GOEL INSTITUTE OF PHARMACY

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M. PHARM (PHARMACOLOGY)

Laboratory Manual

Pharmacology Practical-I (MPL 105P)

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CONTENTS

EXPERIMENT NO. 01

OBJECT: To study basic Instruments used in Experimental Pharmacology.

REFERENCES:

- Kulkarni S.K. "A hand book of Experimental Pharmacology", third edition, Reprint 2005, Vallabh Prakashan, page No. 3-10.
- Kale S.R., Kale R.R. "Practical Pharmacology and Toxicology", 9th edition, August 2004, Nirali Prakashan.
- Pillai K.K., "Experimental Pharmacology" first edition, 2008, CBS Publication.

THEORY:

INSTRUMENTS

1. **DALE'S ORGAN BATH-** or isolated organ bath. It is an apparatus used for studying the effects of drugs/ chemical substances on isolated tissues in vitro. The apparatus consist of
   - ❖ An inner glass tube or organ bath containing PSS and tissue and connected to reservoir through polythene or rubber tube.
   - ❖ Aeration cum tissue holder tube to hold tissue and supply O_2 / air to perfusion fluid.
   - ❖ An outer bath made up of glass/perspex filled with water and the temperature checked with the help of thermometers & maintained at 37°C for all mammalian experiments.
   - ❖ A lever for recording the responses of the tissue on a kymograph drum.
   - ❖ The isolated organ bath meant for research purpose also possesses an inbuilt warming device the thermostat, a fluid warming cell and a stirrer meant for automatic temperature control of the water of the outer bath.
   - ❖ The entire assembly is mounted on tripod stand.

2. **SHERRINGTON'S RESEARCH KYMOGRAPH**

   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

- ❖ base loofs (legs): with adjustable leveling screw to keep drum horizontal on the uneven surface.
- ❖ side hoofs- to turn the drum on its side so that shaft become horizontal.
- ❖ Gear rods- arrangement with gear & clutch to obtain desirable speed of drum.
- ❖ Drum cylinder- is a brass or iron cylinder around which paper is wrapped and smoked for recording of tracings

3. LEVERS: It is the device by virtue of which response of isolated tissue can be recorded and magnified.

Principle:

(i) Fulcrum- the point around which the lever moves on the lever holder is the fulcrum.

(ii) Stylus- is the writing point which records the tracing on the smoked paper of the drum. It is either made of celluloid parchuments paper, aluminium foil or thin photographic or x-ray film. Magnification- The fulcrum (F) should be so placed that there is some magnification of the actual contraction (response). In order to achieve this, the distance between the writing point and the fulcrum (F) should always be greater than the distance between fulcrum and point of attchment of tissue (T). By adjustment of these relative distances from the fulcrum any degree of magnification can be obtained. Therefore lesser the inherent contractility of tissue higher the magnification needed or vice-versa Name of tissue magnification

1. Guinea pig ileum 5-10 times
2. Rat uterus 4-6 times
3. Frog rectus abdominis muscle 10 times
4. Rat fundic strip 16 times

Adjustment for the load or 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 after wards. This is achieved by the following way:- Select the proper length of longer and shorter arm after fixing magnification for particular tissue and fix the fulcrum. Balance the lever by putting the wt (plasticin) at the end or shorter arm and mark the point of tissue attachment.

At equidistance i.e. the distance between the F &T from the (F) on the longer arm of lever the desired load required for particular tissue. The tension (load) prescribed for

various commonly used tissues is as follows:

- ✓ guinea pig ileum 1 gm
- ✓ guinea pig trachea 0.2 gm
- ✓ guinea pig vas deferens 0.5gm
- ✓ rat colon 0.5 gm
- ✓ rat uterus 1.0 gm
- ✓ rat fundus 1.0 gm
- ✓ frog rectus abdominis muscle 1.5 gm

The writing levers are light in weight, rigid and are generally made up of wood (straw), light aluminum or stainless steel. The levers are of 2 types

a. Isotonic type- i.e. change in length due to contraction is recorded while the tension on the muscle remains the same e.g. simple lever frontal writing lever.
b. Isometric type-These levers are used under special circumstances for instance, when a twitch is produced by stimulating a muscle suspended between two rigid points, one being a strong spring, the muscle does not shorten but only creates a force or tension which is recorded; the twitch is also much faser in action. e.g. Paton's auxotonic lever will serve purpose well.

Different types of lever

a. Simple lever- (sideway writing): It is simplest type of lever made up wood (straw), stainless steel or aluminium. A celluloid writing tip (stylus) is attached at the end of the longer arm. The contractions are recorded as curved lines.
b. Frontal writing lever (writes frontally)- This lever is designed in such a way that the writing point rotates freely about its axle. This helps in reducing the tension between the smoked paper and the recording tip. The contraction are recorded as straight line.
c. Starling's or Heart Lever:-This lever is used to record the contraction of heart. The differences between this & other isotonic levers is that fulcrum lies at one end beyond the point of attachment. It consist of a frame carrying a light lever arm with holes and notches supported by a fine adjustable nickel silver spring attached to an adjustable hook.
d. Universal lever (Brodies): It is a lever of versatile utility there is an adjustable spring support which counters the pull on the writing arm. The spring helps in bringing the

pulled writing arm back to its original position. This lever is mainly used for recording sudden repetitive contraction of muscles/movements of a part of the body e.g. contraction of a gastrocnemius muscle in response to sciatic nerve stimulation.

4. **ONCHOMETERS:** These are used to study volume changes of organ due to the effect of drug (i.e. measure blood volume changes in a particular organ). These have to be connected to Marey's tambour or piston recorder.

Principle: works on displacement of air.

Types :-(1) Spleen on oncometer (2) Kidney oncometer (3) Heart oncometers (4) Intestine oncometer

They are made of metal according to organ shape & are likewise called spleen kidney etc. When an organ relaxes in the oncometer then the piston of the piston recorder moves up, where as it moves down when the organ contracts.

5. **JACKSON'S ENTEROGRAPH:** It is the device used for recording the movements of an isolated segment of intestine "in situ" This has been named after its discoverer. It consist of

a) Hollow metallic tube about 15 cm in length and 15 cm in diameter.

b) hook welded at its lower end

c) a pulley fixed at the lower end.

One end of an isolated segment of intestine “in situ” is hooked at its lower end and the other end is connected to a writing lever with there of a thread which passes over the pulley. Thus, the contraction of longitudinal muscles of this segment of intestine is transmitted to the recording lever.

(Since the two ends of isolated segment of intestine are tied up in length wise fashion, it records the responses of the longitudinal muscles of intestine without any record of the contractions of the circular muscle.)

6. **MAREY'S TAMBOUR:** It is an apparatus used for recording delicate pressure changes in a gaseous column.

- It is particularly useful for recording the respiratory excursions in anaesthetized animals.
- It consist of –

a. Metallic hollow tube (about 3-4 mm in diameter and about 10 mm in length connected internally to a hollow disc/cup.)

b. Tightly fitted soft rubber diaphragm covering the entire upper opening of disc/ cup.

c. a writing device which moves with each up & down movement of the diaphragm and marks it on the kymograph paper.

- ✓ The open end of the metallic tube is connected to the tracheal cannula with the help of polythene tubing.
- ✓ The pressure changes in the hollow passage of the system are transmitted by the diaphragm to the writing lever whose magnification can be adjusted by changing the position of the writing tip of the lever.
- ✓ Changes in the resp. rate and depth are easily recorded by Marey's tambour.

7. PISTON RECORDER: It is used to record the slightest blood volume changes in the organ easily. It consists of an internal ground glass tube in which an aluminum piston works very smoothly throughout its entire stroke. The piston is further attached anteriorly to the lever assembly. The lower end of the piston recorder is connected to the oncometer by means of an air tight pressure tube.

8. ARTERIAL CANNULA: It is a small glass apparatus used in animal experiments to cannulate an artery (usually common carotid or femoral) for recording the blood pressure in anesthetized animals e.g. dog, cat, rabbit & rats.

- ❖ It consists of hollow bulb connected to three arms, open into the cavity of bulb.
- ❖ The small globular arm is beveled at its outer end to help in its insertion into the artery.
- ❖ The size (length & thickness) of this arm depends on the size of the artery to the cannulated.
- ❖ The larger thicker arm is used for connecting the cannula with the Hg manometer.
- ❖ The small thicker arm is used for flushing out and for removing the blood clot from the cavity of the cannula if it occurs otherwise it is clamped with the help of punch clip.
- ❖ During the experiment the entire cannula is filled up with a solution containing sodium citrate or heparin as anticoagulant.

9. VENOUS CANNULA: It is small hollow glass tube about 4-5 cm in length & 3-4 mm in diameter and is used for cannulating a vein for I.V. administration of drugs/ fluids during experiment on dog, cat, & rabbit etc.

- At one end there is small globular projection with beveled outer end help its insertion into the vein.
- The base of this projection is thinner to retain the tied thread in place & prevent slippage of the cannula out of the vein.
- A piece of rubber tube is slipped on the other thicker end of the cannula and is clamped with a punch clip to prevent back flow of blood.
- The cannula is filled up with saline after cannulating the vein.

10. TRACHEAL CANNULA: It is a small metallic tubular device meant for cannulating trachea for - Artificial respiration, recording of respiration in animals.

They are of following types

a. Straight

b. Z- shaped

c. "Y" shaped

- "Straight" and "Z" shaped tracheal cannula are used whenever respiration is to be recorded.
- One end of these cannula is inserted into lumen of trachea and another end is connected to Marey's tambour with the help of rubber tube.
- These cannulas have adjustable slit, this is used to adjust the entry of air.
- "Y" shaped tracheal cannula is used whenever artificial respiration is to be given. Stem of this "Y" cannula is inserted into the lumen of trachea out of the remaining two arms one is connected to the outlet of the respiration pump and another one to the inlet of the pump with the help of rubber tubing.
- Depending on the size of trachea, small or large tracheal cannula may be used in animals like dog, cat, rabbit guinea pig & rat.

12 MURPHY'S DRIP: It is small glass hollow sealed apparatus used for assessing the rate of flow of a liquid (blood or drug solution) in a transfusion system.

- § It consist of a closed cylindrical body about 4cm in length and 1 cm in diameter and is connected to two thinner tubes at its two ends.
- § The upper input tube projects into the body cavity of the Murphy's drip. Such that the rate of input of fluid can be easily observed.
- The upper end is connected to the bottle containing blood or perfusion fluid

while the lower end is connected to needle with the help of polythene tubing. This lower end is used to drain out the entire content of the body of Murphy's drip.

- ❖ This instrument has been replaced by polythene sets with similar design and function in disposable IV perfusion sets.

13. BULL DOG CLAMP: As the name implies is a small clamp meant for tightly compressing a hollow tube/structure just like the jaws of bull dog.

- ❖ Usually employed for occluding an isolated artery/vein during arterial and venous cannulation in animal experiments.
- ❖ It is made up of stainless steel consist of 2 sturdy prongs connected with each other at the base in a crossed fashion such that the jaws of the clamp open up with this crossed portion is pressed with fingers.
- ❖ The inner flat surface of each prong has serration for tight grip of the vessel and also to minimize tissue damage to the vessel wall.
- ❖ For more precautionary measure these 'jaws' of the clamp may be covered with rubber tubing to prevent tissue damage.

14. VON FREY'S HAIR ASTHESIOMETER: It consists of hair preferably of horse or nylon hair which is adjustable to increase or decrease the tension. It is used to see touch, deep touch for touch sensation in local anesthesia.

15. Syringes: Simple Glass Syringe

- ❖ It is also known as B&D syringe. B&D stand for Beckton & Dickinson, who manufactured it.
- ❖ It is glass syringe with hollow floating piston.
- ❖ Available in 2cc, 5cc, 10cc, 50cc & 100 cc capacity.
- ❖ Sterilized by autoclaving.

Needle- the B & D needle has a bevel, body & shoulder. The needle thickness varies. The lower the number the thicker the needle.

- ❖ Sterilized by boiling for half an hour or by autoclaving.
- ❖ In the BD syringes the needle can be locked by applying a metallic lever lock to the nozzle.
- ❖ These BD glass syringes are largely replaced by plastic disposable syringe and needle.

