Experiment No. 4

1.0 Title :

To study the common instruments and equipments used in experimental Pharmacology.

(Refer the Experiment No.1 in the CD of MSBTE).

2.0 Prior Concept :

Instruments for Dissection.

3.0 New Concept :

Proposition 1 : Tracing of graph requires recording device.

Concept Structure :

Recording device.

Levers
- Frontal writing lever
- Simple lever
- Universal lever
- Gimbal lever
- Starling heart lever
- Fixit lever
- Isometric lever
- Pendulum Auxotonic lever

- Sherrington's drum and device
- Kymograph Paper
- Organ bath and Organ tube
- Symes cannula.
- Mariott's Bottle

4.0 Learning Objectives :

4.1 Intellectual skills :

To understand working and use of equipments.

4.2 Motor Skills :

Ability to handle and adjust the equipments.

Ability to setup the assembly.

5.0 Requirements :

Sherrington's recording drums and device, Organ bath and Organ tube, Symes cannula, Kymograph paper, Levers, Forceps, Scissors, Arrows, Dissection box.

6.0 Diagram :

6.1 Sherrington's recording drum and device :

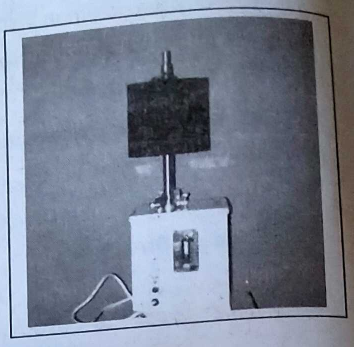

Fig. 4.1

It is used to move the kymograph at a desired speed. The drum (152 mm in diameter) on which the kymograph is fixed on the shaft of sherrington's revolving drum machine and this drum revolves at a fixed speed around the shaft. The shaft has a groove in which movable metal block is adjusted. This movable metal block is used to elevate or lower the position of drum. This metal block is moved up and down by rotating the screw meant for horizontal leveling of the machine. The gears in the basal part are for controlling the speed of rotating drum. The clutch on the backside of the machine is to start and stop the rotation of the drum during recording of responses. Generally the rotation speed of this machine ranges from 0.12mm/sec to 50mm/sec.

6.2 Organ bath assembly :

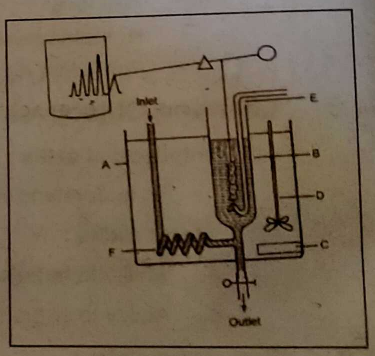

Fig. 4.2

The tissue bath used to put animal tissue for studying drug action is called as students organ bath. The organ bath essentially consist of (A) An outer Jacket (water bath) made up of a steel, glass or Perspex; (B) The inner organ or tissue bath made up of glass with a capacity varying from 10ml to 50ml; (C) Thermostatically controlled heating rod; (D) Stirrer to keep the water in the outer jacket at uniform temperature; (E) Oxygen and delivery glass tube which also serves as tissue holder; and (F) glass coil, one end of which is connected to the lower end of the organ bath and the other to the container having the physiological salt solution. The glass coil is usually of double the capacity of inner organ bath to ensure warming up of the solution before it enters the organ bath.

The student organ bath having two units of inner tissue bath is called double unit organ bath.

6.3 Recording levers :

They are used to record the contraction or relaxation of the isolated tissue preparation. The recording is done on smoked papers fixed on Sherrington's recording drum. The speed of the drum is adjusted depending upon nature of experiment.

1. Simple lever (Side way writing) :
It is the simples type of lever made up of wood, stainless steel or Aluminium. A celluloid writing tip is attached at the end of the longer arm.

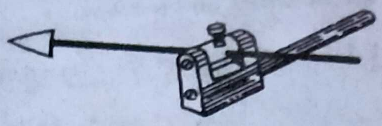

Fig. 4.3

2.Frontal writing lever(Writes frontally):
This lever is designed in a such a way that the writing point rotates freely about its axis. It is used to record the contractions of isolated muscle preparations.

