TECHNOCRATS

Lab Work Book of

Pharmacology-I

(BP - 408 P)

Department of Pharmacy

Lab Manual of

Pharmacology-I

(BP - 408P)

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TECHNOCRATS
PUBLICATIONS

Lab Work Book
of

Pharmacology-I

(BP-408 P)

(Strictly According to RGPV Syllabus)

Name : .....

Enrollment No. : .....

Institute : .....

Academic Session : .....

Department of Pharmacy

TECHNOCRATS
PUBLICATIONS

Vision of the Institute

To grow as an institute of Excellence for Pharmacy Education and Research and to serve the humanity by sowing the seeds of intellectual, cultural, ethical, and humane sensitivities in the students to develop a scientific temper, and to promote professional and technological expertise.

Mission of the Institute

M 1: To inculcate ethical, moral, cultural and professional values in students

M 2: To provide state of art infrastructure facilities to the staff and students so as to enable them to learn latest technological advancements

M 3: State of art learning of professionalism by the faculty and students

M 4: To produce well learned, devoted and proficient pharmacists

M 5: To make the students competent to meet the professional challenges of future

M 6: To develop entrepreneurship qualities and abilities in the students

PROGRAM OUTCOMES (POs)

1. **Pharmacy Knowledge:** Possess knowledge and comprehension of the core and basic knowledge associated with the profession of pharmacy, including biomedical sciences; pharmaceutical sciences; behavioral, social, and administrative pharmacy sciences; and manufacturing practices.
2. **Planning Abilities:** Demonstrate effective planning abilities including time management, resource management, delegation skills and organizational skills. Develop and implement plans and organize work to meet deadlines.
3. **Problem analysis:** Utilize the principles of scientific enquiry, thinking analytically, clearly and critically, while solving problems and making decisions during daily practice. Find, analyze, evaluate and apply information systematically and shall make defensible decisions.
4. **Modern tool usage:** Learn, select, and apply appropriate methods and procedures, resources, and modern pharmacy-related computing tools with an understanding of thelimitations.
5. **Leadership skills:** Understand and consider the human reaction to change, motivationissues, leadership and team-building when planning changes required for fulfillment of practice, professional and societal responsibilities. Assume participatory roles as responsible citizens or leadership roles when appropriate to facilitate improvement in health and well- being.
6. **Professional Identity:** Understand, analyze and communicate the value of their professional roles in society (e.g. health care professionals, promoters of health, educators, managers, employers, employees).
7. **Pharmaceutical Ethics:** Honour personal values and apply ethical principles in professional and social contexts. Demonstrate behavior that recognizes cultural and personal variability in values, communication and lifestyles. Use ethical frameworks; apply ethical principles while making decisions and take responsibility for the outcomes associated with the decisions.
8. **Communication:** Communicate effectively with the pharmacy community and with society at large, such as, being able to comprehend and write effective reports, make effective presentations and documentation, and give and receive clear instructions.
9. **The Pharmacist and society:** Apply reasoning informed by the contextual knowledge to assess societal, health, safety and legal issues and the consequent responsibilities relevant to the professional pharmacy practice.
10. **Environment and sustainability:** Understand the impact of the professional pharmacy solutions in societal and environmental contexts, and demonstrate the knowledge of, and need for sustainable development.
11. **Life-long learning:** Recognize the need for, and have the preparation and ability to engage in independent and life-long learning in the broadest context of technological change. Self-assess and use feedback effectively from others to identify learning needs and to satisfy these needs on an ongoing basis.

PEOs

PEO 1: To inculcate quality pharmacy education and training through innovative Teaching Learning Process.

PEO 2: To promote professionalism, team spirit, social and ethical commitment with effective interpersonal communication skills to boost leadership role assisting improvement in healthcare sector.

PEO 3: To enhance Industry-Institute-Interaction for industry oriented education and research, which will overcome healthcare problems of the society.

PEO 4: To adapt and implement best practices in the profession by enrichment of knowledge and skills in research and critical thinking

PEO 5: To generate potential knowledge pools with interpersonal and collaborative skills to identify, assess and formulate problems and execute the solution in closely related pharmaceutical industries and to nurture striving desire in students for higher education and career growth.

Course Outcomes (COs):

Student will be able to:

- CO1: Describes about basics of experimental pharmacology.
- CO2: Demonstrates usage of animals in experimental pharmacology and gives knowledge about animal handling, routes of drug administration and bleeding techniques.
- CO3: To interpret various drug actions on experimental animals.
- CO4: To distinguish in-vitro and in-vivo experiments.
- CO5: Understands ethical considerations while housing and handling of experimental animals.

Index

Experiment No. 1

OBJECT:

To study the introductional part of experimental pharmacology**.**

REFERENCE:

Kulkarni S.K "Hand book of Experimental Pharmacology" , Vallabh Prakashan Ninths edition Page No. 4-8

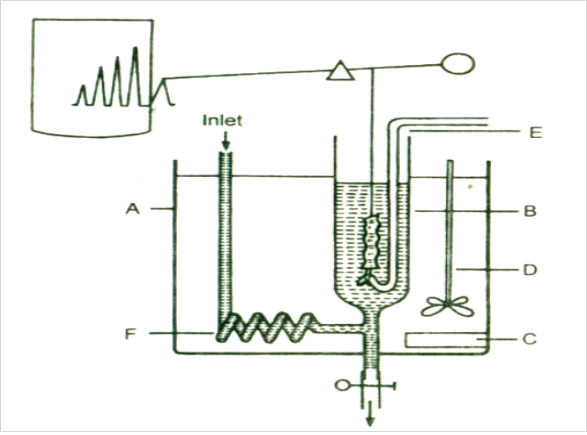
REQUIREMENTS:

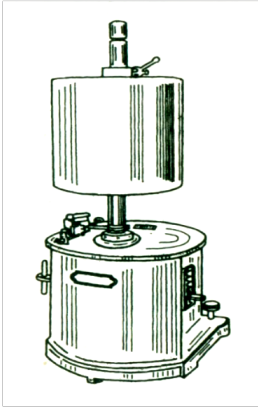
Student organ bath, Rotating Drum, Levers and standard drugs.

THEORY:

Organ bath:

The tissue bath used to put the animal tissue for studying the drug actions is called student organ bath. The organ bath essentially consists of (A) an outer jacket (water bath) made up of steel , glass or Perspex ; (B) the inner organ or tissue bath made up of glass with a capacity varying from 10 ml to 50 ml ; (C) thermostatically controlled heating rod ; (D) stirrer to keep the water in the outer jacket at uniform temperature ; (E) oxygen or delivery glass tube which also serves as tissue holder ; and (F) glass coil, one end of which is usually of double the capacity of inner organ bath to ensure warming up of the solution before it enters the organ bath**.** The student organ bath having two units of inner tissue bath is called double unit organ.





Recording levers:

They are used to record the contractions or relaxations of the isolated tissue preparation. The recording is done on smoked papers fixed on circular cylinders (of different diameters) and run at different speed using electrical recording drums. The speed of the drum is adjusted depending upon the nature of the experiment.

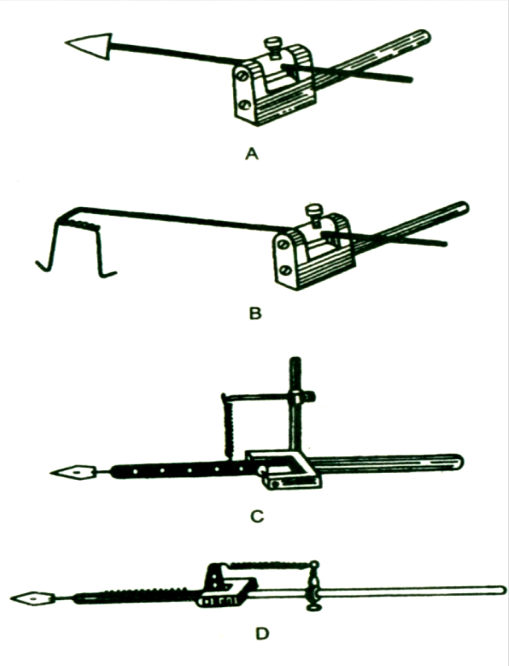
WRITING LEVERS

The writing levers area light in weight. Rigid and are generally made up of wood (straw), light in weight, light aluminium or stainless steel. The levers are of two types-

(i) **Isotonic type**, i.e. change in length due to contraction is recorded while the tension on the muscle remains the same. The examples of isotonic levers are simple, lever, frontal writing lever.

(ii) **Isometric type**, i.e. isometric recording measures increase in tension of the tissue when the length of the tissue is kept constant. These are used in special circumstances such as recording muscle twitches produced by electrical stimulation. For recording such observations isometric strain-gauge transducer may be preferred.

