Laboratory Manual: Animal Physiology & Behavior updated (ZOO502)

Virtual University

Virtual University of Pakistan

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Practical No: 1
Determination of hemoglobin content, determination of hematocrit and cell counting

General information

Hemoglobin is a conjugated protein composed of the basic protein globin linked to 4 heme molecules. Ninety-eight percent of all the iron found in the blood is contained in hemoglobin. Hemoglobin transports oxygen and carbon dioxide. This important substance reacts with oxygen to form oxyhemoglobin. In the tissues, oxygen is released and reduced hemoglobin is formed. Hemoglobin can react with acids, bases, and oxidizing and reducing agents. It also can exist in a variety of forms. The normal values for hemoglobin determinations are as follows:

	Grams per 100 ml blood	Percent
Woman	12.5 to 15	83 to 110
Men	14 to 17	97 to 124
Newborn infants	17 to 23	97 to 138

Fig 1.1: The volume of hemoglobin in male and female

Compounds of hemoglobin

- **Oxyhemoglobi**n. Oxygen combines loosely with iron (ferrous state) in hemoglobin. The loosely attached oxygen diffuses into the tissues for oxidative processes. The hemoglobin then binds carbon dioxide and exists as reduced hemoglobin.
- **Carboxyhemoglobin**. Hemoglobin combines with carbon monoxide to form carboxyhemoglobin. Carbon monoxide has an affinity 200 times greater for hemoglobin than oxygen does. Hemoglobin in this combination is incapable of oxygen transport.
- **Methamoglobin**. This compound is formed when the ferrous state of the heme is oxidized to the ferric state. This compound is incapable of oxygen transport.
- **Sulfhemoglobin**. This compound results from the combination of inorganic sulfides and hemoglobin. This compound is incapable of oxygen transport. This is an irreversible reaction.
- **Cyanmethemoglobin**. This compound results when methemoglobin combines with the cyanide radical. This compound is used in hemoglobinometry.

Hemobloginometry

The hemoglobin concentration is directly proportional to the oxygen-combining capacity of blood. Therefore, the measurement of the hemoglobin concentration in the blood is important as a screening test for diseases associated with anemia and for following the response of these diseases to treatment. There are four basic ways to measure the hemoglobin concentration:

1. Measurement of the oxygen-combining capacity of blood (gasometric)
2. Measurement of the iron content (chemical method)
3. Colorimetric measurement of specific gravity (gravimetric method)
4. The cyanmethemoglobin method is the method of choice and is most widely used

Cyanmethemoglobin (hemoglobin-cyanide) method for estimation of hemoglobin

This is the method of choice for estimation of hemoglobin and is recommended by International Committee for Standardization in hematology. This is because

i. All forms of hemoglobin are converted to cyanmethemoglobin (except sulfhemoglobin)
ii. A stable and reliable standard is available.

Principle

Blood is mixed with a solution of potassium ferricyanide, potassium cyanide and a non-ionic detergent (Drabkin's solution). Erythrocytes are lysed producing an evenly distributed hemoglobin solution. Potassium ferricyanide converts hemoglobin to methemoglobin, and methemoglobin combines with potassium cyanide to form cyanmethemoglobin (hemiglobincyanide). All forms of hemoglobin present in blood are completely converted to a single compound, cyanmethemoglobin. When the reaction is completed, absorbance of the solution is measured in a spectrophotometer at 540 nm. At this wavelength, cyanmethemoglobin has a broad absorbance peak. To obtain the amount of hemoglobin in the unknown sample, its absorbance is compared with that of the standard cyanmethemoglo-bin solution (the hemoglobin concentration of which is known) by using a formula or a previously prepared graph/table.

Equipment

1. Photoelectric colorimeter or spectrophotometer
2. Sahli's pipette marked at 20 µl (20 cmm)
3. Pipette 5 ml

Reagents

1. Drabkin's solution (ph 7.0-7.4): Potassium

ferricyanide 200 mg Potassium cyanide 50 mg

Potassium dihydrogen phosphate 140 mg Non-ionic detergent 1 ml

Distilled water to 1000 ml

2. Cyanmethemoglobin standard solution with known hemoglobin value.

Specimen: EDTA-anticoagulated venous blood or blood obtained from skin puncture.

Method

1. In a test tube, take 5 ml of Drabkin's solution and to it add 20 µl of blood (1:251 dilution). Stopper the tube, mix by inverting several times, and allow standing for at least 5 minutes. This time is adequate for conversion of hemoglobin to cyanmethemoglobin.
2. Transfer the test sample to a cuvette. Read the absorbance in a spectrophotometer at 540 nm or in a photoelectric colorimeter using a yellow-green filter. Also take the absorbance of the standard solution. Absorbance should be read against reagent blank (Drabkin's solution).
3. Hemoglobin value is derived from the formula given below or from the previously prepared graph or table.

$$\text{Hemoglobin in gm/dl} = \frac{\text{Absorbance of test sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard} \times \frac{\text{Dilution factor}}{100}$$

Preparation of graph and table: If a graph or a table is prepared which correlates absorbance with hemoglobin concentration, result can be obtained quickly. This is particularly suitable when a large number of samples are regularly processed on the same instrument. Diluted cyanmethemoglobin standards are available commercially for preparation of a calibration graph. Alternatively, standard cyanmethemoglobin solution is diluted serially with Drabkin's solution. On a linear graph paper, hemoglobin concentration (horizontal axis) in each dilution is plotted against the absorbance (vertical axis). A straight line joining the points and passing through the origin is obtained. From this graph, a table can be prepared relating absorbance to hemoglobin concentration.

Determination of hematocrit in bloodsample

Introduction

The hematocrit or packed cell volume (PCV) determines the percentage of red blood cells (RBCs) in whole blood.

The normal hematocrit value for men is 42% to 52%; for women, 37% to 47%; and for newborns, 53% to 65%. When hematocrit determinations are below normal, medical conditions such as anemia and leukemia may be present. Above-normal hematocrit determinations indicate medical conditions like dehydration, such as occur in severe burn cases.

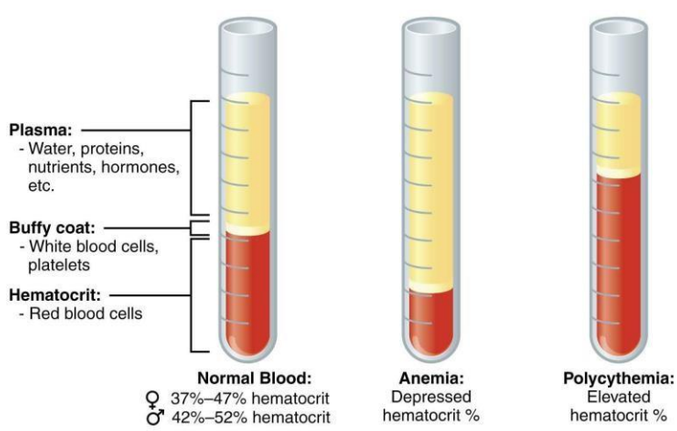


Fig 1.2: Detail of blood parts

Currently, automated hematology analyzers supply most hematocrits. However, when hematology analyzers are not available, hematocrit determinations can be manually performed by two methods,

1. Macrohaematocrit
2. Microhaematocrit

Both methods call for the blood to be centrifuged, and the percentage of packed red cells is found by calculation. The microhematocrit method is the most accurate manual method of determining blood volume and should be used whenever feasible.

Fig 1.3: Parts of blood

1. Macrohaematocrit

A large volume of blood is required in this method. Approximately 2 to 4 ml is required.

Principle:

Anticoagulated blood is taken in a Wintrobe tube. Fill upto the uppermost mark and then rotate for desired length of time. The packed cell volume (PCV) of red cells is directly read from the graduated tube as %.

Requirement:

1. Blood specimen: EDTA or double oxalated anti-coagulated blood is used in this method. Determine P.C.V. within six hr. of blood collection.
2. Wintrobe Tube: It is 110 mm in length and 2.5 mm in diameter. The lower 100 mm are graduated or marked, from 100 at top and 0 (zero) at bottom for PCV.
3. Long necked pasture pipette or a special type of syringes is used for filling Wintrobetube.
4. Centrifuge machine with known speed.

Procedure:

1. Mix 0.4 ml of EDTA with 2 ml blood.
2. Fill the Wintrobe tube up to upper most mark with the help of pasture pipette or syringe.
3. Fill the Wintrobe tube to balance first one. If the blood sample is not available, fill the tube with water.
4. Place the Wintrobe tube in opposite side in centrifuge. Turn the centrifuge to slow speed, then slowly increase the speed to 3,000 rpm. Centrifuge for 30 min. at 3,000 rpm. After 30 min. switch off the centrifuge and allow it to stop by itself.
5. Take out the Wintrobe tube and read PCV directly with the help of graduation mark given on the tube.

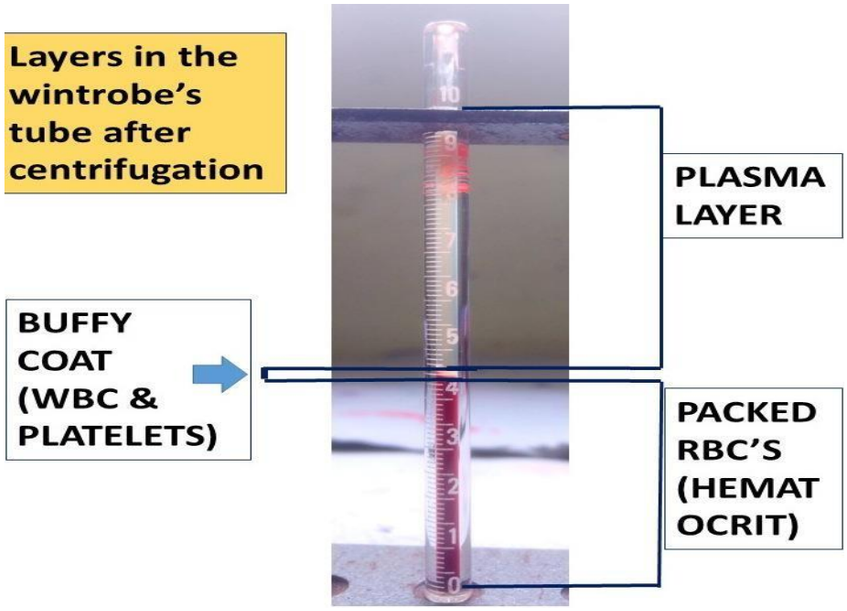


Fig 1.4: View of macrohaematocrit

2. Microhaematocrit

This method requires small amount of blood, 2 to 3 drops only. The blood can be obtained by finger puncture.

Principle:

Anti-coagulated blood is centrifuged in a sealed capillary tube, and then PCV is determined by a special haematocrit reader.

Requirement

1. **Blood Specimen:** Blood from finger puncture may be used or EDTA or double oxalate venous blood can also be used.
2. **Capillary Tube:** Use plain capillary tube for anti-coagulated venous blood and use heparinised capillary tube (Coated with heparin internally) for blood obtained from finger puncture. The capillary tube is approximately 75 mm in length.
3. **Microhaematocrit Centrifuge:** This is a special type of centrifuge. It has speed about 15,000 r.p.m. The top of centrifuge is flat with grooves. The centrifuge also has timer, which is usually set for 5 min.
4. **Haematocrit Reader:** There are several types of readers used for reading hct. The simplest method is use of card reader, which can be made by hand.
5. **Clay:** This is used to seal the end of capillary tube.

Procedure:

1. Draw the blood sample into appropriate capillary tube with capillary action. Use plain tube for anti-coagulated blood and heparinized tube for plain blood. In case of finger puncture, the blood should flow freely with little pressure. Now wipe off the first drop and then collect the blood specimen.

2. Fill the tube about 3/4th length with blood. Seal the end of the tube with clay or wax or ultimately by heating. The sealing should be about 2 mm deep. Place two hct tubes in the groove of centrifuge exactly opposite to each other.

3. It is not necessary that the capillary tube have exact amount of blood level. In case, if there is no filled capillary tube to balance we can use an empty capillary tube. Centrifuge at 13,000 ± 2000 rpm for 15 minutes. If the hematocrit exceeds 50 percent, centrifuge for an additional 3 minutes.

4. Remove capillary tube from centrifuge. It will show three layers. Top layer is of plasma or serum; the middle layer is thin creamy white in color and is known as Buffy coat. It is a layer of WBC; the last layer is the column of RBC. Use the hct reader for finding the value of hct.