(i) Tuberculin Syringe

- It was used initially for the tuberculin test (PDP or pure protein derivative test) therefore the name tuberculin was given.
- Now days it is used for many other sensitivity test
  e.g. lepromin test.
- It is 1 ml capacity syringe and divided into:
- 100 parts having further sub divisions i.e. minimum.01 ml drug can be given 50 equal further divisions 0.02 ml minimum drug can be given.

16. **RAT HOLDER:** It is a metal instrument used in experiments where rat tail is used to screen the Analagesic activity of drugs and Collection of blood from rat tail. In consist of hollow cylindrical tube at one end there is small rounded door with a hole through which the tail of the rat comes out. The other end of the holder is closed and perforated which provides air for respiration.

RESULTS:

EXPERIMENT NO. 02

OBJECT: To study the physiological salt solutions used in experimental pharmacology & various drug dilutions.

REFERENCES:

- Kulkarni S.K. "A hand book of Experimental Pharmacology", third edition, Reprint 2005, Vallabh Prakashan, page No. 3-10.
- Kale S.R., Kale R.R. "Practical Pharmacology and Toxicology", 9th edition, August 2004, Nirali's Prakashan.
- Pillai K.K., "Experimental Pharmacology" first edition, 2008, CBS Publication.

THEORY:

As animal experiments have to be done with isolated organs, it is necessary to use a certain no. of physiological solution of different ionic concentration which almost act as a substitute to the tissue fluid. They provide isotonicity, nutrition and acts as a buffer when drugs are added. It was "Ringer" who Ist introduced the idea that tissue could be kept alive by providing proper nutrition, O2 & temperature. PSS can be defined as artificially prepared solution to keep isolated tissue alive under experimental conditions.

The content of these solutions carries according to tissues & animals taken. These solutions provide food material i.e., energy, O2, electrolytes as in the same proportion as that present in tissue fluid. They exert same osmotic pressure as that of interstitial fluid i.e., isotonic with body fluids. Any variant from the principle will lead to shrinkage or blotting depending on hypertonicity & loss of physiological function. For these two points should keep in mind.

Solution should be prepared carefully with pure material. They can be kept for about 24 hrs. as they are good media for the growth of microorganisms they must be refrigerated and should be freshly prepared after 24 hrs. Following points should be carefully noted at the time of preparation of solution;

i). Balance of cations: Absolute quantity of each ion and preparation among each

other especially with ca^{+2} & K^{+} must be maintained.

The common cations and their significance are;

a) Na^{+} ion: Responsible for maintenance of excitability, contractibility rhythmicity of Muscles and nerves.

b) K^{+} ion: responsible for increase relaxation of heart increased neuromuscular transmission and excitability of nerves.

c) Ca^{++} ions: increase force of contraction & tone of heart and decrease excitability of nervous tissues.

d) Mg^{+2} ions- responsible for contraction of smooth muscle.

i). pH of solution / reaction of solution- pH of various PSS varying from 7.3-7.8 depending upon organ. At lower pH value tone of preparations tend to decrease & effect of drug is also altered. pH affects tissue directly and by ionization. At higher pH ionization is less and leads to alkalinity & thus improves cardiac & smooth muscle activity. During experiment there can be accumulation of metabolite which may change the pH. Buffering agents like HCO 3 & PO-4 are add in saline solution and solutions are changed frequently.

ii). Glucose: Introduced by "Locke" and serves as an energy source, increases contractility of tissue. It is not essential constituent for amphibians' tissue but indispensable for mammalian tissues.

iii). Distilled Water: It acts as a vehicle to dissolve various ingredients.

iv). Control of temperature: In order to get consistent effect, it is important to maintain the temperature of PSS, particularly for mammalian tissue. For instance, when temperature of solution is below 37°C tone of intestine is decreased, increased the contracts become smaller & contracts and relaxation time increases. Whereas, amphibian tissues survive for longer time at room temperature only.

v). Aeration: Air, O_2 or O_2+5% CO_2 is needed for the proper functioning of the tissues. Besides providing O_2 for the tissues, the stream of gas bubbles also stirs the solutions in the bath thereby facilitating diffusion of the drugs.

The solution in the bath should be changed frequently because prolong aeration tends to alter pH. Different physiological salt solutions and their uses:

i). Ringer lock's solution: It is used in isolated rabbit heart perfusion.

ii). Frog's Ringer's solution: Used in frog's rectus abdominis muscle and leech dorsalis

muscle preparation.

iii). Tyrode's solution: It is used in experiment of rabbit intestine & guinea pig ileum.

iv). De- Jalon's solution: Used in rat uterus, duodenum colon experiments.

v). Krebs's Henseleit solution: Used in guinea pig tracheal chain prep. & rabbits aortic strip preparation.

Components of Physiological Salt Solution:

S. No. Salts in g/l Ringer solution Frog's ringer solution Tyrode's solution DeJalon's solution Krebs's-Henseleit solution Mc Ewen's solution

1.	Nacl	9.00	6.50	8.00	9.00	6.9	7.60
2.	KCl	0.42	0.14	0.20	0.42	0.35	0.42
3.	$CaCl_2$	0.24	0.12	0.20	0.06	0.28	0.24
4.	$NaHCO_3$	0.50	0.20	1.00	0.50	2.10	2.10
5.	$MgCl_2.6H_2O$	-	-	0.10	-	-	-
6.	$MgSO_4.7H_2O$	-	-	-	-	0.29	-
7.	NaH_2PO_4	-	0.01	0.05	-	-	0.14
8.	KH_2PO_4	-	-	-	-	0.16	-
9.	Glucose	1.0	1 or 2	1.00	0.50	2.00	2.00
10.	Aeration O_2 air O_2 / air O_2+5% CO_2 O_2+5% CO_2 O_2						

Drug Dilutions: In pharmacological experiments drugs are used in very minute quantities i.e., can be fractions of mg or mg. These small amounts can't be weighed accurately even in analytical balance and hence substance is usually weight in larger quantities and dissolved in solvents. Generally stock solutions are prepared which are 1%. Solution & further diluted according to dose required 1%. solution is prepared by dissolving 100mg. of the drug in 10ml of distilled water.

Drug Dilutions:
% of solution Strength mg/ mg
1% 1:100 100mg/10ml or 10mg/ml
10% 1:10 100mg/ml.
100% 1:1 1000mg/ml or 1g/ml.
0.1% 1:1000 1 mg/ml
0.01% 1:10,000 0.1mg/ml or 100µ g/ml.
0.001% 1:100,000 0.01mg/ml or 10 mg/ml.
0.0001% 1:10,00,000 0.001mg/ml or 1µ g/1ml.

RESULTS:

EXPERIMENT NO. 03

OBJECT: To study the various handling techniques of laboratory animals in Experimental Pharmacology lab.

REFERENCES:

- Kulkarni S.K. "A hand book of Experimental Pharmacology", third edition, Reprint 2005, Vallabh Prakashan, page No.
- Kale S.R., Kale R.R. "Practical Pharmacology and Toxicology", 9th edition, August 2004, Nirali Prakashan.
- Pillai K.K., "Experimental Pharmacology" first edition, 2008, CBS Publication.

THEORY:

These are those animals which can be used and reared (maintain) in the laboratory under suitable conditions. The common laboratory animals are rat, mice, guinea pig, rabbit frog and hamster. Other animals used for experimental purpose are cat, dog, monkey, pigeon etc. since large animal experiments have been a mile stone in advanced medical research. Every animal is not suitable for experimental work, because of the selection based on the following criteria;

- ***Size:*** Smaller animals are preferred because they are easy to handle and less quantity of drug is required.
- ***Availability:*** Animals which are commonly available should be selected e.g., frogs, rats, rabbits and dogs.
- ***Sensitivity:*** Animals which are sensitive to drugs under trial e.g., guinea pig is sensitive to effect of histamine.
- ***Species:*** In rabbits intra-cerebroventricular injection of 5-HT induces a lowering of temperature, but in cats, it induces fever.

  Ethacrynic acid is almost inactive in rats, except at high doses, but quite active in the dog. Following the same dose of hexobarbitone per unit body weights, the average sleeping time of rats is about seven times that of mice, and in the dog its effects lasts for hours. Guinea pig and humans are 500 more times

sensitive to histamine than are rats & mice. Histamine powerfully contracts the uterus of guinea pig while relaxes that of rat. In rodents it produces stronger arteriolar constriction in cat slight constriction, while in dog, monkey and man arteriolar dilation.

Isolation or group housing: Dose of pentobarbitone or phenobarbitone, which produce full hypnosis in isolated mice, produce marked stimulation when the animals are grouped. The CVS becomes more responsive to isoprenaline in solitary housed (6-8 weeks) rats compared to group housed rats.

CHARACTERSTICS AND ADVANTAGES OF DIFFERENT ANIMALS

(i) RAT (*Rattus norvegicus*):

Albino rat is one of the commonest laboratory animals suitable for experimental work because of its because of its small size & greater sensitivity to most drugs. It is also the most standardized of all laboratory animals. It can be used to obtain pure and uniform strains and is found to be very sturdy to withstand long periods of experimentation under anesthesia. It is small in size compared to other animals so drugs are required in small quantity. Vomiting center is absent and so drug can be administered orally. Gall bladder and tonsils are absent. Because of the absence of gall bladder in rat there is continuous flow of bile into the intestine. This facilitates the study of drugs acting on bile, cholesterol reabsorption. Pancreas is diffused, therefore difficult to produce pancreatomy. In stomach, fundus & pyloric parts have clear lining between them. The gastric acid secretion is continuous.

Experimental uses: Adult wt. 180-200 gm., age suitable for most of the experiment 1.5 months old used for following studies;

1. Psychopharmacological studies
2. Study of analysis of anticonvulsants
3. Bioassay of various hormones, such as insulin oxytocin, vasopressin etc.
4. Study of estrus cycle, mating behavior and lactation
5. Studies on isolated tissue preparation like uterus, stomach, vasa deferens, anococcygeal fundus strip, heart, etc.
6. Chronic study on blood pressure
7. Gastric acid secretion studies

8. Study of hepatotoxic and antihepatotoxic compound
9. Acute & chronic toxicity studies

B. GUINEA PIG (*Cavia porcellus*):

It is docile animal, highly susceptible to TB & anaphylaxis and also highly sensitive to histamine and penicillin. It requires exogenous ascorbic acid in diet.

Experimental use: Adult Wt. 400-600 gm, age suitable for experiments 3 months;

1. Evaluation of bronchodilators
2. Anaphylactic and immunological studies
3. Study of histamine and antihistamines
4. Bioassay of digitalis
5. Hearing experiments because of sensitive cochlea
6. Studies on isolated tissues specially, ileum tracheal chain, vas deferens, etc.
7. Study of tuberculosis and ascorbic acid metabolism

C. MOUSE (*Mus musculus*):

Swiss albino mice are commonly used. They are smallest cheap & easy to handle.

Experimental uses*:* Adult weight:20-25 gm., age suitable for experiment, 2 months;

- ❖ Toxicological studies, especially acute and subacute toxicities.
- ❖ They are also used in teratogenicity (fetal abnormalities).
- ❖ Bio assay of insulin
- ❖ Screening of analgesics & anti-convulsant
- ❖ Screening of chemotherapeutic agents
- ❖ Studies related to genetics & cancer research
- ❖ Study of drugs acts on CNS

D. RABBIT (*Oryctolagus cuniculus*):

Rabbit are also docile animals with large ears. Usually, New Zealand white rabbits are used. It has huge cecum and large appendix. The enzyme atropine esterase is present in rabbit liver and plasma, so it can tolerate large doses of belladonna (atropine). Cardio aortic nerve forms a separate depressive nerve. Vasodilator nerves are absent and so vasomotor reversal phenomenon can't be demonstrated. Histamine causes increase in blood pressure. Ovulation is related to the release of luteinizing hormone and occurs 10 hours after coitus.

Experimental uses: Adult weight 1.5-3.0 Kg. age suitable for experiment 5-6 months.

1. Pyrogen testing.

2. Bio assay of antidiabetics, curare form drugs and sex hormones.
3. Screening of agents affecting capillary permeability.
4. Irritancy tests.
5. Screening of antitoxic agents and teratogens.
6. Studies related to reproduction (antifertility agents).
7. Isolated preparation like heart, duodenum, ileum, Finkelman preparation.
8. Study of local anesthetics (surface anesthesia).
9. Study of miotics & mydriatics.

E. HAMSTER (*Mesocriceitus auratus and Cricetulus griseus*):

They have short body with short legs & tail. The skin is loose and covered with dense short soft fur. The cheeks pouches are prominent & extend up to the shoulder region.

