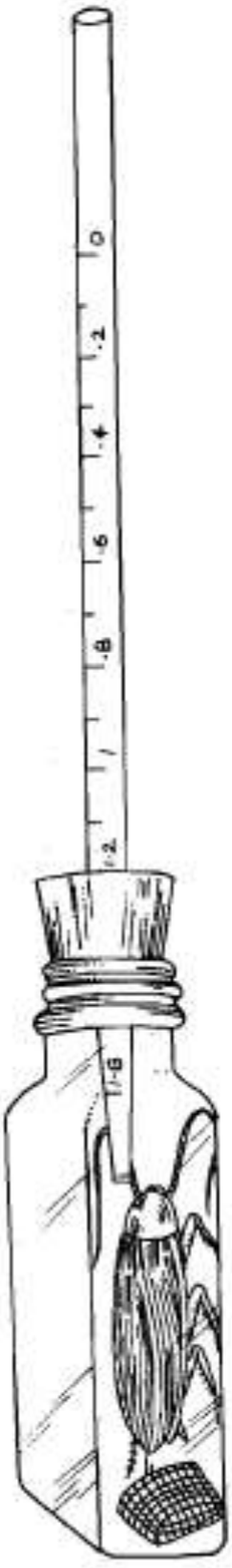


Fig. 18.1: A respirometer.

Experiment aim: To Prepare Haemin crystals with your own Blood (haematin **test/ Teichmann test**)

Requirement: Glass slides, Cover slips, Pricking needles, Dropper, Spirit lamp, Cotton, Compound microscope 90% Alcohol, sodium chloride and glacial acetic acid.

Principle: Haemoglobin is present in RBCs which imparts red colour to the blood and helps in the transport of gases. Haemoglobin is a conjugated chromoprotein which is composed of two parts- non-protein haem and protein globin. Haem is made up of four pyrrole rings and a central iron ion in ferrous state.

The haemin crystals are prepared by heating of the blood with Teichmann reagent (The reagents typically used are sodium chloride and glacial acetic acid). This ruptures the RBCs and the haemoglobin is released. Also, in this process the ferrous form of iron is converted to ferric form. The globin protein gets denatured by heating with acetic acid; while, heme is converted to oxidized heme called haematin. The haematin combines with halogens such as chloride ions to form insoluble haemin which appear as rhombic crystal of chocolate brown colour. The haemin crystals or hydrochloride of heme (Fig. 1.1) are characteristic of blood and are thus, used for identification of blood stains.

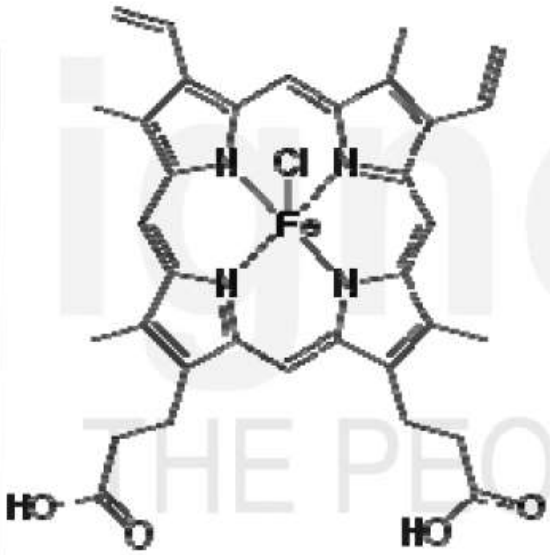


Fig. 1.1: Haemin.

Haemin was first crystallized by **Ludwik Karol Teichmann** in 1853. Therefore, they are also referred to as '**Teichmann Crystals**'.

PROCEDURE

1. Sterilize the tip of finger with cotton swab soaked in 90% alcohol.

2. Prick the finger with the sterilized pricking needle.

3. Place a drop of blood on a clean slide and spread it to form a thin film.

4. Let the slide dry and with the help of scalpel scratch the blood film and collect the dried powdered form of blood in the centre of the slide.

5. Add a pinch of NaCl and few drops of glacial acetic acid over the powdered blood.

6. Gently heat the slide over low flame until the reagent starts boiling.

7. Cool the slide and observe under the microscope, first under low magnification (10x) and then under high magnification (40x).

OBSERVATIONS

Rhomboid, chocolate brown crystals as shown in Figure 1. 2. are observed. Crystals are homogeneous solids, bounded by plane faces and having a geometric shape.

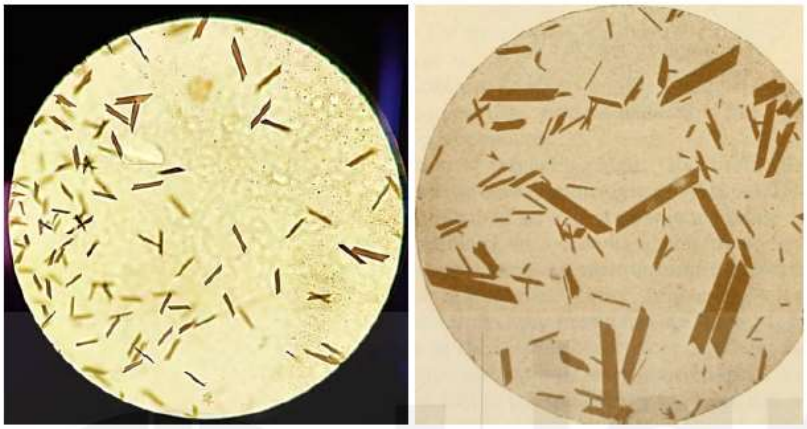


Fig. 1.2: Haemin Crystals of Human Blood.

Advantages

Haemin crystals are used in medico-legal practices to distinguish fresh or dried blood stains from other red-coloured stains. The shape of haemin crystals varies in different species and thus, blood stains of human can be confirmed. Another advantage of the haemin test is that it can be performed with the dried blood stains.

Teichmann test: A confirmatory test for blood based on the formation of distinctive haematin crystals that are viewed under a microscope. The reagents typically used are sodium chloride and glacial acetic acid.

PRECAUTIONS

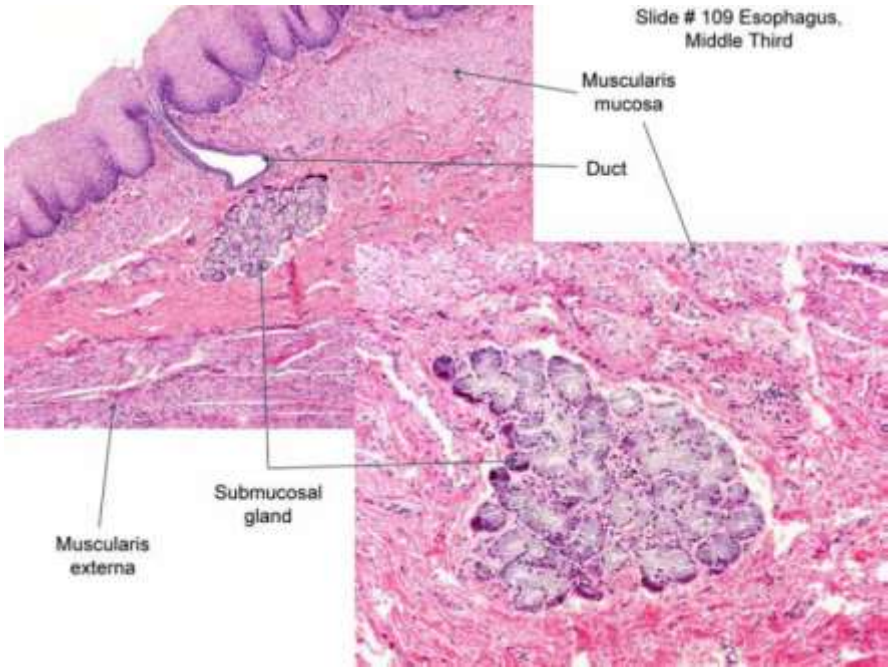
• Clean the finger with alcohol before pricking.

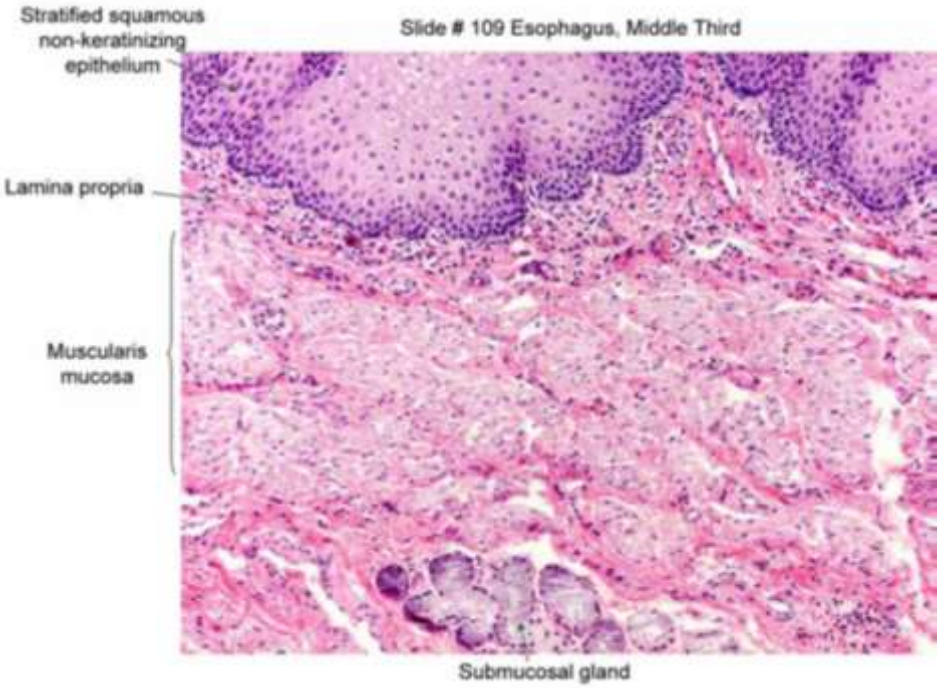
• Discard the first drop of blood.