Card Reader:

The reader is used as, hold the tube against Scale so that the bottom of red cell is matched with 0 (zero) line of the card. Move the tube across the card until the uppermost line of plasma is matched to 100% line of card.

Check to make sure that bottom of red cell column is still in the line of zero and the tube should be straight and vertical. The line that passes through the top of the column of RBC gives the hct value.

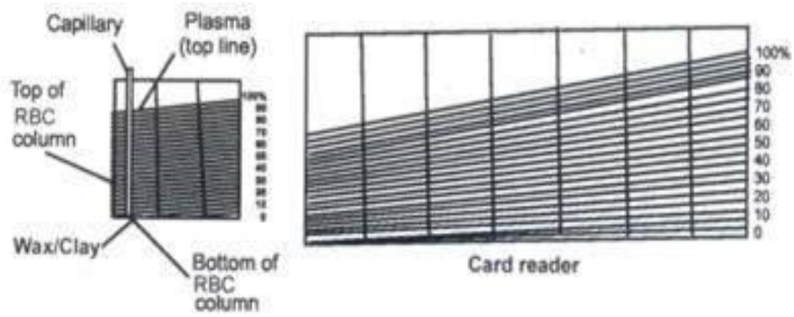


Fig 1.5: View of microhaematocrit

RBC Counting by usingHemocytometer

Introduction

Although a variety of automated cell counting instruments have been developed, Hemocytometer remains the most common method used for cell counting around the world. The most frequently used hemocytometer is the Neubauer (or 'Improved Neubauer') chamber. Other hemocytometers include the Burker, Thoma and Fuchs-Rosenthal. Using these, the particles (e.g., leucocytes, erythrocytes, thrombocytes, bacteria, fungus spores, pollen) are visually counted under a microscope. Neubauer's chamber is a thick glass plate with the size of a glass slide (30x70x4mm). The counting region consists of two square shaped ruled areas. There are depressions or the moats on either side or in between the areas on which the squares are marked thus giving an "H" shape.

The ruled area is 3mm2 divided into 9 large squares each with a 1 mm^2 area. The large central square (which can be seen in its entirely with the 10X objective), is divided into 25 medium squares with double or triple lines. Each of these 25 squares are is again divided into 16 small squares with single lines, so that each of the smallest squares has an area of 1/400 mm^2.

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 ruled area is 0.1 mm lower than the rest of the chamber. So that when a cover slip is kept on the counting region, there is a gap of 0.1 mm (1/10mm) between the cover slip and the ruled area.

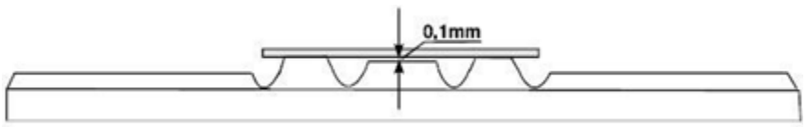


Fig 1.6: View of hemocytometer

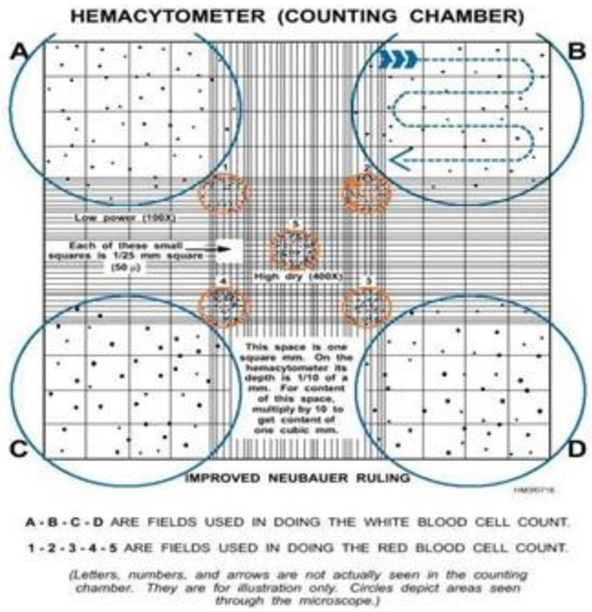


Fig 1.7: Detail of hemocytometer

The counting can be done either in the central large square or in the corner squares, depending on the size of the cells under study.

WBC Counting Area

The four large sqaures placed at the corners are used for white blood cell count. Since their concentration is lower than red blood cells a larger area is required to perform the cell count.

RBC Counting Area

The large center square is used for RBC counts. As already stated, this area is subdivided into 25 medium sqaures, which in turn are each divided into 16 squares. Of the 25 medium sqaures, only the four corner squares and the center square within the large center square are used to perform RBC counts.

Materials:

Biologicals: 1. Droplet of blood from capillary

Glass/plastic ware:

1. RBC diluting pipette with hose and bulb
2. Trash bin for lancets
3. Plastic droppers

Chemicals

1. RBC diluent 3.2 or 4% w/v Sodium Citrate ($Na_3C_6H_5O_7$) /d.H_2O/ Normal Saline (Saline maintains the normal disk shape of RBC and prevents auto agglutination)
2. 95% Alcohol for rinsing

Instruments

1. Sterile lancet
2. Hemocytometer for RBC and WBC counting
3. Microscope

Others

1. Tissue paper
2. Gloves for use while staining

Method

1. Sample Preparation

1. As before make a pin-prick using a fresh unused lancet on the index- or ring finger. If you have been pricked

before on that finger, use another finger.

2. Bring a cleaned RBC dilution pipette tip close to the droplet of oozing blood. Using the bulb allow (by capillarity and pressure) blood to enter up to the 0.5 mark.
3. Using a small tube of Sodium-Citrate solution, additionally aspirate this solution to reach the mark 101 (1:200 dilution)
4. Gently turn the dilution tube in your hand.

2. Preparing Neubauer Chamber

Clean the Neubauer chamber and the cover slip with 70% EtOH. Put the glass cover on the Neubauer chamber central area. Use a flat surface to place the chamber, like a table or a workbench.

3. Introducing the sample into the Neubauer chamber

With a pipette, carefully draw up around 20 ml of the cell mixture (dilution). Place the pipette tip against the edge of the coverglass and slowly expel the liquid until the counting chamber is full. Capillary action will help to ensure that the counting chamber is full, but care should be taken not to overfill the chamber. A volume of 10 ml is sufficient to fill one counting chamber.

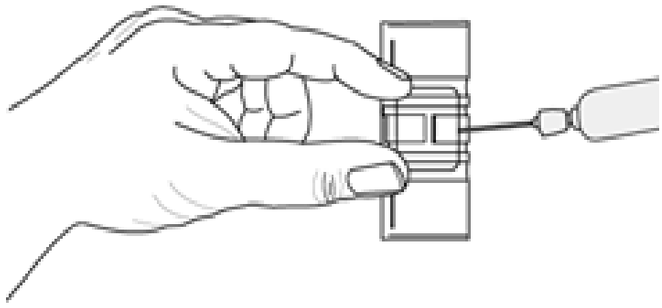


Fig 1.8: Set sample on hemocytometer

4. Microscope focusing and Cell Counting

1. Place the Neubauer chamber on the microscope stage. Using the 10X objective, focus both onto the grid pattern and the cell particles.
2. To count the RBCs and Platelets, the microscope must be switched to 40X objective. Count the cells in the respective areas as stated early.
3. Write down the amount of cells counted
4. If cells are touching the 4 perimeter sides of a corner square, only count cells on 2 sides, either the 2 outer sides or 2 inner sides.

Calculations:

- Count the number of cells in the small 5 squares in the center of hemocytometer
- These 5 squares equals to 1/5 of the whole center square → $\frac{\text{no. of cells counted X dilution factor (200)}}{1/5 \text{ X volume (0.1)}}$

OR: $\frac{\text{number of cell counted x dilution factor x 5}}{\text{volume (0.1)}}$

= cell/µL

Practical No: 2
Preparation of blood smears

Introduction

Blood is the only tissue that flows throughout your body. This red liquid carries oxygen and nutrients to all parts of the body and waste products back to your lungs, kidneys and liver for disposal. It is also an essential part of your immune system, crucial to fluid and temperature balance, a hydraulic fluid for certain functions and a highway for hormonal messages.

Blood cells develop in the bone marrow from a common stem cell in the process known as hematopoiesis. Once mature, cells are divided into groups that reflect their morphological and functional characteristics including the erythrocytes, or red blood cells, the granulocytes, agranulocytes and the megakaryocytes.

The megakaryocyte is found in bone marrow samples, and develops into thrombocytes (platelets) prior to release into the blood stream. The granulocytes are white blood cells, or leukocytes that have a granular cytoplasm and polymorphic nuclei; this group includes neutrophils, eosinophils, and basophils. The neutrophils are the most common cell in this group, and they function together with the other granular cells as part of the nonspecific natural immune response to infection and the inflammatory response to tissue injury.

The agranulocytes include lymphocytes and monocytes. Lymphocytes differ from granulocytes and monocytes as they form part of the acquired immune response, dividing into B or T cells to fight attacks by foreign cells, bacteria and viruses. Monocytes leave the blood stream to enter tissues where they become macrophages, which engulf bacteria and tissue debris by phagocytosis. Other cell types found in tissues include mast cells, which are found in mucosal and connective tissues and are granular in nature.

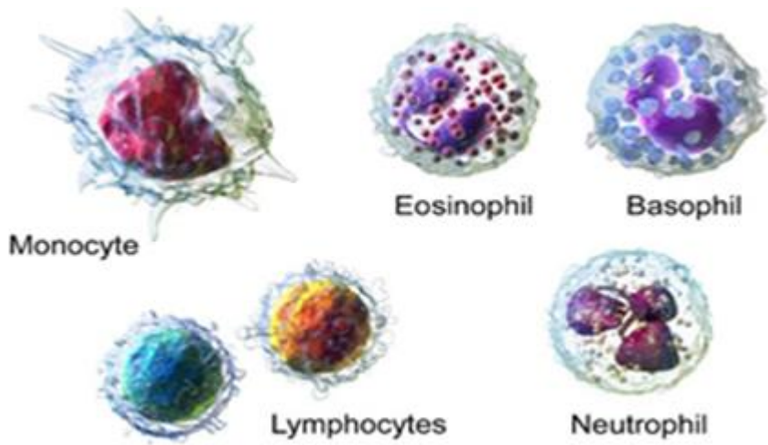


Fig 2.1: Different types of White blood cells

Materials/Equipment:

Following material and instruments are required.

1. Droplet of blood from capillary
2. 50 ml beaker for waste material
3. Trash bin for lancets
4. Plastic droppers
5. 95% Alcohol for rinsing
6. 99% Methanol for fixation
7. Giemsa stain (1:20, vol/vol from stock)

8. Sterile lancet
9. Microscope
10. Tissue paper

Procedure

A. Making blood smear

1. Place a clean slide on a piece of tissue paper and write your unique initials in one corner, using the glass-marker pen.
2. Using a cotton swab dipped in 75% ethanol, clean the middle- or ring-finger with it. This both disinfects the surface as well as causes a slight increase in blood flow due to the evaporation and resultant compensatory blood-rush.
3. Carefully open the protective covering of a sterile Stab gently (vertical w.r.t. the length of the finger) the finger-tip and wait for a drop of blood to come up.
4. Make this drop fall ~1 cm from one of the short-edges of a clean slide.
5. Using the other slide, make contact at 30-40 degrees with the slide on which the blood droplet is. Drag the droplet towards the other end. As a result you should have a comet-like appearance of the blood smear.
6. The smear should have a 'comet' like appearance- thick initially and becoming very thin at the end. The comet tail area is the one we will observe under the microscope.

B. Staining the smear

1. Fix the smear in ~99% methanol 3-5 minutes by dipping in Coplin Jars.
2. Stain in Giemsa (methylene blue and eosin mixture) by dipping in Coplin Jar containing stain for a total time ~15 minutes.
3. Rinse the slide with tap water at room temperature (ensure rinsing doesn't wash away your sample).
4. Drain off the water by leaning it at ~45 degrees and leave it to air-dry.

C. Microscopy

Observe under 10 x magnifications to see that the cells are stained. Move to the 40x lens to see further details (be careful to prevent lens and slide collisions). Erythrocytes are the most numerous cells with a diameter of ~6-7 micrometers. Some larger macrocytes (d>9 um) and smaller microcytes (d<6 um) have been seen. Purple diskettes around 3 um diameter are platelets. Leukocytes show nuclear stains purple in colour. The different granule patterns and nuclear morphologies allow a classification.