Experimental uses: adult weight 80-90 gm average age suitable for experiments 1 month;

- ❖ Chinese hamsters have low chromosome number making it useful for cytological genetics tissue culture & radiation research
- ❖ Research on diabetes mellitus
- ❖ Research related to virology, immunology and implantation studies
- ❖ Bioassay of prostaglandins

F. FROG (*Rana tigrina*):

One of the most commonly used experimental animals in physiology, pharmacology and toxicology. It has been used in experiment science 200 yrs. It is easily available during rainy reason. It is an amphibian animal and safe to handle. It can't be used in laboratory. Adrenaline is neuro transmitter in the sympathetic system.

Experimental uses:

- ❖ Study of isolated tissue such as rectus abdominis muscle and heart preparation.
- ❖ Study of drugs acting on CNS
- ❖ Study of retinal toxicity of drugs, light bleaches rhodopsin in eye within one hour and is regenerated within one hour in dark.
- ❖ Study of drugs acting on neuromuscular junctions (using gastrocnemius, sciatic muscle nerve preparation).

G. CAT:

It is carnivorous animal relatively easy to obtain and to use for experimental purpose. The physiology of circulatory & neuromuscular system is very much similar to that of man. It has highly developed nictating membrane which is contracted by sympathetic nerves. Morphine produces excitation of central nervous system in cat.

Experimental uses:

- ❖ Acute experiments for the drugs affecting BP
- ❖ Bioassay of NA (using spinal cat)
- ❖ Studies on ganglions blockers (using nictitating membrane in vivo)
- ❖ Studies on neuromuscular system (using gastrocnemius, sciatic muscle nerve preparation.)
- ❖ Toxicity studies of compound like acetanilide.

H. DOG:

Commonly Mongrel or Beagle dogs are used. It is easily available & large sized animal. Dogs can be easily tamed as well as trained. It has a small stomach and short intestinal tract resembling those of human beings. It can be conditioned to carry a stomach cannula. The cervical sympathetic and Vagus nerve run together inseparably in the trunk.

Experimental uses:

- ❖ Gastric acid secretion studies (Pavlov pouch).
- ❖ Acute experiments for drug affecting BP and intestinal movements etc.
- ❖ Studies on antidiabetic agents.

I. MONKEY AND APES:

These are the primates belonging to the highest order of the mammals. The anatomy & physiology of these animals are closely related to that of man. The studies done in monkeys are directly translated to man. Considering the human respects, tests in primates should be done only in last stage of evaluation of drugs before clinical trials. They are used in the fields of psychopharmacology, urology, immunology, nutrition, reproduction, parasitology, etc.

RESULT:

EXPERIMENT NO. 04

OBJECT: To Study the Various Anesthetic Agents Used in Experimental Pharmacology Laboratory.

REFERENCES:

- Kulkarni S.K. "A hand book of Experimental Pharmacology", third edition, Reprint 2005, Vallabh Prakashan, page No.
- Kale S.R., Kale R.R. "Practical Pharmacology and Toxicology", 9th edition, August 2004, Nirali Prakashan.
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ANAESTHETIC AGENTS:

General anesthetic agents bring about loss of all sensation particularly pain along with reversible loss of consciousness. Consciousness is regained after the agent is metabolized or excreted. General anesthetics fulfill the following requirements:

a) Induction of anesthesia should be quick & pleasant

b) It must have longer duration of action

c) There should be adequate muscle relaxation

d) It should not interfere with effect of drug under study

e) It should be cheap & non-inflammable

ANAESTHETICS OF TWO TYPES:

1. **Volatile general anesthetics:** These could be liquid like ether, halothane, ethyl chloride, trichlorethylene, gases as N_2O, ethylene & cyclopropane. They are not used commonly in experimental animals, because they require constant monitoring and costly instrument set up. Ethyl chloride is rarely used in experiments of rats & cats.

2. **Non-volatile anesthetics:** These agents are more commonly used for producing anesthesia in various animals because it is easy to administer these agents and no complicated technique is required. Commonly used agents are-chloralose, urethane, barbiturates, $MgSO_4$, paraldehyde etc.

(i) Chloralose: It is the compound of chloral and glucose prepared by heating equal parts of

anhydrous glucose and chloral when both a chloralose (active form) and b chloralose (inactive form) are formed. Chloralose is the active form (a-chloralose) freely soluble in hot water in alcohol & in ether & slightly solute in cold water.

Dose: 80-100 mg/kg body wt. 1% solution used to give 1.V routes.

Advantages:

- ❖ It produces surgical anesthesia lasting for 3-4 hrs.
- ❖ Respiration and BP are not depressed so can be used in conditions where they have to be recorded.
- ❖ Reflexes are not depressed.

Disadvantage:

- ❖ Poor water solubility so solution is prepared in luke warm water or 10% propylene glycol or ether.
- ❖ Large volume is needed.
- ❖ Jerky movements are seen.
- ❖ Unsuitable for rabbits where it produces narcosis instead of anesthesia.

ii) URETHANE (ETHYL CARBAMATE): It is readily soluble in water giving a neutral solution. Usually, 25% solution is used I.M. This is also suitable for acute non recovery type of experiments in dog, cat, rabbit & rats. Duration of action is 3-4 hrs. Frogs can be anaesthetized by keeping them covered in beaker containing 5-10% solution.

Advantages:

- ❖ It does not affect reflexes, CVS, & respiration.
- ❖ It is readily soluble in water.
- ❖ Anesthesia remains for 3-4 hrs.

Disadvantage:

- ❖ Induction is slow so, injection of morphine 2mg/kg body wt. is given, IM 30 min before giving urethane as pre anesthetic agent so induction is quick and smooth.
- ❖ It has irritant nature so animal feels pain.
- ❖ Amount of solution injected to be large so has to be given at various sites.
- ❖ It has delayed toxic effect on liver & may also cause agranulocytosis & pulmonary adenomas. Mice develop high incidence of lung tumors regardless of route of administration.

iii. BARBITURATES: These are most commonly used agents.

Advantage:

- ❖ Induction is very rapid and smooth with minimal excitation.
- ❖ No preanesthetic medication ion is required.

Disadvantage:

- ❖ It produces depression of cardiovascular & spinal reflex, by interfering with nerve impulse transmission both in CNS and ganglia.
- ❖ Muscle relaxation is inadequate therefore, mucle relaxants have to be given simultaneously.
- ❖ Anesthesia produced is of short duration, therefore small doses of these agents are to be injected at regular intervals.

They can be classified as:

a) Long-acting barbiturates: Phenobarbitone 10% aqueous solution is used. Dose 120-180 mg/kg body weight i.p.

b) Short acting barbiturates: Thiopentone sodium 2.5% aqueous solution in dose of 15-25 mg/kg body weight is given i.v. duration of action is 20-30 minutes.

PARALDEHYDE: It has wide margin of safety as it depresses only the cerebrum and not medullary centers. Dose 1 mg/kg. body wt. I.P. or 2 mg/kg body wt. i.m. in dogs & cats.

MAGNESIUM SULPHATE: 20% solution in a dose of 5 ml/kg. intravenously produces anesthesia for one hour, Calcium gluconate is used I.V. to counteract the depressant effect immediately. Its principal use is in producing euthanasia (mercy killing).

Euthanasia: (Painless killing)- When animals are killed at the end of the experiment. It should be done by a human method.

Methods of euthanasia:

a. Chemical method:

i. It is the painless death produced by administration of chemical poisons; certain chemicals are injected & there are;

ii. Magnesium Sulphate i.v. intra cardiac (15gm/kg body wt.)

iii. Chloroform.

iv. A large volume of air (mg/kg body wt.)

v. Sodium citrate in large quantity (anticoagulant) (5) Paraldehyde & MgCl2 can also be used.

vi. In open chest operations adrenaline can be touched at the apex of ventricular - arrhythmias will be started - death.

b. **Mechanical Method:**

i. The quickest & commonest method for killing mice, rats, guinea pigs & rabbits is Stunning is carried out by striking the dorsal part of head against the edge of table.

ii. This lead to stiffening of all muscles followed by a series of convulsions and then gradual relaxation of the limbs and the body.

iii. As a result of stunning animals get sudden shock and temporarily becomes semiconscious one should have practice to stun by a single hard stroke only.

iv. Multiple strokes do not comply the principle of euthanasia.

RESULT:

EXPERIMENT NO. 05

OBJECT: To study the different types of Bioassay methods used in Experimental Pharmacology Laboratory.

REFERENCES: -

- Tripathi K.D. "Essentials of Medical Pharmacology", 5th edition, Jaypee Brothers Medical Publishers (P) Ltd., New Delhi,
- Kulkarni S.K. "A hand book of Experimental Pharmacology", third edition 1999, Reprint 2007, Vallabh Prakashan,
- Pillai K.K., "Experimental Pharmacology" first edition, 2008, CBS Publication,

BIOLOGICAL INDICATOR:

Whole Animal: Cats, Dogs, Mice, Guinea Pigs, Pigeons, Rabbits.

Isolated Tissue: Guinea Pig ileum, Rat ileum, Rat Uterus, Rectus Abdominus Muscle of Frogs.

Bioassays are performed by using official procedures in I.P. /B.P.

***Bioassays are performed when*:**

- A chemical assay for the substance is not available.
- The substance gets inactivated by interacting with the chemical.
- When the quantity of sample is too small.
- In order to estimate the concentration of principles present in tissue extracts like Acetylcholine, 5HT, prostaglandins.
- When bioassay is more sensitive than chemical assay.

Classification:

Bioassays are of two types:

I. QUANTAL

II. GRADED are of following types:

1) Matching
2) Interpolation
3) Bracketing
4) Multiple Point
    a) Three Point
    b) Four Point
    c) Six point

QUANTAL BIOASSAY:

This assay is based All or None response.

E.g.: Insulin induced hypoglycemic convulsions in mice, cardiac arrest caused by digitalis.

GRADED BIOASSAYS:

1. **Matching Bioassay:** Response of the test substance is taken first and the observed response is matched with the response that is contained with standard drugs, several responses of standard drug are recorded till a close matching response to that of the test is observed.

2. **Interpolation Bioassay:** Concentration response curve of the standard is recorded first followed by 2-3 responses of the test substance. The test response is selected in such a way that it should lie on the linear portion of the concentration response curve of the standard.

3. **Bracketing Bioassay:** Here the response of the test substance is bracketed between the greater and smaller response of the standard substance.

4. ***Multiple Point Bioassay:***

   Three Point Bioassay: The concentration response for the standard is recorded first after which the concentration response for the unknown sample is recorded. This is followed by recording two responses of the standard and one response of the test in three permutation and combination.

Selection of Standard:

- The two standard responses should lie on the linear portion of the concentration response curve.
- They should be used in a ratio of 1:2.

Selection of Test:

- The test response should lie on the linear portion of test concentration response curve. Responses are recorded randomly using Latin square design:

$$\text{Concentration of test} = \frac{n_1}{t} \times \text{antilog} \left[\frac{T - S_1}{S_2 - S_1} \right] \times \log \frac{n_2}{n_1} \times Cs$$

n_1= Lower dose of standard n_2= Higher dose of standard

t= Test dose S_1= Response of n_1

S_2= Response of n_2 T= Response of test

C_s= Concentration of Standard

Latin square design:

S_1	S_2	T
S_2	T	S_1
T	S_1	S_2

i. Four Point Bioassay: The concentration response for the standard is recorded first followed by concentration response for the unknown sample. This is followed by recording two responses of the standard and two responses of the standard and two responses of the test.

Selection of standard response:

i. The two standard responses should lie on the linear portion of the concentration response curve of the standard.

ii. They should be in ratio of 1:2 or 2:4

Selection of test:

i. The two-test response should lie on the linear portion of the concentration response of standard sample.

ii. They should be used in a ratio of 1:2 or 2:4.

iii. The heights of the test responses selected should be in between the heights of the standard response.

Responses are recorded randomly by Latin Square Design

S_1	S_2	T_1	T_2
S_2	T_1	T_2	S_1
T_1	T_2	S_1	S_2
T_2	S_1	S_2	T_1

Concentration of unknown: Potency ratio x standard concentration

Potency ratio:

$$\text{Potency ratio} = \frac{s_1}{t_1} \times \text{antilog}\left[\frac{T_2 - S_2 + T_1 - S_1}{T_2 - T_1 + S_2 - S_1}\right] \times \log \frac{s_2}{s_1}$$

s_1= Lower dose of standard s_2= Higher dose of standard

t_1= Lower dose of test t_2= Higher dose of test

S_1= Response of s_1 S_2= Response of s_2

T_1= Response of t_1 T_1= Response of t_2

RESULTS:

Experiment No. 06

OBJECT: To study the various routes of drug administration for laboratory animals.