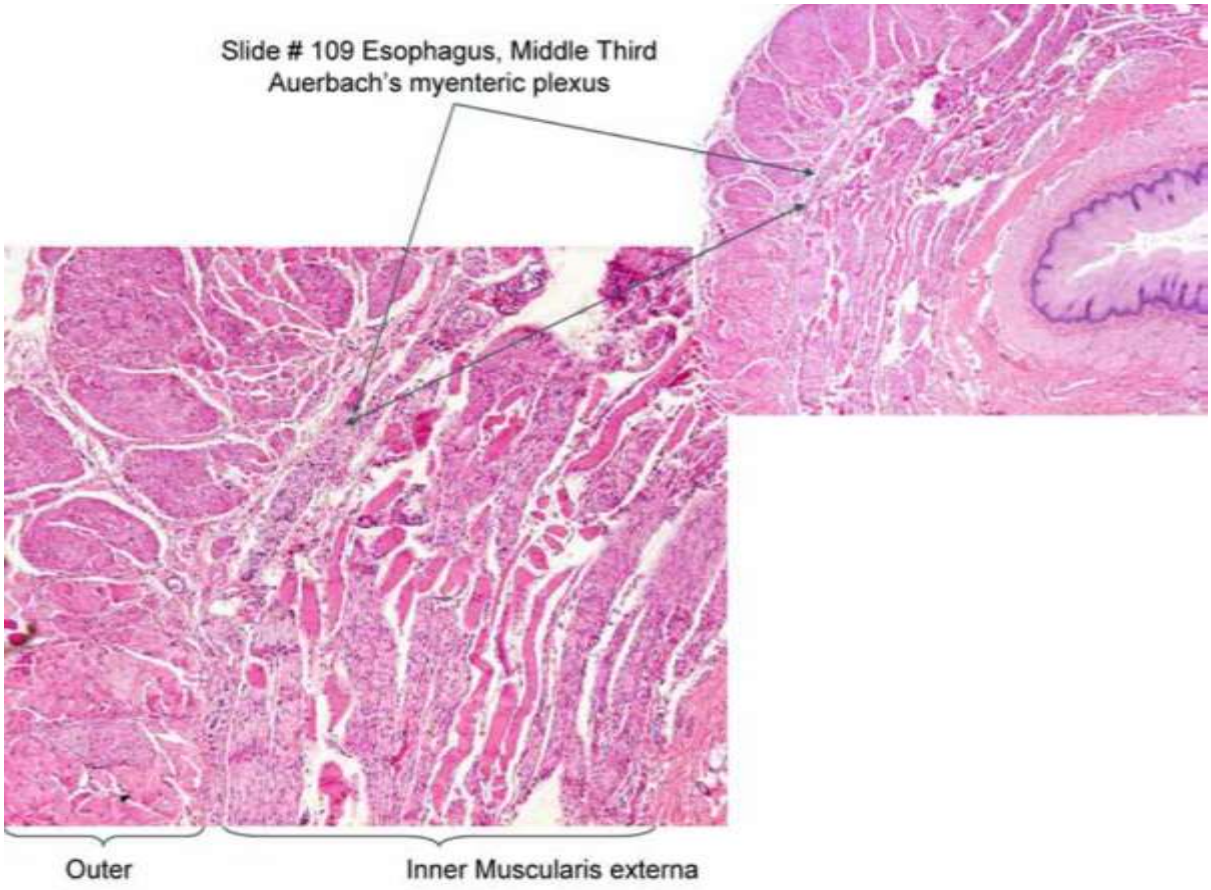
• Avoid overheating of the slide.

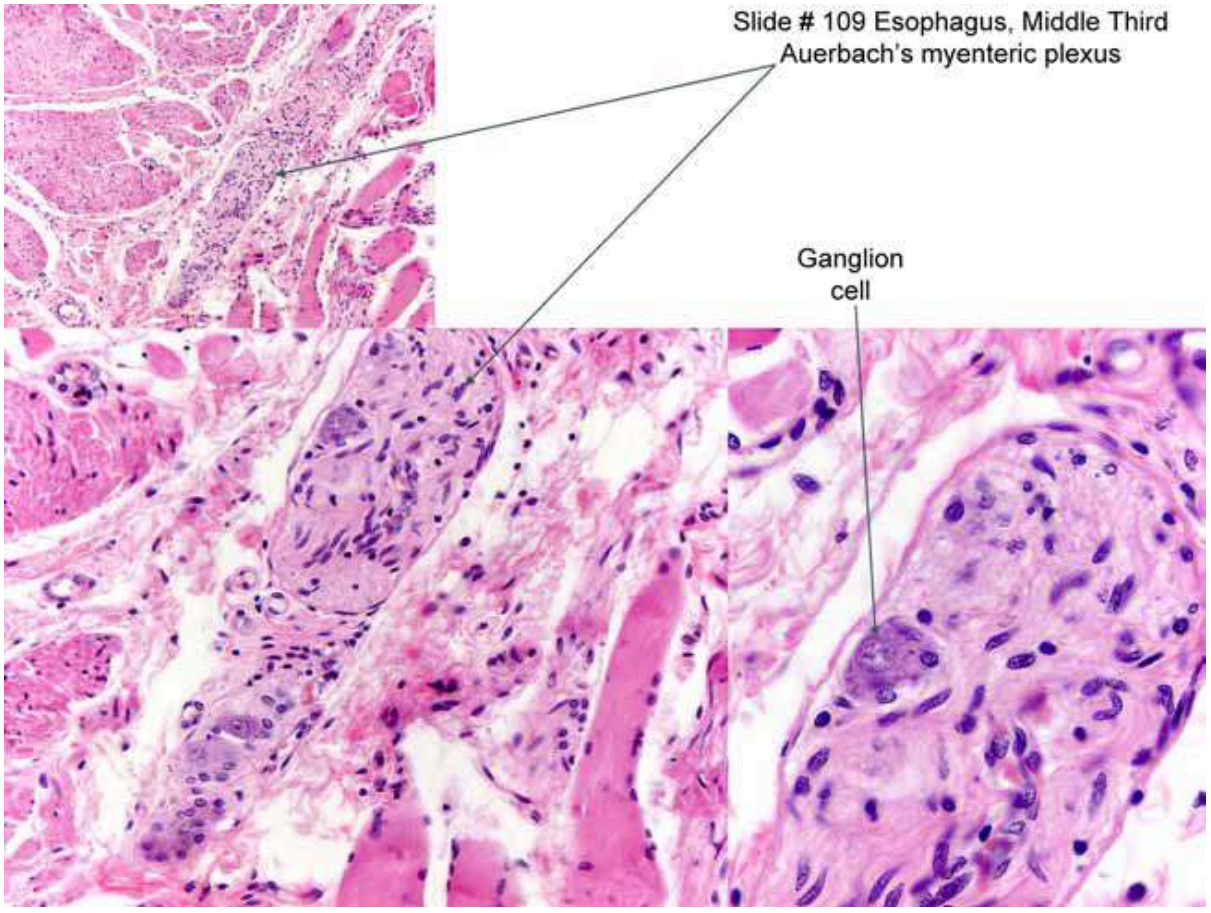
• Keep the slide undisturbed at the time of cooling