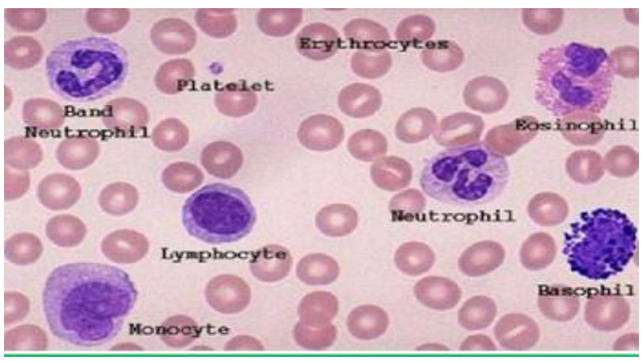


Fig 2.2: Types of blood cells

Practical No: 3 Nerve muscle preparation, Muscle twitch, Comparison of muscle and nerve irritability, effect of stimulus strength, effect of stimulus frequency (tetany), effect of load or stretch, effect of prolonged activity (fatigue), neuromuscular fatigue, stimulation of motor points in frog

Introduction

The functional unit of skeletal muscle activity is the motor unit. Interposed between the axon branches and the muscle fibers is a structure called the neuromuscular junction (motor end-plate). The most convenient way of examining the properties of the motor unit is by use the nerve-muscle preparation.

Purpose

1. To make a sciatic nerve & gastrocnemius (SNG) preparation
2. To measure the effects of different stimulus intensities on the muscle response
3. To measure a single twitch, incomplete tetanus and complete tetanus

Chemicals

Ringer's solution is a solution of recently boiled distilled water containing NaCl 6.5g, KCl 0.14g, CaCl2 0.12g, NaHCO3 0.20g, NaH2PO4 0.01g per liter

Equipment

- RM6240 biological signal collecting system
- Force transducer

Procedure

For intact Nerve-Muscle preparation, pith the toad, resulting in the toad not feeling pain. As soon as the brain and spinal cord destroyed, the toad should become flaccid. Then peel the skin of one leg. Fix the toad on the frog board with the dorsal surface uppermost. Identify the gastrocnemius muscle. Tie a knot around the Achilles tendon using suture thread. Cut the Achilles tendon as close to the bottom of the foot as possible. Locate the sciatic nerve in the thigh region. Use the glass hooks to separate the sciatic nerve. Pass a piece of thread beneath the sciatic nerve.

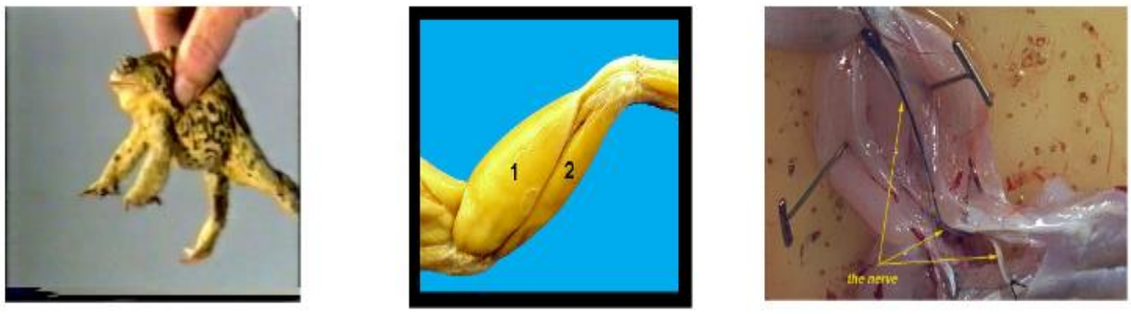


Fig 3.1: Nerve-Muscle

Hold knee in place by pushing down two T-pins. Tie the free end of the thread to the hole on the end of the tongue of the force transducer. Place the stimulating electrode on the sciatic nerve.

Warning:

- Do not touches the nerve with any object that is made of metal; this will render the nerve useless.
- Avoid stretching or otherwise damaging the nerve. Keep the tissue moist with frog ringer's solution.

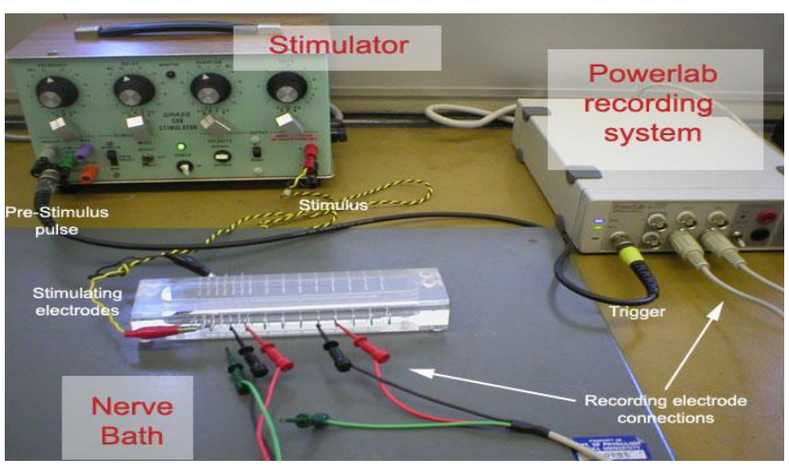


Fig 3.2: View of electrocardiogram

Effect of stimulus intensity on twitch amplitude

Set record parameters. The recording trace should be set to zero.

Apply a light load to the muscle by raising the force transducer with the tension adjuster until the trace moves approximately 2 g above the baseline. Warning: Make sure to lift the muscle vertically to an angle that is perpendicular to the rest of the frog leg.

Display the stimulation mark. Change the stimulus amplitude to 0.2V. Determine the threshold voltage by increasing the stimulus voltage in 0.01V increments. Stop and save the recording trace.

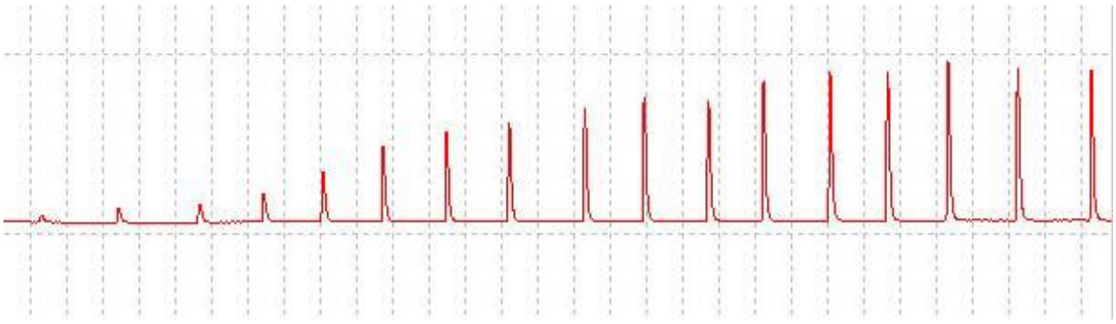


Fig 3.3: Threshold stimulus maximal stimulus

Table 3.1: Measure the following values

	Intensity stimulus (V)	**Tension of contraction (g)**
Threshold intensity		
Maximal intensity		

Table 3.2: Record the following values in a single twitch induced by maximal stimulus

	Duration (ms)
Latent period	
Contraction	
Relaxation	

Effect of stimulus frequency on twitch amplitude

Set record parameters. Click on the record button. Select the frequency ascending mode. Display the stimulation mark. Begin the recording. Set the stimulus intensity to the lowest value that will give strong contraction as during the twitch amplitude calculation. Stop and save the recording trace.

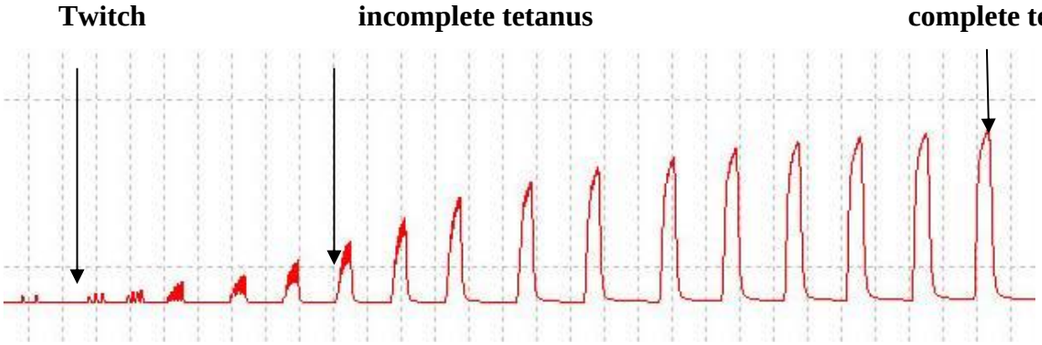


Table 3.3: Determine the following values

	Frequency of stimulus (Hz)	**Tension of contraction (g)**

Single twitch		
Incomplete tetanus		
Complete tetanus		

Practical No: 4
Recording of action potential by oscilloscope and demonstration of its various features. Experiments to demonstrate characteristic of reflex arc, experiment in human (students themselves) to demonstrate some aspect of sensory physiology

Background

Neurons are cells in the nervous system that are used to conduct signals at high speed from one part of the body to another. This enables rapid, precise responses to occur in order to compensate for changes in the environment.

Neurons are able to send signals at high speed due to their ability to generate and conduct an electrical signal called an action potential down the length of their axons. An action potential is a brief reversal of the membrane potential, so that for a brief interval at a segment of the axon the intracellular fluid just inside of the plasma membrane is more positive than is the extracellular fluid just outside the plasma membrane. This signal is typically generated at the axon hillock of the neuron, and requires the opening of voltage-gated ion channels — specialized pore-like transmembrane proteins that open to allow ion passage in response to changes in the relative charge difference across the plasma membrane.

There are two different types of voltage-gated ion channels important for the generation action potentials: those specific for sodium ion (Na+), and those specific for potassium ion (K+). In the intervals between action potentials (i.e., when the neuron is "resting") the two types of ions are kept at different concentrations across the plasma membrane. Na+ is maintained at higher concentrations outside the cell than inside the cell. Conversely, K+ tends to be accumulated at higher concentrations inside the cell than outside the cell. The potential for movement of these ions across the cell membrane is thus influenced by the concentration gradients for each ion. Moreover, charge differences across the cell membrane affect the potential for diffusion of these ions. The interior of cells is typically more negatively charged than is the outside of the cell, due to negative charges on certain side-chains of the amino acids of proteins inside the cell, phosphorylated compounds (e.g., ATP), etc. As a result, under resting conditions, there is a strong electrochemical gradient favoring the flow of Na+ into the cell, and a weak electrochemical gradient favoring the flow of K+ out of the cell. Ion concentrations are maintained at relatively constant levels, however, due to the normally low permeability of the plasma membrane to Na+ and low-level activity of the Na+/K+ pump, which pumps Na+ back out into the extracellular fluid and K+ back into the intracellular fluid. The distribution of charged particles across the cell membrane at rest generates the resting potential of the cell membrane, which is variable among different neurons, but typically around -70 mV. The membrane potential (the difference in overall charge across the plasma membrane) of the neuron can change if the relative difference in charges across the membrane is changed. The action potential is generated by just such a redistribution of charged particles across the membrane. By opening large numbers of voltage-gated channels, the permeability of the membrane to Na+ and K+ is increased markedly, allowing the ions to flow along their respective electrochemical gradients from one side of the membrane to the other.

Experimental Procedures

We will be running a simulation of experiments conducted on whole nerve segments using PhysioEx™ (Benjamin Cummings). The software should be loaded for you and the screen illustrated in Figure.