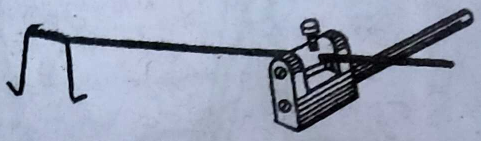

Fig. 4.4

3. Starling heart lever :
This lever is used to record the contraction of the heart.

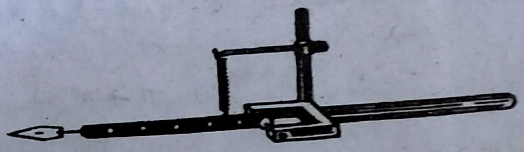

Fig. 4.5

4. Brodies Universal lever :
It is general utility lever.

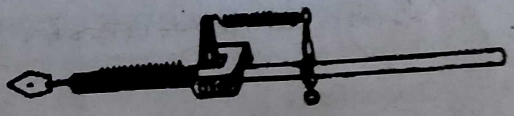

Fig. 4.6

5. Fixit lever :
It is similar to Brodies lever but the central spindle has an ebonite roller on which three pins are set at right angle.

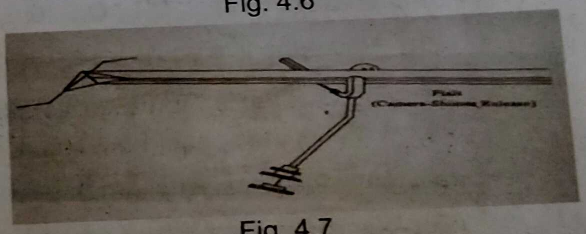

Fig. 4.7

6. Gimbal lever :

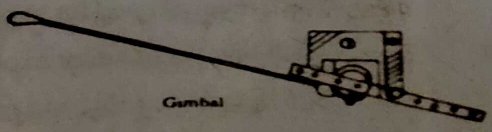

Fig. 4.8

7. Isometric lever :
It consist of heavy brass frame with spring steel wire fixed to each limb and carrying the lever from its center.

8. Pendulum Auxotonic lever :

6.4 Scissors and forceps :



Fig. 4.9

Curved Scissor

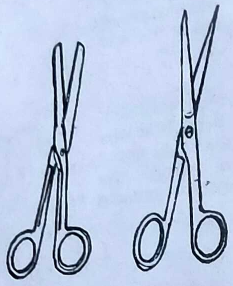

Fig. 4.10

Blunted and Pointed Scissors

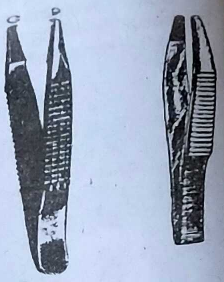

Fig. 4.11

Blunted and Pointed Forceps

6.5 Mariottes bottle :

It is employed as a convenient device for maintaining a steady pressure head since the pressure always corresponds to the lower lever of the glass tube.

7.0 Questions :

Write answers to Q.... Q.... Q.... Q.... (Questions to be allotted by subject teacher)

1. Name the five equipments used in experimental pharmacology.
2. Name the five levers used in experimental pharmacology.
3. What is the speed requirement for Sherringtons recording device.
4. How the capacity of organ tube is measured?
5. Name the five parts of organ bath assembly.
6. Which lever is used for experiment of isolated heart of frog?
7. Which lever is used for experiment of rectus abdominus muscle of frog?
8. List five instruments used for dissection of frog.
9. What is the use of Marriott's bottle?
10. What is the use of Symes Venous Cannula?

(Space for answers)

1) Equipments used in experimental pharmacology
   1) Sherrington's rotating drug
   2) organ both assembly
   3) simple lever
   4) starling heart lever
   5) curved scissor

8) Instrument
of frog—
1) Disse
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9) Use of
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(Space for answers)

Levers used in experimental p'cology →
1) Simple lever
2) Starling heart lever
3) Frontal writing lever
4) Brondies universal lever
5) fracit lever.

Speed requirements for sherringtons rotating device - 0.12 mm/sec to 50 mm/sec.

organ tube capacity - organ tube is completely filled with fluid, the fluid is then withdrawn and measured in caliberated measuring cylinder.