Histology of gastrointestinal tract

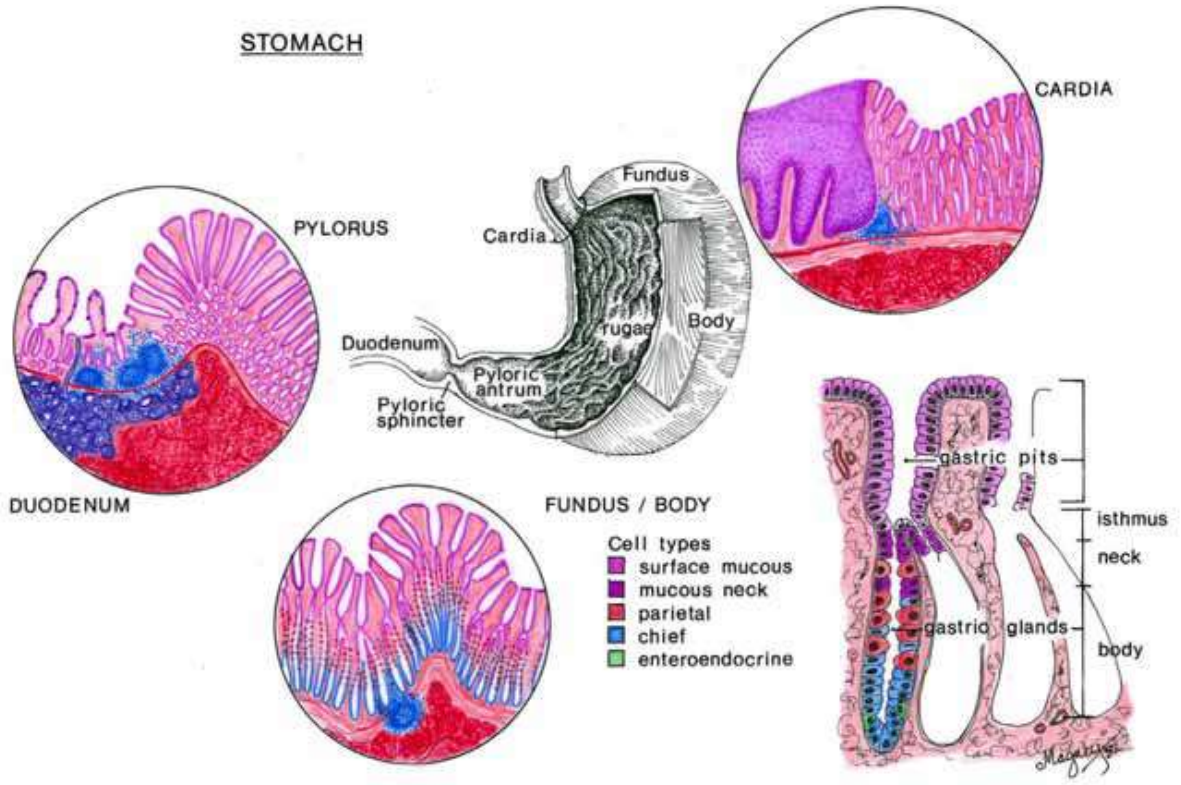




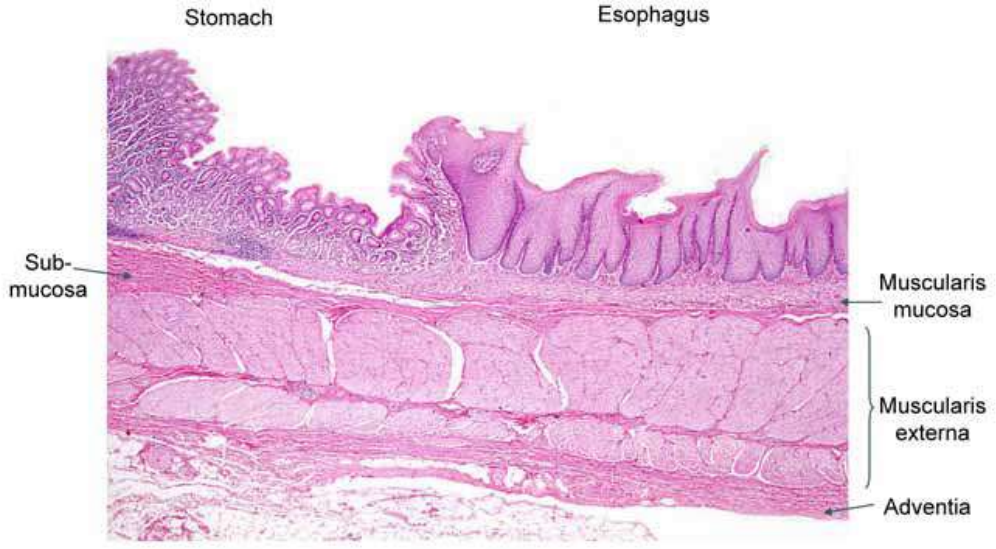

Slide # 109 Esophagus, Middle Third
Auerbach's myenteric plexus
Outer
Inner Muscularis externa