The common recording levers used in the laboratory are:



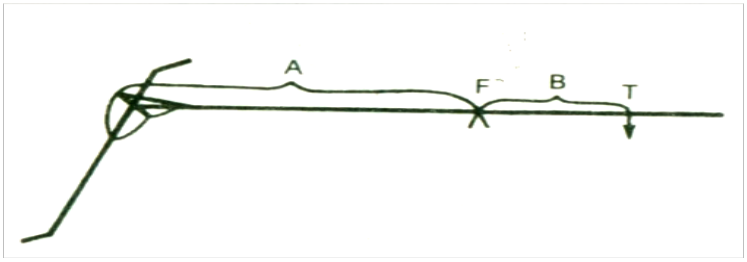
- **Simple lever** (side-way writing) - It is the simplest type of lever made up of wood, stainless steel or aluminium. A celluloid writing tip (stylus) is attached at the end of the longer arm. The contractions are recorded as curved lines.
- **Frontal writing lever** (writes frontally) - This lever 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 contractions are recorded as straight line.

- **Starling's heart lever** - This lever is used to record the contraction of the heart. The difference between this and other isotonic levers is that the fulcrum lies at one end beyond the point of attachment.
- **Boride's universal lever** - It is a general utility lever.

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

RECORDING PROCEDURES

1) 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 tissue showing less contractility need more magnification and the reverse is true with tissues which have higher inherent rhythmic contractility. For example while recording the effect 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 **.**



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

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.

2) Application of load (tension) - The muscle preparation has to be properly relaxed 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 (1g); guinea pig trachea (0.2 g); guinea pig vas deferens (0.5g); rabbit duodenum (1-3g); rat uterus (1g); rat colon (0.5g); rat fundus (1g) and frog rectus abdominis (1-5g), respectively.

3) 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.

4) Fixing of the tracing - The recordings on the smoked drum (trancings) are preserved by properly fixing them with the help of fixing solution. The commonly used resins to prepare the fixing solution of shalle are prepared in alcohol, and it 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.

5) Contact time - The time that is allowed for 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'.

6) 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 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

7) Standard drugs - The commonly used agonists in isolated tissue preparations in practical pharmacology class are acetylcholine, adrenaline, histamine and serotonin.

OBSERVATION:

RESULT AND DISCUSSION:

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VIVA QUESTIONS

Q.-1. How to fix the tracing ?

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Q.-2. What is the Magnification value ?

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Q.-3. Explain the type of levers ?

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Q.-4. How to Smoke kymograph drums ?

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Q.-5. Define Pharmacodynamics ?

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Experiment No. 2

OBJECT:

To study the common laboratory animals and anaesthetics used in animal studies.

REFERENCE:

Kulkarni S.K "Hand book of Experimental Pharmacology" , Vallabh Prakashan Ninths edition Page No. 12-18

REQUIREMENTS:

Laboratory animals, Alaesthetics -Barbiturates, Chloralose, Urethane (Ethyl Carbamate), Paraldehyde, Magnesium Sulphate

THEORY:

Experimental Animals

1) Guinea pig- Guinea pigs (400-600 g) are the commonly used experimental animals. They are very docile and easy to raise and maintain. They are highly sensitive to histamine. They are therefore used in experimental asthma to study bronchodilators. They are also used to study local anaesthetics and as models in experimental amoebiasis and cholera as they are sensitive to these micro-organism. Guinea pig tissue (organs) such as ileum is the most common tissue preparation used to study spasmogens and antispasmodics. Guinea pig isolated heart and trachea are sensitive to histamine.

2) Albino rat- White rat (wistar strain) is the commonest laboratory animal used in experimental pharmacology. Rats are easy to breed and maintain. Rats being small in size (200 g) resembles man in several organ function and nutrition and sensitive to most of the drugs make them very useful experimental animals. However they do not have vomiting centre and hence it is not possible to use them study emetics. The various rat tissues used are colon, stomach (fundus), uterus, caecum and vas deferens. Besides these organs rat brain tissue (whole or desecrate areas) is extensively employed in radio-receptor ligand studies. The other strains of rats are Sprague-Dawlely and porton.

3) Albino mouse - White mice (Swiss strain) are the smallest laboratory animals used. Mice are also easy to breed and maintain. They are small in size (25-30 g) and therefore, easy to handle. They are sensitive to most of the drugs used in experimental pharmacology. Mice are used extensively in toxicity study, bioassay of drugs (insulin), testing of analgesics, CNS active drugs and chemotherapeutic agents. More recently mouse brain tissues as well as primary cell culture of mouse spinal cord neurons are used in neuropharmacology for studying neurotransmitter receptor functions. The other strains of mice used are Laca and Bulb/C.

4) **Rabbit -** Domestic rabbits (2-3 kg) are generally used for pyrogen testing. Some of the tissue or organs from rabbits used are heart, aorta, duodenum and ileum. One unusual thing about rabbits is that they are resistant to the actions of atropine as they contain atropinesterase anzyme, the presence of which is genetically determined.

5) Frog - Frogs (150-200 g) are also extensively used in experimental pharmacology. Isolated frog heart, rectus abdominis muscle preparation, study of muscle nerve preparation and ciliary movements are some of the organs of frog used. Frog is also used for the study of nerve block type of local anaesthesia. Frogs are inexpensive and easily available.

Cats, dogs (Mongrel, stray dogs) and monkeys are used for pharmacological investigations of drugs. Cats and dogs are commonly used to study blood pressure experiments. Chronically prepared fistula and pounches of dogs are used to study the gastric secretary function. Monkeys are not used for routine pharmacological testing. Since they aspects, the toxicity study on monkeys forms an essential requirement for developing a new drug for human use. Monkeys are quite expensive and difficult to breed and maintain.

LABORATORY ANAESTHETICS

1. Barbiturates:

Barbiturates are the most commonly used anaesthetics for laboratory animals. Pentobarbital sodium (Nembutal) is the anaesthetics of choice. It produces surgical anaesthesia in a dose of administration. The onset of action is almost immediate and the duration of anaesthesia lasts for 45-60 min. If longer action is needed maintenance dose may be administered. It produces reversible anaesthesia. When the drug is given by intravenous route, special precaution should be taken to administer it slowly otherwise it may cause respiratory paralysis. Pentobarbitone anaesthesia may not be suitable for the study of cardiovascular drugs as it affects the basal activity of cardiovascular system.

2. Chloralose:

It is a compound of chloralose and glucose prepared by heating equal parts of anhydrous glucose and chloral, when both α-chloralose (active form) and β-chloralose (inactive form) are formed. Chloralose is the active form (α-chloralose) freely soluble in hot water, alcohol and ether and slightly so in cold water. It is prepared as one percent solution by boiling in 0.9% NaCl (saline) or in distilled water and administered intravenously or intra-peritoneally at a temperature of 30 to 40^0 C before the chloralose comes out of solution.

It is suitable only for acute (nonrecovery) experiments, usually in dogs and cats, inducing surgical anaesthesia for 3 to 4 hours or longer. It has the advantage of greater constancy of the depth of anaesthesia. The respiration and circulation are not depressed and the blood pressure is well maintained usually on the higher side. Chief disadvantage is its low water solubility, but can make up 10 per cent solution in polyethylene glycol. Autonomic reflexes are disturbed in an unpredictable manner. It is not a suitable anaesthetic for rabbits since animals are narcotized rather than anaesthetized, large volume needed and may produce convulsion on slight stimulation.

3. It is readily soluble in water giving a neutral solution. Usually 25 per cent solution in water is used. It is suitable only for acute experiments since it has delayed toxic on liver and may also cause agranulocytosis and pulmonary adenomata; has little or no effect on nerve transmission and produces little reflex depression. Basal blood pressure is uniformly lower than with chloralose in dogs. Pressure response to catecholamines is most reduced compared to other anaesthetics. It is especially suitable for rabbits and rats, duration of anaesthesia being three to four hours or more. Mice develop an exceptionally high incidence of lung tumours regardless of the route of administration. Frogs can be anaesthetized by placing them in a covered beaker containing 5 to 10 per cent urethane solution.

4. Paraldehyde

It has a wide margin of safety because it depresses only the cerebrum and not the medullary centres. Intravenous injection is likely to produce cardiac dilatation and pulmonary congestion and oedema. Under its influence the basal blood pressures as well as the response to vasopressor and depressor drugs are low. Bilateral carotid occlusion produces poor pressor response or even a depressor.

5. Magnesium Sulphate

A 20% magnesium sulphate solution 5 ml/kg intravenously produces anaesthesia for about an hour; calcium gluconate intravenously will counteract its depressant effect immediately. Its principal use is in producing euthanasia.

OBSERVATION:

RESULT AND DISCUSSION:

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VIVA QUESTIONS

Q.-1. Why we use rodent animals for experimental studies?

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Q.-2. What is the importance of barbiturates?

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Q.-3. Why anaesthetics used in animal studies?

