

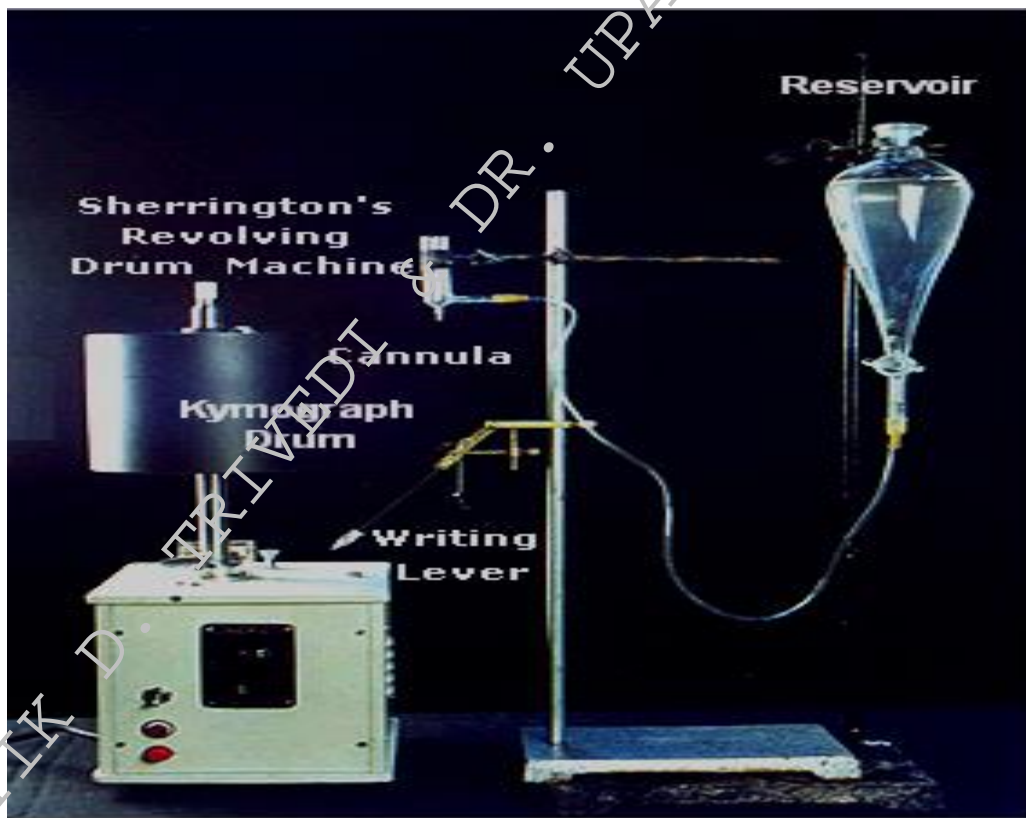
EXPERIMENT NO.: 2

DATE:

AIM: INSTRUMENTS USE IN EXPERIMENTAL PHARMACOLOGY

A. AIM: TO STUDY THE EQUIPMENTS USED FOR ISOLATED AND PERFUSED FROG HEART IN EXPERIMENTAL PHARMACOLOGY

Equipment 1: Equipment used for isolated and perfused frog heart



1. **Reservoir:** an ideal reservoir has an arrangement to deliver the physiological solution at a fixed rate and with constant pressure.

For practical purpose even glass separators can be used as reservoirs.

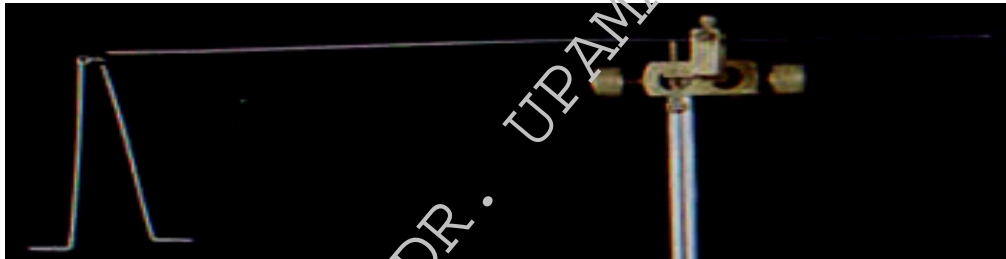
2. **Margate bottle:** it consists of an aspirator bottle fitted with tight stopper perforated by a glass tube reaching nearly the bottom of the bottle. It is used as a steady pressure had since the pressure always corresponds to the lower level of the glass tube no matter how much liquid there is above this level in the bottle.