Parts of organ bath assembly →
1) organ bath 2) organ tube 3) Glass coil
4) Frontal writing lever 5) Heating root
6) perfusion bottle

Lever used for experimental isolated heart of frog → starling heart lever is used for experiment of isolated heart of frog.

For experiment of rectus abdominus muscle of frog → Frontal writing lever is used for experiment of rectus abdominus muscle of frog.

8) Instrument used for dissection of frog →

1) Dissecting tray
2) Curved scissor
3) Blunted scissor
4) Arrow needle
5) Curved forcep.

9) Use of marriott's bottle →

It is used for maintaining a steady pressure head since pressure always corresponds to lower of glass tube.

10) Uses of Symes venous cannula →

It is placed in sinus venous cannula of frog heart is holds the heart after isolation, It also supply frog ringer solution to heart.

Date-

Experiment No. 5

1.0 Title :

To study the various laboratory techniques used in experimental Pharmacology.

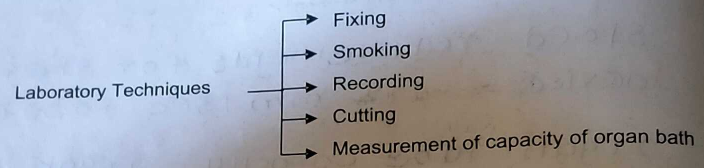
2.0 Prior Concept :

Effect of drug on animal preparation.

3.0 New Concept :

Proposition 1 : Graphical record of response.

Concept Structure :



4.0 Learning Objectives :

4.1 Intellectual skills :

1. To understand the composition of fixing solution.

4.2 Motor Skills :

1. Ability to Cut, Attach, Smoke, Kymograph paper to the drum.
2. Ability to fix Kymograph Paper in solution.
3. Ability to measure and fix the capacity of organ bath.

5.0 Requirements :

Sherrington's recording drum and device, Organ bath and Organ tube, Kymograph paper, Levers, Scissors, Dissection box.

6.0 Stepwise procedure :

Recording procedure :

1. Adjustment for magnification :

1. Adjust the magnification of response in order to get a proper recording of the observed physiological response.

   The tissue showing less contractility need more magnification and the reverse is true with the tissues, which have higher inherent rhythmic contractility.

2. Adjust for magnification by properly adjusting the distance between righting tip and fulcrum and the distance between the point of attachment to the tissues and the fulcrum by adjusting the relative distances desire degree of magnification is obtained.

$$\text{Magnification Value} = \frac{\text{Distance between fulcrum and Writing Point. (A)}}{\text{Distance between fulcrum and the point of attachment to the tissue (B)}}$$

2. **Application of load (Tension) :**
   1. Select the proper length of longer and shorter arms depending upon magnification for the tissue, which is under study, and fix the fulcrum.
   2. Balance the lever by putting the weight at the end of the shorter arms and mark the point of tissue attachment.
   3. At equidistance i.e. Distance between fulcrum and the point of tissue attachment, from the fulcrum on the longer arms of the lever fix the desired load required for the particular tissue.
   4. The tension for various tissue preparation are
      - Guinea pig ileum (1 gm.)
      - Guinea pig trachea (0.2 gm.)
      - Guinea pig vas deferens (0.5 gm.)
      - Rabbit duodenum (1 – 3 gm.)
      - Rat uterus (1 gm.)
      - Rat colon (0.5 gm.)
      - Rat fundus (1 gm.)
      - Fog rectus abdominus (1 gm.)
3. **Smoking of kymograph drums :**
   1. Fix the kymograph paper tightly to the drum.
   2. Smoke the drum uniformly with the black smoke of benzene and kerosene or the mixture of the two. (Uniform smoking is essential for proper recording. The recording can be done directly on the white paper with the help of pen attached to the tip of the lever.)
4. **Fixing of the tracings :**
   1. Preserve the recordings on the smoke drum by properly fixing them with the help of fixing solution.

   (The commonly used resins to prepare fixing solution are shellac and colophony. A saturated solution of shellac is prepared in alcohol and it is allowed to stand for a week. The clear supernatant is decanted and is used for fixing the tracings. The solution may be reused several times and should be kept in a well-closed bottle to prevent evaporation of the solvent. One hundred fifty grams resin is dissolved in two litres of spirit.)
5. **Contact time :**
   1. Allow the contact time of 90 seconds for the rectus abdominus muscle of the frog. (The time allowed for the drug to remain in contact with the tissue is called as contact time.)
6. **Time cycle :**

   Follow the time cycle given below for the recording of response of frogs rectus abdominus muscle.