REFERENCES: -

- Tripathi K.D. "Essentials of Medical Pharmacology", 5th edition, Jaypee Brothers Medical Publishers (P) Ltd., New Delhi,
- Kulkarni S.K. "A hand book of Experimental Pharmacology", third edition 1999, Reprint 2007, Vallabh Prakashan,
- Pillai K.K., "Experimental Pharmacology" first edition, 2008, CBS Publication,

THEORY:

ROUTES OF DRUG ADMINISTRATION

INSTRUCTIONAL OBJECTIVES

At the end of the period of instruction, the student should be able to;

a) List various routes of administration of drug.

b) Select or make a choice of route of a drug depending on clinical condition of patient.

c) Vividly describe advantages and disadvantages of various routes of drug administration.

Introduction

To administer a drug is to make the drug accessible to the patient's body where the effect is desired. The drug therefore, is desired by the therapist to elicit or manifests an effect where it is desired. For this to occur, the drug must come in contact with the tissues of organs and cells of tissues by one way or the other, the way the drug comes in contact or is made accessible to the tissue fluids tissues, cells, extracellular and intra cellular fluids is the route of administration of drug.

Choice of Route of Administration

1. This is based on the way the drug is preferred for administration i.e. based on the drug dosage forms. Drugs are administered in various dosage forms: as solid – e.g. oxytetracycline capsule. Paracetamol tablet

2. Dimenhydrinate pill: as solution - codeine syrup and as suspension – insulin, penicillin procaine. Aerosol – beclomethasone in Volatile liquid - halothane or nitrous oxide as ointments, lotions, pastes etc.

3. Based on the nature of the drug, oil based, organic, polar, non-polar solvent etc.

4. The desired bioavailability of the therapist.
5. Desired onset of action - how fast the therapist wants to see the manifest effect of the drug. This is important, especially in life threatening conditions or circumstances that require
6. Immediate onset of action are shock, circulatory collapse, the nature of the disease and its location of the disease.
7. Duration of action – If a duration is required to be long; the drug is administered 2-4 times daily. This could be done in a depot form as a patch on the skin, another example is treating anaplasmosis, the aqueous oxytetracycline is administered 2-3 days by intramuscular, subcutaneous or intravenous injection. The long acting oxytetracycline, which is designated for slow absorption over 4-5 days.

LOCATION OF DESIRED EFFECTS OF THE DRUG

Routes of drug administration: The routes of drug administration for systemic effect may be divided into two major groups: Oral (enteral) and parenteral (systemic). When the gastrointestinal tract is by-passed by injection or introduction into the lungs (inhalation). When the drug is effect is desired locally it is administered topically, that is on the skin.

ORAL OR ENTERAL ADMINISTRATION OF DRUG

Oral ingestion is the most ancient method of drug administration, another organ where the substance or drug to be administered is placed is the rectum. Intravectally The drug could be placed in the mouth, under the tongue, that is (subliquel). The drug could be administered directly into the stomach using intragastric tube.

Advantages: Safe, sterility is not required, danger of acute drug reaction is minimal.

Disadvantages: Ingestion of drug could cause gastric irritation, nausea, vomiting (in animals like dog, pig), complexes formed with ingesta could prevent the drug absorption, the drug could be destroyed by low gastric pH or by the digestive and liver enzymes before entry into the circulation.

PARENTERAL ADMINISTRATION

Parenterally “par” means beyond “enteral” means intestinal. This is the route of administration of drug without crossing the intestinal mucosa. This is possible when the drug is directly into the blood or tissue fluid using needle and syringe. It is important to note that the man that lead to the introduction of the hypodermic needle and syringe is Alexander wood.

The most important and most frequently used parenteral routes are I.V. (intravenous route), intramuscular route and SC (subcutaneous route) respectively.

Other less frequent routes are:

□ Tissue infiltration

□ Intra articular

□ Intradermal

□ Epidural

□ Subarachnoid

□ Intra-arterial

□ Intrathecal

□ Intrathoracic

□ Intracardiac

□ Intramedullary

□ Intratesticular

□ Intralesional

□ Subconjunctival

□ Intramammary

Advantages

1. Bioavailability is faster and more predictable.
2. Gastric irritation and vomiting are provoked.
3. Parenteral routes could be used in unconscious, uncooperative and vomiting patient.
4. There are no chances of interference by food or digestive enzymes.
5. Liver enzymes are by-passed.
6. It essential sometimes in the absorption of the active form of the drug.

Disadvantages

□ It is generally more risky

□ The preparation must be sterile

□ The technique is intensive and painful.

□ Drug administered by all routes except intra-arterial might still be eliminated by first pass elimination in liver prior to distribution to the rest of the body.

INTRAVENOUS ROUTE

The drug is injected slowly, sometimes it could be infused rapidly as bodies.

This method provides accurate, reliable dosage of drug directly into the circulation. It means that the bioavailability of drug is 100% when administered intravenously. **Advantages**

□ This route is often used in drug administration in life threatening situations.

□ The drug would have rapid onset of action.

□ Irritating and non-isotnoic solutions can be administered intravenously, since the intima of the vein are insensitive.

Disadvantages

□ The drugs administered by this method have short duration of action.

□ Thrombophlebitis of veins

□ Necrosis of adjoining tissue.

□ Severe adverse effect especially when organs such as liver, heart, brain are involved in toxicity.

INTRAMUSCULAR

The drug is injected deep in the belly of a large skeletal muscle. The muscles that are usually used are detoid, triceps, Gluteus, Maximus, rectus, femurs depending on the specie of animal. The muscle is less richly supplied with sensory nerves, hence injecting a drug 1m is less painful.

Advantages

1. It is convenient route in administering drugs in animals that are difficult to restrain.
2. It is used in administering aqueous or oleaginous suspensions or solutions.
3. Muscles are highly vasculorized thus, the drug could be absorbed haematologenously or through the lymphatic fluid.

Disadvantages

1. Intermuscular injection into fascia might lead to erratic absorption of the drug.
2. There is a possibility of improper deposition of drug preparation in nerves, fats, blood vessels or between muscle bundles in connective sheaths.

SUBCUTANEOUS

The drug is deposited in the loose subcutaneous tissue that is richly supplied with nerves but less vascular. The rate of absorption is slower than the intramuscular route.

Advantages

□ It is a good route of administration especially in skin infections.

□ It is relatively safer than I.M. and I.V.

□ Absorption is slower thus, it is a good route of a prolonged effect is to be achieved.

Disadvantages

□ If the drug is irritating it might cause the sloughing off of the skin epithermal tissue. Other forms of subcutaneous route include;

- pellet implantation and dermoject.

Pellet implantation – The drug dosage form of the drug is in solid pellet and is implanted subcutaneously suing a trocher and cannula under the skin.

Dermoject: In this method, needle is not used, rather a high velocity jet of drug solution is projected from a microfine orifice of gun-like implement.

The solution passes through the superficial layer of the skin and gets deposited in the subcutaneously. This method is used in Mass inoculation.

INTRA-ARTERIAL ROUTE

Drugs and diagnostic agents are administered via this route. The diagnostic media e.g. (Contrast media in angiography) is injected directly into the artery. This is also of great use in treatment of limb malignancies.

Advantages

- The first pass and cleansing effects are by-passed.
- Bioavailability is 100%.
- It is of great clinical value in administering anticancer drugs for example, in limb malignancies, the drug is administered into the brachial artery or femoral artery.

Disadvantages

- Intra-arterial injection requires great and expertise
- If the drug is of adverse effect there might be great danger.

INTRAPERITONEAL

The peritoneum possesses a cavity that offers a large absorptive area for drugs. The perioneum is highly vascularized. This route is used in laboratory animal's administration and large animal practice for administration of large volumes of fluid.

Disadvantages: Irritating compounds may produce peritonitis or adhesion.

INTRATHECAL

This is a route of administration of drug in which the effects of the drug is desired in the C.N.S. The blood brain barrier and the blood cerebrospinal fluid barrier often slow the entrance of drug into the C. N. S.

The drug will be accessible to the meninges and cerebrospinal axis. The injection made in the

lumbar area or in the cisterna magna.

These routes are primarily for diagnostic procedures (e.g. myel; myography), and treatment of meningoencephalitis. Local anesthetics are sometimes administered intrathecally to produce region or spinal anesthesia.

Intradermal: the drug is injected into the skin raising a bleb. This route is used in diagnosis of tuberculosis (tuberculin testing in cattle) and (allergen sensitivity testing).

Intra-articular: Intra-articular injection of anti-inflammatory preparation (e.g. steroids) may be justified in some forms of lameness due to acute inflammation or trauma e.g. (swollen bursa or tendon sheath) Other routes of drug injection include intra-medullary, which is used for blood transfusion directly into the bone marrow. This is done in neonates when other is difficult.

PULMONARY ROUTE (Inhalation)

Gases, volatile liquids, and aerosols (fine droplets ion air). Some drugs such as Ventolin are administered using a nebulizer or inhalers. Anesthesia such as halothane, sevoflurane are vaporized and made to be atomized by a process atomization – This is delivered into the respiratory passages with the aid of anesthetic machine or vaporizer. The vaporized anesthesia is inhaled to cause anesthesia and thus is eliciting its effect.

RESULTS:

Experiment No. 07

OBJECT: To study the various blood sampling techniques anesthesia and euthanasia for laboratory animals.

REFERENCES: -

- Kulkarni S.K. "A hand book of Experimental Pharmacology", third edition 1999, Reprint 2007, Vallabh Prakashan.
- Parasuraman, R Raveendran, and R Kesavan. Blood sample collection in small laboratory animals. Journal of Pharmacology and Pharmacotherapeutics, 2010; 1(2):87–93.

THEORY:

Collection of blood from small laboratory animals is necessary for a wide range of scientific research and there are a number of efficient methods available for that. It is important that blood sample collection from experimental animals should be least stressful because stress will affect the outcome of the study. Various regulatory agencies and guidelines have restricted the use of animals and the techniques used for blood collection in laboratory animals. This article deals with the approved blood collection techniques for laboratory animals like rodents, lagomorphs and nonrodents. Permission of the Institute Animal Ethics Committee has been obtained for the use of animals for demonstrating the techniques.

GENERAL PRINCIPLES

It should be least painful and stressful. Blood sample may be collected under anesthesia [Table 1] or without anesthesia.

Atropine (0.02 mg/kg s.c./i.m.) is used as a preanesthetic medication for all the species to reduce salivation, bronchial secretion and protect heart from vagal inhibition.

- Adequate training is required for blood collection using any method in any species.
- In general, blood sample is withdrawn from venous, arterial blood vessels or heart chambers.
- Frequency of blood collection is important. Once in two weeks is ideal for nonrodents. If the study needs multiple blood samples, lagomorphs (e.g., hares and rabbit) can be used.
- All nonterminal blood collection without replacement of fluids is limited up to 10% of total circulating blood volume in healthy, normal, adult animals on a single occasion and

collection may be repeated after 3 to 4 weeks. In case repeated blood samples are required at short intervals, a maximum of 0.6 ml/kg/day or 1.0% of an animal's total blood volume can be removed every 24 hour.

Table 1: Commonly recommended anesthetic agents for laboratory animal experiments

Animal species	**Short anesthesia**	**Medium anesthesia**	**Long anesthesia**
Mice	Isoflurane (inhalation)	Xylazine + ketamine	Xylazine + ketamine
	Halothane (inhalation)	(5 mg + 100 mg i.m.)	(16 mg+60 mg i.m./i.p.)
		Xylazine + ketamine	Xylazine + ketamine
		(5 mg + 100 mg i.m.)	(16 mg +60 mg i.m./ i.p.) or
			Urethane (1200 mg/kg i.p.)
Guinea pig	Isoflurane (inhalation)	Xylazine + ketamine	Xylazine + ketamine
		(2 mg + 80 mg i.m.)	(4 mg + 100 mg i.m.)
Rabbits	Isoflurane (inhalation)	Xylazine + ketamine	Xylazine + ketamine
		(5 mg + 15 – 30 mg i.m.)	(5 mg + 100 mg i.m.)