3. **Writing Levers:**

Levers are meant for recording and magnifying the responses of isolated tissues to drugs. The levers are attached to the isolated tissues and are used to record various types of contractions in them.

Levers: Side way writing, Frontal Writing, Sterling's, Broodie's Universal, Gimbal, Auxotonic.

- a. **Frontal Writing levers:** this is used for recording of isotonic contraction of the isolated tissues. In this lever the writing end (stylus) can freely rotate around its axis. This minimizes the friction between the stylus and the kymograph. With frontal writing lever, the contraction of the isolated tissues are recorded as straight lines.



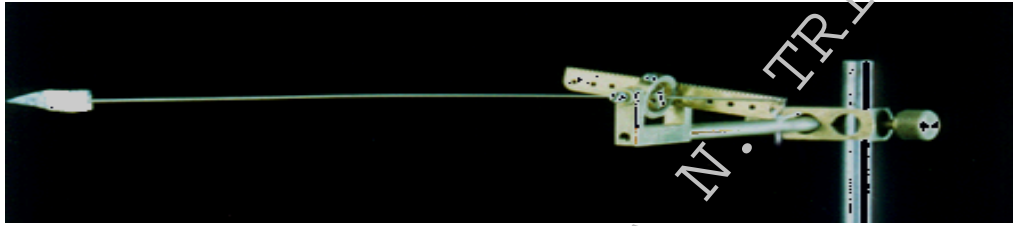
- b. **Simple/Side way writing lever:** this is used for recording of isotonic contractions of the isolated tissues. The responses recorded by simple lever are curvilinear. Uncontrolled friction between the writing end (stylus) and the kymograph is a major disadvantage of simple writing lever.



- c. **Starling's heart lever and broodie's Universal lever:** This is used for recording of isometric contractions of the isolated tissues. In this, the horizontal arm of the lever is suspended to a rigid point with a spring. This type of lever is used for recording of rapid and multiple contractions in the isolated tissues.



- d. **Gimble lever:** The friction between the writing end and the kymograph is minimum in the Gimble lever because the pressure of stylus on the kymograph depends on gravity.



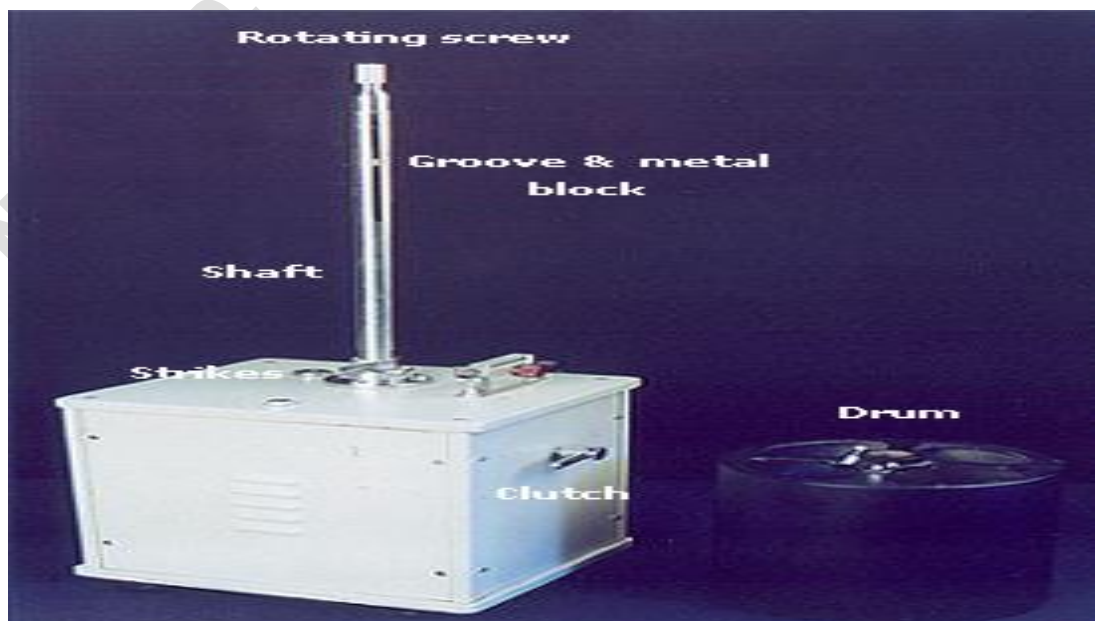
e. **Paton's Auxotonic lever:** it is designed in such a way that the load on the tissue goes on increasing as tissue contracts.