   5 minutes time cycle.

1. 30 seconds : take the base line record.
2. 90 seconds : Allow the drug to contact with tissue.
3. 120 seconds : Give the first wash. (Give three subsequent washings at the interval of 1min.)
4. 300 seconds : Give the second dose.

7.0 Questions :

Write answers to Q.... Q.... Q.... Q.... (Questions to be allotted by subject teacher)

1. What is the composition of fixing solution used for kymograph paper?
2. How the fixing solution is prepared?
3. Write the time cycle, which should be followed for recording of response.
4. What is contact time?
5. How the magnification is adjusted?
6. By which method the response is recorded on kymograph?
7. Write the tension applied for preparations of guinea pig ileum and frogs rectus abdominus muscle?
8. Which laboratory techniques you learnt from this experiment?
9. What is the use of Kymograph paper?
10. What is smoking of drum?

(Space for answers)

1) Composition of fixing solution used for kymograph paper →
It contains shellac and colophony is a saturated solution of shellac is prepared in alcohol and it is allowed to stand for decuned and clear supernatent is (weak) is used for fixing the trainings the solution may be reversed served times and should be kept in well closed bottle to prevent evaporation of solvent. one hundread fifty grams resins is dissolved in 2 litre of spirit.

6) Method recorded By unifo for pro can be paper attach

7) Tensio 1) for 2) for -1gm

8) Labo ① Fi ④ c

9) use ky dru re gro tion

10) ①

(Space for answers)

3) Time cycle - 5 minute time cycle
1) 30 second - Take the base line record
2) 90 second - Allow the drug contact with time.
3) 120 second - Give the first wash
4) 300 second - Give the second dose.

4) Contact time →
The time that allowed for drug to remain in contact time with tissue is called contact time depends upon the type of tissue used.

5) Magnification of response is adjusted to get proper recording of observed physiological response-
Adjust for magnification by properly adjusting the distance between righting tip and fulcrum and the distance between of point attachment to tissues and function by adjusting relative distance desire degree of magnification is obtained.

Magnification value - $\frac{\text{Distance beth fulcrum and writing}}{\text{Distance beth fulcrum and point of attachment the tissue (8)}}$

6) Method by which response is recorded in kymograph →
By uniform smocking is essential for proper recording the recording can be done directly on white paper with the help or pen attached to tip of lever.

7) Tension of various preperation →
1) for Guinea pig ilium - 1gm
2) for frogs rectum abdomen- - 1gm

8) Laboratory techniques -
① Fixing ② Smoking ③ Recording
④ cutting ⑤ measurement of capacity of organ bath

9) use of kymograph paper →
kymograph paper is tightly to drum and used for kymographic recording.
The lever is touched to kymograph paper to record the contraction of muscles

10) Smoking of drum-
① Fix the kymograph paper tightly to the drum
② Smoke the drum uniformly

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7.0

Pharmacology and Toxicology (0813)

3) Time cycle
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Experiment No. 6

1.0 Title :

To prepare the Physiological salt solution.

2.0 Prior Concept :

Composition of ringer solution.

3.0 New Concept :

Proposition 1 : Function of composition of Ringer Solution.

4.0 Learning Objective :

4.1 Intellectual skills :

1. To understand the Mechanism of action of ions.

4.2 Motor Skills :

1. Ability to weigh the chemicals
2. Method of preparation of Physiological salt solution.

5.0 Requirements :

5.1 Apparatus :

Weighing balance, beaker, stirrer, spatula, butter paper.

5.2 Chemicals :

Sodium Chloride (NaCl)

Potassium Chloride (KCl)

Calcium Chloride ($CaCl_2$)

Sodium Bicarbonate ($NaHCO_3$)

Sodium Hydrogen Phosphate (NaH_2PO_4)

Glucose

Distilled water

6.0 Stepwise procedure :

1. Weigh all the chemicals accurately.
2. Dissolve alkalies and sodium hydrogen phosphate, glucose, calcium chloride separately in Distilled water in beaker.
3. Add the required amount by sodium bicarbonate by dissolving in sufficient volume of distilled water.
4. Add Sodium bicarbonate at the time of setting up of the experiment (since calcium carbonate is liable to be precipitated if the calcium and bicarbonate are kept long together).