- If the study involves repeated blood sample collection, the samples can be withdrawn through a temporary cannula. This may reduce pain and stress in the experimental animals.
- The estimated blood volume in adult animals is 55 to 70 ml/kg body weight. Care should be taken for older and obese animals. If blood collection volume exceeds more than 10% of total blood volume, fluid replacement may be required. Lactated Ringer's solution (LRS) is recommended as the best fluid replacement by National Institutes of Health (NIH). If the volume of blood collection exceeds more than 30% of the total circulatory blood volume, adequate care should be taken so that the animal does not suffer from hypovolemia.

GENERAL METHODS FOR BLOOD COLLECTION

Blood samples are collected using the following techniques:

- Blood collection not requiring anesthesia

- Saphenous vein (rat, mice, guinea pig)
- Dorsal pedal vein (rat, mice)
- ***Blood collection requiring anesthesia*** (local/general anesthesia)
    - Tail vein (rat, mice)
    - Tail snip (mice)
    - Orbital sinus (rat, mice)
    - Jugular vein (rat, mice)
    - Temporary cannula (rat, mice)
    - Blood vessel cannulation (rat, guinea pig, ferret)
    - Tarsal vein (guinea pig)
    - Marginal ear vein/artery (rabbit)
- **Terminal procedure**
    - Cardiac puncture (rat, mice, guinea pig, rabbit, ferret)
    - Orbital sinus (rat, mice)
    - Posterior vena cava (rat, mice)

PROCEDURE FOR SAPHENOUS VEIN BLOOD SAMPLE COLLECTION

Requirements include animal, rodent handling gloves, towel, cotton, sample collection tubes and 20G needle.

- Lateral saphenous vein is used for sampling while taking aseptic precautions.
- The back of the hind leg is shaved with electric trimmer until saphenous vein is visible. Hair removal cream can also be used.
- The animal is restrained manually or using a suitable animal restrainer.
- Hind leg is immobilized and slight pressure may be applied gently above the knee joint.

- The vein is punctured using a 20G needle and enough volume of blood is collected with a capillary tube or a syringe with a needle. The punctured site is compressed to stop the bleeding. While collecting blood:
    - the local anesthetic cream may be applied on the collection site
    - no more than three attempts are made
    - continuous sampling should be avoided and
    - Collecting more than four samples in a day (24-hour period) is not advisable.

PROCEDURE FOR DORSAL PEDAL VEIN BLOOD SAMPLE COLLECTION

Requirements include animal (rat or mice), rodent handling gloves, cotton, capillary tube, 23G/27G needle and blood sample collection tubes.

- The animal is kept in a restrainer.
- The hind foot around ankle is held and medial dorsal pedal vessel is located on top of the foot.
- The foot is cleaned with absolute alcohol and dorsal pedal vein is punctured with 23G/27G needle.
- Drops of blood that would appear on the skin surface are collected in a capillary tube and a little pressure is applied to stop the bleeding.

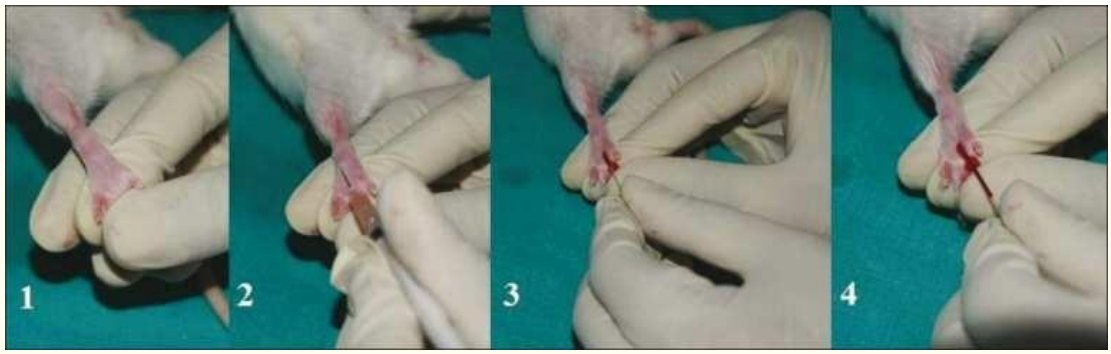

Figure 1: Blood sample collection from rat dorsal pedal vein

PROCEDURE FOR TAIL VEIN BLOOD SAMPLE COLLECTION

Requirements include animal, rodent handling gloves, towel, cotton, sample collection tube and animal warming chamber.

- This method is recommended for collecting a large volume of blood sample (up to 2ml/withdrawal)
- The animal is made comfortable in a restrainer while maintaining the temperature around at 24 to 27°C.
- The tail should not be rubbed from the base to the tip as it will result in leukocytosis. If the vein is not visible, the tail is dipped into warm water (40°C).
- Local aesthetic cream must be applied on the surface of the tail 30 min before the experiment.
- A 23G needle is inserted into the blood vessel and blood is collected using a capillary tube or a syringe with a needle. In case of difficulties, 0.5 to 1 cm of surface of the skin is cut open and the vein is pricked with bleeding lancet or needle

and blood is collected with a capillary tube or a syringe with a needle.

- Having completed blood collection, pressure/silver nitrate ointment/solution is applied to stop the bleeding.
- If multiple samples are needed, temporary surgical cannula may be used.
- Restrainer is washed frequently to avoid/prevent pheromonally induced stress or cross infection [Figure 2].

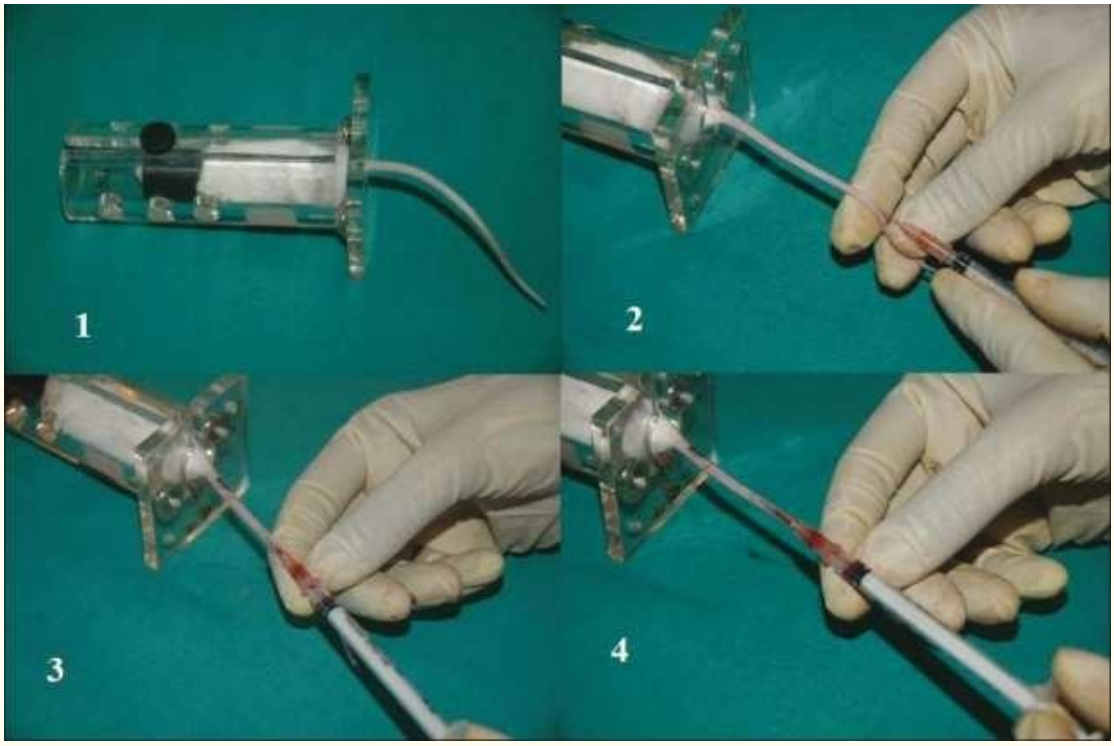

Figure 2: Blood sample collection from mouse tail vein

PROCEDURE FOR TAIL SNIP BLOOD SAMPLE COLLECTION

Requirements include animal, anesthetic agent, cotton, surgical blade and blood sample collection tubes.

- This method is recommended for blood collection only in mice.
- This method should be avoided as far as possible because it can cause potential permanent damage on the animal tail. If needed, it should be done under terminal anesthesia only.
- Before collecting the blood, local anesthesia is applied on the tail and a cut is made 1 mm from the tip of the tail using scalpel blade.
- Blood flow is stopped by dabbing the tail tip.

PROCEDURE FOR ORBITAL SINUS BLOOD SAMPLE COLLECTION

Requirements include animal, anesthetic agent, cotton, capillary tube and blood sample collection tubes.

- This technique is used with recovery in experimental circumstances and this method is also called periorbital, posterior-orbital and orbital venous plexus bleeding.
- Blood sample is collected under general anesthesia.
- Topical ophthalmic anesthetic agent is applied to the eye before bleeding.
- The animal is scruffed with thumb and forefinger of the nondominant hand and the skin around the eye is pulled taut.
- A capillary is inserted into the medial canthus of the eye (30 degree angle to the nose).
- Slight thumb pressure is enough to puncture the tissue and enter the plexus/sinus.
- Once the plexus/sinus is punctured, blood will come through the capillary tube.
- Once the required volume of blood is collected from plexus, the capillary tube is gently removed and wiped with sterile cotton. Bleeding can be stopped by applying gentle finger pressure.

- Thirty minutes after blood collection, animal is checked for postoperative and periorbital lesions [Figure 3 and 4.