4. **Cannula:** Cannula is generally made of glass or steel. They are used to infuse the physiological salt solution or drug solution in to an isolated organ (tissues) or for administration of physiological salt solution or drug solution to the experimental animal. Specifically devided cannula is also used for providing artificial respiration to anaesthetized animal or to measure the rate of respiration.



5. Sherrington Recording Drum and Drum Cylinder:

It is the instrument on which physiological responses such as contraction and relaxation of muscle are recorded. It consists of a *heavy base* and a vertical *shaft*. Heavy base gives stability to drum. It has;



- a) **Base hoofs** (legs) with adjustable **leveling screws** to keep drum horizontal if surface of the table is uneven.
- b) **Side hoof** to turn the drum on its side so that shaft becomes horizontal.
- c) **Gear rod** arrangement with fast, slow and neutral gears and clutch (starter). The gear rod is attached to a cone wheel which has 4 pulley grooves. Desirable speed of drum can be obtained by changing gear position and shaft drum pulley connections.
- d) **Contact screw** on the surface. A wire can be fixed from main plug to convey the current through base and
- e) **Contact foil** with a contact screw mounted on an insulated material on the superior surface of the base. Second wire can be connected here.

Drum cylinder is a brass or iron cylinder around which a paper is wrapped and smoked. Drum with smoked paper is fitted on vertical shaft. At the base of vertical shaft, there are two projecting strikers which can be drawn apart to set any desired angle between them. When the striker makes the contact with foil, the make the circuit occurs.

These days electrical drum is more commonly used. This is similar to Sherrington recording drum but speed is controlled electrically with the help of gear.

CONTRACTIONS:

1. **Isotonic contractions:** in this type, there is change in the length of isolated tissues when it contracts. The levers used for recording isotonic contraction are called type-1 levers. In such levers the fulcrum lies between the writing end (stylus) and tissue tying position. Eg.: Contraction of guinea pig ileum in response to histamine.



2. **Isometric Contractions:** in this type there is changes in force of contraction rather than change in length when the tissue contractions. The levers used for recording the isometric contractions are called as type-2 levers. In such levers the fulcrum lies at one end beyond the tissue tying position.

The isolated tissue is tied between two rigid points, one of which is a spring. Type-2 levers are used for recording of the rapid and multiple contractions.

Eg.: electrically stimulated muscle twitches.



3. **Auxotonic Contraction:** In certain cases, while recording contractions in the isolated tissues, the restoring force on the tissue is increased as the tissue contracts. Thus, a record of change in force of contraction with respect to change in length is obtained. Such type of recording is called as recording of auxotonic contractions. Strain gauge coupler and paton's lever are used for such type of recordings.

Magnification of response: the lever has to adjusted so that contraction recorded on the kymograph is magnified at least five times that of the actual contraction of the tissue. The magnification of response depends on the ratio of the distance between stylus and fulcrum (X) to the distance between fulcrum and the tissue tying position (Y).