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Q.-4. Why Guinea pig are used in asthma study?

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Q.-5. What varities of rat are used in Experiments?

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Experiment No. 3

OBJECT:

To study different routes of drug administration in mice. Study the onset and duration of action of pentobarbital sodium in mice when given by different routes of administration.

REFERENCE:

Kulkarni S.K "Hand book of Experimental Pharmacology" , Vallabh Prakashan Ninths edition Page No. 111

REQUIREMENTS:

Mice (20-25g), Pentobarbital sodium (Dose: 45 mg/kg. Make stock solution containing 4.5 mg/ml of the drug and inject 1 ml/100 g body weight of mouse by any route), 1 ml syringe having 100 divisions per ml, hypodermic (26 no.) and oral feeding needles.

THEORY:

The onset and duration of action of a drug depends on the route of administration. For example when a drug is given by intravenous route the effect is seen instantaneously as compared to oral administration of drug when it takes up to show the effect.

Most drugs can be administered by a variety of routs. The choice of appropriate route in a given situation depends both on drug as well as patient related factors. Mostly common sense considrations, feasibility and convenience dicate the route to be used.

I. Local Routes

These routes can only be used for localized lesions at accessible sites and for drugs whose systemic absorption from these sites are minimal or absent.

i. **Topical**

This refers to external application of the drug to the surface for localized action. It is aften more convenient as well as encouraging to the patient. Drugs can be efficiently delivered to the localized lessions on skin, oropharyngeal/nasal mucosa, eyes, ear canal, anal canal

ii. **Deeper tissues**

Certain deep areas can be apporoached by using a syringe and needle, but the drug should be such that systemic absorption is slow.

iii. **Arterial supply**

Close intra-arterial injection is used for contrast media in angiography; anticancer drugs can be infused in femoral or bronchial artery to localise the effect for limb malignancies.

II. Systemic Routes

The drug administered through systemic routes is intended to be absorbed into the blood stream and distributed all over, including the site of action, through circulation.

i. **Oral**

Oral ingestion is the oldest and commonest mode of drug administration. It is safer, more convenient, does not need assistance, noninvasive, often painless, the medicament need not be sterile and so is cheaper.

ii. **Sublingual (s.i.) or buccal**

The tablet or pellet containing the drug is placed under the tongue or crushed in the mouth and spread over the buccal mucosa. Only lipid soluble and non-irritating drugs can be so administered.

iii. **Rectal**

Certain irritant and unpleasant drugs can be put into rectum as suppositories or retention enema for systemic effect. This route can also be used when the patient is having recurrent vomiting or is unconscious.

iv. **Cutaneous**

Highly lipid soluble drugs can be applied over the skin for slow and prolonged absoption. The liver is also bypassed.

v. **Inhalation**

Volatile liquids and gases are given by inhalation for systemic action, e.g. general anaesthetics.

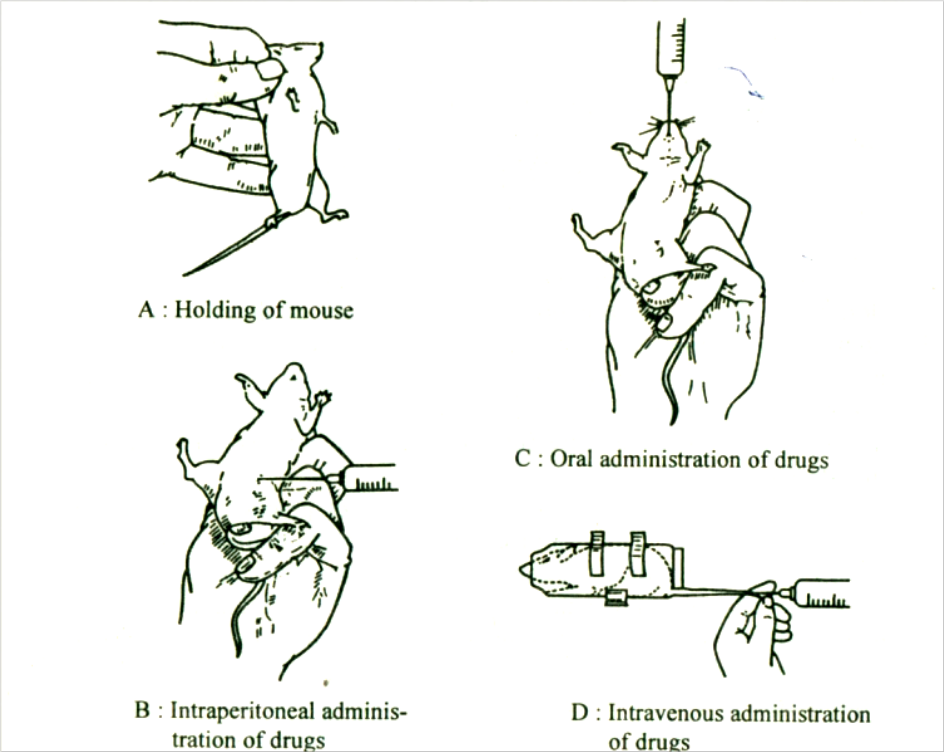
vi. **Nasal**

The mucous membrane of the nose can readily absorb many drugs; digestive juices and liver are by pass.

vii. **Parenteral** (*par*- beyond, *enteral*- intestinal)

This refers to administration by injection which takes the drug directly into the tissue fluid or blood without having to cross the intestinal mucosa.

- **Subcutaneous (s.c.)**
- **Intramuscular(i.m.)**
- **Intravenous(i.v.)**
- **Intradermal injection**



Pentobarbital sodium is chosen for the present experiment because it shows a clear cut onset of action (animals go to sleep by losing their righting reflex i.e. ability to maintain upright posture) and end point is also clear when the animals recover from sleep. Moreover, its effects can be seen when administered by all routes of administration. For the present purpose, three routes are chosen i.e. intra-peritoneal, intravenous and oral routes for the administration of drug.

PROCEDURE

1. Weigh the animal and number them by marking them on the tail.
2. Divide them into three groups each consisting of 5 mice. For intraperitoneal, intravenous and oral routes of administration, respectively.
3. Intraperitoneal injection is made in the abdomen whereas the dorsal tail vein is used for intravenous administration. For oral route, hold the mouse by its neck muscle and place the feeding needle on the tongue and gently push the solution in the mouth. Observe that no drug comes out of the mouth or nose. If the solution is coming out from the nose it is sure that the solution is not going in stomach but entering trachea, if not stopped immediately the mouse may die .
4. Note the time of injection and time of onset of sleep (loss of righting reflex).When the animal sleeps, put it on its back so that when it regains consciousness it will turn over to its normal posture (end point).
5. Note the time of recovery and calculate the duration of action.

6. Compare the onset of action and duration of sleep due to pentobarbital sodium when given by different routes of administration.

OBSERVATION TABLE:

Effect of Different Routes of Administration on the onset and Duration of Action of Pentobarbital sodium (45 mg/kg) in mice

S. No.	Body Wt.(g)	Route of administration	Time when injection (Ti)	Time when Asleep (Ts)	Time when wake (Tw)	Onset of action (sec) (Ts-Ti)	Duration of action (min) (Tw-Ts)	Mean duration (min)
1.		IV						
2.		IV						
3.		IV						
4.		IV						
5.		IV						
1.		Oral						
2.		Oral						
3.		Oral						
4.		Oral						
5.		Oral						
1.		IP						
2.		IP						
3.		IP						
4.		IP						
5.		IP						

INFERENCE

Intravenous route produces quick onset of action and long duration of action as compared to oral route of administration of pentobarbital sodium.

OBSERVATION:

RESULT AND DISCUSSION:

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VIVA QUESTIONS

Q.-1. What is the basic principle route of administration?

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Q.-2. What do you mean by duration of action?

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Q.-3. What is the advantage of oral routes?

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Q.-4. What are various routes of drug administration?

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Q.-5. What are the advantage of Parentral route?

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Experiment No. 4

OBJECT:

To prepare and submit various physiological salt solutions.

REFERENCE:

Kulkarni S.K "Hand book of Experimental Pharmacology" , Vallabh Prakashan Ninths edition Page No. 85

REQUIREMENTS:

Analytical grade reagents, beaker, Weighing Box, distilled water, pH strips.

THEORY :

The physiological salt solutions are used to keep isolated tissue or organ preparations surviving as long as the experiments are 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 carbon dioxide (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 compositions are listed in the Table.