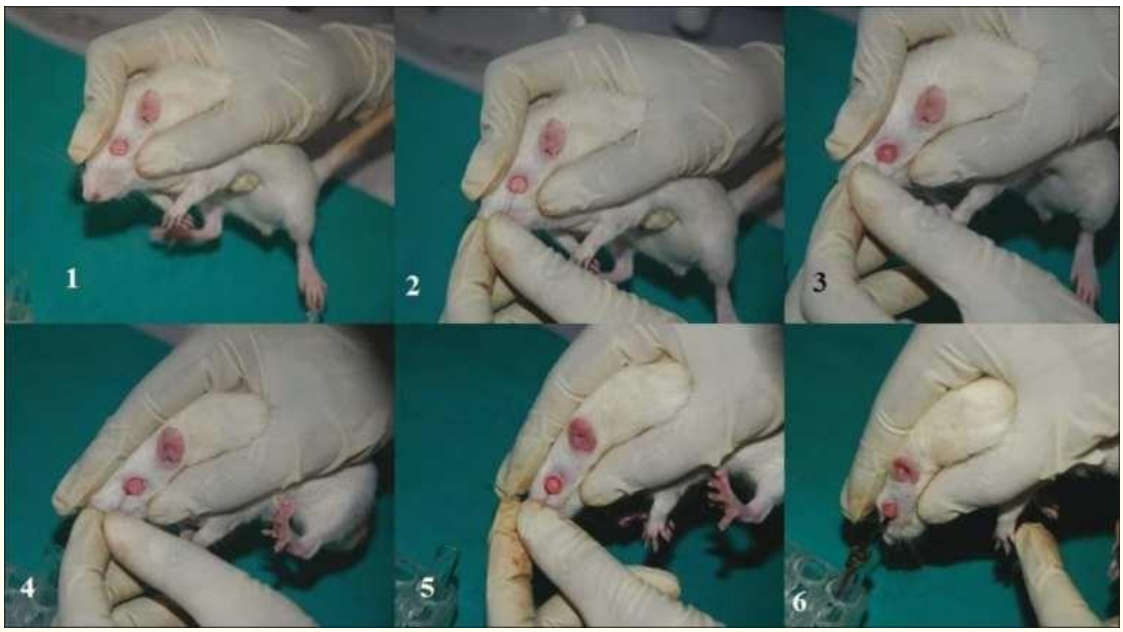

Figure 3: Blood sample collection from rat orbital sinus

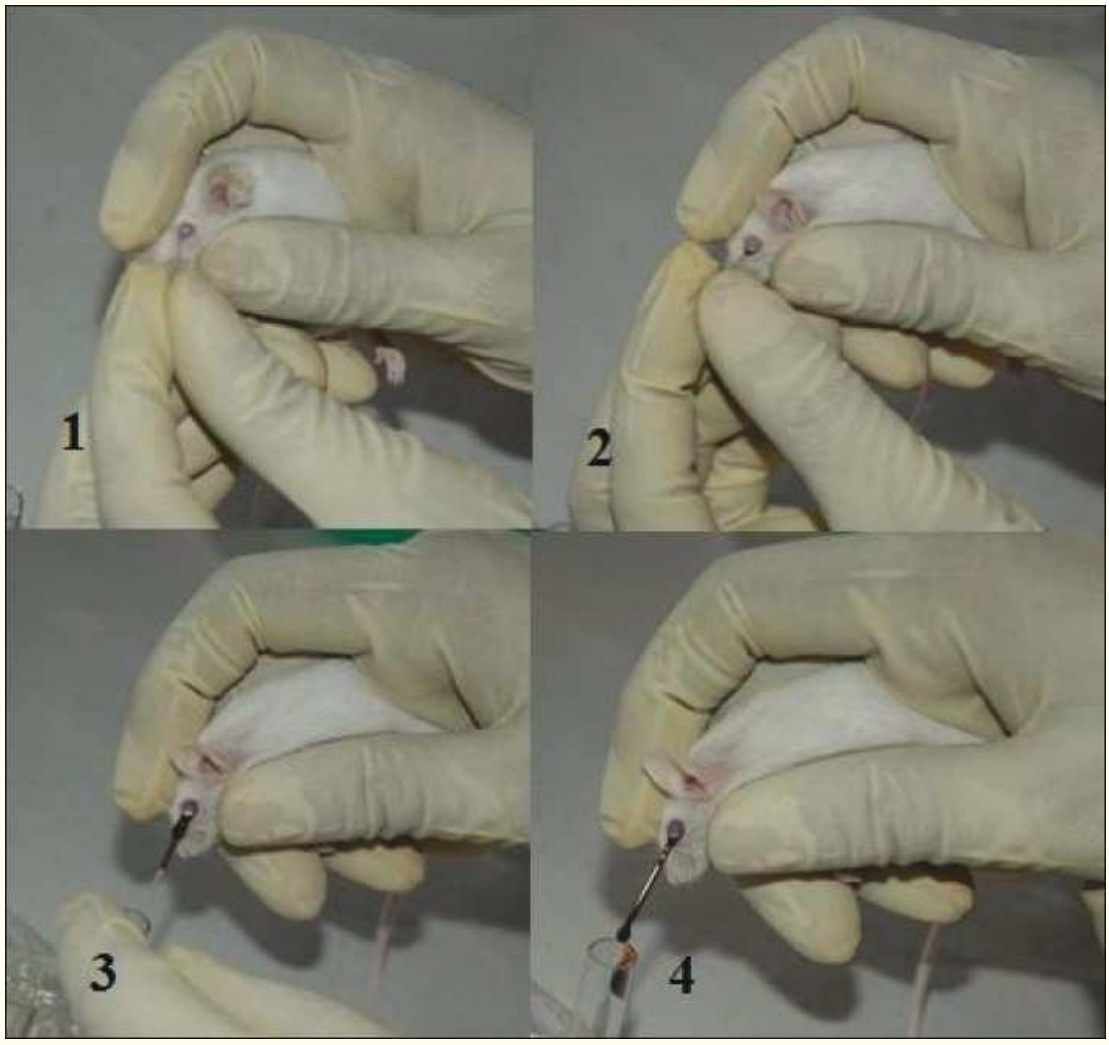

Figure 4: Blood sample collection from mouse orbital sinus

- Caution:
    - Repeated blood sampling is not recommended.
    - Skill is required to collect blood.
    - Even a minor mistake will cause damage to the eyes.
    - Two weeks should be allowed between two bleedings.
- Adverse effects reported from this method is around 1 to 2% which includes hematoma, corneal ulceration, keratitis, pannus formation, rupture of the globe, damage of the optic nerve and other infraorbital structures and necrotic dacryoadenitis of the harderian gland.

PROCEDURE FOR JUGULAR VEIN BLOOD SAMPLE COLLECTION

Requirements include animal, anesthetic agent, cotton, 25G needle and blood sample collection tubes.

- In this method, warming of the animals is not required and is used to collect micro volumes to one ml of blood sample.
- This method has to be carried out under general/inhalation anesthesia and two persons are needed to collect blood sample.
- One person has to restrain the animal and monitor the animal. Another person is required to collect the blood sample from the animal.
- The neck region of the animal is shaved and kept in hyperextended position. The jugular veins appear blue in color and is found 2 to 4 mm lateral to sternoclavicular junction. A 25G needle is inserted in the caudocephalic direction (back to front) and blood is withdrawn slowly to avoid collapse of these small blood vessels. Animal has carefully handled and not more than 3 - 4 mm of needle is to be inserted into the blood vessel.
- If the attempt to collect blood fails, the needle is slowly removed and the site is monitored for bleeding. If there is no bleeding, one more attempt can be made. Further attempts should be avoided in case of bleeding as it may collapse the vein.
- Finger pressure is applied to stop bleeding.
- Caution: Number of attempts is limited to three and applies local anesthetic cream 30 minutes prior to sampling.

PROCEDURE FOR COLLECTION WITH TEMPORARY CANNULA

Requirements include animal, anesthetic agent, cotton, 25G needle, animal warming chamber and blood sample collection tubes.

- Usually a temporary cannulation is made in the tail vein and used for a few hours.

- The animal is restrained and local anesthetic cream is applied on the tail (1 – 2 cm above the tail tip).
- The tail is either cannulated or a 25G needle is used.
- Tail bleeding normally requires the animal to be warmed in order to dilate the blood vessels (37 – 39°C for 5 – 15 min).
- After cannulation, animal has to be housed individually in large cages.

PROTOCOL FOR BLOOD VESSEL CANNULATION

Requirements include animal, anesthetic agent, cotton, 25G needle, i.v. cannula, surgical blade, heparin (or any anticoagulant) and blood sample collection tubes.

- This method involves continuous and multiple sampling in the experimental animal.
- This method requires close and continuous monitoring of the animal.
- Usually blood vessel cannulation is done in the femoral artery, femoral vein, carotid artery, jugular vein, vena cava and dorsal aorta.
- Surgery is required with appropriate anesthesia and analgesia used to minimize the pain.
- After surgical cannulation, animal should be housed singly in a large and spacious cage.
- Blood sample may be collected over 24 hour at the volume of 0.1 to 0.2 ml/sample.
- After withdrawing the blood, the cannula is flushed with an anticoagulant and the withdrawn volume may be replaced with LRS and closed tightly [Figure 5].

Figure 5: Blood vessel cannulation of rat femoral vein

- Caution: The experiment has to be conducted fully under aseptic precautions. Infection, hemorrhage, blockage of cannula and swelling around the cannulation site should be looked for. The needle size and maximum blood volume to be collected are given in Table 2.

Table 2: Needle size used for blood vessel cannulation in different species

Species	Needle to be used	Maximum collection volume
Mice	23 – 25G	1 ml
Rat	19 – 21G	10 – 15 ml
Rabbit	19 – 21G	60 – 200 ml
Guinea pig	20 – 21G	1 – 25 ml

PROTOCOL FOR TARSAL VEIN BLOOD SAMPLE COLLECTION

Requirements include animal, anesthetic agent, cotton, 22G needle, hair remover and blood sample collection tubes.

- Tarsal vein is identified in one of the hind legs of large animals. This method is commonly recommended for guinea pig.
- One person has to restrain the animal properly. Tarsal vein may be visible in blue color.
- The surface hairs are removed by applying a suitable hair remover. A local anesthetic cream is applied on the collection site.
- After 20 to 30 minutes, blood sample is collected slowly by using 22G needle.
- Maximum three samples can be taken per leg and 0.1 to 0.3 ml of blood can be collected per sample.
- After the sample collection, gentle pressure is applied with finger for 2 minutes to stop bleeding [Figure 6].

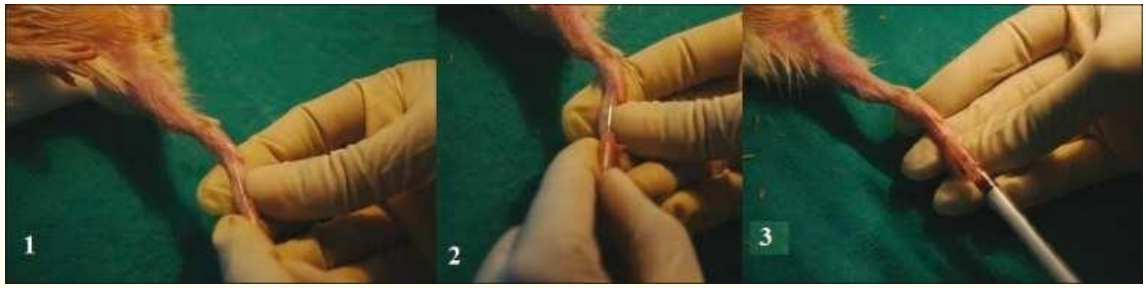

Figure 6: Blood sample collection from guinea pig tarsal vein

- Caution: Not >6 samples from both hind legs are taken and the number of attempts is 3.

PROTOCOL FOR MARGINAL EAR VEIN/ARTERY BLOOD SAMPLE COLLECTION

Requirements include animal, anesthetic agent, cotton, 26G needle, 95% v/v alcohol, o-Xylene, surgical blade and blood sample collection tube.

- This method is commonly adopted for rabbits.
- The animal should be placed in a restrainer.
- Ear is cleaned with 95% v/v alcohol and local anesthetic cream is applied on the collection site 10 min prior to sampling. (If required, the o-Xylene/topical vasodilator may be applied topically on the collection site to dilate blood vessels).
- Size 11 surgical blade is used to cut the marginal ear vein and blood is collected in a collecting tube. Otherwise, a 26G needle may be used to collect blood from animal marginal vein.
- After collecting blood, clean sterile cotton is kept on the collection site and finger pressure is applied to stop the bleeding [Figure 7 and 8].

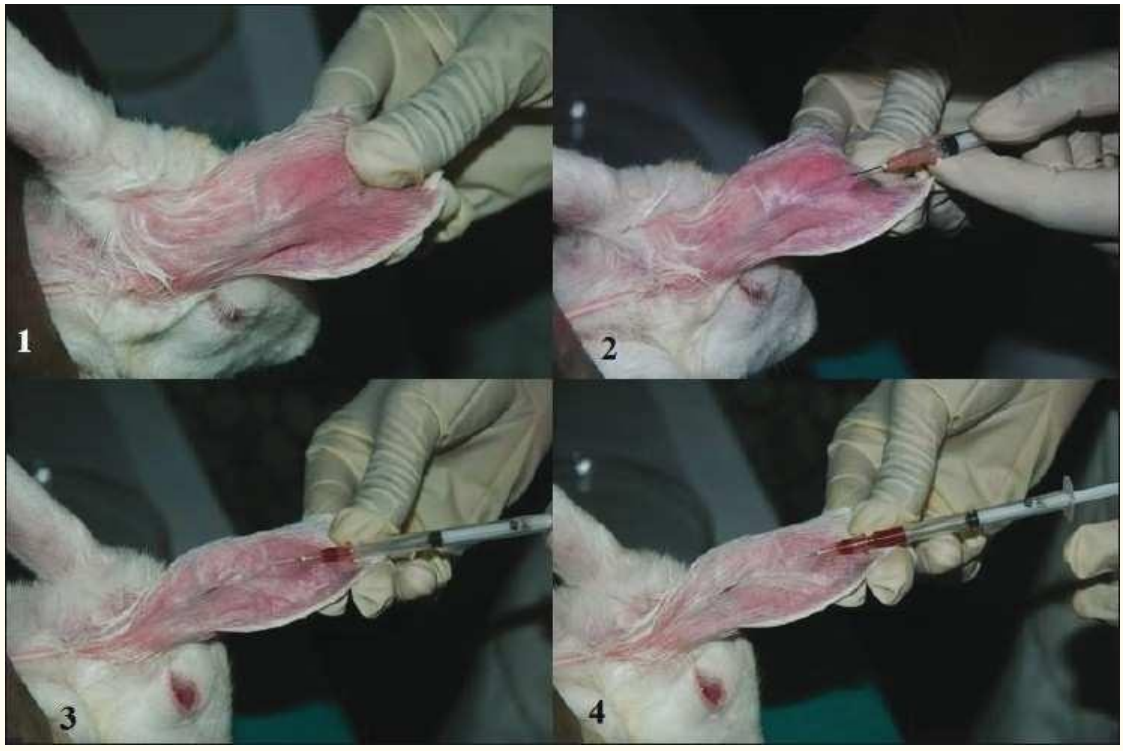

Figure 7: Blood sample collection from rabbit marginal ear vein using 26 G needle