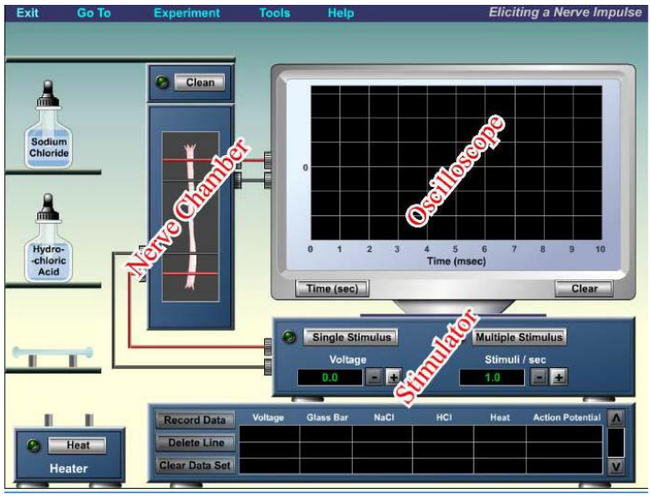


Fig 4.1: PhysioEx™ software

There are three different pieces of equipment displayed on the screen which you should note. First, there is a nerve chamber. A segment of a nerve has been dissected and suspended over a series of metal bars that act as electrodes. The two electrodes at the bottom are stimulating electrodes and are connected to an electrical stimulator. The stimulator can be used to apply electrical current to the nerve at different voltages and frequencies to try to elicit an action potential. The other set of electrodes are called recording electrodes, and they are connected to an oscilloscope. Differences in charge between the two recording electrodes (such as those caused by an action potential passing by) cause the line traced on the oscilloscope screen to deflect. Thus, we can observe any action potentials forming in the nerve.

Stimulation of an Action Potential

A. Electrical Stimulation – Observation of Threshold

Initially, the voltage on the electrical stimulator is set to 0.0 V. Increase the voltage to 1.0 V by clicking on the + button next to "Voltage". Then click on "Single Stimulus". Notice that a flat-line tracing is recorded on the oscilloscope, meaning no action potential was generated. Increase the voltage by 0.3 V using the + button next to "Voltage" and click on "Single Stimulus" again. A second tracing should appear in a different color. Repeat this procedure, increasing the voltage by 0.1 to 0.2 V increments until an action potential is recorded. Determine and record the minimum amount of voltage needed to evoke the action potential. This value is the threshold stimulus. Record this voltage on your data sheet.

B. Electrical Stimulation – Observation of Compound Action Potential and Determination of Maximal Stimulus

Continue increasing the voltage above threshold by 0.1-0.2 V increments. Notice that as you increase the stimulus strength, the amplitude of the action potential increases slightly (Fig 6.12). This is because as you increase stimulus strength you are reaching the threshold of more individual neurons in the nerve, more neurons undergo action potentials, and thus the compound action potentials strength increases. Eventually, though, you will reach a point where no further increase in the amplitude of the compound action potential will occur. This is because the stimulus strength is now strong enough for all the neurons in the nerve to undergo action potential. The lowest stimulus strength required to cause all the neurons in the nerve to undergo action potentials is called the maximal stimulus. Determine and record this voltage.

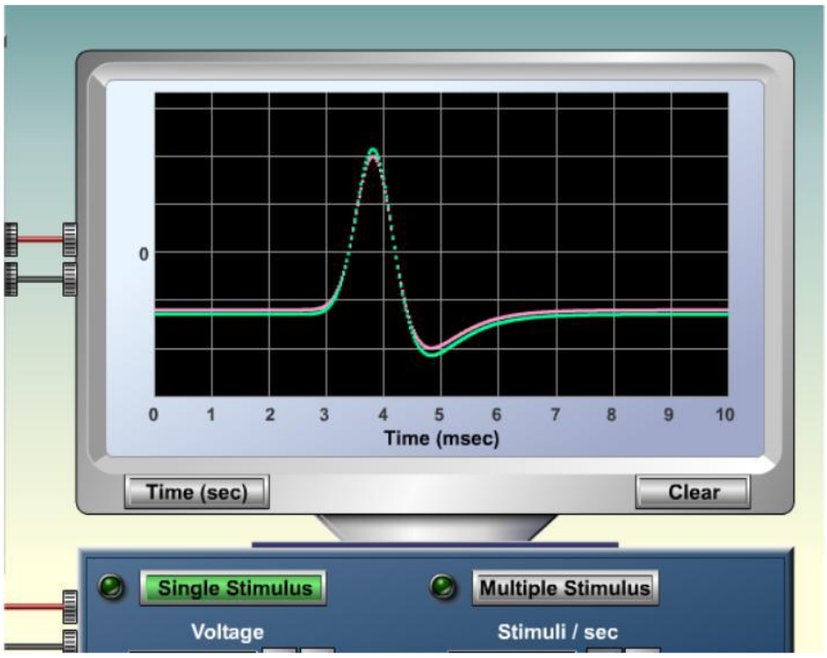


Fig 4.2: A pair of compound action potentials recorded on the PhysioEx oscilloscope.

Notice that the green tracing (produced when the nerve was stimulated at a higher voltage) has higher amplitude than does the pink tracing. This illustrates that through the submaximal range of stimuli strengths, action potential amplitude will increase with increased stimulus intensity.

C. Chemical Stimulation

Click on the "Clear" button on the oscilloscope to remove previous tracings. Move your pointer over to the dropper bottle on the left labeled "Sodium Chloride", left click on the dropper and hold, then drag the dropper over the nerve chamber and release the left mouse button to apply the NaCl solution onto the nerve. Notice what happens on the oscilloscope. If we assume that the NaCl concentration in this solution is greater than that normally found in extracellular fluid, explain why the application of NaCl to the nerve caused this response. Click the "Clean" button on the nerve chamber to wash the NaCl solution from the nerve. Then apply a dropper from the bottle labeled "Hydrochloric Acid" to the nerve. Again, note the response of the nerve, and provide an explanation for this response. Click on the "Clean" button of the nerve chamber when you are finished.

D. Heat Stimulation

Place your pointer over the glass rod on the lower left portion of the screen, left click, and drag it down to the heater just below it. Left click on the heated rod, drag it over the nerve chamber, and release the left click to apply the stimulus. Note the response of the nerve on the oscilloscope, and provide an explanation for this response.

Practical No: 5
Normal cardiac activity in amphibian model, effect of temperature, effect of drug, heart block, tantalization of heart

Introduction

Kymograph is an instrument for recording variations in pressure, as of the blood, or in tension, as of a muscle, by means of a pen or stylus that marks a rotating drum. A kymograph (which means 'wave writer') is a device that gives a graphical representation of spatial position over time in which a spatial axis represents time. It basically consists of a revolving drum wrapped with a sheet of paper on which a stylus moves back and forth recording perceived changes of phenomena such as motion or pressure.

It was invented by German physiologist Carl Ludwig in the 1840s and found its first use as a means to intrusively monitor blood pressure, and has found several applications in the field of medicine. Its primary use was to measure phenomena such as changes in muscular contractions or other physiological processes, including speech sounds. Kymographs were also used to measure atmospheric pressure, tuning fork vibrations, and the functioning of steam engines.

Fig 5.1: View of kymograph

Cardiac activity

The heart primary function is simply to act as a pump that provides pressure move blood to its ultimate destination – the tissues, the control of cardiac contractility is complex and represent a balance of intrinsic (within the heart) and extrinsic (from outside the heart) factor. An isolated heart beats regularly without any extrinsic stimulation from nerves or hormones. This mechanical automaticity reflects the fact that the action potentials which are conducted throughout the heart (the signals which lead to contraction) are generated spontaneously within the cardiac muscle itself. The cells responsible for spontaneous action potential production are often referred to as pacemaker cells because they determine the rate at which the heart beats. Several cell types in the heart are capable of pacemaker activity, but in the intact organ it is the fastest pacemaker which drives the rest of the heart.

Normally the pace making frequency is highest in a group of specialized cells called the sinoatrial node (SN\SAN), which also have a different shape of action potential. These cells dictate the rate of electrical events in the rest of the heart.

The Sino-atrial node in human is located in the wall of the right atrium close to the superior vena cava. In the frog or turtle heart the pacemaker region is the sinus venous, an enlarged region between the vena cava and the right atrium.

Purpose

To study frog heart beat records and examine some of these intrinsic and extrinsic factors that make the heart such a unique and versatile pump (To record the relative force and frequency of contraction in a resting heart).

Materials/Chemicals

- Gloves,
- Frog, Formaldehyde (or any Anesthetic) ,
- Kymograph,
- Dissection tools,
- Dissection dish,
- Ringer's Solution (10.4 g NaCl , 0.32 g KCL , 0.48 g $CaCl_2$, 0.32 g $NaHCO_4$; Make up in 11 distilled wate

Procedure

In clean aseptic work area:

1. Double pith a frog and fasten it to a frog board, ventral side up.
2. Use scissors to make a Iongitudinal incision through the skin and body wall of the thoracic region to expose the heart.
3. Hold the pericardium with forceps and carefully cut away the sac from the heart, using scissors. From this point on make sure that the heart is periodically moistened with frog ringer's solution.
4. Using forceps, gently lift the apex of the heart upward.
5. Insert a bent insect pin or small fish hook through the tip of the ventricle, being careful not to puncture the ventricle.
6. Tie a thin thread to the hook and connect the ventricle to the transducer as you connected the gastrocnemius muscle to the transducer in:
7. If physiograph type of transducer is used, attach the thread to the transducer hook and adjust the tension on the ventricle until the recording pen is raised slightly above the baseline.

Results

1. Normal heartbeat

Obtain a recording of the normal cardiac rhythm, using a medium to fast paper speed so as to distinguish the atrial and ventricular. Run a 1-second time line while recording so that the duration of systole and diastole of the

ventricle can be determined.

2. Temperature

Record the heart contractions at room temperature. Then drop warm (40°C) frog ringer's solution on the heart until significant changes are seen in rate and contractility.

Record contraction at this time finally, drop cold (5°C) ringer's on the heart and record when changes are observed.

Then rinse the heart with room temperature ringer's to return the beat to normal before continuing the experiments

Practical No: 6
Measurement of blood pressure

Introduction

A sphygmomanometer is a device that measures blood pressure. It is composes of an inflatable rubber cuff, which is wrapped around the arm. A measuring device indicates the cuff's pressure. A bulb inflates the cuff and a valve releases pressure. A stethoscope is used to listen to arterial blood flow sounds.

As the heart beats, blood forced through the arteries cause a rise in pressure, called systolic pressure, followed by a decrease in pressure as the heart's ventricles prepare for another beat. This low pressure is called the diastolic pressure.

The sphygmomanometer cuff is inflated to well above expected systolic pressure. As the valve is opened, cuff pressure (slowly) decreases. When the cuff's pressure equals the arterial systolic pressure, blood begins to flow past the cuff, creating blood flow turbulence and audible sounds. Using a stethoscope, these sounds are heard and the cuff's pressure is recorded. The blood flow sounds will continue until the cuff's pressure falls below the arterial diastolic pressure. The pressure when the blood flow sounds stop indicates the diastolic pressure.

Systolic and diastolic pressures are commonly stated as systolic 'over' diastolic e.g. 120 over 80. Blood flow sounds are called Korotkoff sounds.

Instrument required

- Sphygmomanometer

Fig 6.1: View of Sphygmomanometer

Procedures

- To begin blood pressure measurement, use a properly sized blood pressure cuff. The length of the cuff's

bladder should be at least equal to 80% of the circumference of the upper arm.

- Wrap the cuff around the upper arm with the cuff's lower edge one inch above the antecubital fossa.
- Lightly press the stethoscope's bell over the brachial artery just below the cuff's edge. Some health care workers have difficulty using the bell in the antecubital fossa, so we suggest using the bell or the diaphragm to measure the blood pressure.
- Rapidly inflate the cuff to 180mmHg. Release air from the cuff at a moderate rate (3mm/sec).
- Listen with the stethoscope and simultaneously observe the sphygmomanometer. The first knocking sound (Korotkoff) is the subject's systolic pressure. When the knocking sound disappears, that is the diastolic pressure (such as 120/80).
- Record the pressure in both arms and note the difference; also record the subject's position (supine), which arm was used, and the cuff size (small, standard or large adult cuff).
- If the subject's pressure is elevated, measure blood pressure two additional times, waiting a few minutes between measurements.
- Then ask the subject to exercise (running, jumping, etc.) for about 30 minutes and record the blood pressure.
- Now check and record the differences in blood pressure during normal conditions and after exercise. What is the difference you recorded and why is that so?

Precautions

1. Use a larger cuff on obese or heavily muscled subjects.
2. Use a smaller cuff for pediatric patients.
3. For pediatric patients a lower blood pressure may indicate the presence of hypertension.
4. Don't place the cuff over clothing.
5. Flex and support the subject's arm.
6. In some patients the Korotkoff sounds disappear as the systolic pressure is bled down. After an interval, the Korotkoff sounds reappear. This interval is referred to as the "auscultatory gap." This pathophysiologic occurrence can lead to a marked under-estimation of systolic pressure if the cuff pressure is not elevated enough. It is for this reason that the rapid inflation of the blood pressure cuff to 180mmHg was recommended above. The "auscultatory gap" is felt to be associated with carotid atherosclerosis and a decrease in arterial compliance in patients with increased blood pressure.