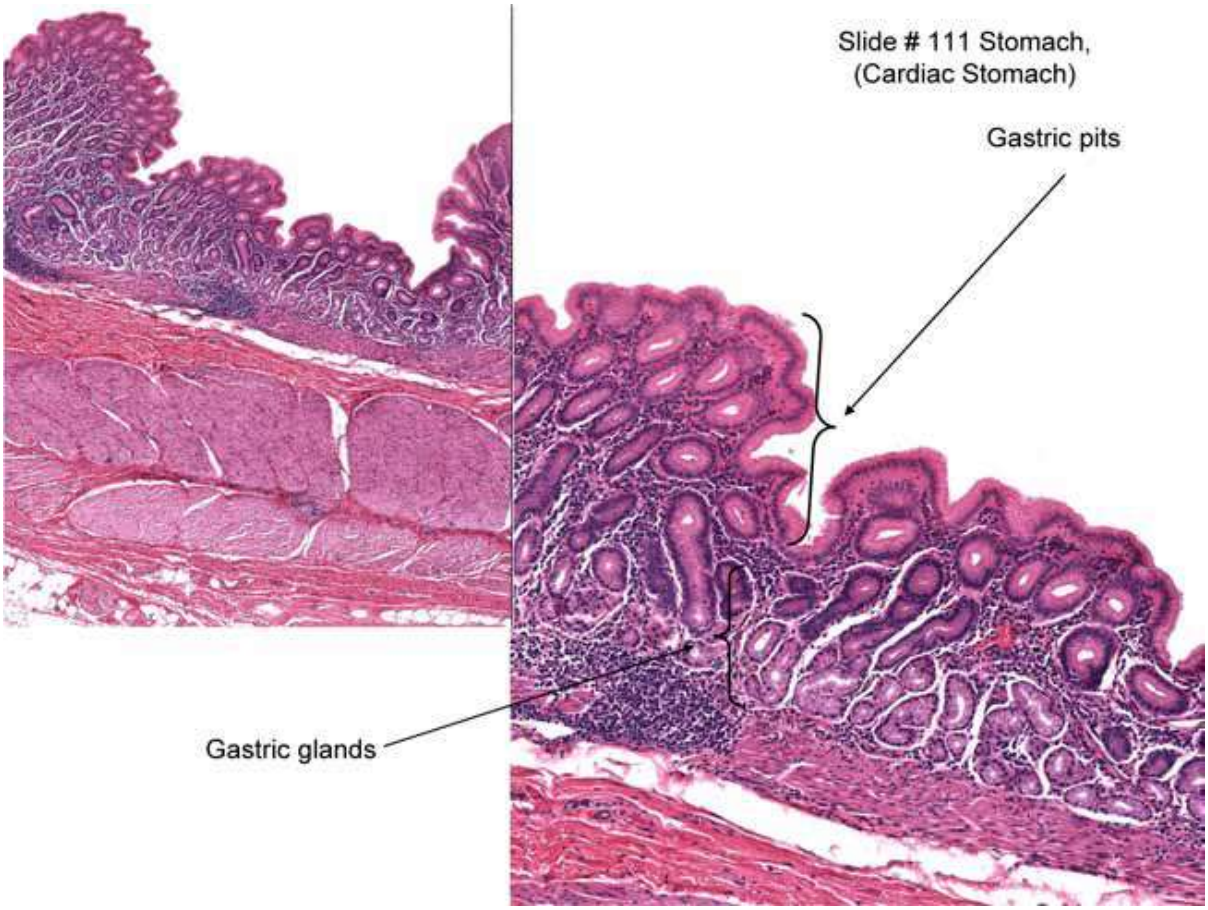

Slide # 109 Esophagus, Middle Third
Auerbach's myenteric plexus
Ganglion
cell

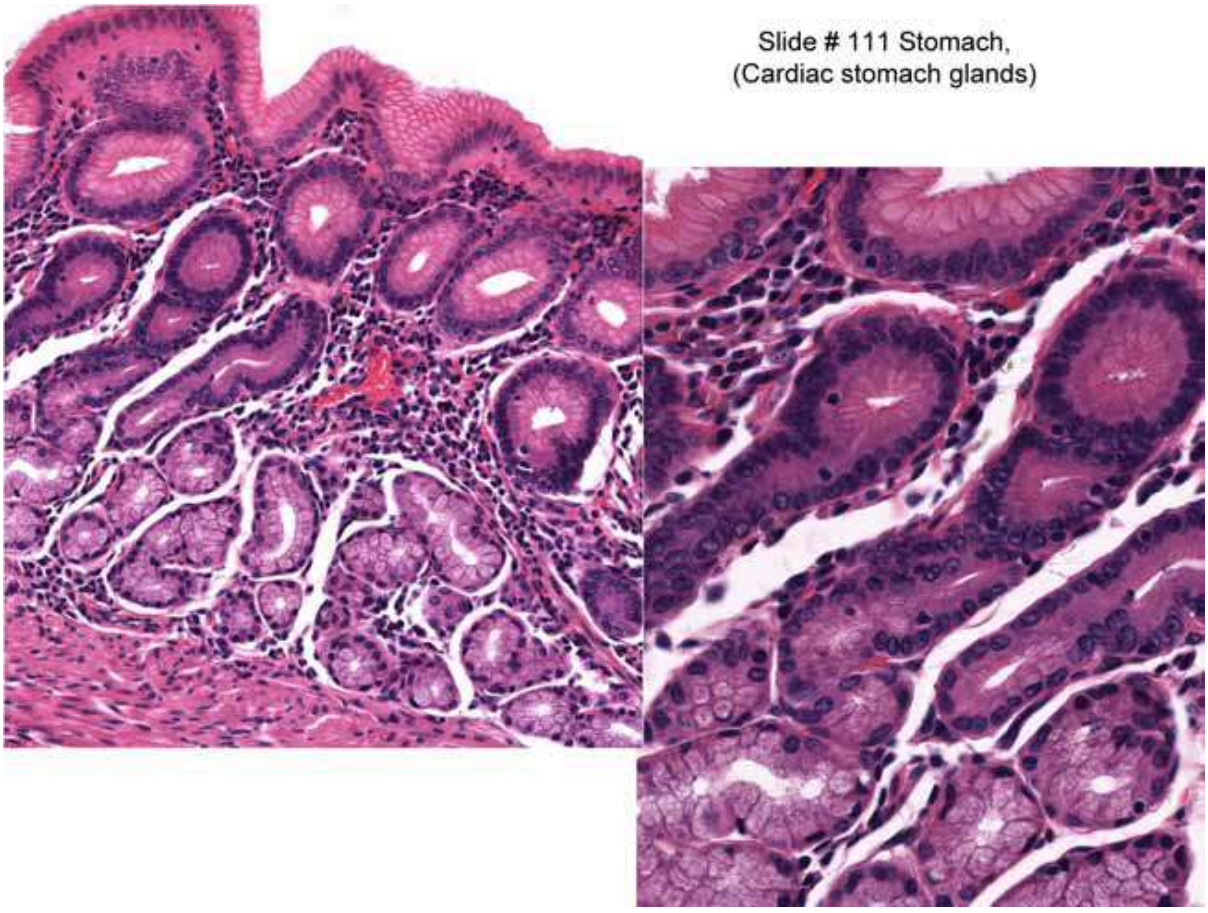
Organization of Stomach



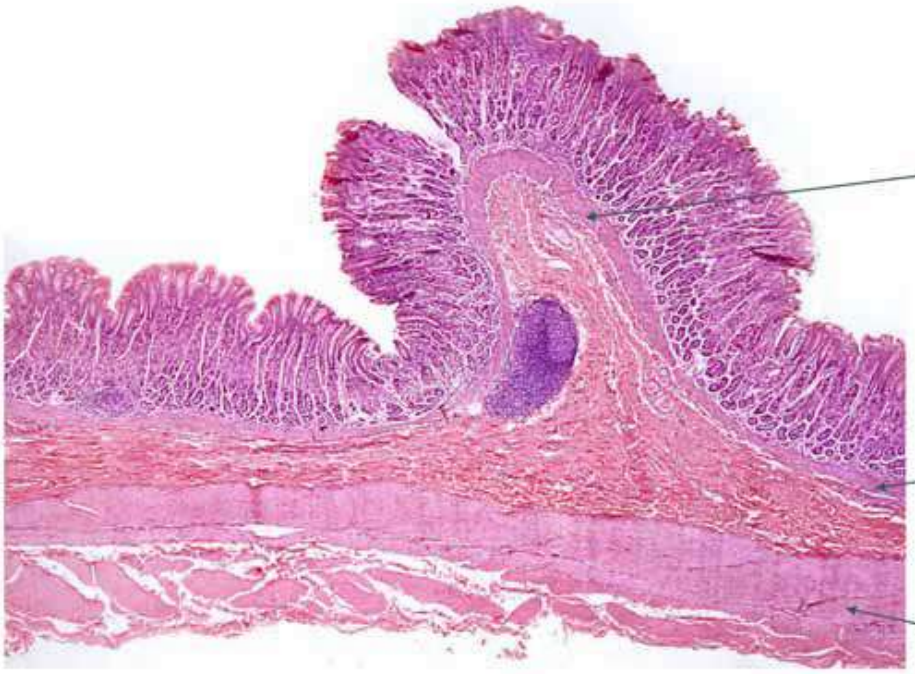
Slide # 111 Stomach, Cardio-esophageal Junction (H&E)



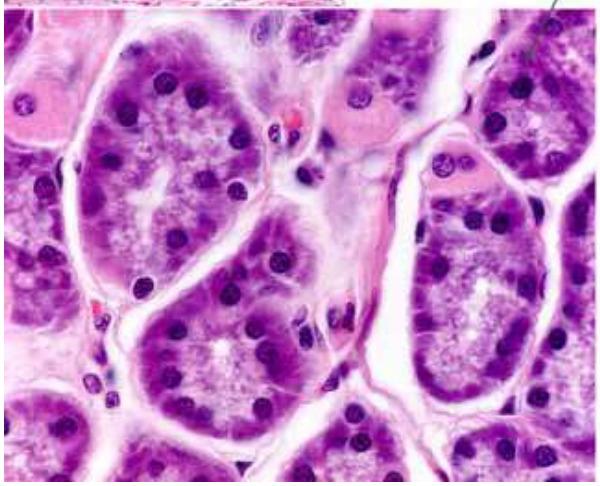

Slide # 111 Stomach,
(Cardiac Stomach)
Gastric pits
Gastric glands

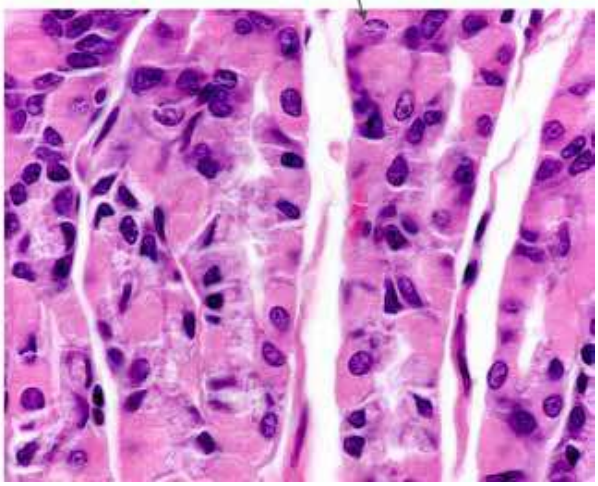

Slide # 111 Stomach,
(Cardiac stomach glands)

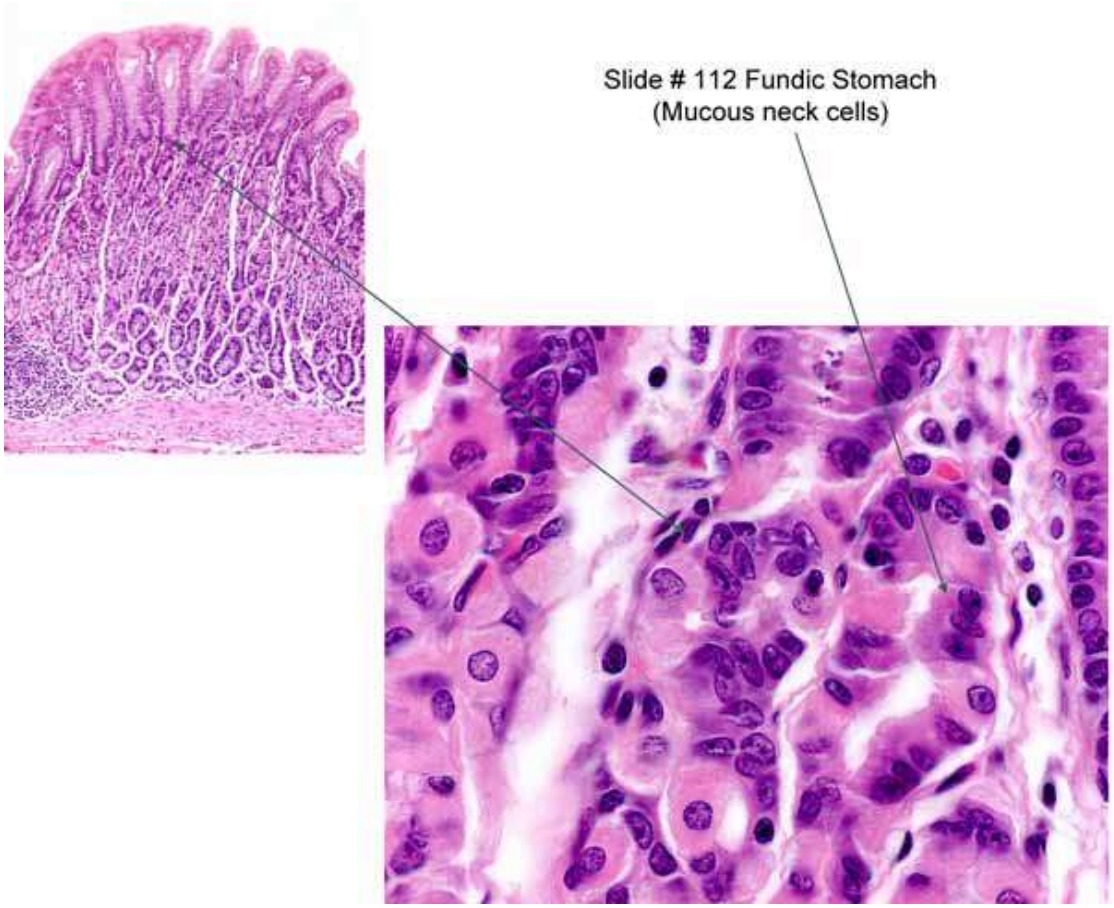
Slide # 112 Fundic Stomach (H&E)