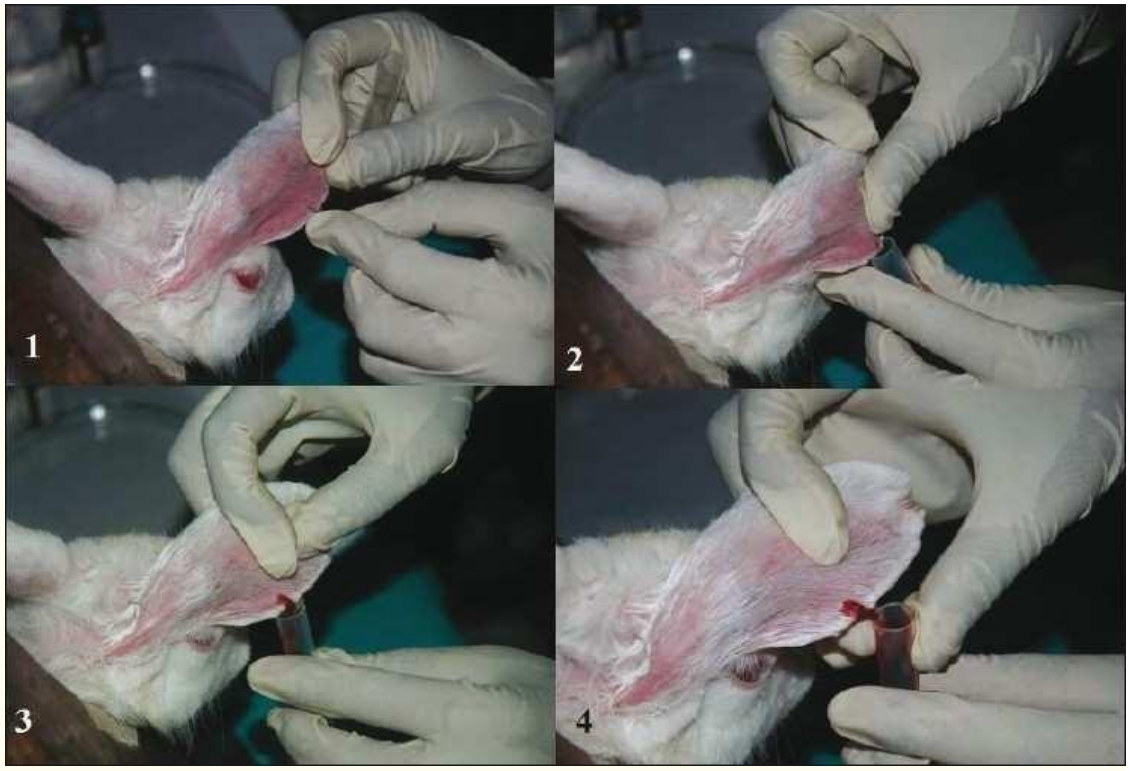

Figure 8: Blood sample collection from rabbit marginal ear vein using incision method.

PROTOCOL FOR CARDIAC PUNCTURE

Requirements include animal, anesthetic agent, towel, cotton, 19 to 25G needle with 1 to 5 ml syringe, surgical blade, tube (internal diameter of 0.1 to 0.3 mm) for thoracotomy, plastic disposable bag and blood sample collection tubes.

- In general, cardiac puncture is recommended for terminal stage of the study to collect a single, good quality and large volume of blood from the experimental animals.
- During blood sample collection, animal will be in terminal anesthesia.
- Appropriate needle is used for blood sample collection with or without thoracotomy. Blood sample will be taken from the heart, preferably from the ventricle slowly to avoid collapsing of heart [Figure 9].

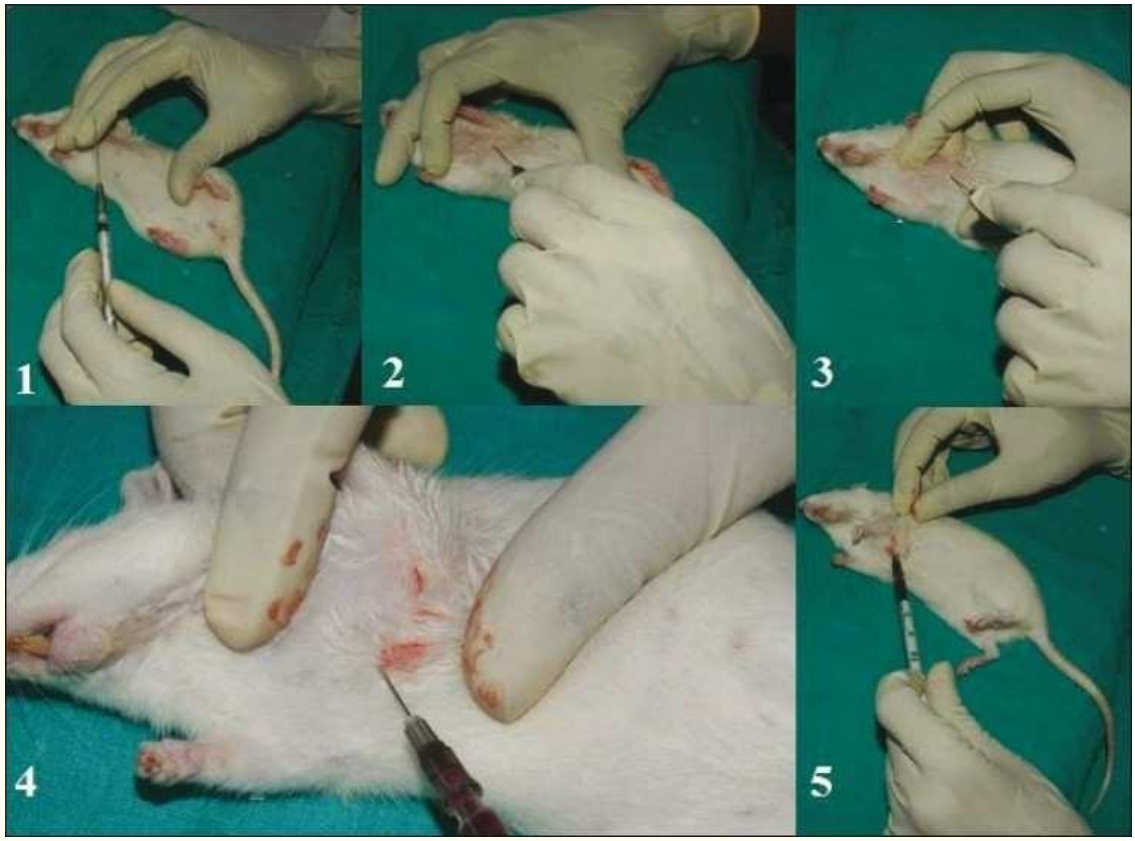

Figure 9: Blood sample collection through cardiac puncture in rat

- Caution: If animal has dextrocardia, sampling may fail.

PROTOCOL FOR COLLECTION THROUGH POSTERIOR VENA CAVA

Requirements include animal, anesthetic agent, surgical blade, small glass rods, surgical scissor, 21 to 25G needle with 1 to 5 ml syringe and blood sample collection tube.

- In general, posterior vena cava blood sample is recommended for terminal stage of the study.
- Animal have to be anesthetized and 'Y'- or 'V'-shaped cut in the abdomen is made and the intestines are gently removed.
- The liver is pushed forward and the posterior vena cava (between the kidneys) is identified.
- 21 to 25G needle is inserted to collect blood from the posterior vena cava.
- This procedure will be repeated three to four times to collect more volume of blood sample.

RESULTS and DISCUSSION:

Blood collection from the experimental animals is one of the important procedures in biomedical research. Even a small error in the collection procedure may lead to a lot of variation in the results.

Points to be remembered

- Before starting any kind of blood sample collection, it must be ensured that all chemical, surgical, fluid requirements are available in the working site.
- Not more than two to three attempts should be made to collect any kind of *in vitro* biological sample (excluding biological secretion).
- The blood collection tube must be labeled before starting the experiment and blood sample collected in the appropriately labeled collection tube.

EXPERIMENT NO. 08

OBJECT: To study muscle relaxant property of diazepam in rat using Rota rod apparatus.

REFERENCE: Kulkarni, S.K., "Hand book of experimental pharmacology", Published by Vallabh Prakashan, Page no. 122.

REQUIREMENTS: Animal – Rat

Drug - diazepam

Apparatus – Rota Rod

PRINCIPLE: One of the important pharmacological actions of antianxiety agent of benzodiazepines class is muscle relaxant property. These agents reduce anxiety and tension. The loss of muscle grip is an indication of muscle relaxation. These agents can easily study in Rotarod apparatus.

Rota rod

The Rota rod apparatus consists of a metal rod (3 cm diameter) coated with rubber attached to a motor with the speed adjusted to 2 rotations per minute. The rod is 75 cm in length and is divided into 3sections by metallic discs, allowing the simultaneous testing of 3 mice. The rod is in a height of about 50 cm above the tabletop in order to discourage the animals from jumping off the roller. Cages below the section serve to restrict the movements of the animals when they fall from the roller.

PROCEDURE

- Weighed the animals.
- Turn on the Rota rod and select an appropriate speed (20-25 rpm).
- Placed the animals on rata rod and noted the fall down time when the mice falls from rotating rod.
- Inject Diazepam to the animal, after 30 min repeated the experiment and noted the fall down time.
- Compare the falloff time of animals before and after diazepam treatment

Dose Calculation:

Observation Table:

S.No.	**Body wt.**	**Dose**	**Fall down time**		**% in time**
			Before	**After**	
1					
2					
3					
4					
5					

Comments:

RESULT:

EXPERIMENT NO. 09

OBJECT: To study the analgesic effect of Diclofenac sodium in rat using Tail Flick method.

REFERENCE: Kulkarni S.K. "Hand book of experimental pharmacology" 3rd Edition, 2005. Published by Vallabh Prakashan, Page No-123-124.

REQUIREMENTS:

Animal Rat

Equipment Analgesiometer

Drug Diclofenac sodium (Dose 10mg/kg).

PRINCIPLE:

Analgesia is defined as a state of reduced awareness to pain. Analgesics are substance which decreases pain sensation by increasing threshold to painful stimuli. The commonly used analgesic is aspirin, paracetamol (non-narcotic) and morphine (narcotic type).

Painful reaction in experimental animals can be produced by applying noxious stimuli such as;

- Thermal
- Chemical
- Physical pressure

But the major procedure used in tail flick method using analgesiometer, hot plate and acetic acid induced writhing method

PROCEDURE:

- Weigh and number the animals.
- Take basal reaction time to radiant heat by placing the trip of tail on radiant heat source, the tail withdrawal from the heat (flicking response) is taken as the end point.
- Normally rat withdrawals its tail within 3-5sec. A cut off period of 10-12 sec is observed to prevent damage to the tail.
- Any animal falling to withdrawals its tail within 3-5sec is rejected from the study.

- Take a basal 3-5 basal reaction time for each mouse at gap of 5 min to conform normal behavior of the animal.
- Injected Diclofenac sodium and noted the time at 5, 10, 15 and 30 min after the drug. As the reaction time reaches to 1o sec .it is considered maximum analgesia and the tail is removed from the source of heat to avoid tissue damage.
- Calculate the percentage the increase in reaction time.

Dose Calculation:

Observation Table:

S. No.	**Body wt.**	**Dose**	**Analgesic activity**		**Average percentage increase**
			Before	**After**	
1					
2					
3					

Comments:

RESULT:

EXPERIMENT NO. 10

OBJECT: To study the anxiolytic (antianxiety) effect of diazepam in rat using elevated Plus Maze Apparatus.

REFERENCE: Kulkarni S.K. "Hand book of experimental pharmacology" 3rd edition, 2005. Published by Vallabh Prakashan, page no-135-136.