Practical No: 7
Oxygen consumption in fish and effect of temperature (by dissolved oxygen meter)and terrestrial animal (mouse)

Background

Dissolved oxygen concentration (DO) is considered the most important water quality variable in fish culture. In the broadest sense, however, dissolved oxygen concentration is no more important than other environmental variables because any factor that is outside the range tolerated by fish can cause stress or death. What makes dissolved oxygen concentration so important in intensive fish culture is the speed with which it can change. Over a matter of hours, sometimes even minutes, DO can change from optimum to lethal levels. No other critical environmental variable in fish culture is so dynamic. The dynamic nature of dissolved oxygen results from the interaction of three factors. First, oxygen is not very soluble in water so water has only a limited capacity to "hold" oxygen. Second, the rate of oxygen use by fish, plankton and organisms living in the pond mud can be high. Third, oxygen diffuses very slowly from the atmosphere into undisturbed water. The combination of these three factors—limited solubility, rapid use and slow replenishment—can cause rapid changes in dissolved oxygen concentrations. Dissolved oxygen levels can be managed with aeration, but the response time for taking corrective measures is short. This makes it critical to have a rapid and reliable method of measuring dissolved oxygen concentrations so that aeration devices can be activated when needed.

There are a number of ways to measure dissolved oxygen concentration. Select a method based on 1) the number of ponds or tanks to be measured, 2) the level of accuracy required, and 3) the cost of the measurement technique. The titration-based "drop count" method fairly rapidly assesses whether or not there is sufficient dissolved oxygen in water. The drop count method is inexpensive and appropriate if DO concentration is to be measured infrequently in a few ponds or tanks. However, on commercial fish farms or in any other situation where DO measurement of many ponds or culture units is routine, a dissolved oxygen meter is an indispensable piece of equipment.

What is an oxygen meter?

An oxygen meter has two components—the sensor (sometimes called the probe) and the meter. The sensor reacts with oxygen and an electrical signal is produced in proportion to the oxygen concentration. The signal is then amplified, translated into concentration units, and displayed by the meter. The meter circuitry also compensates the reading for changes in temperature, altitude or salinity.

DO meters do not measure oxygen concentration directly, but measure a voltage that is produced by the

chemical reactions of oxygen with the various components of the sensor.

Materials

- Dissolved oxygen meter
- Beakers
- Thermometer
- Fishes and mouse

Procedure

Making dissolved oxygen measurements

The process of measuring dissolved oxygen concentration with an oxygen meter is simple, although it involves a bit more than simply putting the sensor in the water and reading a number on the display. Accurate measurement of DO concentration with polarographic sensors requires moving the sensor in the water. Oxygen is consumed across the membrane, so failure to move the sensor will create an oxygen-depleted microzone around the sensor tip and show a measurement that is too low. Move the sensor up and down or side to side about 1 foot per second to prevent this problem. The response of dissolved oxygen sensors is not instantaneous. Measured values will change rapidly at first and then begin to stabilize within 15 to 20 seconds, although it is rare that measured values in ponds will fully stabilize.

Dissolved oxygen measurements made near the pond bank are very different (usually lower) than those made in open areas of the pond because of the effects of sediment respiration and pond bank vegetation. The dissolved oxygen meter should not be turned off between measurements to avoid depolarizing the probe. Recalibrate the meter when it is used again. If a large meter adjustment is necessary during recalibration it may be an indication of problems with the membrane or sensor.

Dissolved oxygen monitoring in ponds

Dissolved oxygen concentrations in fish ponds vary with depth, from one side of a pond to the other and particularly from one time of day to another. In general, dissolved oxygen concentrations are lowest at dawn and highest at dusk; lowest near the bottom and highest near the surface; and lowest near the upwind side and highest near the downwind side.

Effect of temperature on oxygen consumption

Take pond water in beakers at different temperature ranges from 10-30 degrees. Place gold fishes in these beakers (one each). Let the fishes settle. Take a stop watch and record the oxygen consumption of fishes in water at different time intervals.

In the same way use an enclosed glass chamber and record the oxygen consumption of mouse.

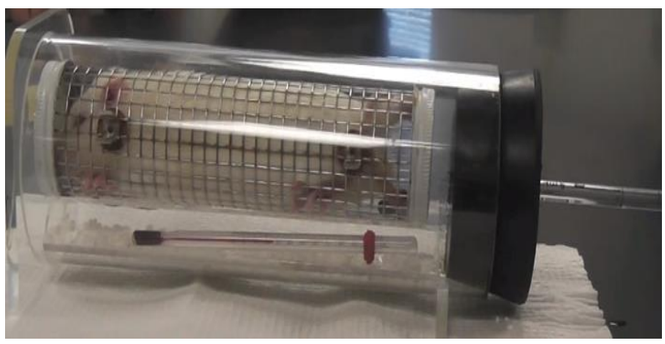


Fig 7.1: Oxygen consumption by rat

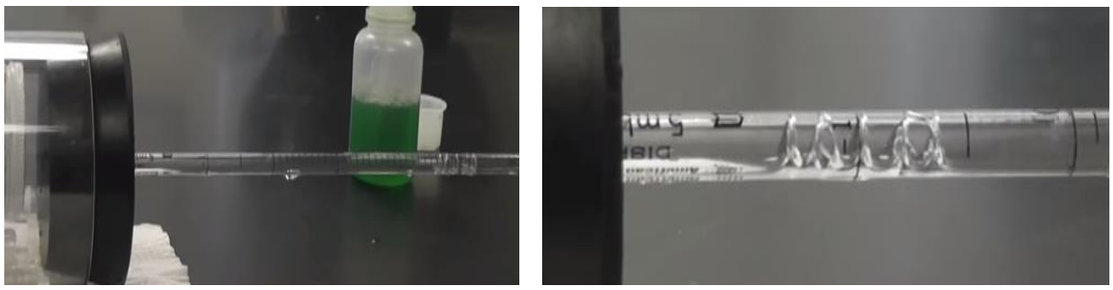


Fig 7.2: Comparison of bubble movement in tube at different time intervals

Practical No: 8
Oxygen consumption in fish and mouse by Respirometer

Introduction

A respirometer is a device used to measure the rate of respiration of a living organism by measuring its rate of oxygen consumption. They allow investigation into how factors such as age, chemicals or the effect of light affect the rate of respiration. There are various different types of respirometer. One type is shown in the diagram.

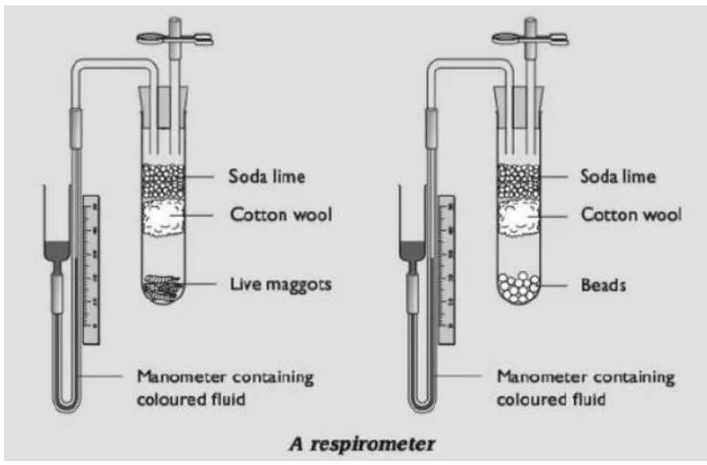


Fig 8.1: A view of Respirometer

Using a respirometer to measure the rate of uptake of oxygen

The organisms to be investigated are placed in one tube, and non-living material of the same mass in the other tube. Soda lime is placed in each tube, to absorb all carbon dioxide. Cotton wool prevents contact of the soda lime with the organism.

Coloured fluid is poured into the reservoir of each manometer and allowed to flow into the capillary tube. It is essential that there are no air bubbles. You must end up with exactly the same quantity of fluid in the two manometers. Two rubber bungs are now taken, fitted with tubes as shown in the diagram. Close the spring clips. Attach the manometers to the bent glass tubing, ensuring an airtight connection. Next, place the bungs into the tops of the tubes. Open the spring clips. (This allows the pressure throughout the apparatus to equilibrate with atmospheric pressure.) Note the level of the manometer fluid in each tube. Close the clips. Each minute, record the level of the fluid in each tube.

As the organisms respire, they take oxygen from the air around them and give out carbon dioxide. The removal of oxygen from the air inside the tube reduces the volume and pressure, causing the manometer fluid to move towards the organisms. The carbon dioxide given out is absorbed by the soda lime. The distance moved by the fluid is therefore affected only by the oxygen taken up and not by the carbon dioxide given out. You would not expect the manometer fluid in the tube with no organisms to move, but it may do so because of temperature changes. This allows you to control for this variable, by subtracting the distance moved by the fluid in the control manometer from the distance moved in the experimental manometer (connected to the Living organisms), to give you an adjusted distance moved.

Calculate the mean (adjusted) distance moved by the manometer fluid per minute. If you know the diameter of the capillary tube, you can convert the distance moved to a volume:

Volume of liquid in a tube - length x πr^2

This gives you a value for the volume of oxygen absorbed by the organisms per minute.

Using a respirometer to investigate the effect of temperature on the rate of respiration

The respirometer can be placed in water baths at different temperatures. You can use the same respirometer for the whole experiment. Or you could have different ones for each temperature. (In each case, there are difficulties with controlling some variables — you might like to think about what these are.) At each temperature, you need a control respirometer with no organisms in it.

If you are simply comparing the rates of respiration at different temperatures, then you do not need to convert the distance moved by the manometer fluid to a volume. You could just plot distance moved on the y-axis of your graph and time on the x-axis. The rate of respiration is represented by the gradient of the graph.

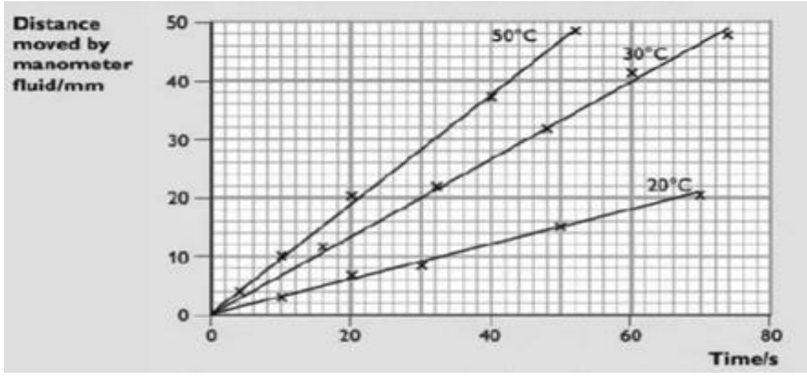


Fig 8.2: Comparing rates of respiration at different temperature

Practical No: 9 Study of stages in estrous cycle

Introduction

The estrous cycle or oestrus cycle is the set of recurring physiological changes that are induced by reproductive hormones in most mammalian therian females. Estrous cycles start after sexual maturity in females and are interrupted by anestrous phases or by pregnancies. Typically, estrous cycles continue until death.

Stages of estrus cycle

A four-phase terminology is used in reference to animals with estrous cycles.

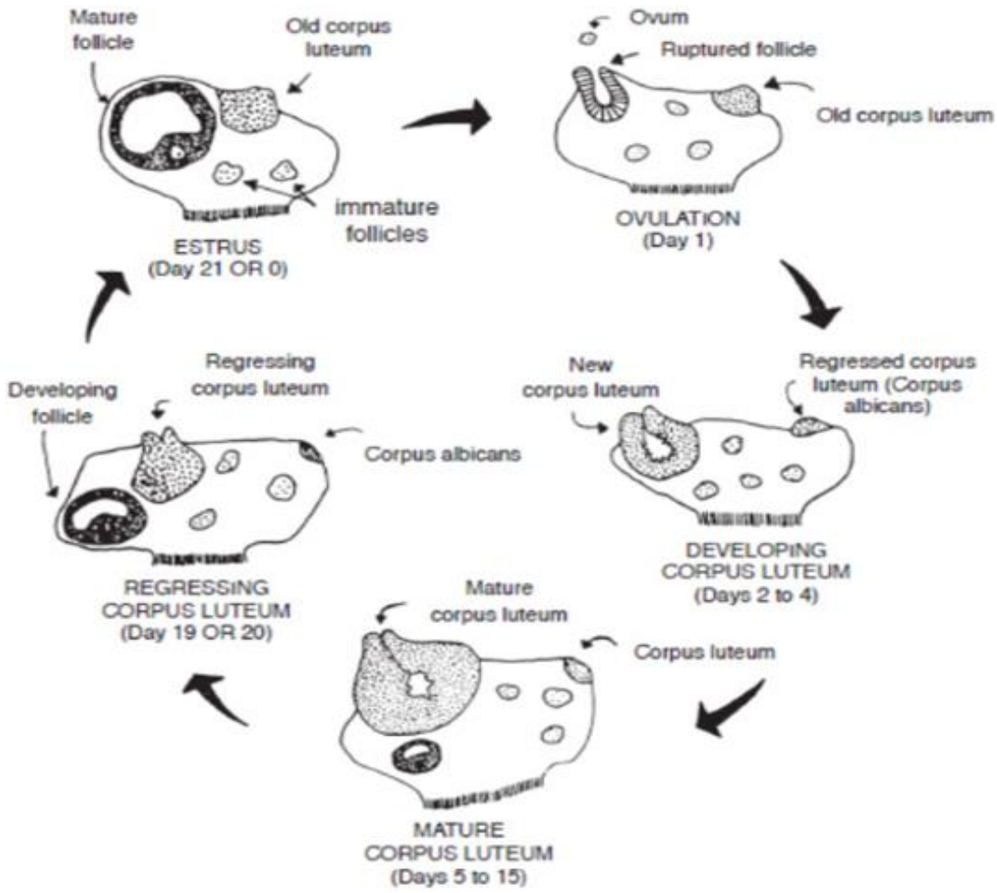


Fig 8.2: Stages of estrus cycle

1. In proestrus stage one or several follicles of the ovary start to grow. Their number is species specific. Typically this phase can last as little as one day or as long as three weeks, depending on the species. Under the influence of estrogen, the lining in the uterus (endometrium) starts to develop.
2. Estrus or oestrus refers to the phase when the female is sexually receptive. Under regulation by gonadotropic hormones, ovarian follicles mature and estrogen secretions exert their biggest influence. The female then exhibits sexually receptive behavior. Estrus is commonly seen in the mammalian species, including primates.
3. Metestrus or diestrus phase is characterized by the activity of the corpus luteum, which produces progesterone. The signs of estrogen stimulation subside and the corpus luteum starts to form. The uterine lining begins to appear. In the absence of pregnancy the diestrus phase (also termed pseudo-pregnancy) terminates with the regression of the corpus luteum. The lining in the uterus is not shed, but is reorganized for the next cycle.
4. Anestrus refers to the phase when the sexual cycle rests. This is typically a seasonal event and controlled by light exposure through the pineal gland that releases melatonin. Melatonin may repress stimulation of reproduction in long-day breeders and stimulate reproduction in short-day breeders. Melatonin is thought to act by regulating the hypothalamic pulse activity of the gonadotropin-releasing hormone.

After completion (or abortion) of a pregnancy, some species have postpartum estrus, which is ovulation and corpus luteum production that occurs immediately following the birth of the young. For example, the mouse has a fertile postpartum estrus that occurs 14 to 24 hours following parturition. Estrous cycle variability differs among species, but cycles are typically more frequent in smaller animals. Even within species significant variability can be observed, thus cats may undergo an estrous cycle of 3 to 7 weeks. Domestication can affect estrous cycles due to changes in the environment.

Practical No: 10
Locomotory behavior of small animals, earthworm, garden snails

Introduction

Earthworm behavior is simple, and most every aspect is understood with the exception of coming to the surface following a rain. Most of earthworm behavior consists of moving through the soil for food and reproduction.

Earthworms move by contracting the muscles in their segments in a wave motion. This makes the body shorten and lengthen, pushing the earthworm ahead with each cycle. While the earthworm is shortened, it extends small bristles called setae, anchoring it in position. The anterior of the earthworm remains unanchored, so when it extends, the front portion moves ahead, after which, the setae contract so the posterior portion of the body can move ahead, also. Earthworm locomotion is aided by the secretion of lubricating mucus that can help soften the soil and help the earthworm slide through it.

As earthworms move through the soil, they push air through it. This has prompted some scientists to call them biological pistons. As the soil is aerated and the earthworms deposit their excretions in it, the soil is made more fertile. Some earthworms actually move near the surface, where nutrients are more abundant, to feed and then, move back down to process the food, pulling nutrients further down into the soil. Many gardeners introduce earthworms to their soil because of this. In fact, Charles Darwin theorizes in one of his books that no other animal has been so important to world history than the earthworm.

Apparatus:

1. Mustard (optional)
2. Glass sheet
3. To collect the worms: Plastic tub (1 litre plus) plastic tub with small air holes in lid and sides containing potting compost

Health and safety and technical notes

1. In choosing a site to dig, be aware of the risk of picking up infections from animal faeces, and the risks from sharp buried objects. If you expose broken glass or metal objects, choose another site immediately. Ensure everyone who has handled the soil washes their hands with soap and hot water on return to the laboratory and before leaving the lesson.
2. If you use a sheet of glass it should have milled or taped edges. Students should handle it with care. Be aware of your laboratory procedures for dealing safely with broken glass in case of accident.
3. Handling worms safely (from the worms' perspective): keep the worms in a humid, dark container, with some sphagnum moss (not sphagnum moss peat).
4. After handling soil, wash hands with soap and hot water.

Procedure:

Preparation

Collect worms by digging in the soil in some part of your school grounds or a domestic garden. If you find only a few worms, the following two methods might encourage more to surface.

Method 1 Rhythmic rocking

Gather a group of 8-20 students in a circle in the area where you want to collect worms. Ask them to rock gently back and forth on the soles of their shoes, from heel to toe and back again. There is no need to stamp or make vigorous movements, just rock steadily for a couple of minutes. Worms will surface.

Method 2 Using mustard

Dig a pit 10 cm deep and 20 cm x 20 cm wide. Collect the worms from the soil you extract. Then add the

contents of a sachet of mustard (as supplied in restaurants) to 750 cm^3 of water and pour it into the pit. This encourages deep-dwelling worms to surface. This is not toxic and does not destroy grass or other plants. The mustard is a slight irritant to the worms, but preferred to other chemicals (including washing up liquid) which are more harmful.

Investigation

1. Put a worm in the middle of a large sheet of paper. Observe its movements and try to describe them. Listen carefully for the sounds the worm makes on paper.
2. Transfer the worm to a sheet of glass. Observe how it moves now. Try to describe any differences.

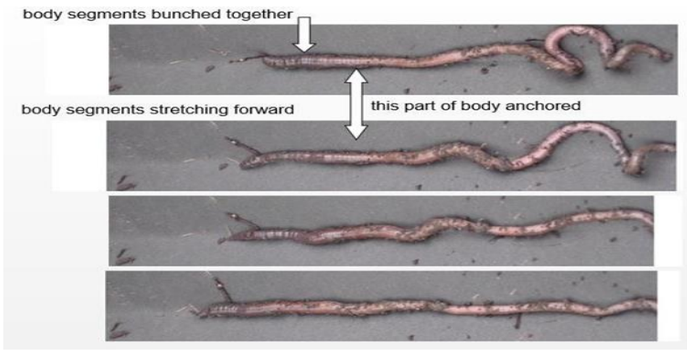


Fig 10.1: Locomotory behavior of earthworm

If there is no background noise, you can hear worms moving on paper – a faint scratching sound as their bristles (called chaetae, chetae or setae) engage with the paper. Worms cannot get a grip on glass and so cannot move over it.

The sequence of movement – engaging the chetae in one segment, followed by muscular contraction to pull the rest of the body to that point – is fascinating to observe.

Locomotory behavior of Garden snail (Helix aspersa)

Introduction

The Helix aspersa is an air-breathing snail, which has a single lung. They have a brownish soft body, covered with slimy mucus and yellow or cream-colored shells with brown spiral stripes. The garden snail is a terrestrial species with nocturnal habits; it does most of its day-to-day activities at night or very early in the morning, but if it rains during the day, it usually goes out.

In hot or drought periods, Helix aspersa retracts in its shell and covers the opening with a layer of mucus called epiphragm, which helps to keep the moisture inside and avoid the attack of animals that can kill it, like some ants. After doing this, it enters a state of inactivity that compares to hibernation. But not all snails hibernate. For example, in Southern California, young individuals are often active throughout the year.Some of these snails hibernate during winter months, especially when they are mature, but they return to activity with the spring.

Garden Snails move by gliding along on their muscular foot, which is lubricated with mucus and covered with epithelial cilia. This motion is powered by succeeding waves of muscular contractions that move down the ventral of the foot. This muscular action is clearly visible when a snail is crawling on the glass of a window or aquarium. Snails move at a proverbially low speed (1 mm/s is a typical speed for an adult). They produce mucus to aid locomotion by reducing friction, and the mucus also helps reduce the snail's risk of mechanical injury from sharp objects. This means that they can crawl along sharp objects like a straight razor and survive without injury.

Fig 10.2: Close up to garden snail on green background

Activity 1

Study of Snail's locomotion

Material required

1. Sheet of clear plastic or acrylic
2. Books or a brick
3. Garden snails
4. Acrylic paint
5. Small paintbrush
6. Notebook
7. Pen or pencil

Procedure

1. Get a pane of clear plastic or acrylic. Prop one end of the plastic on books or a brick. Position it so that you can look up through the glass from underneath. Now you have a transparent runway for watching snails.
2. Gather a few snails from the garden. Mark each snail with a small dot of acrylic paint on the shell. Use a different color for each snail to tell them apart. Wet the glass runway and place a snail in the middle.
3. Once it begins moving, watch from underneath. The foot can grip the glass while rippling muscles move it forward. The slime layer lubricates the surface so the foot doesn't get injured.
4. Now put several marked snails in the middle of the glass. Line them up so they face the same direction. Draw their positions in your notebook at the start.
5. Draw their new positions every five minutes. Do the snails move at random, or do you detect patterns in their motion?
6. If you don't have land snails in your area, try a similar experiment with aquarium snails. Mark some snails with paint, then put them back in the aquarium. Watch them crawl up the sides of the glass and note whether they move in a pattern. If you let algae grow on the sides of the aquarium, snails will leave trails as they eat the algae.

Activity 2

Making tracks of snail's locomotory pathway

Where they crawl, snails leave behind a characteristic, proverbial slime trace, because a large gland at the foot sole's front end produces large amounts of mucus the snail crawls on. The mucus reduces friction between the foot sole and the ground, enabling the snail to perform such astonishing acts as to crawl unharmed over a knife's edge.

Material/Specimen required

1. Garden snails
2. Black construction paper
3. Talcum powder

Procedure

1. Have a snail hunt and see if you can collect several snails. (Be gentle! Those shells may be fragile!) Snails are nocturnal, which means they like to sleep during the day and come out at night.
2. You can usually find them sleeping in damp dark places, such as under a rock. One way to catch snails is to put a large clay flower pot upside down, with one side propped up, overnight in a garden.
3. Once you've found some snails, put them on a sheet of black construction paper and let them do what they do -- crawl around. You'll be able to see the slimy trail they leave.
4. When the paper is criss-crossed with snail tracks, carefully put the snails back where you found them. Sprinkle talcum powder on their tracks. Tap off the excess talcum powder, and admire your snail art.

Practical No: 11 **Ear**

pinna reflex responses in domestic cats

Introduction

Cats are very sensitive to sound, with a range of hearing both above and below the range of frequencies that can be detected by humans. They can hear better than people and even better than most dogs. The ear is an organ of hearing and an organ of balance. It consists of the outer, middle, and inner ear.

Parts of Ear

The outer ear includes the pinna (the part you see that is made of cartilage and covered by skin, fur, or hair) and the ear canal. The pinna is shaped to capture sound waves and funnel them through the ear canal to the eardrum. In cats, the pinnae are mobile and can move independently of each other. The ear canal of cats is deeper and more tapered than in people, creating a better funnel to carry sound to the eardrum. This deeper canal is subject to buildup of dirt and wax that can lead to inflammation and secondary infection, although to a lesser degree than in dogs.

The middle ear includes the eardrum and a small, air-filled chamber that contains 3 tiny bones: the hammer, anvil, and stirrup. It also includes 2 muscles, the oval window, and the eustachian tube (a small tube that connects the middle ear with the back of the nose, allowing air to enter the middle ear).

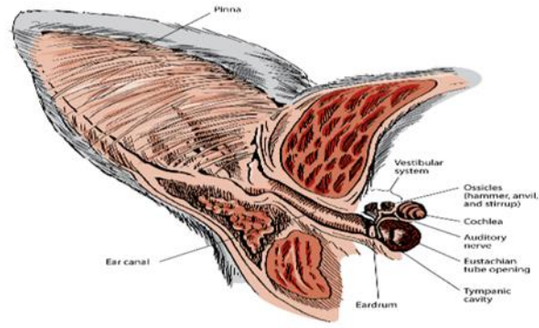


Fig 11.1: View of interior of ear

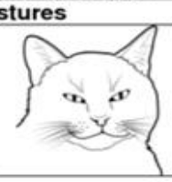
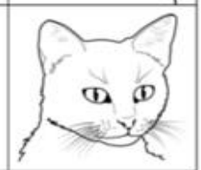
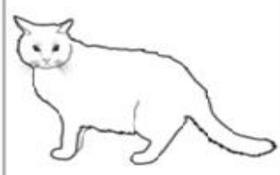
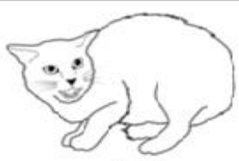
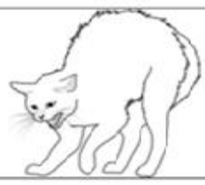
The inner ear is a complex structure that includes the cochlea (the organ of hearing) and the vestibular system (the organ of balance). The semicircular canals, which are found within the inner ear, are filled with fluid and are important for maintaining balance. These are highly developed in the cat, accounting for its agility and excellent sense of balance.

Ear pinna reflex responses in domestic cats

Bring a domestic cat in laboratory and play different types of sounds near it and observe the movement and position of its ear in response to that sounds. The ear pinna reflex of a cat can be compared with the ear position of cat shown in chart and its behaviour can be understood.

Reading your cat's "body language"

Cats use different body postures to communicate their emotions. Below are some typical postures you may observe in your cat When observing your cat, try to get an idea of its usual attitude when alone and in contact with other animals, including people. As cats become more anxious about their surroundings, they will try to avoid contact with threats. Their score may change very quickly depending on the seriousness of the threat. The highest scores usually are seen only when escape is not possible.

Score	Body Postures		Head Postures	
1 Relaxed	**Activity** – sleeping or resting, alert or active, may be playing **Body** – lying on side, on belly or sitting; if standing or moving, back horizontal **Breathing** – slow to normal **Legs** – bent, hind legs may be laid out; when standing extended **Tail** – extended or loosely wrapped; up or loosely down when standing		**Head** – laid on surface or over body, some movement **Eyes** – closed to open, pupils slit to normal size **Ears** –normal to forward **Whiskers** – normal to forward **Sounds** –none, purr	
2 Alert	**Activity** - resting, awake or actively exploring **Body** – lying on belly or sitting; if standing or moving the back is horizontal **Breathing** – normal **Legs** – bent; when standing extended **Tail** – on body or curved back; up or tense downwards when standing; may be twitching		**Head** – over the body, some movement **Eyes** – open normally, pupils normal **Ears** – normal or erected to front or back **Whiskers** – normal to forward **Sounds** –none or meow	
3 Tense	**Activity** – resting or alert, may be actively exploring, trying to escape **Body** – lying on belly or sitting; if standing or moving the back of the body is lower than the front ("slinking") **Breathing** – normal **Legs** – bent, hind legs bent and front legs extended when standing **Tail** – close to body; tense downwards or curled forward, may be twitching when standing.		**Head** – over the body or pressed to body, little or no movement **Eyes** – wide open or pressed together, pupils normal to partially dilated **Ears** – erected to front or back **Whiskers** – normal to forward **Sounds** – none, meow, or plaintive meow	
4 Anxious	**Activity** – alert, may be actively trying to escape **Body** – lying on belly or sitting; if standing or moving the back of the body is lower than the front **Breathing** – normal or fast **Legs** – under body, bent when standing **Tail** – close to the body; may be curled forward close to body when standing. The tip may move up and down or side to side.		**Head** – on the plane of the body, little or no movement **Eyes** – wide open, pupils dilated **Ears** – partially flattened **Whiskers** – normal to forward or back **Sounds** – none, plaintive meow, growling, yowling	
5 Fearful	**Activity** – motioniess, alert or crawling **Body** – lying on belly or crouched directly on top of all paws, may be shaking; if standing the whole body is near to the ground, may be shaking **Breathing** – fast **Legs** – bent; when standing bent near to surface **Tail** – close to the body; curled forward close to the body when standing.		**Head** – near to surface motionless **Eyes** – fully open, pupils fully dilated **Ears** – fully flattened **Whiskers** – back **Sounds** – none, plaintive meow, growling, yowling	
6 Terrified	**Activity** – motioniess alert **Body** –crouched directly on top of all paws, shaking. Hair on back and tail bushy. **Breathing** – fast **Legs** – stiff or bent to increase apparent size **Tail** – close to body		**Head** – lower than the body **Eyes** – fully opened, pupils fully dilated **Ears** – fully flattened, back on head **Whiskers** – back **Sounds** – none, plaintive meow, growling, yowling, hissing	

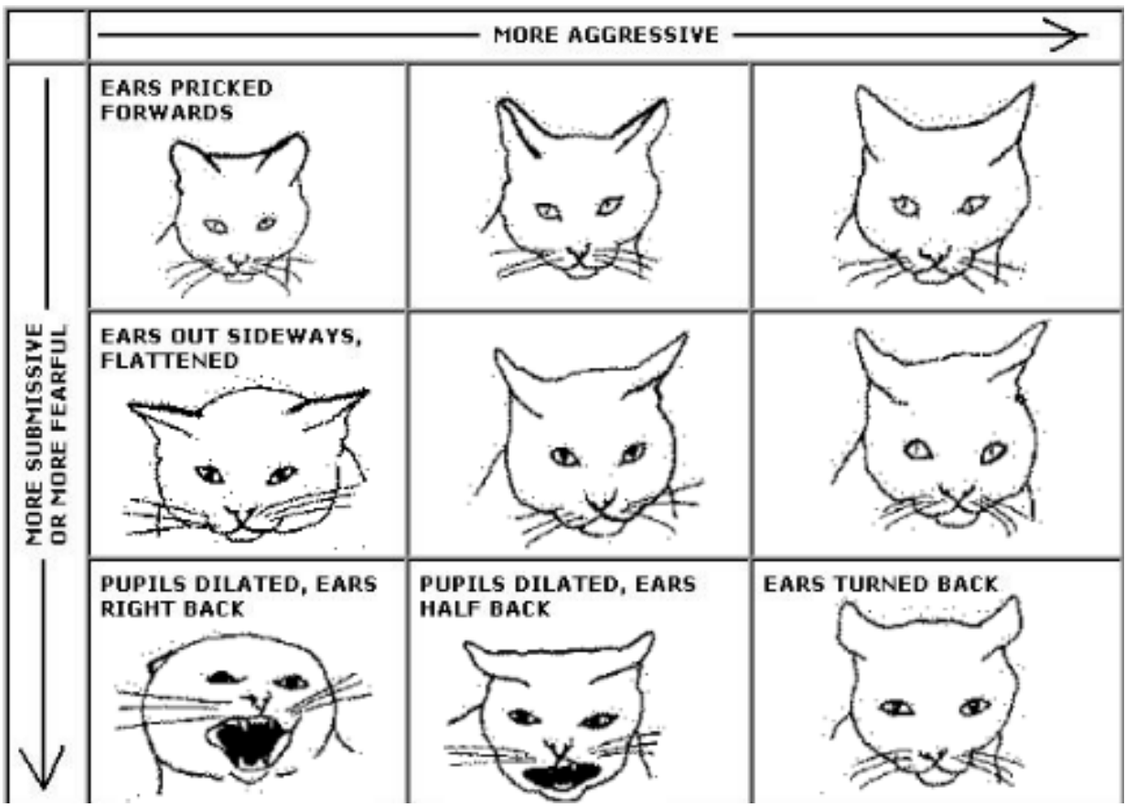


Fig 11.2: Ear pinna reflex responses in domestic cats

Practical No: 12
Preparation of Skinner box or maze for study of mouse or rat behavior

Background

The theory of Operant Conditioning **was** proposed by Burrhus Frederic Skinner, commonly known as B.F. Skinner. Skinner based his theory in the simple fact that the study of observable behavior is much simpler than trying to study internal mental events. He is also called the father of Operant Conditioning Learning, but he based his theory known as "Law of Effect", discovered by Edward Thorndike in 1905.

Operant Conditioning Learning

B.F. Skinner proposed his theory on operant conditioning by conducting various experiments on animals. He used a special box known as "Skinner Box" for his experiment on rats. As the first step to his experiment, he placed a hungry rat inside the Skinner box. The rat was initially inactive inside the box, but gradually as it began to adapt to the environment of the box, it began to explore around. Eventually, the rat discovered a lever, upon pressing which; food was released inside the box. After it filled its hunger, it started exploring the box again, and after a while it pressed the lever for the second time as it grew hungry again. This phenomenon continued for the third, fourth and the fifth time, and after a while, the hungry rat immediately pressed the lever once it was placed in the box. Then the conditioning was deemed to be complete.

Here, the action of pressing the lever is an operant response/behavior, and the food released inside the chamber is the reward. The experiment is also known as Instrumental Conditioning Learning as the response is instrumental in getting food. This experiment also deals with and explains the effects of positive reinforcement. Upon pressing the lever, the hungry rat was served with food, which filled its hunger; hence, it's a positive reinforcement.

The electric current reacted as the negative reinforcement, and the consequence of escaping the electric current made sure that the rat repeated the action again and again. Here too, the pressing of the lever is an operant response, and the complete stop of the electric current flow is its reward.

Both the experiment clearly explains the working of operant conditioning. The important part in any operant conditioning learning is to recognize the operant behavior and the consequence resulted in that particular environment.

Material

- Cardboard box with a lid (this one is 33x11x25 cm)
- Paper cutter
- Hard plastic the same dimensions as the box lid

- Safety pin
- 16 gauge wire (2 times the width of the box plus 10 cm)
- Small bell
- Plastic jug with a hollow handle (we used a cat litter jug)
- Clear scotch tape
- Glue
- Double-sided poster tape
- Framing square
- Handheld saw

Procedure

1. Draw an outline of your box on a large piece of construction paper to determine the dimensions of each side. The simplest box to make will have four sides and a bottom but the fourth side should be covered by the clear plexiglass piece. If your box is meant for a small rodent, make it big enough to allow the rodent to turn around comfortably.
2. Measure each side of the intended box equally and draw an outline in the wood. Cut each side of the box from the wood template. Reserve one piece of the cut wood for the lever you will make. Cut a one inch by one inch slit in this piece of wood. The slit should be big enough to stick a wooden ruler, tongue depressor or popsicle stick through but also allow a seesaw like movement.
3. Construct the lever. Before securing the walls of the box, construct the lever. Place the lever material through the slit of the side of the box. Glue walls on the left and right side of the lever. Construct the walls from tongue depressors and secure them with wood glue. Seal the end of the lever on the outside of the box but leave the end that will be inside the box open. Do this so that you can place food pellets on the closed side of the lever and when your lab subject successfully pushes the lever, they can obtain the reward inside the box.
4. Glue the walls of the box together and let the glue dry. Once the glue dries, ensure security by nailing the four points of the sides of the box together. Use small tacking nails to avoid splitting the wood. The top of the box should be open. Glue the plexiglass or plastic to the side opening of the box. The clear side allows you to observe the experiment from a distance.
5. Load the closed end of the lever with a food pellet. Place the lab animal in the box and slowly condition the animal to seek the lever for reward.