6. Rotating Drum:

- a) **Smoke Drum:** The responses are recorded on smoked drum which is prepared as follows:

The glazed paper is laid on the table, keeping glazed surface downward. One end of the paper is gummed. The drum cylinder is placed in the middle of the paper. The proximal ungummed end is rolled around the drum and held tightly between the thumbs. The other end is also rolled on other side and the gummed and is pasted on the proximal ungummed end.

The cylinder with paper is passed over a rod fixed in smoking rack. A shooty flame is obtained by passing the gas through benzene or using a mixture of benzene and kerosene in the ratio of 1 : 9. The burner is brought nearer to the drum which is rolled uniformly at the maximum possible speed. The outer orange zone of flame should touch the paper. The uniform deposit of shoot.

- b) **Fixing the graph (Varnishing of the Graph):**

The paper is cut after obtaining the recording and then it is dipped in a solution resin (colophony) in methylated spirit. This solution is prepared by dissolving 150 gm of

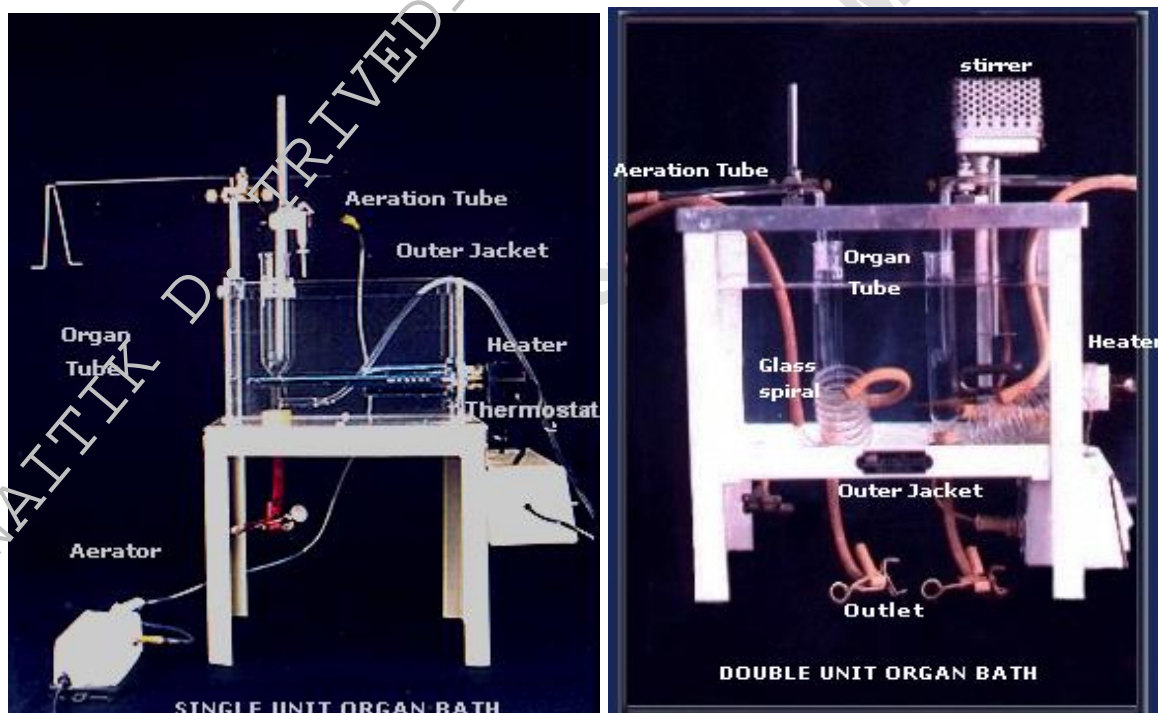
resion in two liters of sprite. After passing the paper through the solution, it is drained and then allowed to dry.

c) **Recording of responses on drum cylinder without smoking (Sketch-pen tip):**

The responses with the help of frontal writing levers can be recorded on drum cylinder using unsmoked paper. Simple sketch-pen tip can be tied with the help of cotton thread with very small amount of wool and a drop of ink (or eosin) can be placed before start of recording. This avoided the trouble of smoking as well as varnishing of the graph.