REQUIREMENTS:

Animal	Rat (150-200gm).
Drug	Diazepam (2mg/kg).
Equipment	Plus-maze apparatus (2 open & 2 closed arm)

PRINCIPLE:

Anxiety is the major CNS disorder, different class of benzodiazepines used to treat the anxiety disorder. In experimental way, the elevated plus maize is most simple apparatus to study anxiolytic effect of almost all type of anti-anxiety agents. Exposure to animal to novel maze alley evokes an approach-avoidance conflict which is stronger in open arm as compared to enclosed arm. Rodents have aversion for high and open space and prefer enclosed arm, and therefore spend the greater amount of time in enclosed arm. When animal enter in open arm, they freeze and become immobile, defecate and show fear like movements. The plasma cortisol level is also reported to be increased, as a true reflection of anxiety.

PROCEDURE:

1) Weighed & no the animals, divide them into 2 groups each consisting of 6 mice, one group is used as control & other for drug treatment.
2) Placed animal individually in center of maze , head facing towards open arm and start the stop watch and note following parameter for five minute
   a) First presence of mouse to open and enclosed arm.
   b) Number of entries in open & enclosed arm (an arm entry defined as entry of four paws into the arm)
   c) Average time each animal spend in each arm. (average time=total duration in arm/no. of entries

3) Injected diazepam to test gp .After 30 min place the animal individually in the centre of maze & note all parameters.
4) Compare the preference of the animal to open and enclosed arm. Average time spent in open arm & no of entries in open arm in each group.

Comments:

RESULT:

EXPERIMENT NO. 11

OBJECT: To study the analgesic effect of Diclofenac Sodium in rat by Hot Plate. analgesiometer.

REFERENCE: Kulkarni, S.K., "Hand book of experimental pharmacology", Published by Vallabh Prakashan, Page no. 125.

REQUIREMENTS: Animal - Rat

Drug- Diclofenac-sodium

Equipment – EDDY'S Hot plate

PRINCIPLE: Analgesia is defined as a state of reduced awareness of pain. In this method heat is used as a source of pain. Animals are individually placed on a hot plate maintained at constant temperature (55^0C) and the reaction of animals, such as paw licking or jump response is taken as the end point. Analgesics increase the reaction-time.

PROCEDURE:

- Weigh and number the rat.
- Take the basal reaction-time by observing hind paw licking or jump response (whichever appears first) in animals when placed on the hot plate maintained at constant temperature (55^0C).
- Normally animals show such response in 6-8 sec. A cut off period of 15 sec is observed to avoid damage to the paws.
- Inject morphine to animals and note the reaction time of animals on the hot plate at 15, 30, 60 and 120 min after the drug administration.
- As the reaction time increases with morphine, 15 sec is taken as maximum analgesia and the animals are removed from the hot plate to avoid injury to me paw.
- Calculate percent increase in reaction -time (as index of analgesia) at each time interval.

Dose Calculation:

Observation Table:

S. No.	**Body Weight**	**Dose**	**Basal Reaction Time**		**Basal Reaction Time**	
			Paw Licking	**Paw jumping**	**Paw Licking**	**Paw jumping**
1						
2						
3						
4						

Comments:

RESULT:

Experiment No. 12

OBJECT: To study the anticonvulsant activity of Phenytoin against maximum electroshock induced convulsion in Rats.

REFERENCE: Kulkarni S.K. "Hand book of experimental pharmacology" 3rd Edition, 2005, Vallabh Prakashan, Page No-144-146.

REQUIREMENTS:

Animal	Rats (150-200g)
Drug	Phenytoin (25mg/kg)
Equipment	Electro-Convulsiometer

PRINCIPLE:

Different types of epilepsies are i.e. Grandmal, petitmal, psychomotor type, can be studied in laboratory animals. The max Electroshock-induced convulsion in animal represents grandmal type of epilepsies. Similarly, chemo-convulsion is to pentylenetetrazol which produce a tonic type of convulsion resembles petitmal type of convulsion in man. In MES convulsion electroshock is applied through the corneal electrodes, though optic stimulation cortical excitation is produced. The MES convulsions are divided in five phases such as;

(1) Tonic flexion
(2) Tonic extensor
(3) Clonic convulsions
(4) Stupor
(5) Recovery & Death

PROCEDURE:

1. Weight & number the animals, Divide them into 2 groups as control & other for Drug (phenytoin) treatment.
2. Hold the animal property, Place corneal electrodes on cornea & apply the prescribed current Note diff. stages of convulsions i.e., Note the time (see) spent by each animals in each phase of convulsions. Repeat other animal of control group.

3. Injected phenytoin IP into a group of 4-5 Rats. Wait for 30 min & subjected to animal to electro convulsion as described in step 2.

4. Noted the reduction in time or abolition of tonic extensor phase of MES-convulsion.

Dose Calculation:

Observation Table:

S. No.	**Body wt.(g)**	**Treatment(Dose)**	**Time (sec) in various phases of convulsions**				
			F	**E**	**C**	**S**	**R/D**
		Control					
		Control					
		Phenytoin					
		Phenytoin					

F-Flexion, E- extensor, C- clonic convulsions, S-stupor & R/D recovery or death

Comments:

RESULT:

Experiment No. 13

OBJECT: To study the anti-inflammatory property of indomethacin against carrageenan- induced acute paw oedema in rats.

REFERENCE: Kulkarni S.K. "Hand book of experimental pharmacology" 3rd Edition, 2005. Published by-Vallabh Prakashan, Page No-128-130.

REQUIREMENTS:

Animal:	Rat (150-200g)
Drug:	Carrageenan (dose 20mg/kg)
Equipment:	Plethysmometer

PRINCIPLE:

Inflammation is a tissue-Reaction to infection, irritation or foreign substance. It is a part of host defense mechanism but when it becomes great it is a hopeless condition. Aging is also considered to be inflammatory Response. These are several tissue factor or mechanism that is known to be involved in inflammatory reaction such as Release of histamine, bradykinin & Prostaglandins. The inflammatory Reaction is readily produced in Rats in the form of Paw oedema with the help of irritants. Substances such as carrageenan, formalin, Bradykinin, Histamine, 5-HT, When injected in dorsum of the foot of Rats they produce acute Paw oedema within few min of injection.

PROCEDURE:

- Weighed the animal & number them.
- Make a mark on both the hind Paws, just beyond tibio-tarsal junctions that every time the Paw is dipped in the Hg column up to the fixed mark to ensure constant Paw volume.
- Noted the initial Paw volume of each Rat by mercury displacement method.
- Divided the animals in 2 groups, each compressing of at least 4 Rats. To one group injected saline & second to indomethacin subcutaneously.
- After 30 min injected 0.1 ml of 1% (w/v) carrageenan in the plantar region of the left Paw of control as well as indomethacin treated group.
- The right paw will serve as reference non inflamed paw for comparison.

- ❖ Notes the Paw volume of both legs of control & indomethacin treated Rats at 15, 30, 60 & 120 min after Carrageenan challenge.
- ❖ Calculate the percentage difference in the right & left Paw volume of each animal of control & indomethacin treated groups.
- ❖ Compare the mean percent change in Paw volume in control & drug-treated animals & express as percent oedema inhibition by drugs.

Dose Calculation:

Observation Table:

S. No	**Body wt.(g)**	**Treatment**	**Dose(mg/kg)**	**`Paw volume**		
		Control				
		Control				
		Indomethacin				
		Indomethacin				

Comments:

RESULT:

Experiment No. 14

OBJECT: To study the Central Nervous System depressant property of diazepam on locomotor activity of rat using Actophotometer (Activity cage).

REFERENCE: Kulkarni S.K. "Hand book of experimental pharmacology" 3rd edition, 2005. Published by Vallabh Prakashan, page no-131-133.

REQUIREMENTS:

Animal Rat (150-200 gm).

Drug Diazepam (2mg/kg).

Instruments: Actophotometer

Procedure:

1. Weigh and number the mice.
2. Turn on the equipment (check & make sure that all the photocells are working for accurate recording) & place individually each mouse in the activity cage for 5-10 min. Note the basal activity score of all the animals.
3. Inject Chlorpromazine ,& after 30 min re-test each mouse for activity score for 5-10 min. Note the difference in the activity before & after Chlorpromazine
4. Calculate the percent decrease in motor activity.

Dose Calculation:

Observation Table:

S. No.	Body wt.(g)	Treatment	Dose (mg/kg)	loco motor activity (in 10 min)		% Change in time
				Before drug	After drug	
1.						
2.						
3.						
4.						
5.						

Comments:

RESULT:

Experiment No. 15

OBJECT:-To study the analgesic effect of diclofenac sodium against acetic acid-induced writhing in rat.

REQUIRMENTS-

Animals: Rat (150-150 gm).

Drugs Acetic acid, diclofenac sodium

PROCEDURE:

- Weigh and numbers the animals.
- Divide the animals in to two groups.
- Administer appropriate volume of acetic acid solution to the first group (which serves as disease control), place them individually under glass jar for observation.
- Note the onset on writhes.
- Record the number of abdominal contraction, trunk twist response and extension of hind limbs as well as the number of animals showing such response during a period of 10 mins.
- To the second group of animals inject diclofenac sodium (test drug).
- 15 mins later, administer acetic acid solution to these animals. Note the onset and severity of writhing response as done in step 3.
- Calculate the mean writhing scores in disease control and diclofenac sodium treated groups.
- Note the inhibition of pain response by diclofenac sodium.

Dose Calculation:

Observation Table: Acetic acid-induced writhing

S. No.	Body wt. (g)	Treatment	Number of writhing (10 min)	Mean
		Control (A. acid)		
		Control (A. acid)		
		Treatment (Drug+ Acetic acid)		
		Treatment (Drug+ Acetic acid)		

Comments:

RESULT:

EXPERIMENT No: 16

OBJECT: To study the analgesic effect of Aspirin in rat using tail immersion method.

REQUIREMENTS:

Animal: Rat (150-200 g)

Drugs: Aspirin

Equipment: Analgesiometer (Techno)

PROCEDURE:

- Weigh and number the animals.
- The lower 5 cm portion of tail of each mouse is marked & immersed in a beaker of freshly filled water of 55-60^{O} C.
- Within a few seconds, the rat reacted by withdrawing the tail.
- Note down the withdrawal time. After each determination the tail is carefully wiped.
- Inject drug and note the reaction time at 15, 30 min after the drug.
- As the reaction time reaches 10 sec it is considered maximum analgesia and the tail is removed from the source of heat to avoid tissue damage.
- Calculate percentage increase in reaction time (index of analgesia) at each time interval.

Dose calculation:

Observation Table:

S. No.	Body wt.(g)	Treatment	Dose (mg/kg)	Activity of animal (in 10 min)				% Decrease in activity
				Before drug		After drug		
				15 min	30 min	15 min	30 min	
1								
2								
3								

Comments:

RESULT:

EXPERIMENT No: 17

OBJECT: To study the anxiolytic effect of diazepam using Hole Board test in mice.

REFERENCES:

- Crowley JN. Neuropharmacological specificity of a simple animal model of the behavioral actions of benzodiazepines *Pharmacol. Biochem. Behav,* 1981, 15: 695-699.
- Mohan M, Kasture SB, Balaraman R. Anxiolytic activity of standardized extract of Korean ginseng – a study on exploratory behavior. *Oriental Pharm. Exp. Med,* 2005, 5; 301-307.

REQUIREMENT:

Polypropylene cages, Shock dispenser (0.5 mA for 3 minutes).

RATIONALE: Placing a mouse is on the hole board apparatus, elevated to 25 cm from table, includes anxiety as it is exposed to a new environment. The anxiogenic agents reduce the number of head poking, whereas the anxiolytic agents increase the number of head poking.

PROCEDURE:

- The hole board apparatus consisted of a metal plate floor (40x40 cm) placed 3.5 cm above the ground.
- The metal plate contained six holes (1.5 cm diameter), spaced symmetrically in a diamond pattern.
- A mouse was placed on one corner of the apparatus and observed for the next 5 minutes for the number of head poking.
- Dexamethasone (1 mg/kg s.c.) or diazepam (1 mg/kg i.p.) was administered 30 minutes before the test.
- The effect of these drugs was compared with that of vehicle treated group.

Dose calculation:

Observation Table: Effect of diazepam on number of head poking in hole board apparatus

Treatment (Dose in mg/kg i.p.)	Number of head poking (mean ± SEM)
Vehicle	
Diazepam (1)	
Dexamethasone (1)	

Comments:

RESULT: