Experiment No. : 1 **Date:** 14/01/16

STUDY OF EQUIPMENT USED IN EXPERIMENTAL PHARMACOLOGY

AIM: To study the equipments used in experimental pharmacology.

REFERENCE:

PRINCIPLE:

Experimental Pharmacology makes use of an entire animal or an isolated part of an animal to study the effect of drugs. Each of these experiments makes use of several equipments or instruments so as to qualify their effects. Primary aim of experimental pharmacology is

1. To find out the therapeutic agent suitable for human use.
2. To study pharmacodynamic of drug.
3. To study toxicity of drug.

All animal experiments are integral part of preclinical studies.

In an in vitro experiment which is performed on an isolated part, mounted on a suitable set up and perfused in a warm physiological solution. Maintaining the mammalian body temperature and aerating the tissue are an important aspect. Traditionally in-vitro experiments help to study the effect of agonist and antagonist and to perform bioassays. A suitable recording device (Sherrington's recording unit) is an equally important requirement.

Several advantages of in-vitro experiment are as follows:

- Number of animals used can be reduced
- Results are reproducible and can be repeated many times.
- Relatively small quantity of drug is enough to derive number of conclusions.
- Economical.

Following are the examples of in-vitro experiments:

1. Recording of dose dependant response of an agonist. (Acetylcholine)
2. Effect of antagonist (Atropine) over the effect of agonist (Ach)
3. Effect of drugs on cardiac muscle and blood vessels.
4. To prove the effect of drug is via the receptors.

All in-vivo experiments are performed under controlled condition using whole animal. An in-vivo experiment makes use of equipments that can be relatively simpler to sophisticated instruments.

Following are some of the example of in-vivo experiments

- Analgesic activity.
- Anticonvulsant activity

- Effect of phenobarbitone on righting reflex
- Effect of drugs on locomotor activity
- Effect of drugs on learning and memory.

INSRUMENTS FOR IN-VITRO EXPERIMENTS: (write in small letters)

Student organ bath:

A student organ bath is a basic instrument to perform in-vitro experiments. It is possible to mount the isolated tissue, aerate it and perfuse it with physiological salt solution. A typical student organ bath consists of following parts:

An outer jacket:

Which is usually square or rectangular aquarium tank made up of metal stand.

An inner organ bath:

It is made up of glass of varying capacity of 10-50ml.

Warming coil/condensing coil:

It is made up of glass placed in the outer jacket so as to ensure that physiological salt solution is warmed before it reaches inner organ bath. It can hold upto double the capacity of (20-30 ml) physiological salt solution.

A hollow glass tube:

It is with a curved end which will serve as holder of tissue and to aerate the tissue.

A stirrer:

It is to ensure temperature uniform throughout the bath.

A reservoir:

It contains appropriate physiological salt solution connected to one end of condensing coil.

Heater:

It is to warm the water in the outer jacket.

Thermostatic unit:

It is to maintain the temperature.

Cannula:

It is made of glass. Following are examples of commonly used cannula

1. **Syme's cannula:** it is made up of glass and is used to cannulate isolated heart. It is also possible to perform the heart with it as it has an outlet.
2. **Arterial cannula:** made up of glass and is used to cannulate the artery.
3. **Venous cannula:** made up of glass and used to cannulate veins.
4. **Tracheal cannula:** it is Y shaped made up of steel to cannulate the trachea of higher animals to record the effect of drugs on respiratory system.

Recording levers:

They are used to record charges in the level due to drug. Levers are light in weight, rigid and are generally made of light aluminum or stainless steel levers:

They are of two types:

1. **Isotonic levers:** e.g.: frontal writing lever, simple writing lever, starling's heart lever, Brodie's (universal) levers.

twitchings.

2. **Isometric lever:** e.g.: lever used for recording muscle twitches. In isotonic lever change in the length due to contraction is recorded while the tension is constant. on the contrary isometric lever helps to record increasing tension on muscle while length of tissue is kept constant. following are commonly used levers in lab.

- **Simple lever (sideway writing lever) :** It is made up of stainless steel or aluminum with a writing tip attached to one end of long arm. Recordings using simple lever are in the form of Curved lines.
- **Frontal writing lever:** this lever is designed in such a way that writing point rotates freely above its axis. This helps in reducing the tension between the tip and the glazed paper. The contractions are recorded as straight lines.
- **Starling's heart lever:** this lever is used to record contraction of the heart. Heart is attached with the help of a pin to one of the perforations in the arm of the lever.

→ Brodie's universal lever: General utility lever.

Sherrington's recording unit: This unit of electrically driven gear box with vertical spindle carrying a drum .Gear box has a jack to control movement of drum. Speed ranging from 1.2mm/s to 640mm/s speed (rpm) can be adjusted. Drum always moves in a clockwise direction. The recording on the glossy paper (Kymograph paper) is referred to as Kymograph. A small inkjet with a sharp writing tip attached to one of freely rotating arm of frontal writing lever, which glides smoothly on the glossy paper while recording the changes.

In addition to the above mentioned number of

The other levers and essential equipments used alongwith organ bath are gimbal lever, auxotonic lever, straw or lever holder (fulcrum), different types of X-blocks, clamps, supporting rods, thermometer and surgical instruments.