Table: Composition of Some Physiological Salt Solutions

Compound	Frog Ringer	Ringer or Ringer Locke (Locke)	De Jalon	Tyrode	Kerbs-Hensleit (Kerbs)
NaCl (58.45)*	110 (6.0)**	154 (9.0)	154 (9.0)	137 (8.0)	118 (6.9)
KCl (74.56)	1.9 (0.14)	5.6 (0.42)	5.6 (0.42)	2.7 (0.2)	4.7 (0.35)
$CaCl_2$	1.1 (0.12)	2.2 (0.24)	0.55 (0.06)	1.8 (0.2)	2.5 (0.28)
$MgCl_2$ (95.23)	-	-	-	0 . 1 - 1 . 0 (0.01-0.10)	-
$MgSO_4 7H_2O$	-	-	-	-	1.2 (1.28)
$NaHCO_3$	2.4 (0.2)	6.0 (0.5)	6.0 (0.5)	11.9 (1.0)	25.0 (2.1)
NaH_2PO_4 (119.97)	-	-	-	0.4 (0.05)	-
KH_2PO_4 (136.08)	-	-	-	-	1.2 (0.16)
Glucose (180.16)	11.1 (2.0)	5.55 (1.0) or	2.78 (0.5)	5.55 (1.0) or 11.1 (2.0)	5 . 5 5 (1 . 0) o r 11.1(2.0)

*Value in parenthesis against salts indicates mol. wt.,**Values are in mM (g/l)

PROCEDURE:

1. Weigh the analytical grade reagents according to the type of physiological salt solutions.
2. Add each reagent in a beaker containing 500 ml of distilled water.
3. Dissolve the reagent until the clear solution and makeup the volume upto 1000 ml with distilled water.
4. Adjusting the proper pH of the final solution and aeration with oxygen, (mixture of 95% oxygen and 5% carbon dioxide).

OBSERVATION:

RESULT AND DISCUSSION:

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VIVA QUESTIONS

Q.-1. What is the importance of physiological salt solutions?

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Q.-2. What is the turbidity of solution?

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Q.-3. What is diffrence between Frog Tyrode and Ringer soultion?

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Q.-4. How organ are stilmulated?

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Q.-5. What is role of PH in soultion?

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Experiment No. 5

OBJECT:

To study the CPCSEA guidelines for ethical management of laboratory animals facility.

REFERENCE:

CPCSEA guidelines for laboratory animal facility. Committee for the purpose of control and supervision on experiments on animals. No. 13/1, 3rd Seward Road, Valmiki Nagar, Thiruvanmiyur, Chennai -600041.

THEORY:

The animal house facility is available to boost the teaching, training and research facilities and to meet the growing demand for high quality laboratory animals in emergent field of Experimental Pharmacology. Qualified veterinarians and technical personnel are always at hand to help carry out research and keep it in accordance with the standards established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

GUIDELINES

Animal Care

Cages should be checked First thing every day, to note the condition of the animals. A staff member will check the animal cages daily for visible signs of change or distress, such as leaky bottles, birth of new pups, decrease in food or water consumption, blood in cage, wounds, secretions around the eyes, nose and genital area, respiratory distress, constipation, diarrhoea, swelling, sluggishness, gait, dull coat or loss of hair. All concerns will be reported to the Supervisor and depending on the severity of the concern, the PI/ attending veterinarian will be notified.

Cages should be changed at least once per week or more often as needed. During cage changing, animals are inspected for any abnormal conditions as listed above. Water bottles should be checked every day and fresh water should be added as needed.

Sanitize the water bottles once a week.

Shelves, cage holders, lids and bonnets should be cleaned once a month

Room should be sanitized every three to six months.

Sweep the floor should be and mop weekly or as needed.

Feeding plates should be wiped weekly.

Each cage must have an identification card with the following information: protocol number, investigator's name, date received, strain, sex, date of birth and number of animals per cage.

Only items that are essential to the animal care of that room should be stored in the animal housing room.

The floor drains should be checked every day and flush out if necessary.

Doors should be wiped weekly.

VETERINARY CARE

Adequate veterinary care must be provided and is the responsibility of a veterinarian or a person who has training or experience in laboratory animal sciences and medicine. Daily observation of animals can be accomplished by someone other than a veterinarian; however, a mechanism of direct and frequent communication should be adopted so that timely and accurate information on problems in animal health, behaviour and wellbeing is conveyed to the attending veterinarian.

The veterinarian can also contribute to the establishment of appropriate policies and procedures for ancillary aspects of veterinary care, such as reviewing protocols and proposals, animal husbandry and animal welfare; monitoring occupational health hazards containment and zoonosis control programs and supervising animal nutrition and sanitation. Institutional requirements will determine the need for full-time or part-time or consultative veterinary services.

ANIMAL PROCUREMENT

All animals must be acquired lawfully as per the CPCSEA guidelines. A health surveillance program for screening incoming animals should be carried out to assess animal quality. Methods of transportation should also be taken into account. Each consignment of animals should be inspected for compliance with procurement specifications and the animals should be quarantined and stabilized according to procedures appropriate for the species and circumstances.

QUARANTINE

Quarantine is the separation of newly received animals from those already in the facility until the health and possibly the microbial status of the newly received animals have been determined. An effective quarantine minimizes the chance for introduction of pathogens into an established colony. The duration at quarantine in small lab animals from one week to one month.

STABILIZATION

Regardless of the duration of quarantine, newly received animals should be given a period for physiologic, psychologic and nutritional stabilization before their use. The length of time stabilization will depend on the type and duration of animal transportation, the species involved and the intended use of the animals.

SEPARATION

Physical separation of animals by species is recommended to prevent interspecies disease transmission and to eliminate anxiety and possible physiological and behavioural changes due to interspecies conflict. Such separation is usually accomplished by housing different species in separate rooms, cubicles or cages. If two species have a similar pathogen status and are behaviourally compatible, it shall be acceptable to house different species in the same room.

People should be restricted from entering in to the facilities unless otherwise required and after handling these animals they should not be handling any other animals in the facilities.

SURVEILLANCE, DIAGNOSIS, TREATMENT AND CONTROL OF DISEASE

All animals should be observed for signs of illness, injury or abnormal behaviour by animal house staff daily, but more-frequent observations might be warranted, during postoperative recovery or when animals are ill or have a physical deficit. It is imperative that appropriate methods be in place for disease surveillance and diagnosis.

Post mortem examination and signs of illness, distress, or other deviations from normal health condition in animals should be reported promptly to ensure appropriate and timely delivery of veterinary medical care. Animals that show signs of a contagious disease should be isolated from healthy animals in the colony. If an entire room of animals is known or believed to be exposed to an infectious, the group should be kept intact and isolated during the process of diagnosis, treatment and control. Diagnostic clinical laboratory may be made available.

ANIMAL CARE AND TECHNICAL PERSONNEL

Institutions should employ people trained in laboratory animal science or provide for both formal and on-the-job training to ensure effective implementation of the program.

PERSONAL HYGIENE

It is essential that the animal care staff maintain a high standard of personal cleanliness by using appropriate Personnel Protective Equipment (PPE) e.g. change of uniforms, footwear etc.

Clothing suitable for use in the animal facility should be supplied and laundered by the institution. A commercial laundering service is acceptable in many situations. It is acceptable to use disposable gloves, masks, head covers, coats, coveralls and shoe covers. Personnel should change clothing as often as is necessary to maintain personal hygiene. Outer garments worn in the animal rooms should not be worn outside the animal facility.

Washing facilities appropriate to the program should be available. Personnel should not be permitted to eat, drink, smoke or apply cosmetics and perfumes in animal rooms. They should finish the work with animals as early as possible and sit somewhere else outside and not in the animal rooms / areas.

MULTIPLE SURGICAL PROCEDURES ON SINGLE ANIMAL

Multiple surgical procedures on a single animal for any testing or experiment are not to be practiced unless specified in a protocol only approved by the IAEC.

DURATIONS OF EXPERIMENTS

No animal should be used for experimentation for more than 3 years unless adequate justification is provided.

PHYSICAL RESTRAINT

Brief physical restraint of animals for examination, collection of samples and a variety of other clinical and experimental manipulations can be accomplished manually or with devices be suitable in size and design for the animal being held and operated properly to minimize stress and avoid injury to the animal.

Important guidelines for the use of restraint equipment:

Restraint devices cannot be used simply as a convenience in handling or managing animals.

The period of restraint should be the minimum required to accomplish the research objectives.

Animals to be placed in restraint devices should be given training to adapt to the equipment.

Provision should be made for observation of the animal at appropriate intervals. Veterinary care should be provided if lesions or illness associated with restraint are observed. The presence of lesions, illness, or severe behavioural change should be dealt with by the temporary or permanent removal of the animal from restraint.

PHYSICAL FACILITIES

The physical condition, design and size of an animal facility depend on the scope of institutional research activities, animals to be housed, physical relationship to the rest of the institution and geographic location. A well planned, properly maintained facility is an important element in good animal care.

LOCATION OF ANIMAL FACILITIES TO LABORATORIES

Good animal husbandry and human comfort and health protection require physical separation of animal facilities from personnel areas such as offices, break room, training and education room.

1. Laboratory animals are very sensitive to their living conditions. It is important that they shall be housed in an isolated building located as far away from human habitations as possible and not exposed to dust, smoke, noise, wild rodents, insects and birds.

2. This separation can be accomplished by having the animal quarters in a separate building, wing, floor, or room. Careful planning should make it possible to place animal housing areas adjacent to or near laboratories, but separated from them by barriers such as entry locks, corridors, or floors.

3. While planning an animal facility the space should be well divided for various activities. The animal rooms should occupy about 50-60% of the total constructed area and the remaining area should be utilized for services such as stores, washing, office and staff, machine rooms, quarantine and corridors. The environment of animal room (Macro-Environment) and animal cage (Microenvironment) are factors on which the production and experimental efficiency of the animal depends. Since animals are very sensitive to environmental changes, sharp fluctuations in temperature, humidity, light, sound and ventilation should be avoided.

FUNCTIONAL AREAS

Sufficient animal area required to:

- Ensure separation of species or isolation of individual projects when necessary
- Receive, quarantine and isolate animals
- Provide for animal housing
- Specialized laboratories
- Receiving and storage areas for food, bedding
- Pharmaceuticals and biologics and supplies
- Space for administration, supervision and direction of the facility
- An area for washing and sterilization equipment and supplies
- An autoclave for equipment
- Food and bedding and separate areas
- For holding soiled and cleaned equipment
- An area to store wastes prior to incineration or removal

PHYSICAL FACILITIES

Building materials should be selected to facilitate efficient and hygienic operation of animal facilities. Durable, moisture-proof, fire-resistant, seamless materials are most desirable for interior surfaces including vermin and pest resistance.

Corridor(s) should be wide enough to facilitate the movement of personnel as well as equipment and should be kept clean.

Utilities such as water lines, drain pipes, and electrical connections should preferably be accessible through service panels or shafts in corridors outside the animal rooms.

ANIMAL ROOM DOORS

Doors should not be rust, vermin and dust proof. They should fit properly within their frames and provided with an observation window. Door closures may also be provided. Rodent barriers can be provided in the doors of the small animal facilities.

EXTERIOR WINDOWS

Windows are not recommended for small animal facilities. However, where power failures are frequent and backup power is not available, they may be necessary to provide alternate source of light and ventilation.

FLOORS

Floors should be either monolithic or epoxy smooth, moisture proof, non-absorbent, skid-proof, resistant to wear, acid, solvents, adverse effects of detergents and disinfectants. They should be capable of supporting racks, equipment, and stored items without becoming gouged, cracked, or pitted, with minimum number of joints. A continuous moisture-proof membrane might be needed. If sills are installed at the entrance to a room, they should be designed to allow for convenient passage of equipment.

DRAINS

Floor drains are not essential in all rooms used exclusively for housing rodents. Floor in such rooms can be maintained satisfactorily by wet vacuuming or mopping with appropriate disinfectants or cleaning compounds. Where floor drains are used, the floors should be sloped and drain taps kept filled with water or corrosion free mesh. To prevent high humidity, drainage must be adequate to allow rapid removal of water and drying of surfaces. At the inlet and outlets of the drains should be fitted with wire mesh guard to prevent wild rodent entry

WALLS & CEILINGS

Walls should be free of cracks, unsealed utility penetrations, or imperfect junctions with doors, ceilings, floors and corners. Surface materials should be capable of withstanding scrubbing with detergents, disinfectants and the impact of water under high pressure.

STORAGE AREAS

Separate storage areas should be designed for feed, bedding, cages and materials not in use. Refrigerated storage, separated from other cold storage, is essential for storage of dead animals and animal tissue waste.

FACILITIES FOR SANITIZING EQUIPMENT AND SUPPLIES

An area for sanitizing cages and ancillary equipment is essential with adequate water supply

EXPERIMENTAL AREA

All experimental procedures in small animals should be carried out in a separate area away from the place where animals are housed.

ENVIRONMENT

Temperature and Humidity Control:

Air conditioning is an effective means of regulating these environmental parameters for laboratory animals. Temperature and humidity control prevents variations due to changing climatic conditions keeping in view of the variations in the number of room occupants the range should be within or approximately between 18 to 29°C (64.4 to 84.2oF) all times. The relative humidity should be under control within the range of 30% to 70% throughout the year. During extreme summer appropriate methods e.g. sprinklers should be adopted for cooling.

Ventilation:

In renovating existing or in building new animal facilities, consideration should be given to the ventilation of the animals' primary enclosures. Heating, ventilation, and air-conditioning systems should be designed with 12-15 air cycles per hour so that operation can be continued with a standby system. The animal facility and human occupancy areas should be ventilated separately.

Power and Lighting:

The electrical system should be safe and provide appropriate lighting and with sufficient number of power points lighting system be installed provide adequate illumination for people to work in the animal rooms and a lowered intensity of light for the animals. A time-controlled lighting system should be used to ensure a regular diurnal lighting cycle wherever required. Emergency power should be available in the event of power failure.

Noise Control:

The facility should be provided with noise free environment. Noise control is an important consideration in designing the animal facility. Concrete walls are more effective than metal or plaster walls because their density reduces sound transmission. Preferably less than 85 dB is desirable for rodents.

ANIMAL HUSBANDRY

Caging or Housing System

The caging or housing system is one of the most important elements in the physical and social environment of research animals. It should be designed carefully to facilitate animal wellbeing, meet research requirements, and minimize experimental variables.

The housing system should:

- Provide space that is adequate, permit freedom of movement and normal postural adjustments, and have a resting place appropriate to the species
- Provide a comfortable environment
- Provide an escape proof enclosure that confines animal safety
- Provide easy access to food and water
- Provide adequate ventilation
- Meet the biological needs of the animals, e.g., maintenance of body temperature, urination, defecation, and reproduction
- Keep the animals dry and clean, consistent with species requirements
- Facilitate research while maintaining good health of the animals.

They should be constructed of sturdy, durable materials and designed to minimize cross-infection between adjoining units. Polypropylene, polycarbonate and stainless steel cages should be used to house small lab animals. To simplify servicing and sanitation, cages should have smooth, impervious surfaces that neither attract nor retain dirt and a minimum number of ledges, angles, and corners in which dirt or water can accumulate. The design should allow inspection of cage occupants without disturbing them. Feeding and watering devices should be easily accessible for filling, changing, cleaning and servicing. Cages, runs and pens must be kept in good condition to prevent injuries to animals, promote physical comfort, and facilitate sanitation and servicing. Particular attention must be given to eliminate sharp edges and broken wires, keeping cage floors in good condition.

ACTIVITY

Provision should be made for animals with specialized locomotor pattern to express their natural habitat, especially when the animals are held for long periods. Cages are often used for short-term (up to 3 months) housing for postsurgical care, isolation of sick animal and metabolic studies.

FOOD

Animals should be fed with palatable, non-contaminated, and nutritionally adequate food daily unless the experimental protocol requires otherwise. Feeders should allow easy access, while avoiding contamination by urine and feces. Food should be provided in sufficient amounts to ensure normal growth in immature animals and to maintain normal body weight, reproduction, and lactation in adults. Food should contain adequate nutrition, with proper formulation and preparation; and ensure free from chemical and microbial contaminants; bioavailability of nutrients should be at par with the nutritional

requirements of the animal. The animal feed should contain moisture, crude fibre, crude protein, essential vitamins, minerals, crude fat and carbohydrate for providing appropriate nutrition. Diet should be free from heavy metals (e.g., Lead, Arsenic, Cadmium, Nickel, Mercury), naturally occurring toxins and other contaminants. Areas in which diets are processed or stored should be kept clean and enclosed to prevent entry of insects or other animals. Exposure to extremes of relative humidity, unsanitary conditions, light, oxygen, and insects hasten the deterioration of food. Food hoppers should not be transferred from room to room unless cleaned and properly sanitized.

BEDDING

Bedding should be absorbent, free from toxic chemicals or other substances that cause irritation, injure animals or personnel, and of a type not readily eaten by animals. Bedding should be used in amounts sufficient to keep animals dry between cage changes without coming into contact with watering tubes.

Bedding should be removed and replaced periodically with fresh materials as often as necessary to keep the animals clean and dry. The frequency is a matter of professional judgement of animal care personnel in consultation with the investigation depending on the number of animals and size of cages. In general it is ideal to change the bedding twice a week or whenever requires.

The desirable criteria for rodent contact bedding is ammonia binding, sterillizable, deleterious products not formed as a result of sterilization, easily stored, non - desiccating to the animal, uncontaminated, unlikely to be chewed or mouthed, non - toxic, non - malodorous, nestable, disposable by incineration, readily available, remains stable during use, optimizes normal animal behaviour, non - deleterious to cage - washers, non - injurious and non - hazardous to personnel, non - nutritious and non - palatable. Nesting materials for newly delivered pups should be provided wherever needed (e.g. Paper cuttings, tissue paper, cotton etc.).