7.0 Standard Tab

(Values are in gr

Sr. No.	Compositio
1	Sodium Chloride
2	Potassium Chlo
3	Calcium Chlori
4	Magnesium S
5	Sodium Bica
6	Sodium Dihy Phosphate
7	Potassium Phosphate
8	Glucose

8.0 Quest

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7.0 Standard Table :

(Values are in gram / Litre.)

Sr. No.	Composition	Frog-Ringer	Ringer-Locke	De Jalon	Krebs	Function
1	Sodium Chloride	6.5	9.0	9.0	6.9	To provide Isotonicity, Isomolarity, Contractility and excitability
2	Potassium Chloride	0.14	0.42	0.42	0.35	To provide Ionic balance
3	Calcium Chloride	0.12	0.24	0.06	0.28	To provide contractility.
4	Magnesium Sulphate	----	----	----	0.28	To stabilize the preparation.
5	Sodium Bicarbonate	0.2	0.5	0.5	2.1	To provide alkaline medium.
6	Sodium Dihydrogen Phosphate	0.008	----	----	----	As a buffer.
7	Potassium Dihydrogen Phosphate	----	----	----	0.16	As a buffer.
8	Glucose	2.0	2.0	0.5	2.0	To provide energy.

8.0 Questions :

Write answers to Q.... Q.... Q.... Q.... (Questions to be allotted by subject teacher)

1. Why sodium bicarbonate is added at the time of setting up of the experiment? Give reason.
2. What is the function of each composition in frogs ringers solution?
3. State three physiological salt solutions other than frogs ringer solution.
4. What is the use of ringer solution?
5. Which Physiological Salt Solution is used for experiment of isolated heart and rectus abdominus muscle preparation?
6. Which precaution should be taken while preparing the physiological salt solution?
7. What is contractility?
8. What is Isotonicity?
9. Why there is need of providing isotonic solution?
10. What is the need to provide buffer in physiological salt solution?

(Space for answers)

1) Sodium bicarbonate is added at the time of setting up of the experiment because - Sodium bicarbonate forms precipitation with of calcium chloride

(Space for answers)

2) Functions of composition in frog ringer's soln –
1) Sodium chloride – To provide isotonicity isomolarity, contractivity etc.
2) Potassium chloride – To provide ionic balance.
3) Calcium chloride – To provide contractivity
4) Magnesium sulphate – To establish the preparation
5) Sodium bicarbonate – To provide alkaline nature
6) Sodium dihydration phosphate → As a buffer.
7) Potassium dihydrogen – As a buffer.
8) Glucose – To provide energy.

4) Use of ringer soln →
It is used to keep the isolate time or organ preparation surviving as long as the experiment isover. It is important to choose the particular type of solution in which tissue is known to survive.

5) a. Isolated heart – frog ringer's soln
b. Rectus abdominus muscle – frog ringers, normal saline.

6) Precautions -
1) Adjusting the proper final volume
2) Aireate the soln with oxygen
3) If the solution is not clear soln and prepared freshly

7) Contract quality -
It is reduction in length of muscle producing for when nearer impulse is spread over muscle membrane.

8) Isotonicity -
It means same tone that is the solution having osmotic pressure same as extracellular fluid of body.

9) The providing isotonic solution -
In isotonic soln the isolated organ will survive and give optimum response the cells will neighther shrink nor swell

10) Glucose is physiological salt isolution is metabolised by isolated organ for energy these produces lactic acid or pyruvic acid or water product.

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The acid change pH or
Physiological soln hence buffer
is provided

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Pharmacology and Toxicology (0813)

1.0 **Title**:

To interpret from the graph by
contraction on isolated heart
(Refer the Experiment No.1

2.0 **Prior Concepts:**

Anatomy and Physiology o
Atria, ventricles.

3.0 **New Concepts :**

Proposition 1:

Drugs may infl
heart. An increase in
chronotropic effect
called a **positive ino**
inotropic effect.

Proposition 2 : M

positive chronotr
which are prese
negative chrono
which are prese

Proposition 3

membrane is

divided into