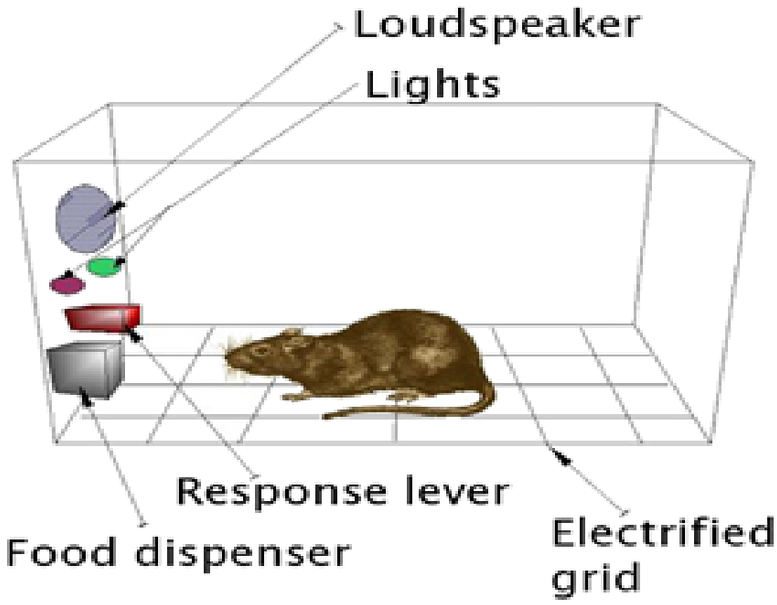


Fig 12.1: Skinner's Box

Practical No: 13
Study of Infant killing Behavior

Infanticide is a powerful tool in ensuring the survival of a species, researchers are increasingly finding. It sounds like the cruellest thing of all - mothers and fathers brutally killing their animal offspring. But for many animal infants, the greatest threat to their survival they face is from their own kind. "It's not like a predation event, which is quiet," says lion expert Professor Craig Packer.

During infanticide, it's growling and violent, and really pretty disturbing," he says, describing how adult lions kill cubs. "They bite them on the back of the head, and neck, crushing their abdomens. Infanticide is an often overlooked way of ensuring the survival of the fittest. It has been recorded in a number of species including mammals such as rodents and primates, and fish, insects and amphibians. Scientific research shows it can provide benefits to the perpetrator, such as increased reproductive opportunities, access to limited resources, direct nutritional benefits, or the prevention of misdirected parental care.

Infanticide is often perpetrated by adult males.

Fig 13.1: Skinner's Meerkats are co-operative breeders but females are known to kill each other's litters

Taxonomic distribution

Infanticide occurs in lots of different kind of birds including common ones like sparrows, and swallows. It also happens in rodents like mice and ground squirrels. You also see it in lions. It's a similar sort of situation- females live in matrilineal groups and only the males disperse. A set of several males (usually related) live with the group and enjoy mating privileges until they're driven out and then the new males kill all the babies. There are plenty of infanticidal primates- lemur catta, red howlers, red colobus, silver leaf monkeys. There are several cercopithecine examples- red-tails, blue monkeys among them. A couple of different savannah baboons, and also chimps and gorillas among the apes.

The two main hypotheses

Population density hypothesis

Infanticide is due to high population densities, and is an aberrant and dysfunctional behavior.

This was the early explanation. It says that infanticide is a pathological behavior. It isn't part of the normal makeup of the species but is because of abnormally high population densities. Like the way when you crowd lab rats together they kill each other. This made sense for the langurs since one of the groups that was studied was being crowded into little areas by deforestation, and in other study areas people were feeding them and this usually makes levels of aggression rise.

Sexual selection hypothesis

- Infanticide is a male reproductive tactic:
- Loss of suckling infant leads to the onset of estrous in the mother
- Males gain a reproductive advantage through earlier conception by females.

Practical No: 14
Pecking behavior of chicken

Chickens are one of the most studied animal species, and researchers observed chicken behavior extensively. The term behavior can be defined as the way in which an animal or person acts in response to a particular situation or stimulus. The term 'pecking order' was first coined in 1921 by Thorleif Schjelderup-Ebbe to describe the hierarchy of flock dynamics and it came into popular usage in the 1930s. Recently hatched chicks do not typically show any competitive behavior until after three days of age. By 16 days of age, fighting to determine the pecking order begins. Research has shown that with groups composed entirely of female chicks, the pecking order is established by the 10th week. In small groups, the order is typically established earlier, around eight weeks. With groups of males, the social order may remain unresolved for many weeks.

What Is The Pecking Order?

So, exactly what is it and how does it relate to you and your flock?

It's a system by which birds arrange their social standing in the flock. The higher ranked birds will get the best food, water and roosts while the lower placed birds will get the leftovers.

This method of organization places each member of the flock on a 'heirachy ladder'. At the top of the ladder will be the head rooster (or hen if no rooster is present). This complex social order is designed to ensure that there is good cohesion between members, and few if any petty squabbles… This sort of co-operation between members of the flock ensures the survival of the flock by giving the best chances to the fittest birds.

It is a flexible structure and within the flock there are usually three different types of social order going on:

- Rooster to Rooster
- Hens to Hens
- Roosters to Hens

A rooster may go up the ladder if he mounts a successful campaign against the leader. He becomes the new chicken-in-charge! And the defeated roosters go down the ladder, as do weak or sickly birds.

Fig 14.1: Roosters that is lower in the order, crow less frequently and rarely mate

Hens have their own 'girls' only ladder. The matriarchs of the flock will be up to the top of the ladder, with less dominant birds at the bottom. In this system the older, stronger and savvier hens will be at the top.

Young pullets just coming to point of lay, will try to 'move up' the social ladder quickly.

If a bird tries to go 'out of turn', she will earn glares, pecks and feather pulling from the higher 'ranked' hens. Usually a 'look' or a quick peck is enough to remind the lower ranking hen she has overstepped the boundary.

The serious games of the pecking order start when chicks are around six weeks of age. Chicks will start rushing at each other, bumping chests and flaring feathers. These are all methods used to intimidate flock mates at any stage of life. By the time they leave the brooder, they will have their own pecking order sorted out.

Pecking Order Problems

Whilst the pecking order can create a sense of harmony within a flock it can also create absolute havoc, with chickens fighting each other for their position within the order. A full on pecking order assault is a violent and terrible thing to see. Older birds can be relentless, drawing blood, causing serious injury even death. There is nothing gentle about the pecking order.

1. Adding New Birds to Your Flock

Adding birds to your existing flock will cause a shift in the pecking order. The older birds will be very suspicious of the new members and can be quite violent about it. If you do add new birds to your flock, it needs to be done slowly and cautiously. You should never add less than two birds to an established flock. The method worked well if it is done in separation pen. Separation pen is an area that the new hens can be put safely without the older girls being able to peck them. They can look, pace around the enclosure but can't get in. This should be done for couple of weeks before opening the enclosure. When you do open up the temporary enclosure you need to have places the new birds can hide or run to if flock members get really mean.

2. Sick or Injured Birds

Chickens rarely show any signs of illness or weakness. If they do, other flock members will pick on them and either drive them from the flock or kill them. This sounds awful, but remember, the flock in the wild is as strong as its' weakest member. It's simply a survival tactic. If you have a chicken that is constantly being pecked at, you will need to isolate her away from harm. The victim will need to be isolated until wounds are healed- now comes the tough part, trying to re-integrate the affected bird. Use the segregation pen which we mentioned earlier on for a few days and then reintegrate her.

3. Bully Birds

Sometimes you have a hen who is a bully to everyone. Often she will be in the middle of the pecking order, rarely at the top. The pecking order will change while she is in isolation, so when she gets re-introduced she will be a 'newbie' and treated accordingly. Once in a while you will get two or more hens that form a 'bully

club'. Use the same treatment for them, except re-introduce them to the flock on separate days- this should break the pattern of bullying.

How to Avoid Pecking Order Problems

1. The good news is that much can be done by the keeper to ensure that old and new flock members integrate fairly peacefully.
2. First and most importantly, each bird needs to have sufficient 'personal space'. There really isn't a 'perfect formula' for space requirements, often quoted is four square foot/bird for floor space. If they are confined within the coop twenty four hours a day. If however, your birds are allowed to free range, coop space doesn't really become an issue until winter.
3. Make sure you provide lots of roosting spots so that a hen can get away if she needs to. Providing places to 'hide' is important- old boxes, straw bales (outside the coop) dark quiet areas in the barn/shed.
4. Don't forget to provide extra feeding and watering stations.

Practical No: 15
Observation of birds' nests and study of parental behavior

Introduction

Parental care is the love, care and protection provided by the parents to ensure development and survival of their offspring. In most birds, parents invest profoundly in their offspring as a mutual effort, making a majority of them socially monogamous for the duration of the breeding season. There are different modes of parental care such as bi-parental care, mono-parental care.

Nesting and Parental Care

Ninety-five percent of bird species are socially monogamous. That is, a male and female bird will pair for at least the length of the breeding season or, in some cases, for several years or until the death of one mate. Monogamy allows for care of the fledglings by both parents. Some birds are polygamous; they have many mates in a breeding season. Among most groups of animals, male parental care is rare. In birds, however, it is quite common — more so than in any other vertebrate class.

Birds usually lay and incubate their eggs in a nest. A nest can also be a shallow depression made in sand, a burrow dug into the ground, a chamber drilled into a tree, or a mud dome with an entrance tunnel. Some species of cave swiftlets make their nests entirely from their saliva, which dries and hardens to form a bracket on the cave wall into which the birds lay their eggs. Eggs are usually incubated by the parents. Most birds have an extended period of parental care after hatching. The length and nature of parental care varies widely amongst different orders and species.

Some bird species have no nests; the cliff-nesting Common Guillemot lays its eggs on bare rock, and male Emperor Penguins incubate their single egg in a brood pouch between their body and feet. The absence of nests is especially common in ground-nesting species, where the newly hatched young are able to take care of themselves after hatching.

Eggs

All birds lay amniotic eggs with hard shells made mostly of calcium carbonate. Hole and burrow nesting species tend to lay white or pale eggs, while open nesters tend to lay camouflaged eggs, as shown in Figure below. Before nesting, the females of most bird species molt feathers from their belly area, developing a bare brood patch. The skin of the brood patch is well supplied with blood vessels and is therefore warm, which helps to incubate eggs.

Incubation, which maintains temperature for chick development, usually begins after the last egg has been laid. In monogamous species, incubation duties are often shared, whereas in polygamous species, one parent is

wholly responsible for incubation. Warmth from the parents passes to the eggs through brood patches. Incubation can be an energetically demanding process; adult albatrosses, for example, lose as much as 83 grams (2.9oz) of body weight per day of incubation. Incubation periods range from 10 days (in woodpeckers, cuckoos, and passerine birds) to over 80 days (in albatrosses and kiwis).

Fig 15.1: View of bird eggs in field

Camouflaged eggs

Not all mother birds are very attentive to their chicks. Cuckoos (Cuculus canorus) leave the raising of their young to other birds - a process called brood parasite behavior. The female cuckoo will lay her eggs in another bird's nest, usually a Reed Warbler (Acrocephalus scirpaceus). The cuckoo's eggs are a similar color to the warbler's eggs, so they are not noticed by the mother warbler. When the young cuckoo hatches, it will push all other nestlings and eggs out of the nest. The Reed Warbler will feed and raise the cuckoo chick as its own.

Hatchlings

There are two general types of hatchlings. Some newly hatched birds are naked and helpless and are called altricial. Others are hatched precocial; they are feathered and able to walk and feed themselves very soon after hatching. Birds that nest on the ground, such as ducks, chickens, and swans, have precocial broods. Birds that nest off the ground, in trees, hedges, or buildings tend to have altricial young that need to be taken care of by the parents for a relatively long time. Male and female parents often share duties involved in raising young. These include territory and nest site defense, incubation, and chick feeding. However, there are species in which one mate undertakes all or most of a particular duty.

Fig 15.2: View of Altricial hatched and Precocial hatched

Practical No: 16
Altrusic Behavior in Monkeys

Introduction

Altruism refers to behavior of an individual that increases the fitness of another individual while decreasing the fitness of the actor. The term altruism was coined by the French philosopher Auguste Comte in French.

Altruistic behaviors appear most obviously in kin relationships, such as in parenting, but may also be evident among wider social groups. They allow an individual to increase the success of its genes by helping relatives that share those genes.

Fig 16.1: Behavioral study of Monkeys

Altruism in Monkeys

Vervet monkeys have four confirmed predators: leopards, eagles, pythons, and baboons. The sighting of each predator elicits an acoustically distinct alarm call. If an individual gives an alarm call for a different predator, group members respond to alarm caller. This suggests vervet monkeys are able to recognize and to respond to not only the individual calling. It is believed that vervet monkeys have up to 30 different alarm calls. In the wild vervet monkeys have been seen giving a different call when seeing a human being approaching, leading researchers to believe that vervet monkeys may have a way of distinguishing between different land and flight predators. Mothers can recognize their offspring by a scream alone.

Observing altruism in an experiment

An experimental method was developed to study possible “altruistic” interactions in monkeys. Two monkeys were placed in adjacent cages. Each monkey could choose to feed itself by pressing one lever or to feed both itself and the other monkey by pressing a different lever. The results demonstrated that several types of reactions including an altruistic one are possible.