WATER

Animals should have continuous access to fresh, potable, uncontaminated drinking water, according to their requirements. Periodic monitoring of microbial contamination in water is necessary. Watering devices, such as drinking nozzles and automatic water should be examined routinely to ensure their proper operation. Sometimes it is necessary to train animals to drink water from automatic watering devices. It is better to replace fresh water bottles every day than to refill them, however, if bottles are to be refilled, care should be taken that each bottle is replaced on the cage properly from where it was removed.

SANITATION AND CLEANLINESS

Sanitation is an essential activity in an animal facility. Animal rooms, corridors, storage spaces, and other areas should be properly cleaned with appropriate detergents and disinfectants as often as necessary to keep them free of dirt, debris, and harmful agents of contamination. Cleaning utensils, such as mops, pails and brooms, should not be transported between animal rooms. Where animal waste is removed by hosing or flushing, this should be done at least twice a day. Animals should be kept dry during such procedures.

Cages should be sanitized before animals are placed in them. Animal cages, racks and accessory equipment, such as feeders and watering devices, should be washed and sanitized frequently to keep them clean and contamination free. Generally this can be achieved by washing solid bottom rodent cages and accessories once or twice a week and cages, racks at least monthly. Wire - bottom cages other than

rodent cages should be washed at least every 2 weeks. It is good practice to have extra cages available at all times so that a systematic cage-washing schedule can be maintained. Cages can be disinfected by rinsing at a temperature of 82.2^{0C} (180^{oF}) or higher for a period long enough to ensure the destruction of vegetative pathogenic organisms.

Disinfection can also be accomplished with appropriate chemicals. Equipment should be rinsed free of chemicals prior to use. Periodic microbiologic monitoring is useful to determine the efficacy of disinfection or sterilization procedures. Rabbits and some rodents, such as guinea pigs, mice and hamsters, produce urine with high concentration of proteins ammonia and minerals. Minerals and organic compounds in the urine from these animals often adhere to cage surfaces and necessitate treatment with acid solutions before washing.

Water bottles, sipper nozzles stoppers, and other watering equipment should be washed and then sanitized by rinsing with water of at least 82.2^{oC} (180^{oF}) or appropriated chemicals agents (e.g. Sodium Hyperchlorite) to destroy pathogenic organisms, if bottles are washed by hand, mechanized brushes at the washing sink are useful, and provision should be made for dipping or soaking the water bottles in detergents and disinfectant solutions. A two – compartment sink or tub is adequate for this purpose.

ASSESSING THE EFFECTIVENESS OF SANITATION

Sanitation practices should be monitored appropriately to ensure effectiveness of the process and materials being cleaned; it can include visual inspection of the materials, monitoring of water temperatures, or microbiologic monitoring. The intensity of animal odours particularly that of ammonia should not be used as the sole means of assessing the effectiveness of the sanitation program.A decision to change the frequency of such bedding changes or cage washing should be based on factors such as the concentration of ammonia, appearance of the cage, condition of the bedding and number and size of the animals housed in the cage. Autoclaving: Chemical Indicator - batch wise assessment; Biological indicator -Periodical assessment.

WASTE DISPOSAL

Wastes should be removed regularly and frequently. All waste should be collected and disposed of in a safe and sanitary manner. The most preferred method of waste disposal is incineration. Incinerators should be in compliance with all central, state, and local Public Health and Pollution Control Board regulations.

Waste containers containing animal tissues, carcasses, and hazardous wastes should be lined with leak - proof, disposable liners. If wastes must be stored before removal, the waste storage area should be separated from other storage facilities and free of flies, cockroaches, rodents, and other vermin. Cold storage might be necessary to prevent decomposition of biological wastes.

PEST CONTROL

Adaptation of Programs designed to prevent, control, or eliminate the presence of or infestations by pests are essential in an animal home environment.

EMERGENCY, WEEKEND AND HOLIDAY CARE

Animals should be cared for by qualified personnel every day, including weekends and holidays, to safeguards their well - being including emergency veterinary care. In the event of an emergency,

institutional security personnel and fire or police officials should be able to reach people responsible for the animals. That can be enhanced by prominently posting emergency procedures, names, or telephone numbers in animal facilities or by placing them in the security department or telephone centre. A disaster plan that takes into account both personnel and animals should be prepared as part of the overall safety plan for the animal facility.

RECORD KEEPING

The Animal House should maintain following records:

- Animal House plans, which includes typical floor plan, all fixtures etc.
- Animal House staff record - both technical and non - technical
- Health record of staff animals
- All SOPs relevant to the animals
- Breeding, stock, purchase and sales records
- Minutes of institute Animals Ethics Committee Meetings
- Records of experiments conducted with the number of animals used (copy of Form D)
- Death Record
- Clinical record of sick animals
- Training record of staff involved in animal activities
- Water analysis report

STANDARD OPERATING PROCEDURES (SOPS) *I* GUIDELINES

The Institute shall maintain SOPs describing procedures / methods adapted with regard to Animal Husbandry, maintenance, breeding, animal house microbial analysis and experimentation records.

A SOP should contain the following items:

- Name of the Author
- Title of the SOP
- Date of preparation
- Reference of previous SOP on the same subject and date (Issue no and Date)
- Location and distribution of Sops with sign of each recipient
- Objectives
- Detailed information of the instruments used in relation with animals with methodology (Model no., Serial no., Date of commissioning, etc)

- The name of the manufacturer of the reagents and the methodology of the analysis pertaining to animals
- Normal value of all parameters
- Hazard identification and risk assessment

PERSONNEL AND TRAINING

The selection of animal facility staff, particularly the staff working in animal rooms or involved in transportation, is a critical component in the management of an animal facility. The staff must be provided with all required protective clothing (masks, aprons, gloves, gumboots, other footwears etc.) while working in animal rooms. Facilities should be provided for change over with lockers, wash basin, toilets and bathrooms to maintain personal hygiene. It is also important a regular medical check-up is arranged for the workers to ensure that they have not picked up any zoonotic infection and also that they are not acting as a source of transmission of infection to the animals. The animal house in-charge should ensure that persons working in animal house don't eat, drink, smoke in animal room and have all required vaccination, particularly against tetanus and other zoonotic diseases. Initial in-house training of staff at all levels is essential. A few weeks must be spent on the training of the newly recruited staff, teaching them the animal handling techniques, cleaning of cages and importance of hygiene, disinfection and sterilization. They should also be made familiar with the activities of normal healthy and sick animals so that they are able to spot the sick animal during their daily routine check up for cages.

TRANSPORT OF LABORATORY ANIMALS

The transport of animals from one place to another is very important and must be undertaken with care. The main considerations for transport of animals are, the mode of transport, the containers, animal density in cages, food and water during transit, protection from transit infections, injuries and stress.

The mode of transport of animals depends on the distance, seasonal and climatic conditions and the species of animals. Animals can be transported by road, rail or air taking into consideration of above factors. In any case the transport stress should be avoided and the containers should be of an appropriate size so as to enable these animals to have a comfortable, free movement and protection from possible injuries. The food and water should be provided in suitable containers or in suitable form so as to ensure that they get adequate food and more particularly water during transit. The transport containers (cages or crates) should be of appropriate size and only a permissible number of animals should only be accommodated in each container to avoid overcrowding and infighting

ANAESTHESIA AND EUTHANASIA

The scientists should ensure that the procedures, which are considered painful, are conducted under appropriate anaesthesia as recommended for each species of animals. It must also be ensured that the anaesthesia is given for the full duration of experiment and at no stage the animal is conscious to perceive pain during the experiment. If at any stage during the experiment the investigator feels that he has to abandon the experiment or he has inflicted irreparable injury, the animal should be sacrificed. Neuromuscular blocking agents must not be used without adequate general anaesthesia. In the event of a decision to sacrifice an animal on termination of an experiment or otherwise an approved method of euthanasia should be adopted and the investigator must ensure that the animal is clinically dead before it is sent for disposal.

Anaesthesia

Unless contrary to the achievement of the results of study, sedatives, analgesics and anaesthetics should be used to control pain or distress under experiment. Anaesthetic agents generally affect cardiovascular, respiratory and thermoregulatory mechanism in addition to central nervous system.

Before using actual anaesthetics the animals is prepared for anaesthesia by overnight fasting and using pre-anaesthetics, which block parasympathetic stimulation of cardiopulmonary system and reduce salivary secretion. Atropine is most commonly used anti-cholinergic agent. Local or general anaesthesia may be used, depending on the type of surgical procedure.