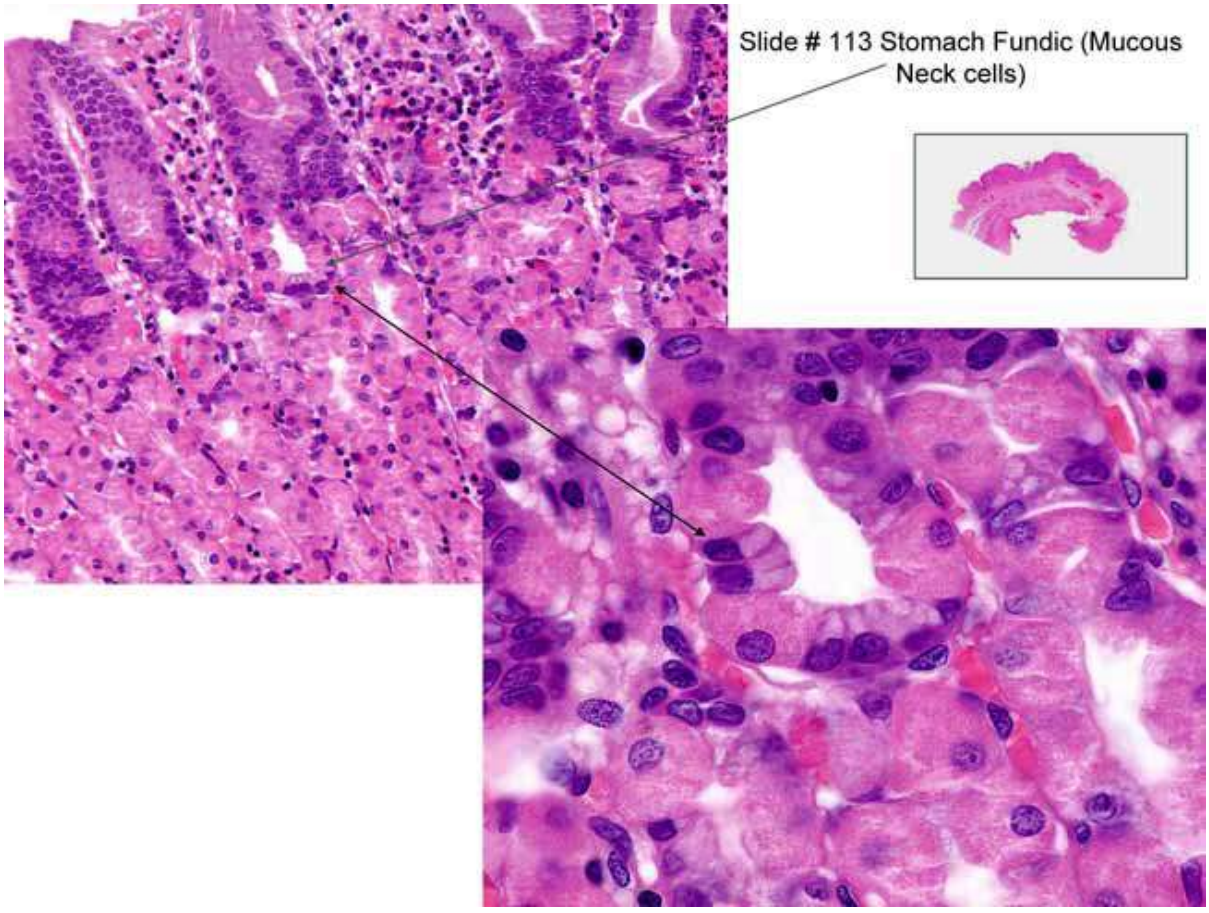


Slide # 112 Fundic Stomach
(Chief & Parietal cells)

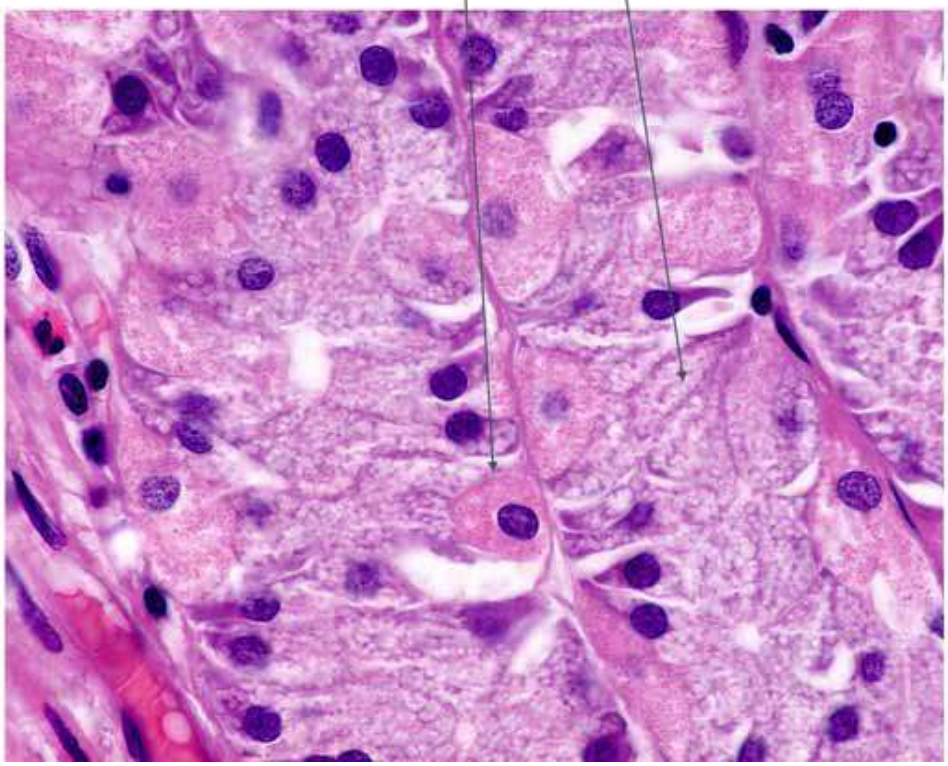


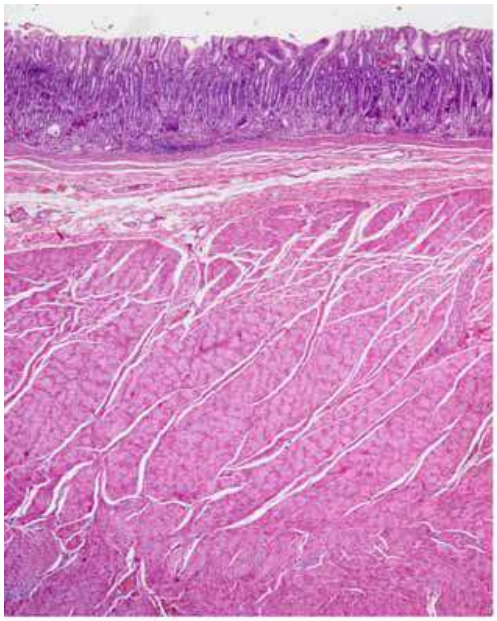



Slide # 112 Fundic Stomach
(Mucous neck cells)

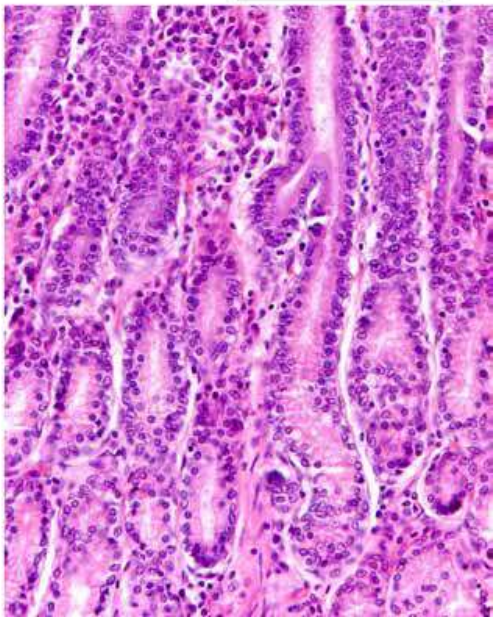

Slide # 113 Stomach Fundic (Mucous
Neck cells)

Slide # 113 Stomach Fundic
(Parietal and Chief cells)

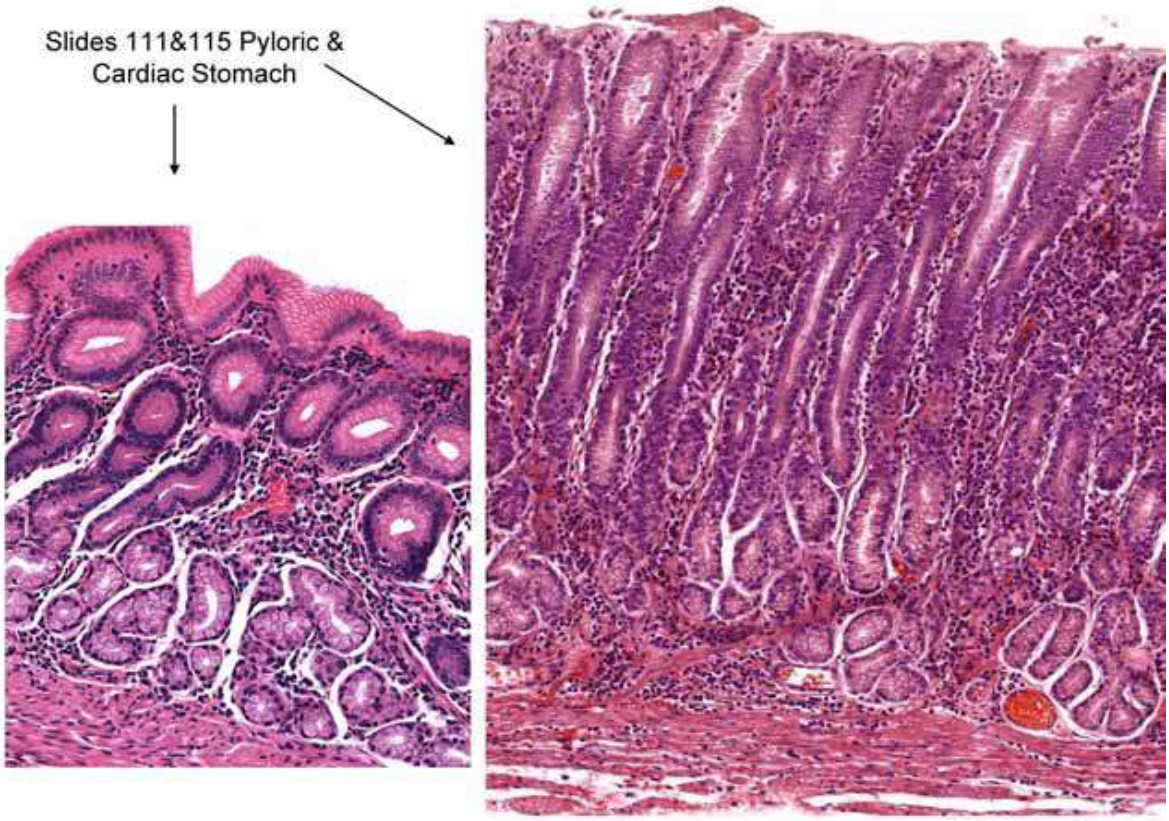




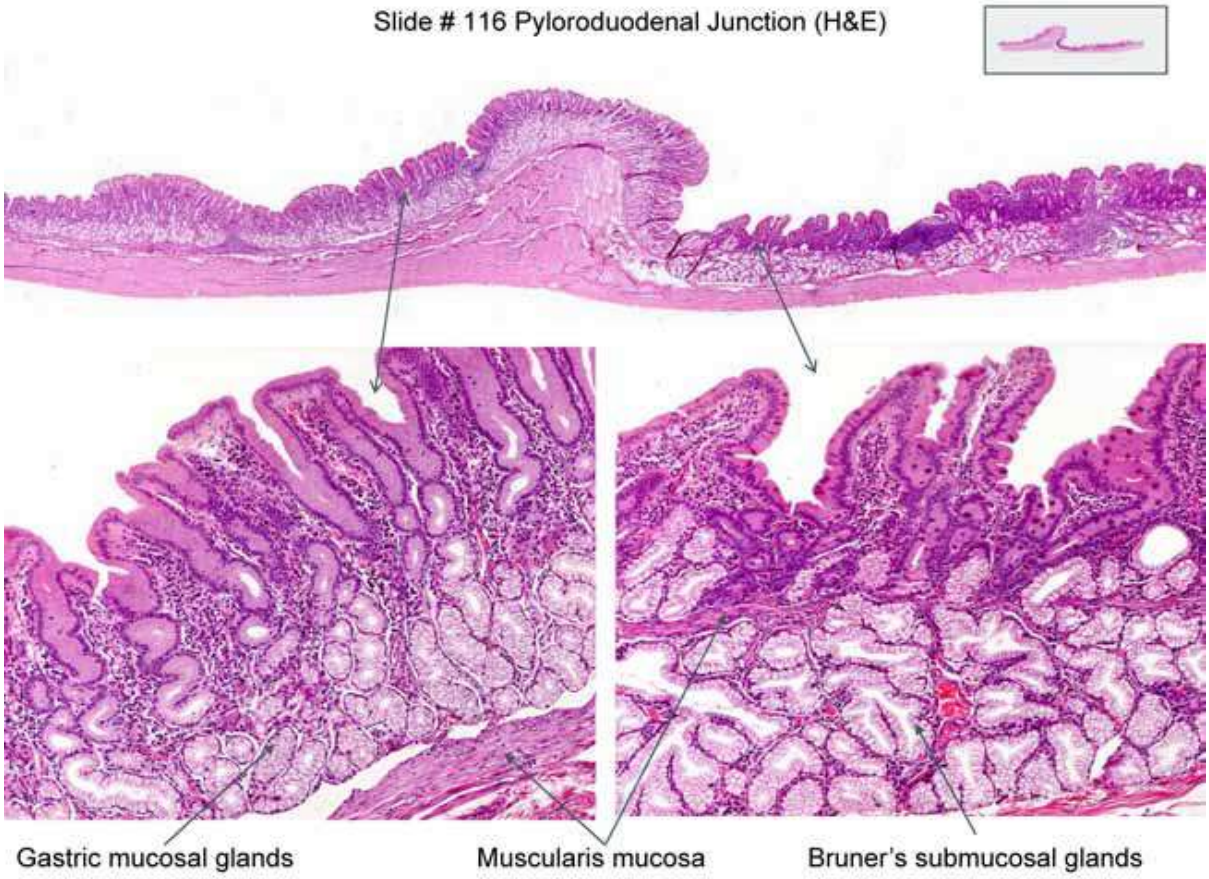
Slide # 115 Pyloric Stomach (H&E)

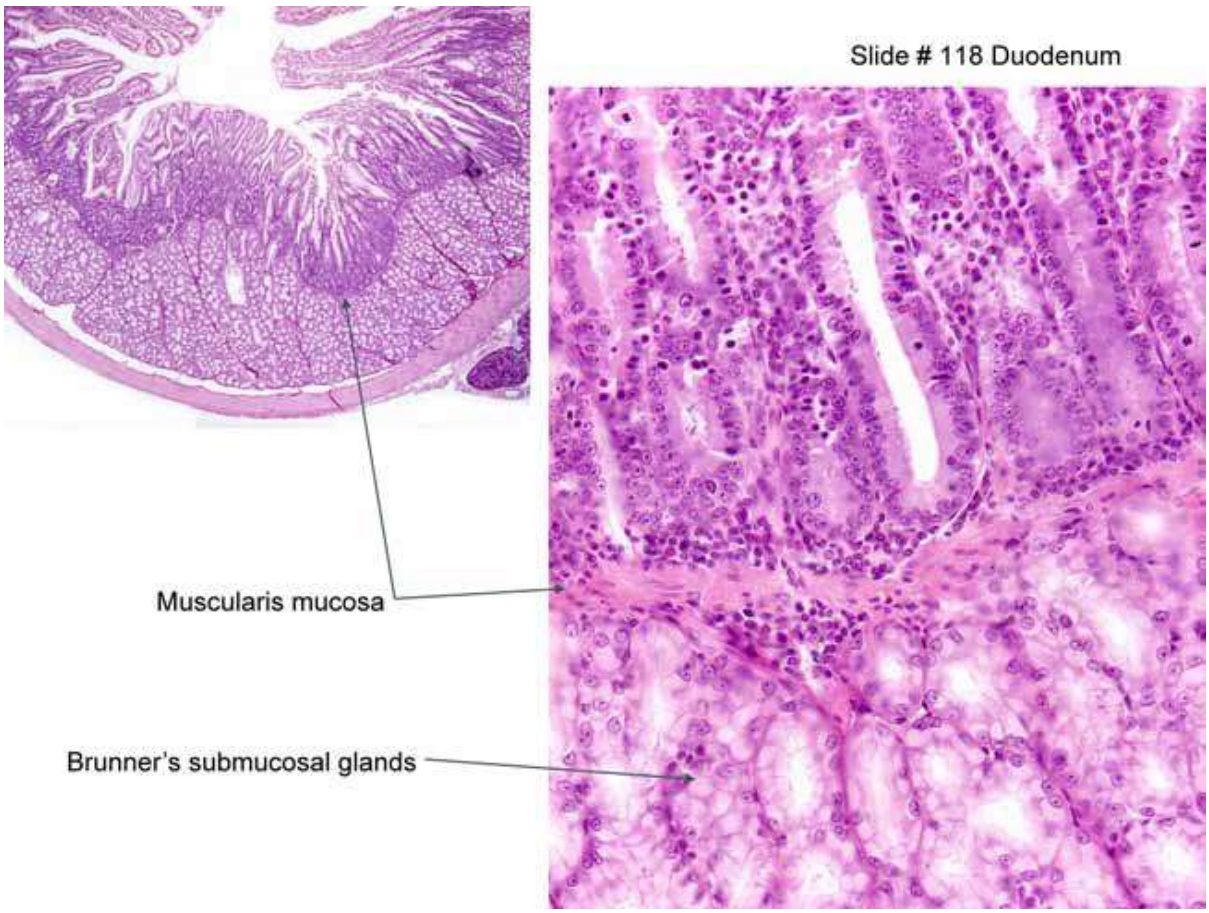


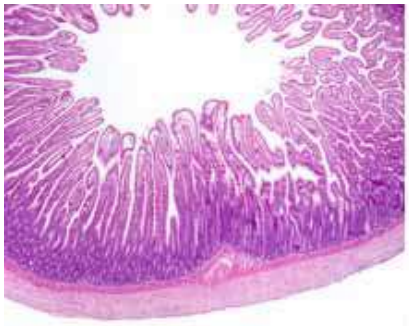
Slides 111&115 Pyloric &
Cardiac Stomach



Slide # 116 Pyloroduodenal Junction (H&E)