B. AIM: TO STUDY THE EQUIPMENTS USED FOR ISOLATED TISSUE PREPARATIONS IN EXPERIMENTAL PHARMACOLOGY

Equipment 2: Equipments used for isolated tissue preparations.



STUDENT'S ORGAN BATH:

- 1) **Outer jacket:** it is generally made of Perspex or glass. It holds tap water warmed thermostatically (at 37°C) and helps to maintain the environment of isolated tissue at physiological temperature.
- 2) **Organ tube:** the isolated tissue is suspended in the organ tube. It has varying----- depending upon the tissue which is to be mounted. It is connected to the reservoir containing physiological salt solution.

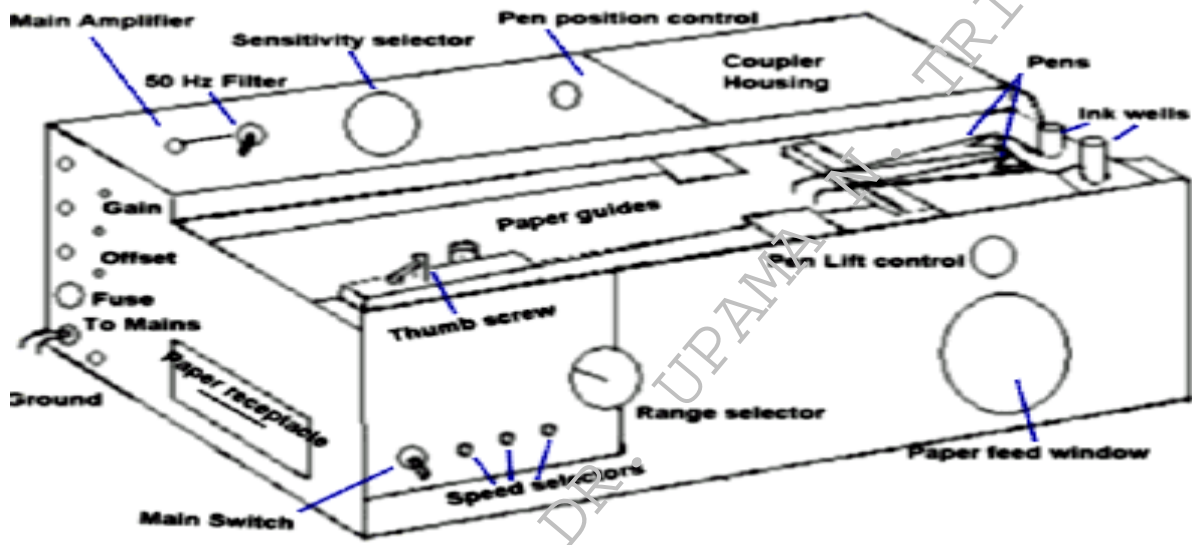
- 3) **Glass coil:** it is also called as preheating coil. This is of about double the capacity of organ tube. The glass spiral is connected in the midway between and organ tube. It holds the physiological salt solution at 37°C, which then enters the organ tube. Thus it avoids fluctuations in the temperature of physiological salt solution during washing of the isolated tissue.
- 4) **Oxygen delivery tube (Aeration tube):** through this tube air or oxygen is supplied to the isolated tissue. At the notch in this tube, one end of the isolated tissue isties. Through an opening in aeration tube, Oxygen (a mixture 95% oxygen and 5% Carbon dioxide) is supplied to the isolated tissue. Generally the speed of aeration is maintained at 1-2 bubbles per second.
- 5) **Thermostat:** maintains the temperature of water in the outer jacket at 37°C
- 6) **Heater:** warms the water in the outer jacket.
- 7) **Stirrer:** Circulates the water held in the outer jacket and helps in distribution of the heat generated by thermostat.
- 8) **Aerator:** it is a device used for supply of the air or mixture of air and oxygen.

C. AIM: TO STUDY THE MODERN INSTRUMENT USED FOR RECORDING THE RESPONSES OF ISOLATED TISSUE OR ORGAN IN EXPERIMENTAL PHARMACOLOGY

Equipment 3: Modern instrument used for recording the responses of isolated tissue or organ

PHYSIOGRAPH AND POLYGRAPH:

In most of teaching institution the responses are recorded on smoked paper, i.e. kymograph which consist of an electrically or pulley driven gear box with a vertical rod carrying a smoked drum. Nowadays students physiograph and multichannel polygraph recorded are also available.



Most of the recorders consist of following three components:

1. **Transducer:** It is a device which converts changes in length, pressure, volume or temperature into electrical potentials are called transducer. Conversion is possible with the help of suitable (appropriate) transducer.
2. **Amplifier:** It is a device which amplifies a very small signal and used it to cause a pen deflection that is directly proportional to the size of the signal. The signal itself may come from an infinite variety of voltage producing sources. It may be generated as another form of energy and translated into an electrical signal by transducer. A wide range of amplifiers are available to meet most requirements.
3. **Recorder:** It is a chart drive device which (with precise speed) moves the chart paper according to required speed with the stylus of a writing element. Student physiograph is a single channel electronic recorder having high sensitivity, precision and accuracy. Its operation is simple as compared to multichannel polygraphs. By changing the type of couplers and matching transducers, number of parameters can be measured like Isometric contraction, Isotonic contraction, blood pressure (E.E.G.), Electromyogram (E.M.G.), Respiratory movements etc.

4. **Students Physiograph is made up of three parts :** Console, Amplifier and coupler:

- (1) **Console:** The console is the main body of the physiograph. The right side of console has three sockets. Upper socket is for the connection of console with the stimulator. The other sockets 'In' and 'Out' are for interconnecting the console with other physiograph to the same experiment.

There are three screw driver controls present on the same side,

- (i) Gain 'C' is to increase the amplitude of recordings beyond the limits of the main amplifier,
- (ii) DAMPING,

- (iii) **OFFSET.** Last two controls should not be used by students.

On the **left side** of console there are three sockets for fuse, earthing and connection to the mains. This side also consists slot (square window) to place the paper stack.

Front side of the console consists of main ON/OFF switch. It also consists of a speed range selector knob (100 to 25 mm/sec. or 10 to 2.5 mm/sec. or 1 to 2.5 mm/sec.) and three speed selector push buttons to get the desired speed. There is one round window for adjusting the chart paper on the console top and pen lift control to lift pen from paper.

On the top of console there are inkwells to fill ink and two recording pens of a 20 mm length and 70 mm wide. The upper pen is for recording the responses through transducer and lower pen is for time / Event recording.

There is a slot for receiving paper, guides to pass the papers and thumb screw and bearing to run or stop the paper movement.

(2) **Main Amplifier** has 3 controls:

- (i) 50 Hz filter ON/OFF. When it is "ON" it filter 50 Hz artifacts.
- (ii) Sensitivity selector for selecting the sensitivity of the amplifier ranging from 50 v to 500 v in 4 steps and from 1 mv to 100 mv in 7 steps.
- (iii) Base line control for adjusting position of pen.

(3) **Couplers:** Couplers can be plugged into coupler housing of physiograph.

Different types of couplers are available for recording various parameters.

- (i) **Strain Gauge Coupler :** This coupler with the help of strain gauge transducer, plethysmography, spirometry , experiments of frog sciaticgastrocnemius preparation (simple muscle curve, successive stimuli, tetanus, fatigue, isometric contractions etc.), experiments on frog, rabbit or rat heart, isolated tissue (ileum, uterus, vas-deferens, anococcygeus etc.) Springs of different tensile strength are available with the transducer.
- (ii) **Biopotential Coupler:** It is a useful for recording of E.C.G. (Electrocardiogram), E.E.G. (Electroencephalogram), E.M.G. (Electromyogram), E.O.G. (Electro-oculogram), Sensory and motor nerve conduction velocities in humans.
- (iii) **Electrocardiogram (EKG) Coupler:** It is used for recording clinical ECG. It consists of a knob to select various leads.

- (iv) **Pulse-Respiration coupler:** It is used for recording arterial pulse with a photoelectric pulse transducer and respiratory movements with a respiration belt transducer.
- (v) **Temperature Coupler:** This is used for recording surface or rectal temp.

➤ **PROCEDURE FOR THE USE OF PHYSIOGRAPHS:**

- (1) Connect the respective transducer. Put the chart papers in proper position. Fill the inkwell and check the free flow of ink from pen. Select the chart speed.
- (2) Never put the instrument "ON" without connecting the transducer.
- (3) Put the instrument's main switch and sensitivity (and not the coupler) to the "ON" position at least for "15 minutes".
- (4) Adjust the position of pen (stylet) with the help of baseline knob as required. The knob of sensitivity usually kept at 200V position. However, it may be changed as required (500V or 1mV in isotonic transducer).
- (5) Put the "Coupler" position to the "ON" position.
- (6) Adjust the balance in other words readjust the original place of the pen with the help of "Balance".
- (7) To change the baseline, put off the balance and then change the baseline and then balance.
- (8) After adjusting the baseline with the balance hang the weight (1gm) on the transducer. See the deflection of pen and it should be 10 mm if not, adjust with the help of "Gain".
- (9) Finer adjustment of sensitivity is done by "Gain" and it should be used in rare circumstances only.
- (10) When you tie the tissue it is advisable to switch off the "Balance".
- (11) Never try to adjust "Damp" or "Offset".
- (12) In case of any problem please ask the concerned teacher.
- (13) Add the different concentration of drugs and record the readings.

➤ **AT THE END OF THE EXPERIMENT:**

- (A) Remove ink from the inkwells and clean with the help of water. Flush the capillary outlet and capillary.
- (B) Clean the pen-writer.
- (C) Cover the instrument.
- (D) Put the pen writer in the locker safely.

- (E) Transducers may be left connected to the physiograph but it must be kept in the box provided.
- (F) Never try to stretch the spring of the transducer it may damage the transducer.

D. SOME OTHER INSTRUMENTS USED FOR EXPERIMENTAL PHARMACOLOGY ON ANIMAL

1. Rota rod

“To evaluate drug effects on motor coordination, balance and motor learning in rodents”



Rotarod test is widely used to evaluate drug effects on motor coordination, balance and motor learning in rodents. The principle of this test is that rats or mice are first trained to walk on a rod rotating at a certain speed. Once the animals have learned this, the effect of a test-compound on their motor performance is evaluated.

Animals experiencing impaired motor coordination are unable to cope with the rotating rod and will drop off when the rotation speed exceeds their motor coordination capacity. When the animal drops from rod safely into its own lane, the time latency to fall is automatically recorded. Rotarod is controlled by an advanced microprocessor which provides precise timing control and accurate speed regulation. Rotation can be electronically set at a constant speed (2 to 60 rpm) using a dial on the front panel.

2. Digital Plethysmometer

“For screening of inflammation or oedema in mouse or rat”



The Digital Plethysmometer is a micro controlled volume meter, specially designed for accurate measurement of the rat / mouse paw swelling. The Digital Plethysmometer is a highly useful tool to evaluate the inflammatory response experimentally induced in small rodents and to screen potential anti-inflammatory or anti-oedema properties of pharmacological substance. The water displacement produced by the immersion of the animal paw in the measuring tube is reflected

into the second tube, inducing the volume displacement. This displacement value is recorded. The control unit can be zeroed between successive readings by pressing the reset key, so as to avoid the error arising due to adherence of water to the paw of the animal.

Foot switch allowing rapid hands-free experiments can be used to control the end point of the measurement.

3. The Digital Tele-Thermometer

“For measuring the temperature of laboratory animals”



The Digital Tele-Thermometer is ideal for continuous monitoring of temperature in laboratory animals for study and research in anaesthesia, cardiac surgery, hyperthermia & pyrogen testing.

4. Pole climb apparatus

For the study of antipsychotic drugs



The pole climb apparatus is one of the most important laboratory models employed for the study of antipsychotic drugs.

Orchid's new improved model with built in solid state buzzer and stimulator provides an electrical stimuli of 100uA -2.8mA for duration controlled digitally. Instrument has also a provision for output for recording on optional kymograph or polygraph.

5. Hot Plate Apparatus

For testing heat induced pain in mice & rat



The Hot Plate Analgesia Meter performs rapid & precise screening of analgesic drug properties on small laboratory animals.

Hot Plate Analgesia Meter has been developed for conventional hot plate pain test. Pain sensitivity to heat is assessed by placing the animal on the top of aluminium plate and starting the timer. The operator stops the timer at the instant the animal lifts its paws from the plate, reacting from discomfort. The front panel timer displays the latency to react, a measurement of the animal's resistance to pain.

6. Photoactometer

To study spontaneous motor activity in open field



Infra-red actophotometer is used to measure the spontaneous activity by means of infra-red beams. It represent an ideal tool for assessing loco-motor activity & exploration in rodents. The system represents a reliable system for easy & rapid drug screening in both day & night light condition.

7. Tail Flick Analgesia Meter

For measuring pain sensitivity in mice & rats



Tail Flick Analgesia Meter measures pain sensitivity in mice and rats as they respond to heat application to a small area of the tails.

Cut off time is 10-12 sec.

8. Maze apparatus

For measuring anxiolytic activity



The elevated plus maze (EPM) is a test measuring anxiety in laboratory animals that usually uses rodents as a screening test for putative anxiolytic or anxiogenic compounds and as a general research tool in neurobiological anxiety research. The model is based on the test animal's aversion to open spaces and tendency to be thigmotaxic. In the EPM, this anxiety is expressed by the animal spending more time in the enclosed arms.

9. Elector Convulsometer

For measuring the anti-epileptic activity



The convulsion in rat and mice can be induced by giving high voltage current near the brain or by suitable CNS stimulants (Eg. Pentylene tetrazol). The screening of antiepileptic agents can be done by experimentally induced convulsion (Seizures) and their prevention by drug under test.

Eye/ear electrode are used.

10. Histamine Chamber

For measuring anti asthmatic/histaminic activity



The chamber is a scientific instrument used to evaluate anti-asthmatic activity of certain drugs or chemicals in laboratory.

E. AIM: TO STUDY THE PHYSIOLOGICAL SALT SOLUTION.

Constituents	Frog's ringer soln.	Mammalian ringer soln.	Tyrode soln.	Krebs Henseleit salt solution	Ringer locke solution	De-jalon solution
NaCl	6.5g	9.2g	8.0g	6.90g	9.15g	9.00g
KCl	1.4g	0.42g	0.2g	0.35g	0.42g	0.42g
MgCl ₂	0.3g	----	0.1g	0.11g	----	---
MgSO ₄ .7H ₂ O	----	----	---	---	---	---
NaH ₂ PO ₄ .2H ₂ O	0.1g	----	0.05g	0.14g	----	----
KH ₂ PO ₄	----	----	----	----	----	----
Glucose	2.0g	----	1.0g	2.0g	1.00g	0.50g
NaHCO ₃	0.2g	0.2g	0.1g	2.10g	0.15g	0.50g
Sodium lactate	-----	----	----	----	3.10g	----
CaCl ₂	0.12g	0.24g	0.2g	0.28g	0.24g	0.06g

PURPOSE OF INGREDIENTS:

Sodium Chloride: To maintain iso-osmolarity, Isotonicity, Excitability and Contractibility of the tissue preparation

Potassium Chloride: It maintain ionic balance of the preparation

Calcium Chloride: It maintain contractility of the tissue

Sodium bicarbonate: It maintain the alkaline pH of the solution

Glucose: It act as an energy source

Sodium or potassium dihydrogen phosphate: It act as a buffer and maintain the pH

Magnesium Chloride: It is useful to stabilize tissue during spontaneous activity.

Note:

- Frog ringer is mainly useful for the heart, rectus abdominis and other preparation of frog.
- Tyrode is useful for rat, rabbit, and guinea pig ileum practical
- De Jalon is useful for rat uterus preparation
- Kreb's solution is useful for rat fundus strip, tracheal chain preparation.

TEACHER'S SIGNATURE