Euthanasia

Euthanasia is resorted to events where an animal is required to be sacrificed on termination of an experiment or otherwise for ethical reasons. The procedure should be carried out quickly and painlessly in an atmosphere free from fear or anxiety. For accepting an euthanasia method as humane it should have an initial depressive action on, the central nervous system for immediate insensitivity to pain. The choice of a method will depend on the nature of study, the species of animal to be killed. The method should in all cases meet the following requirements:

a. Death, without causing anxiety, pain or distress with minimum time lagphase

b. Minimum physiological and psychological disturbances.

c. Compatibility with the purpose of study and minimum emotional effect on the operator.

d. Location should be separate from animal rooms and free from environmental contaminants.

LABORATORY ANIMAL ETHICS

All scientists working with laboratory animals must have a deep ethical consideration for the animals they are dealing with. From the ethical point of view it is important that such considerations are taken care at the individual level, at institutional level and finally at the national level.

MAINTENANCE

Housing, feeding, ventilation, lighting, sanitation and routine management practices for such animals are similar to these for the other animals of the species as given in guidelines. However, special care has to be taken with transgenic/gene knockout animals where, the animals can become susceptible to diseases where special conditions of maintenance are required due to the altered metabolic activities. The transgenic and knockout animals carry additional genes or lack genes compared to the wild population. To avoid the spread of the genes in wild population care should be taken to ensure that these are not inadvertently released in the wild to prevent cross breeding with other animals. The transgenic and knockout animals should be maintained in clean room environment or in animal isolators.

DISPOSAL

A record of animal disposal and the manner of disposal should be kept as a matter of routine.

Experiment No. 6

OBJECT:

To study the common laboratory technique: Methods of Blood Collection in Mouse

REFERENCES:

Janet Hoff, LVT, RLATG. TECHNIQUE: Methods of Blood Collection in the Mouse. Lab Animal, 29 (10); 47-53: 2000.

REQUIREMENTS:

Clippers, petroleum jelly, heating pad, 23–27 gauge needle, Pasteur pipette

THEORY:

Collecting blood from mice is necessary for a wide variety of scientific studies and there are a number of efficient methods available. It is important to remember that blood collection, because it can stress the animals, may have an impact on the outcome of research data. In addition, it is extremely important that those who collect blood become skilled in the techniques they employ and seek to stress the mice as little as possible. Drawing from experience, personal communication and published resources1-4, the following techniques are described:

- Blood collection not requiring anesthesia:
  - –Saphenous vein, Dorsal pedal vein
- Blood collection requiring anesthesia:
  - –Tail vein, Orbital sinus, Jugular vein
- Terminal procedures:
  - –Cardiac puncture, Posterior vena cava, Axillary vessels, Orbital sinus

Methods of Blood Collection Not Requiring Anesthesia

Blood Collection from the Saphenous Vein:

- Warming the mouse immediately prior to blood collection will increase blood flow considerably.
- Place a lamp over the cage for five minutes or place the cage on a heating pad, on the lowest setting.
- Place the mouse in a restraining tube so its head is covered and its hind legs are free. Grasp the fold of skin between the tail and thigh (**Fig. 1**).
- The saphenous vein is found on the caudal surface of the thigh.
- Remove hair from the area with clippers. Apply petroleum jelly (**Fig. 2**) or eye lubricant to prevent migration of blood into the surrounding hair and place a tourniquet around the leg, above the knee (**Fig. 3**).
- Puncture the vein with a 25 gauge needle. Collect drops of blood as they appear.
- The use of collection tubes with capillary action will facilitate blood collection.
- Apply pressure or use a cauterizing agent such as a styptic pencil (silver nitrate) to stop the bleeding.

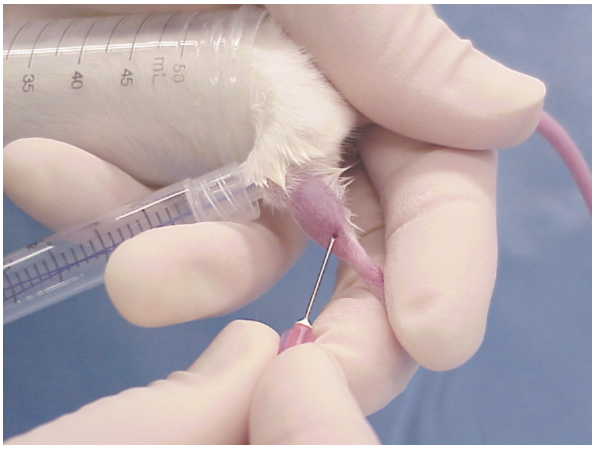

FIGURE 1: Blood collection from the saphenous vein.

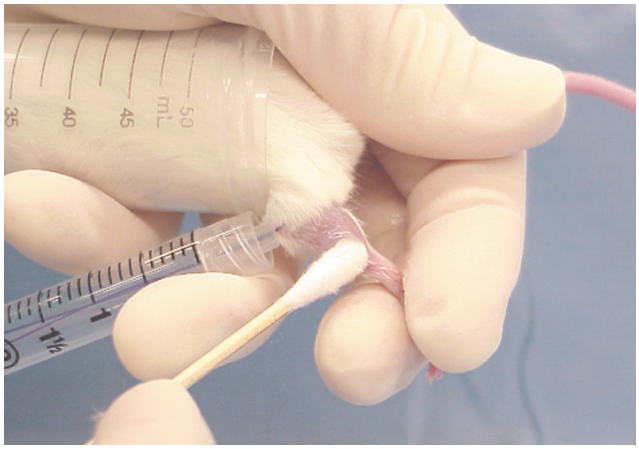

FIGURE 2: Application of petroleum jelly to the blood source.

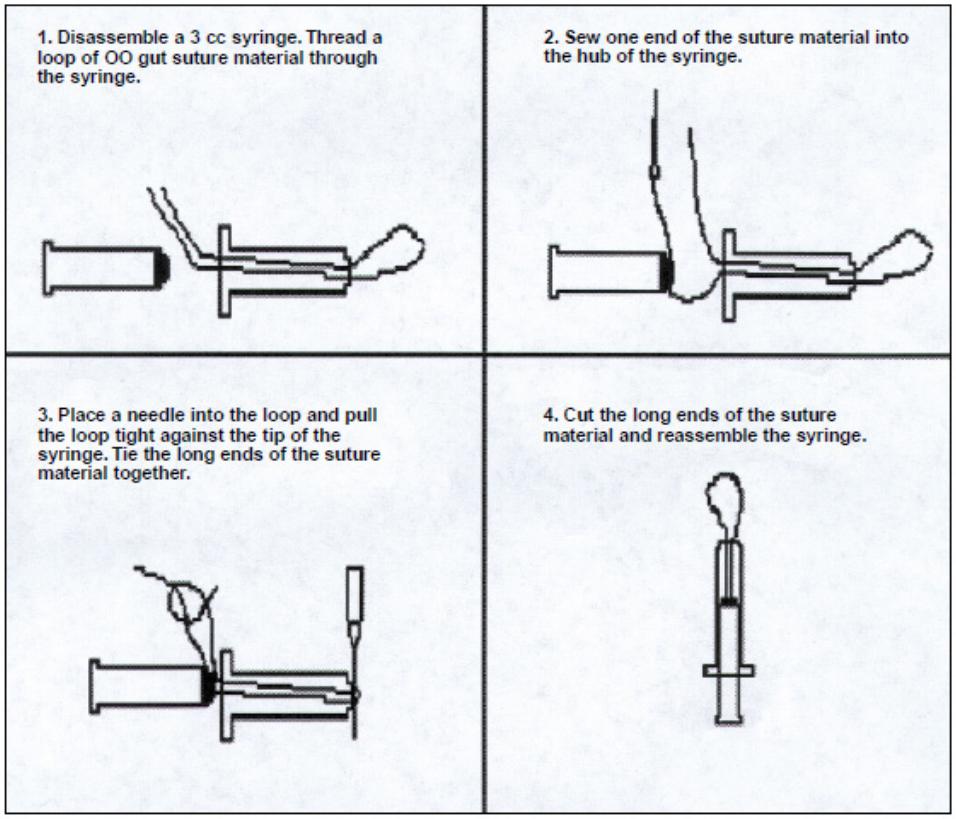

FIGURE 3. Instructions for making a mouse-sized tourniquet.

Blood Collection from the Top of the Foot:

- Warm the mouse and place it in a restraining tube as described above.
- With your thumb and first finger, hold a hind foot around the ankle (**Fig. 4**).
- Your thumb should be on top of the foot.
- The medial dorsal pedal vessel is found on the top of the foot.
- Apply petroleum jelly or eye lubricant to the foot.
- Puncture the vein with a 23–27 gauge needle.
- As drops of blood appear collect them in a capillary tube.
- Apply pressure or use a cauterizing agent such as a styptic pencil (silver nitrate) to stop the bleeding.