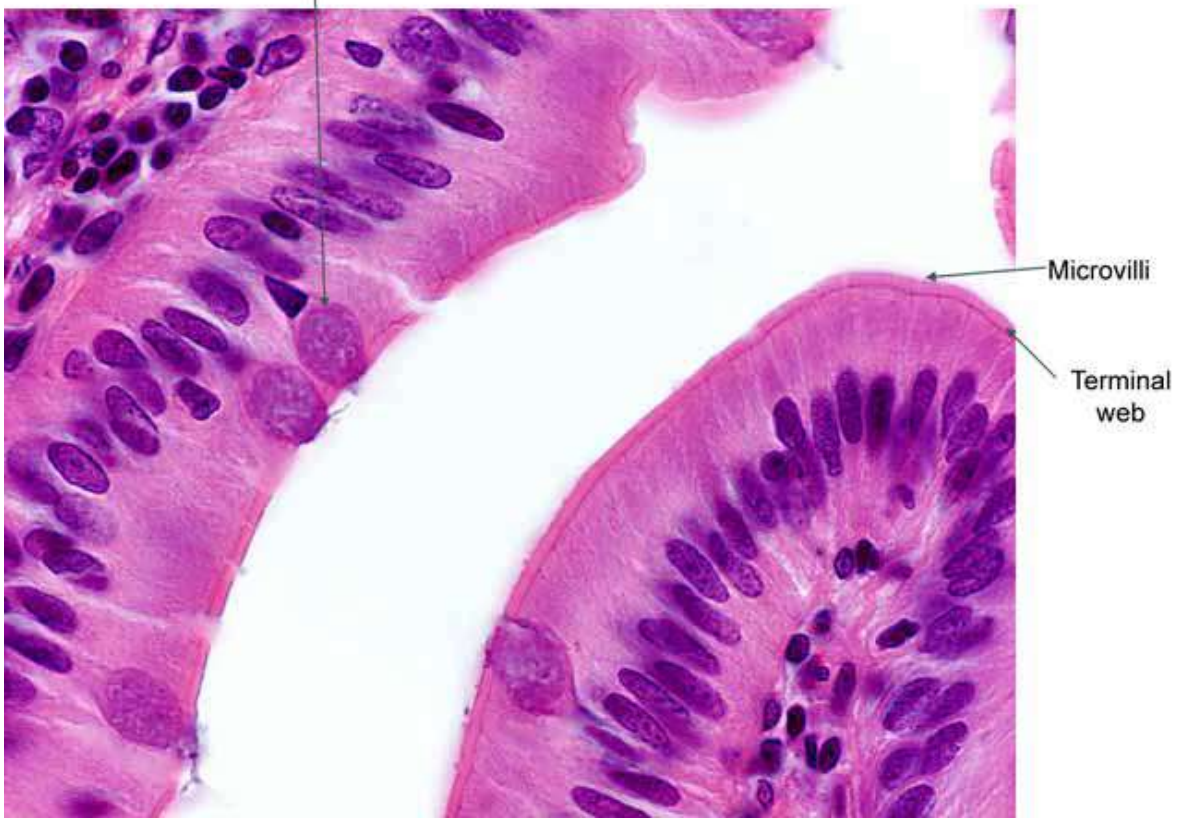

Slide # 118 Duodenum
Muscularis mucosa
Brunner's submucosal glands



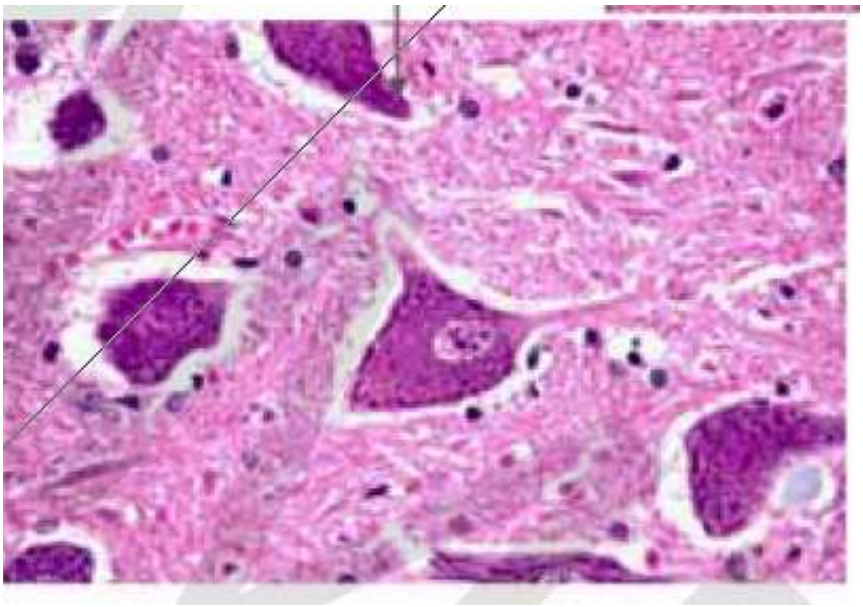
Slide # 118 Jejunum

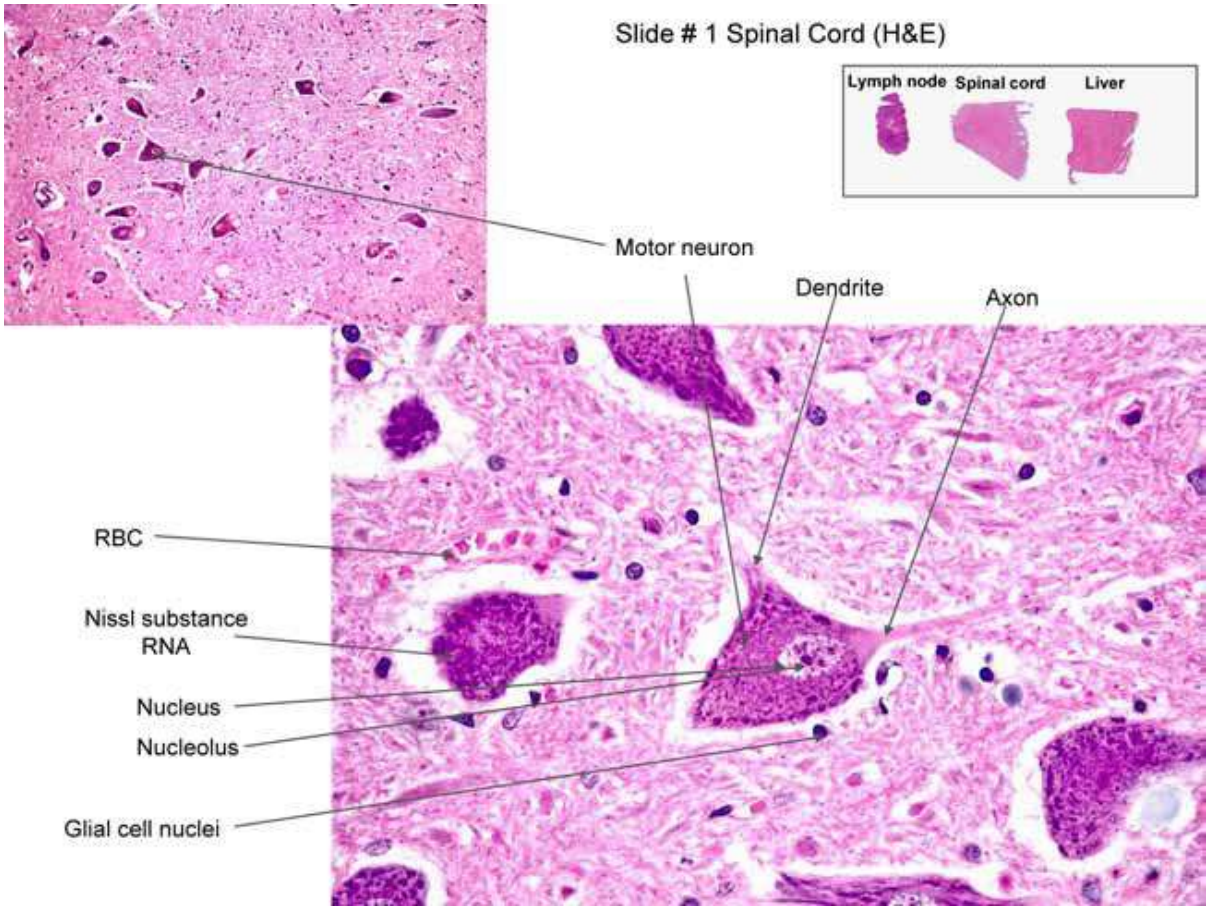
Slide # 118 Illeum (H&E)

Slide # 118 Duodenum, Jejunum and Illeum (surface epithelium)