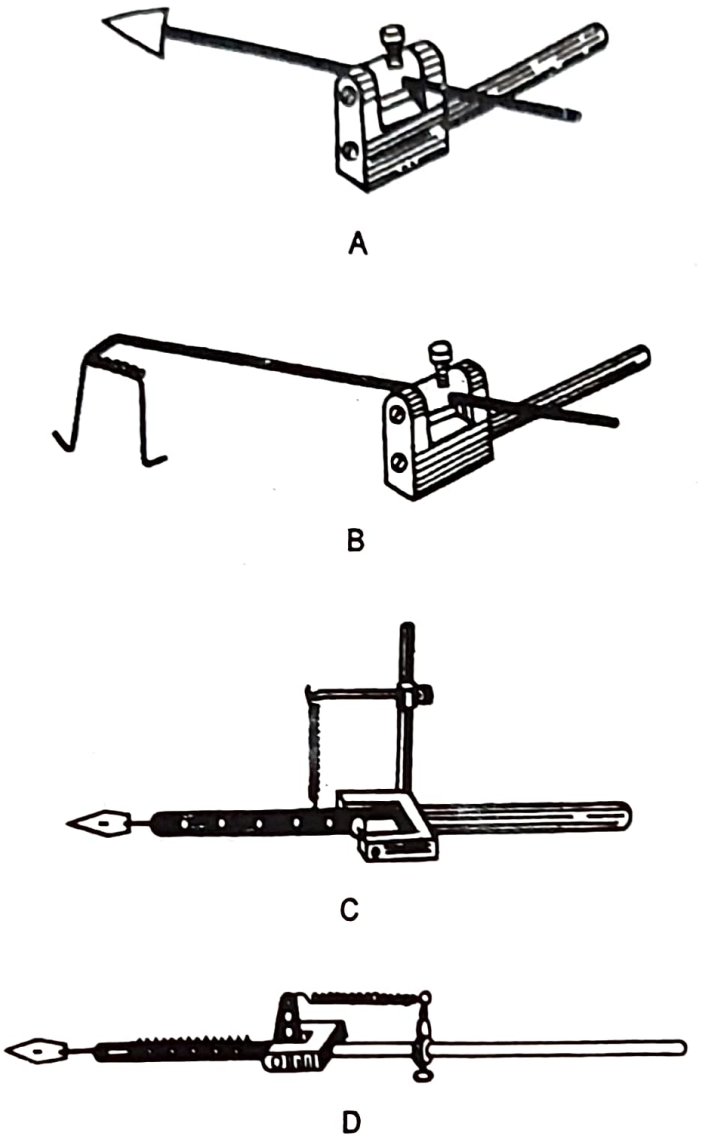

Fig. 1.3 Recording Levers—A : Simple lever; B : Frontal writing lever; C : Starling's heart lever; D : Brodie's lever

Recording Procedures

(*a*) *Adjustment for magnification*—Depending on the inherent contractility of the tissue preparation under study, the magnification of the response should be adjusted in order to get a proper recording of the observed physiological response. The tissues showing less contractility need more magnification and the reverse is true with tissues which have higher inherent rhythmic contractility. For example while recording the effects on guinea pig ileum or rectus abdominis muscle it is desirable to

have 5-10 fold magnification whereas, for rat uterus preparation the magnification needed is only 4-6 times. The adjustment for magnification is done by properly adjusting the distance between the writing tip and the fulcrum, and the distance between the point of attachment to the tissue and the fulcrum. By adjusting relative distances desired degree of magnification is obtained (Fig. 1.4).

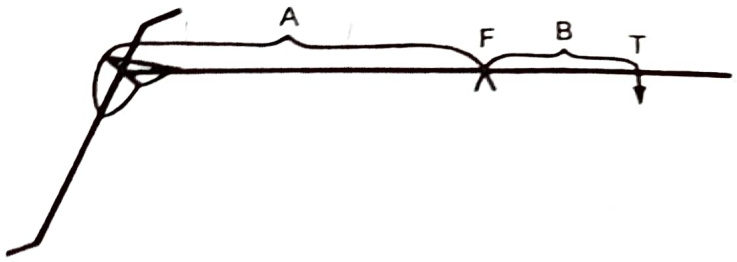

Fig. 1.4 Adjustment of magnification — F : Fulcrum; T : Point of attachment of tissue; A/B = magnifiaction.

$$\text{Magnification value} = \frac{\text{Distance between fulcrum and writing point (A)}}{\text{Distance between fulcrum and the point of attachment to the tissue (B)}}$$

If the distance of the longer arm (A) is 10 cm and that of the shorter arm (B) is 2 cm, the magnification (A/B) will be 5.

(*b*) *Application of load (tension)*—The muscle preparation has to be properly relaxed without affecting the normal tone and rhythmic activity so that efficient contractions are achieved when stimulated, and it also relaxes to its full length afterwards. This is achieved in the following way—(*i*) select the proper length of the longer and shorter arms depending on the magnification for the tissue which is under study, and fix the fulcrum; (*ii*) balance the lever by putting the weight (plasticine) at the end of the shorter arm and mark the point of tissue attachment; (*iii*) at equidistance, *i.e.* the distance between the fulcrum and the point of tissue attachment, from the fulcrum on the longer arm of the lever, fix the desired load (plasticine) required for the particular tissue.

The tension (load) prescribed for various commonly used tissue preparations are—guinea pig ileum (1 g); guinea pig trachea (0.2 g); guinea pig vas deferens (0.5 g); rabbit duodenum (1-3 g); rat uterus (1 g); rat colon (0.5 g); rat fundus (1 g) and frog rectus abdominis (1-5 g), respectively.

(*c*) *Smoking of kymograph drums*—Fix the kymograph paper (glossy surface out) tightly to drum. Then the drum should be uniformly smoked with the black soot (smoke) of benzene or kerosene or the mixture of the two. Uniform smoking is essential for proper recording. The recording can be done directly on white paper (unsmoked paper) with the help of ink-writing device (pen) attached to the tip of the lever.

(*d*) *Fixing of the tracings*—The recordings on the smoked drum (tracings) are preserved by properly fixing them with the help of fixing solution. The commonly used resins to prepare the fixing solution are shallac and colophony. A saturated solution of shallac 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.

(*e*) *Contact time*—The time that is allowed for the drug (agonist) to remain in contact with the tissue is called the contact time. The contact time depends upon the type of the tissue used. For example, a slow contracting tissue such as frog rectus abdominis preparation, the contact time allowed is 90 sec. On the other hand, for guinea pig ileum the contact time is 30 sec. When the drug is in the vicinity of receptors it is called in 'biophase' or 'receptor compartment'.

(*f*) *Time cycle*—A fixed time cycle is used while recording any effect of the drug on the isolated tissue preparation. The fixed time cycle which comprises of starting of the drum, recording the base-line, effect of the drug (contact-time) and washout period. Generally a five minute time cycle is followed *i.e.* 30 sec. of the base line recording, 90 sec. of contact time (response of the drug) and the subsequent three washings at an interval of each minute. It is very essential to follow the fixed time cycle while doing the bioassays to obtain uniform recordings and, it also helps in calculating the approximate time required to complete the experiments depending upon the number of effects to be recorded.

(*g*) *Standard drugs*—The commonly used agonists in isolated tissue preparations in practical pharmacology class are acetylcholine, adrenaline, histamine, 5-hydroxytryptamine (serotonin). ~~The various agonists and their respective antagonists are described in the Table 1.1.~~

(*i*) *Physiological salt solution*—The physiological salt solutions are used to keep isolated tissue or organ preparations surviving as long as the experiment is over. It is important to choose the particular type of solution in which tissue is known to survive. These physiological salt

solutions are prepared carefully using analytical grade reagents and distilled water. The other precautions to be taken are adjusting the proper pH of the final solution and aeration with oxygen, mixture of oxygen and carbondioxide (95% + 5%) or even bubling with air. The physiological solution thus prepared should be clear, and if turbid, it is advised to prepare fresh solutions before the start of the experiment. The various physiological solutions and their composition are listed in

⑤ Arteriole Forceps / Haemostatic Forceps:-
It is used to stop bleeding from the larger blood vessels like arterioles arteries.

⑥ Bull dog clip:-
It is used to occlude the smaller blood vessels and are mainly used for experiments associated with Hypertension.

⑦ Bone cutter:-
It is used to break the bones like ribs, skull etc. of the animals like rabbits, guinea pig etc.

⑧ Scalpel blade and Holder:-
It is used to make small incisions while dissecting or during surgery.

⑨ Scissors:-
These are used as surgical instruments to make various cuts like v-shape cut or straight cut depending on the required during isolation of Organs

⑩ Forceps:-
These are used to hold the tissue or organ and also to seperate the connective tissues like mesenteric plexus, adipose tissue while isolating the Organs and tissues.

⑪ Suturing needle:-
It is used to insert and tie the thread to the

tissues during their mounting in the tissue bath. They are also used to join the skin & tissues after a surgery.

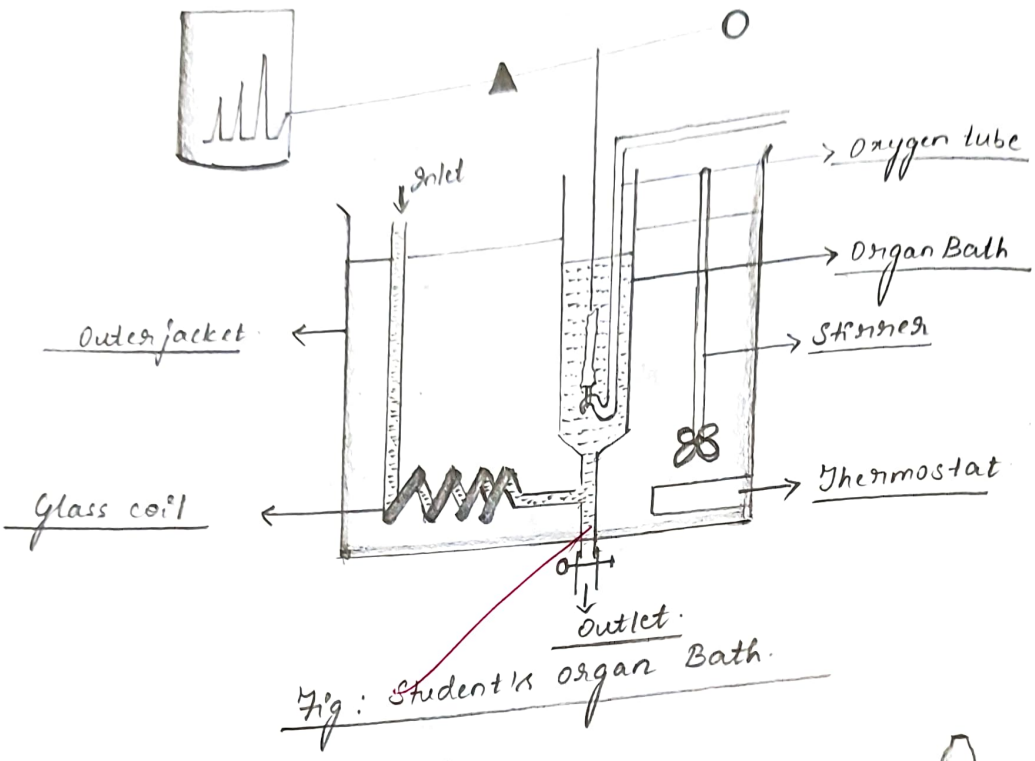

Fig: Student's organ Bath.

Fig: Venous cannula

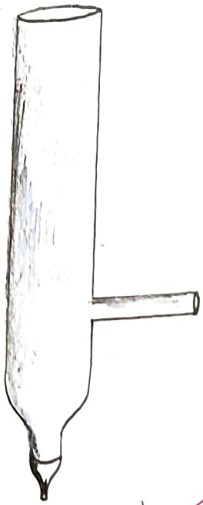

Fig: Syme's cannula

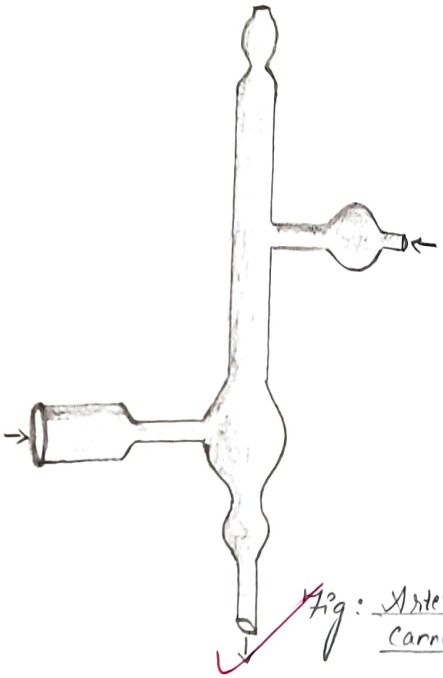

Fig: Asteri... Cannula

Fig: Tracheal cannula.

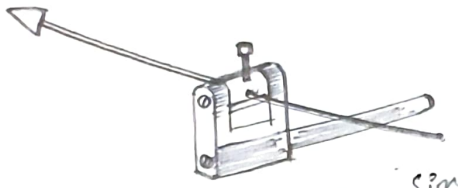

Fig: Simple lever

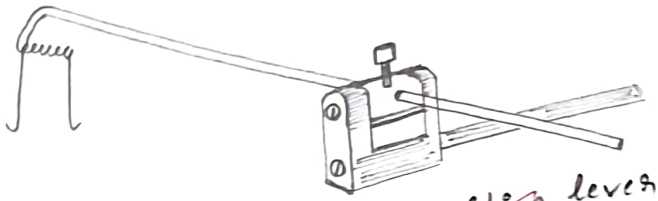

Fig: Frontal writing lever

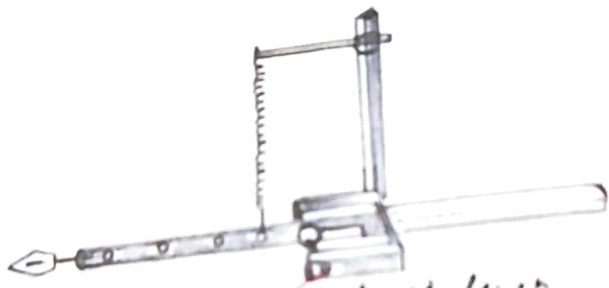

Fig: Starling's heart lever

Fig: Brodie lever

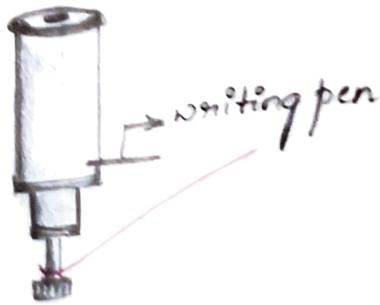

Fig: Ink jet

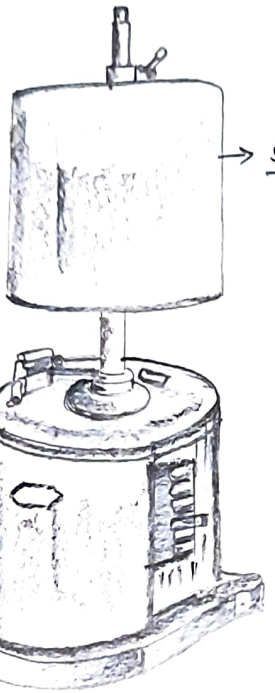

Fig: Sherrington Recording unit

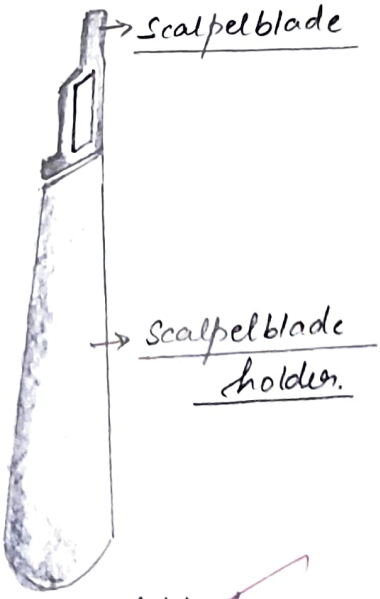

Fig: Scalpel blade.

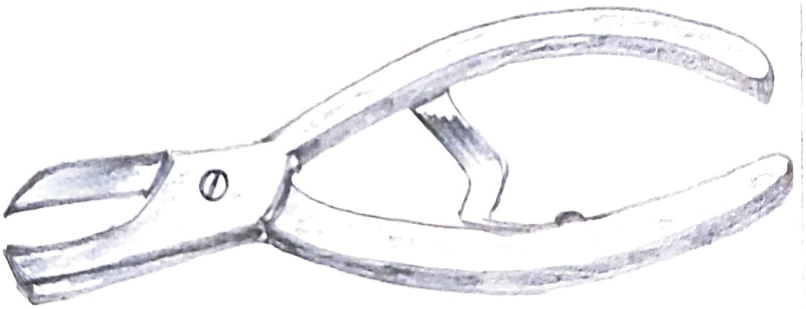

Fig: Bone cutter

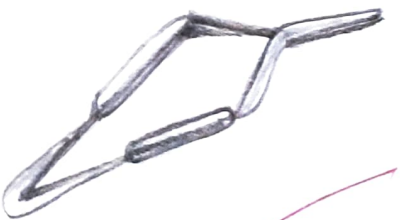

Fig: Bull dog clip

Bull dog clip

Fig : Fulcrum

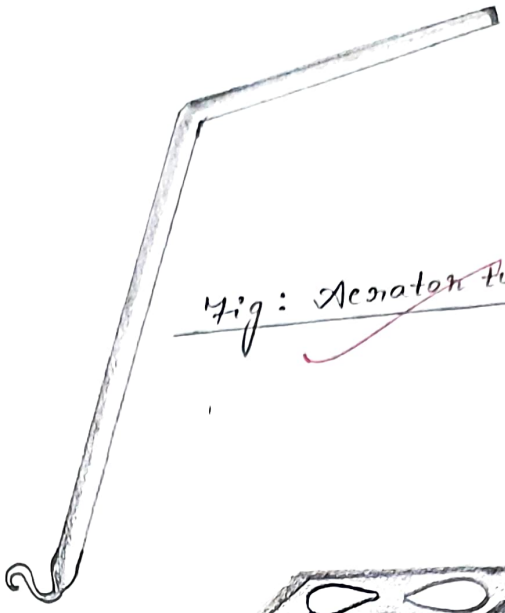

Fig : Aeration tube

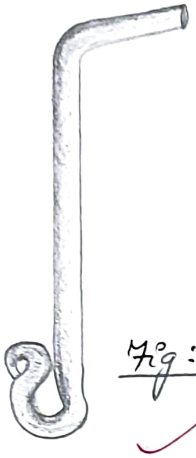

Fig : Tissue holder

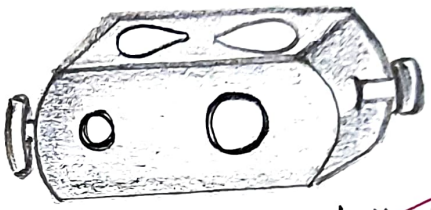

Fig : closed-x-block

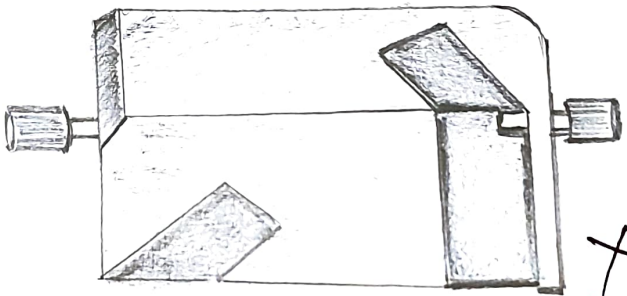

Fig : Open-x-Block

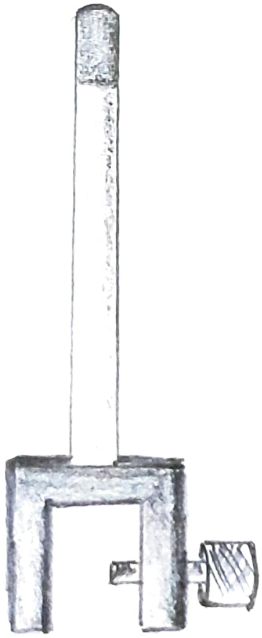

Fig : upright

Fig : Upright

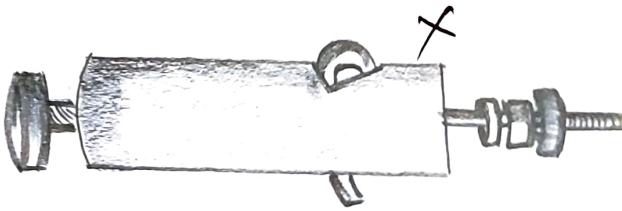

Fig : Upright

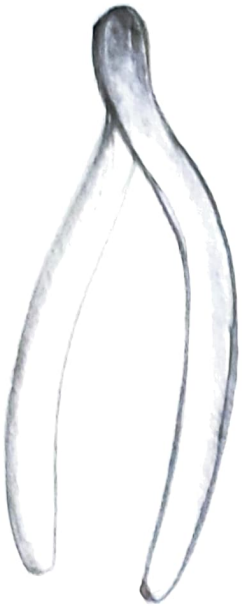

Fig: Blunt Forceps

Fig: Pointed forceps

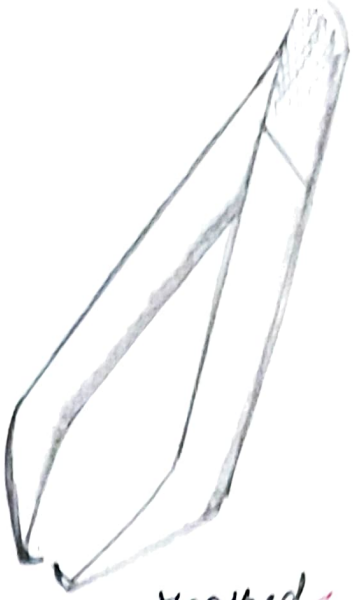

Fig: Toothed forceps

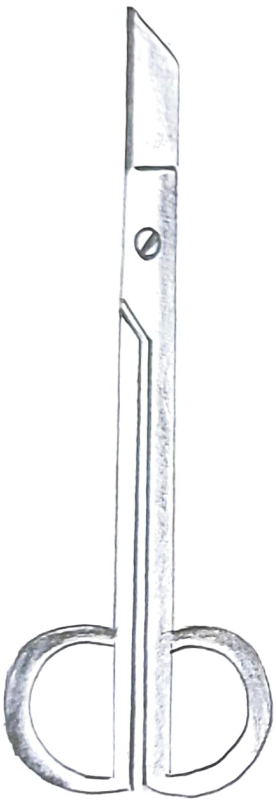

Fig: Large pointed scissors

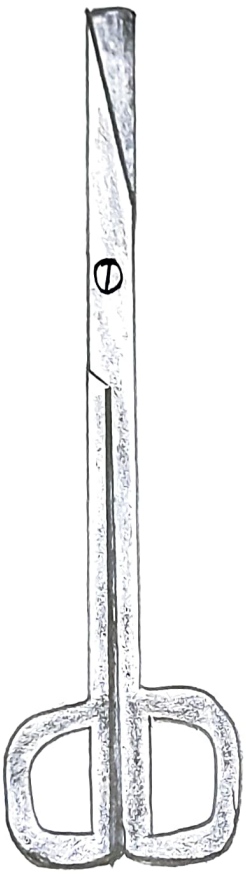

Fig: Blunt Scissors

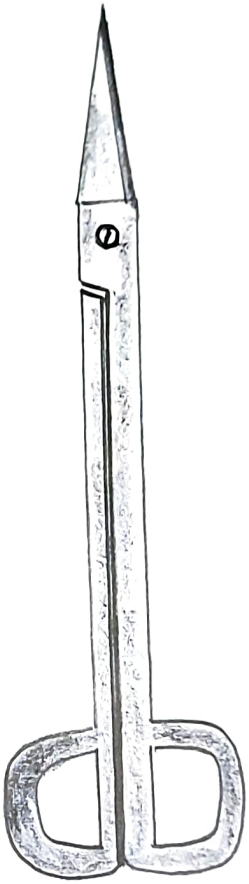

Fig: Small pointed scissors

Fig: Pithing needle

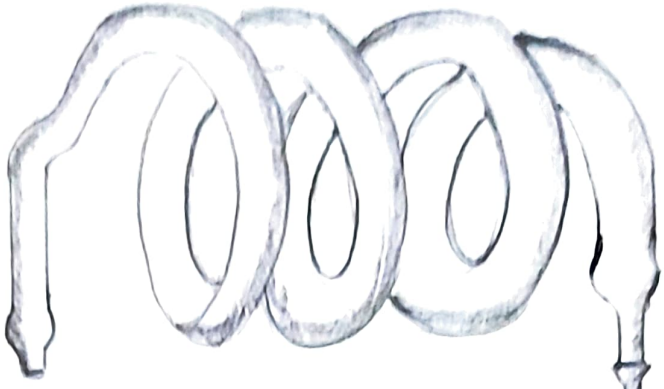

Fig: Preheating glass coil

Fig: Syringe

Fig: Suturing needle

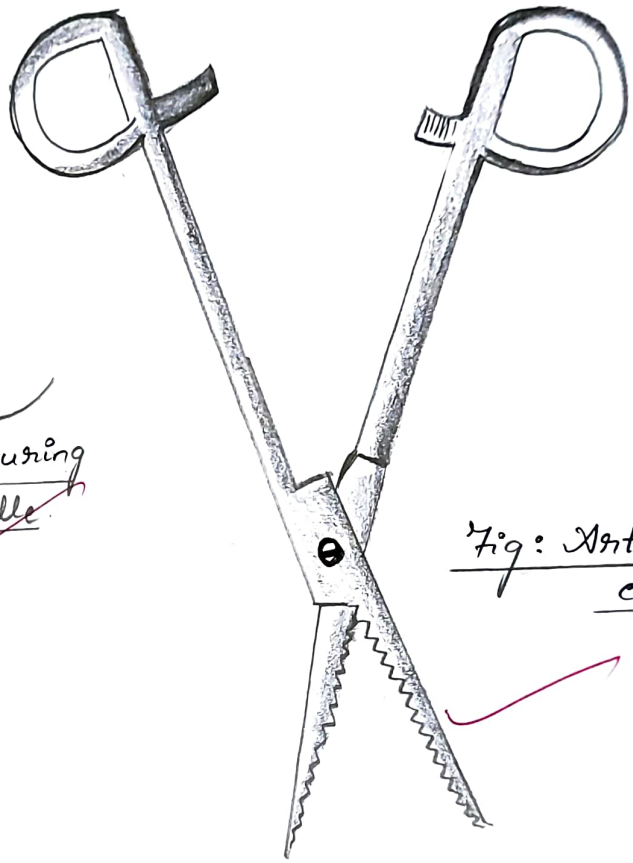

Fig: Arterial cannula

Fig: Reservoir (Marriott bottle)

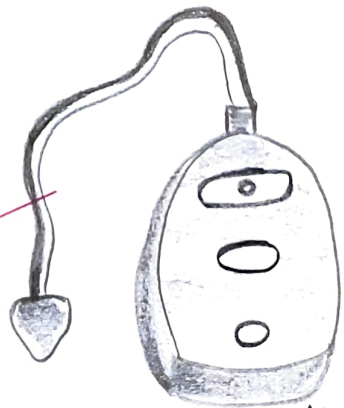

Fig: Aerator

INSTRUMENTS USED FOR IN-VIVO EXPERIMENTS (write in Small letters)

ANALGESIOMETER:

It is an instrument to measure analgesic activity of a compound. Analgesiometer is used to produce analgesia (painful stimulation)

i. **Analgesiometer for Tail flick method :**

In the tail flick method the tail of the test animal is placed on nickel, copper wire which are preheated where analgesic drug animals exhibit delay in flicking the tail from the heat source. Tail flicks in response to a painful stimulus in a normal animal. This instrument can be used for testing non narcotic and narcotic analgesics. This instrument has restrainer (cylinder like) inside which the animal protruding its tail can be placed. It also provides scope for change in strength of the stimulus.

ii. **Eddy's hot plate analgesiometer:**

It is a hot plate in another Analgesiometer used for testing narcotic analgesics heated to 55 C for testing purposes when the test animal is placed on this hot plate, in response to pain, the animalwill jump or licks its own paw (licking response). In test, animal is pretreated with morphine or morphine like analgesic drug. Their is delay in the jump or lick response (latency).

ELECTROCONVULSOMETER:

This instrument is used to study anticonvulsant activity. Electrical current of a particular strength (for rat 130 mA, for sea mice 50 mA for 2 sec)is given with the help of corneal pluna electrodes, current of such a strength produces powerful CNS stimulation manifesting as convulsions. Convulsions consists of various phases such as clonus, tonous, and stupor (like statue), pretreated animals (antiepileptic drugs) do not show any convulsions. This instrument is used in method, called MES electrode method. It is used in frequently because the effects are reversible in nature and it is also possible to control strength of the current.

HISTAMINE CHAMBER:

Histamine chamber is rectangular or a square aquarium tank like apparatus consisting of perforations. It is provided with 2chambers (equally divided with perforations). Histamine is sprayed to the animals in a controlled way (using nebulizer), so as to produce bronchoconstriction. Test animal pretreated with anti histamines fail to exhibit the typical symptoms of breathlessness (bronchoconstriction). Thus bronchodilator effect is studied

ROTAROD:

Rotarod consists of a rough surfaced rotating rod without falling off (recorded as falling of time) due to muscle grip. Animal pretreated with skeletal muscle relaxant or anxiolytic drug fails to remain on the rotating rod and falls off with in short duration (reduction in fall of time). The entire rod is compartmentalized so as to accommodate 3-4 animals at one time, provisions are made to record fall off time. Rotarod is commonly used as a supplementary test while anxiolytic and anti-depressant drugs are tested.

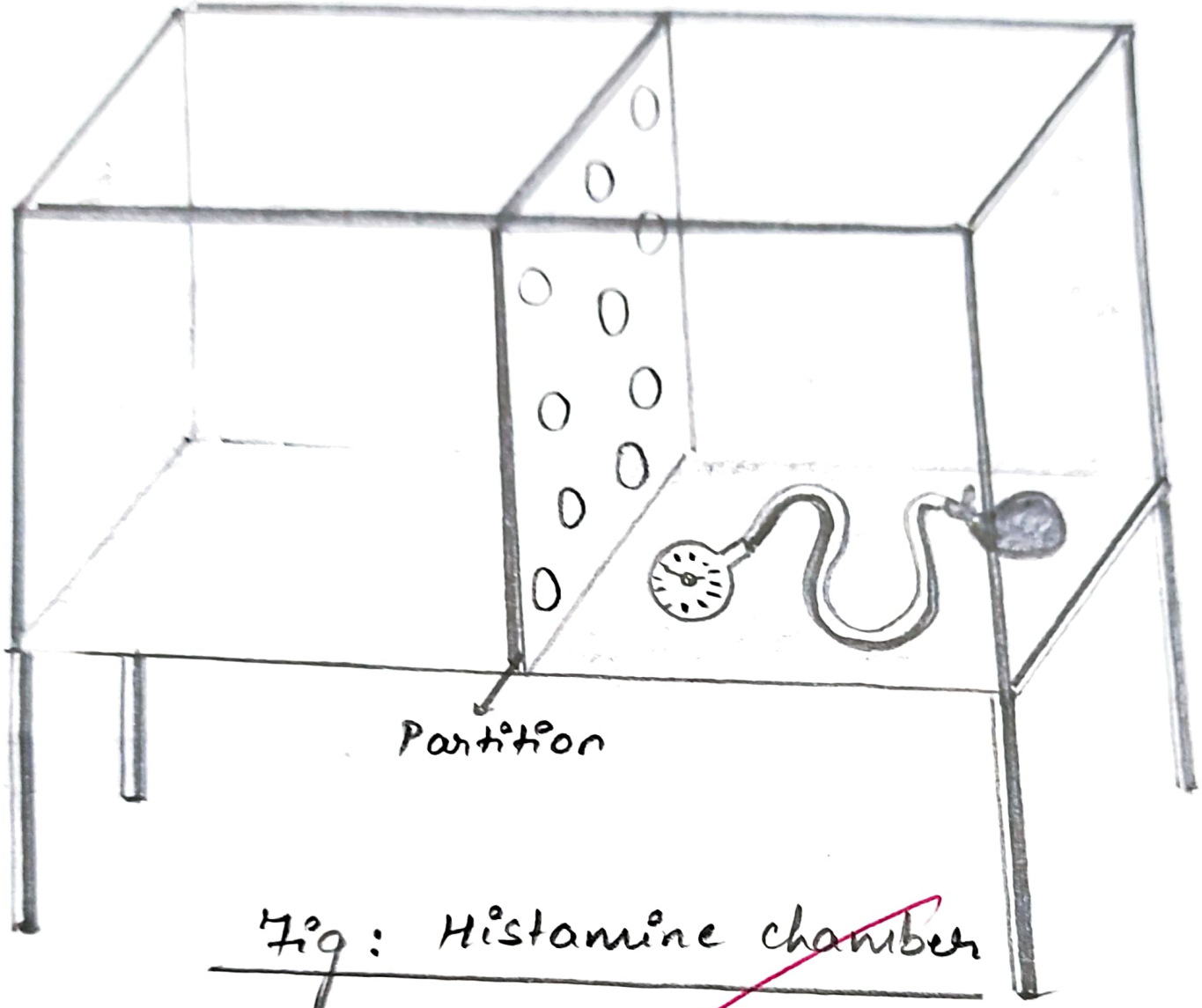

Fig: Histamine chamber

(3) plethysmometer:-

It is used to study antiinflammatory property of drugs. It has two limbs in which the water level is maintained till the marking. The hind paw of the animal is introduced into the limb which has a wider mouth & the level of increase in the water is noted. If the inflammation is more, ~~then~~ the level of the water raises, Based on this the anti inflammatory effect of drugs can be studied.

PHOTOACTOMETER:

Photoactometer consists of an enclosed arena (field) during testing the animal is placed in this central arena and its activity (spontaneous motor activity) is recorded as locomotor activity score (digitally displayed) such an activity indicate its mental alertness. Animals pretreated with CNS depressant drug demonstrates, reduction in locomotor activity score (LMA) (locomotor activity) and those treated with CNS stimulated (Caffeine, amphetamine) will show an increase in LMA score. This is commonly used as a supplementary test of anxiolytic and anti-depressant drugs.

COOK'S POLE CLIMBING RESPONSE APPARATUS:

It is one of the instruments employed in the psycho pharmacological studies to screen and evaluate drugs influencing learning and memory Instrument essentially makes use of condition responses of an animal to a stimulus. A group of trained animals are required to access nootropic effect (effect of learning and memory). Drugs used in treatment of dementia like Alzheimer's disease are studied.

Sl no	Instrument	Animal used	Uses
1	Analgesiometer	Rat (for tail flick method)	To test the analgesic effect.
2	Electro convulsometer	Rat	Anti epileptic
3	Histamine chamber	Guinea pig	Bronchodilators Antihistaminic activity.
4	Rotorod	Mouse	Skeletal muscle Relaxant activity.
5	Photoactometer	Rat and mouse	Effect on mental alertness (CNS stimulant or depressant activity).
6	Cook's pole climbing apparatus.	Rats	Testing of nootropic effect(effect on learning and memory)

REPORT: Various equipments used in the experimental pharmacology were studied.