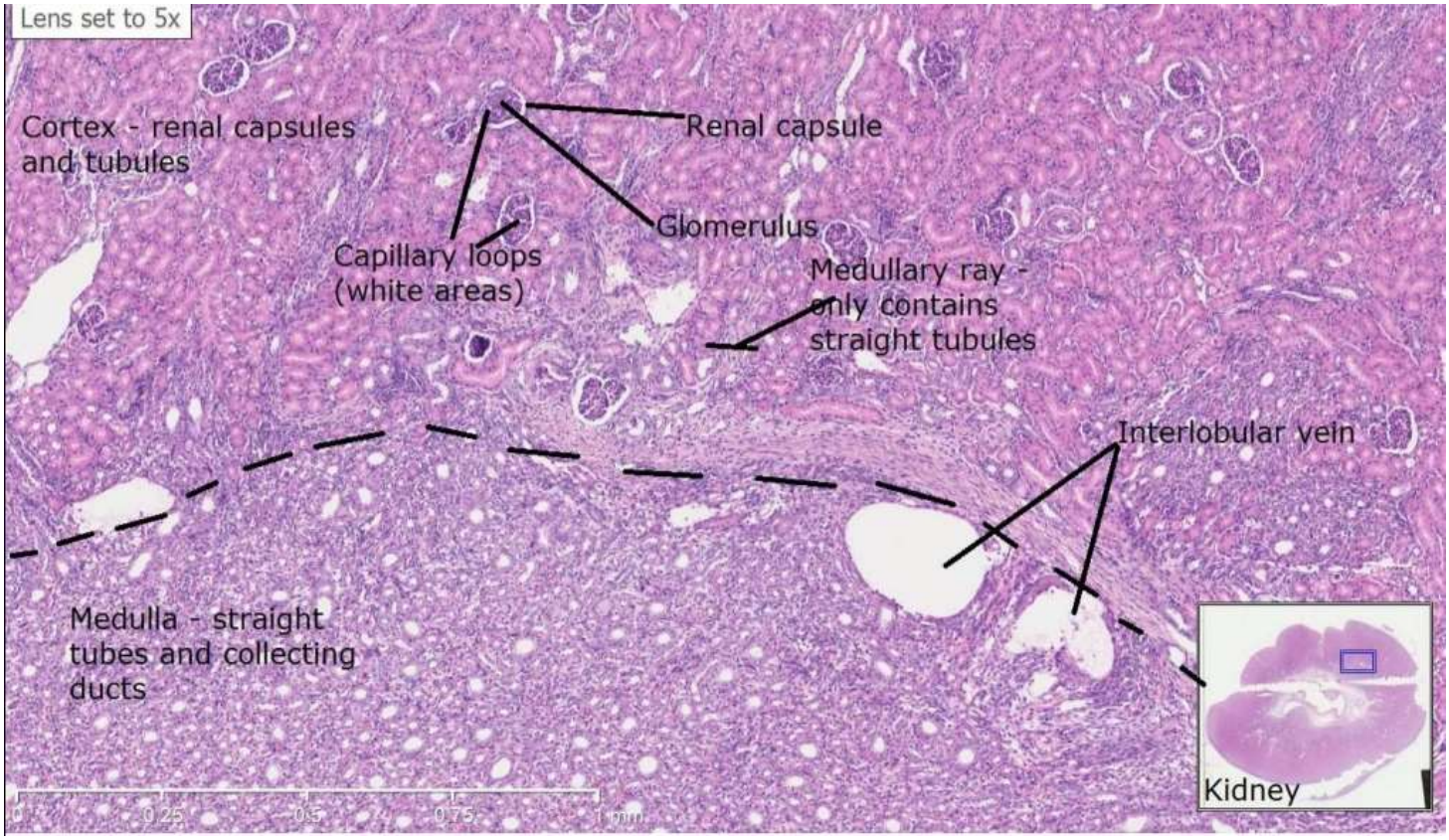


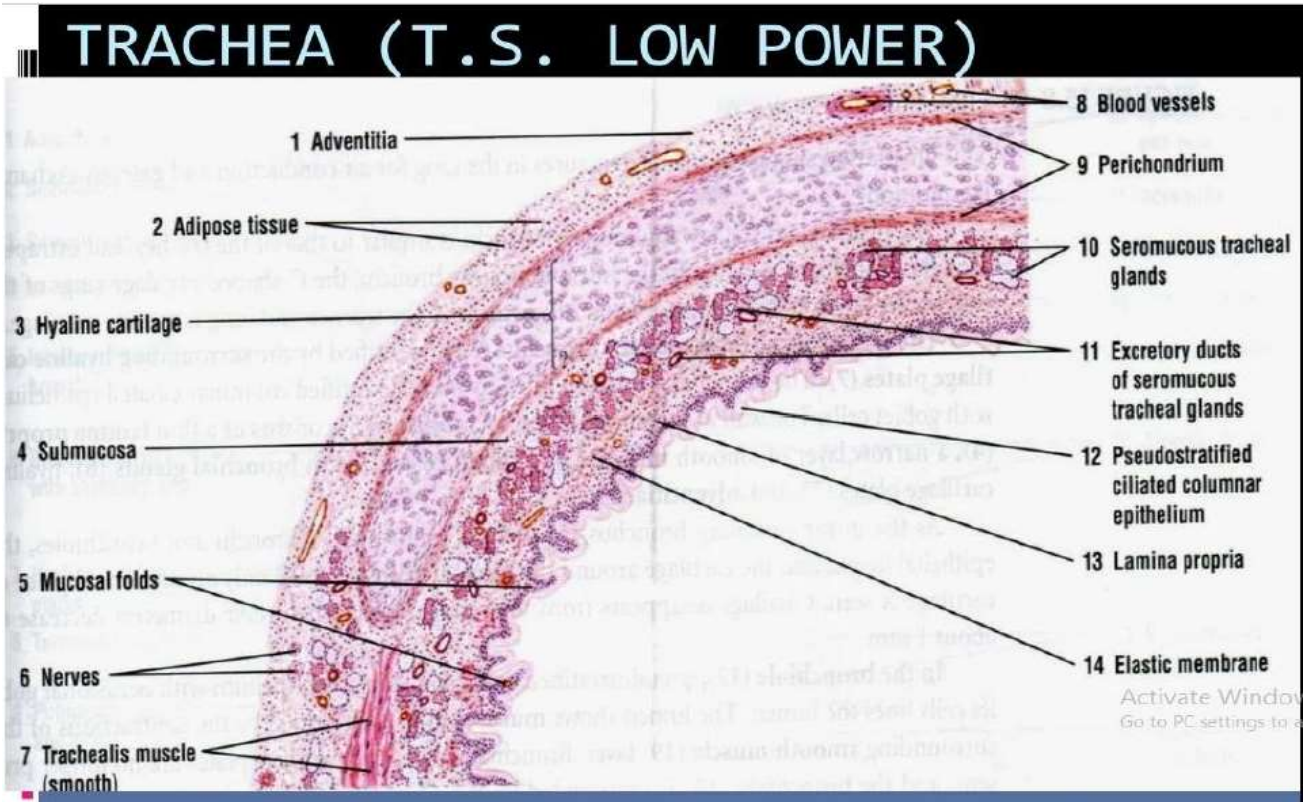
Nerve tissue

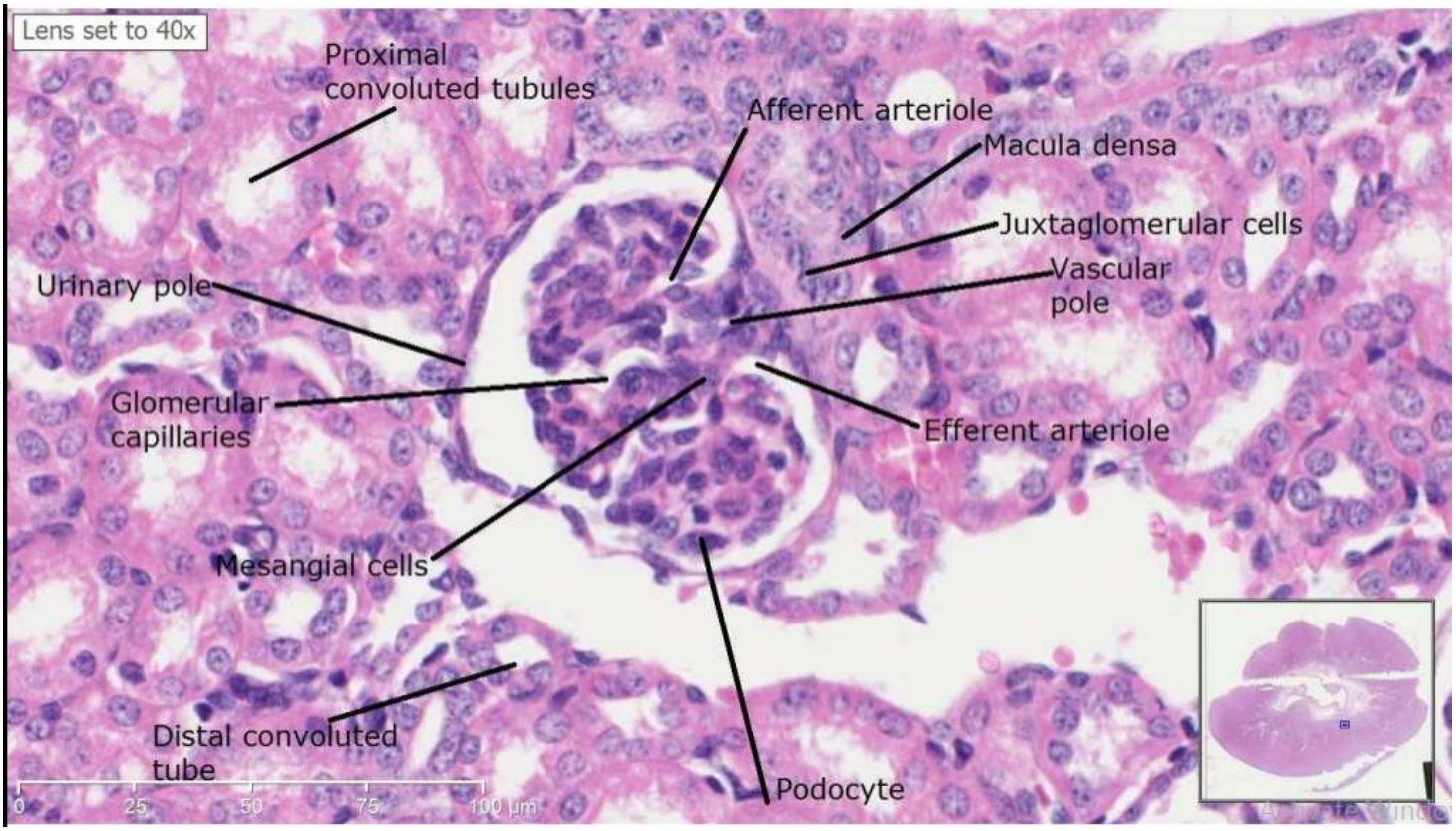




Kidney


Lens set to 5x
Cortex - renal capsules and tubules
Renal capsule
Glomerulus
Capillary loops (white areas)
Medullary ray - only contains straight tubules
Interlobular vein
Medulla - straight tubules and collecting ducts
Kidney


TRACHEA (T.S. LOW POWER)
1 Adventitia
2 Adipose tissue
3 Hyaline cartilage
4 Submucosa
5 Mucosal folds
6 Nerves
7 Trachealis muscle (smooth)
8 Blood vessels
9 Perichondrium
10 Seromucous tracheal glands
11 Excretory ducts of seromucous tracheal glands
12 Pseudostratified ciliated columnar epithelium
13 Lamina propria
14 Elastic membrane


Lens set to 40x
Proximal convoluted tubules
Afferent arteriole
Macula densa
Juxtaglomerular cells
Urinary pole
Vascular pole
Glomerular capillaries
Efferent arteriole
Mesangial cells
Distal convoluted tube
Podocyte
0
25
50
75
100 µm

HISTOLOGY OF LUNG