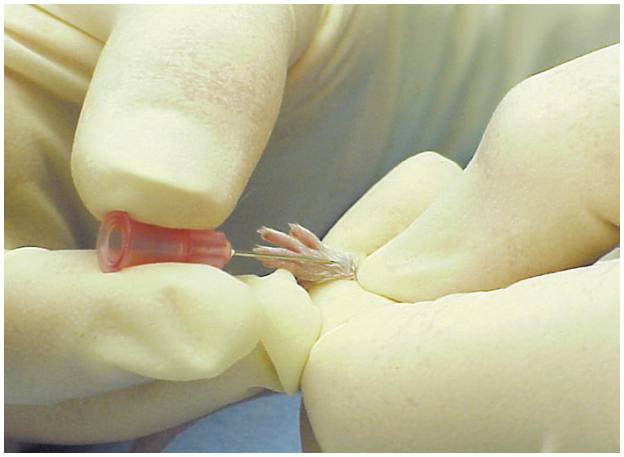

FIGURE 4: Blood collection from the dorsal pedal vein.

FIGURE 5: Retro-orbital blood collection.

Methods of Blood Collection Requiring Anesthesia

Mice can be anesthetized for a short period of time using a drop jar with a tight-fitting lid or a Ziploc bag containing an appropriate amount of anesthetic. When placed in the container, the mouse should become sufficiently anesthetized to perform procedures, without overdosing from the anesthetic.

Blood Collection from the Tail:

- Warm the mouse and place it in a restraining tube as described above.
- Do not attempt to increase blood flow by rubbing the tail from the base to the tip, as this will result in leukocytosis (increased white blood cell count).
- Using a scalpel, straight edge razor, or sharp scissors, quickly removes up to 1 cm of the tail.
- Collect blood in a capillary tube as drops appear.
- Apply pressure or use a cauterizing agent such as a styptic pencil (silver nitrate) to stop the bleeding.
- When several samples are needed within a short time period, the original wound can be reopened by removing the clot.
- When additional samples are needed at a later date, blood samples can be obtained by removing just 2-3 mm of additional tail.
- Cutting the tail too short may result in trauma to the cartilage and ultimately to the coccygeal vertebrae.

BLOOD COLLECTION FROM THE ORBITAL SINUS:

- Lay the anesthetized mouse on its side on a table or hold it in your hand with its head pointing down (**Fig. 5**).
- With your first finger and thumb (finger above and thumb below the eye) pull the skin away from the eyeball, above and below the eye, so that the eyeball is protruding out of the socket as much as possible.
- Take care not to occlude the trachea with your thumb.
- Insert the tip of a fine-walled Pasteur pipette (o.d. of 1-2 mm) or a microhematocrit blood tube into the corner of the eye socket underneath the eyeball, directing the tip at a 45-degree angle toward the middle of the eye socket.
- Rotate the pipette between your fingers during forward passage; do not move it from side to side or front to back.
- Apply gentle downward pressure and then release until the vein is broken and blood is visualized entering the pipette.
- When a small amount of blood begins filling the pipette, withdraw slightly and allow the pipette to fill. Do not let the pipette come out of the eye socket.
- If the pipette is not withdrawn slightly, it may occlude the vein and blood will not flow freely.
- Cover the open end of the pipette with the tip of your finger before removing it from the orbital sinus to prevent blood from spilling out of the tube.
- Bleeding usually stops immediately and completely when the pipette is removed.
- It may be necessary to apply gentle pressure on the eyeball for a brief moment by closing the skin above and below the eye using your first finger and thumb.
- It is recommended that sample collection not be repeated on the same eye for at least two weeks.

Caution: Blindness can occur if the optic nerve is damaged as a result of the blood collection tube coming into contact with the nerve, which attaches to the middle of the ventral surface of the eye. Ocular ulcerations, puncture wounds, loss of vitreous humor, infection, or keratitis may occur as a result of poor technique or uncontrolled movement of the animal.

BLOOD COLLECTION FROM THE JUGULAR VEIN:

Restrain the anesthetized mouse by attaching a loop of string to a gauze square and looping it around the upper incisors.

Pull the head up and back while pulling the gauze across the back of the hand and lock the gauze between two fingers (**Fig. 6**). It may be helpful to place the mouse in your lap.

Wet the fur with alcohol or shave the neck area. In this hyperextended position, the jugular veins appear blue and are found 2-4 mm lateral to the sternoclavicular junction (**Fig. 7**).

Using a 1 ml syringe and 25 gauge needle, approach the vessel in a caudocephalic direction (from back to front).

Insert the needle 1-3 mm deep, 2-4 mm lateral of the sternoclavicular junction, over the sternum; include a small amount of muscle from the sternum to stabilize the needle.

Hold the needle very still when blood enters the syringe (**Fig. 8**).

Withdraw blood slowly to avoid collapse of these small vessels.

If the first attempt to draw blood is unsuccessful, withdraw the needle slightly; it may have been placed too deeply.

If blood stops flowing, do not continue to draw back on the syringe. The vein may have collapsed or the needle may have attached to the vessel wall.

Rotate the needle slightly or apply slight pressure on the needle (either above, below, or to the side of puncture site).

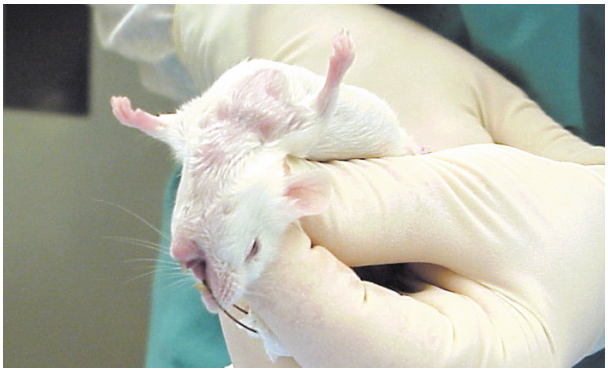

FIGURE 6: Proper positioning of mouse for blood collection from the jugular vein.

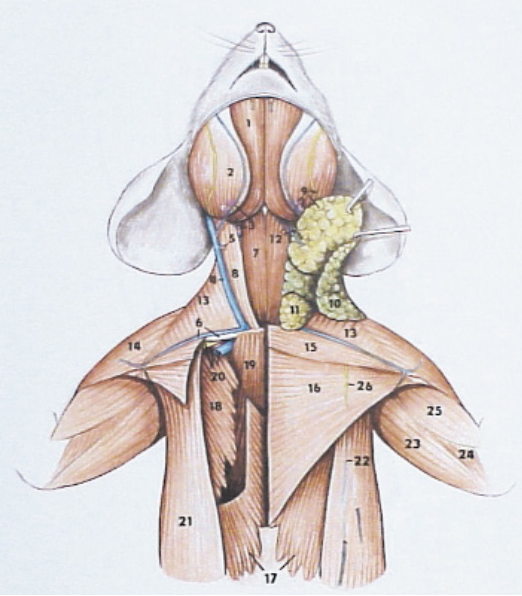

FIGURE 7: Location of the jugular vein in the mouse.

FIGURE 8: Blood collection from the jugular vein.

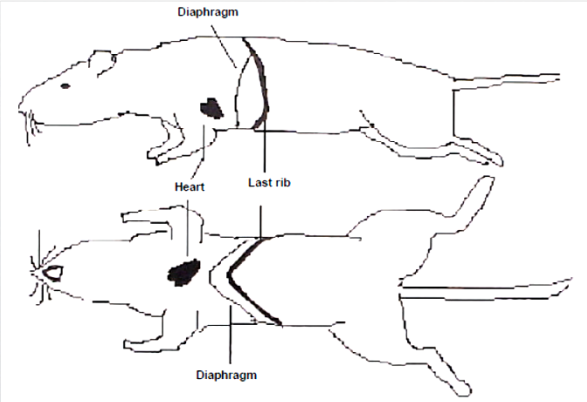

FIGURE 9: Relative locations of the heart and diaphragm in the mouse thorax.

TERMINAL METHODS OF BLOOD COLLECTION

Requiring Deep Anesthesia

After performing a terminal blood collection, always be certain that the animal is dead before placing the carcass in the freezer. Remember that the animal will stop breathing before the heart stops. Either perform a bilateral pneumothorax (puncturing both sides of the thorax) or wait for the animal to become rigid.

Blood Collection by Cardiac Puncture:

Three possible approaches:

First:

- Hold the mouse by the scruff of skin above the shoulders so that its head is up and its rear legs are down.
- Use a 1 ml syringe and a 22 gauge needle.
- Insert needle 5 mm from the center of the thorax towards the animal's chin, 5-10 mm deep, holding the syringe 25-30 degrees away from the chest (**Fig. 9**).

Second:

- Lay animal on back and push syringe vertically through sternum.

Third:

- Lay animal on side and insert the needle perpendicular to chest wall.
- If blood doesn't appear immediately, withdraw 0.5 cc of air to create a vacuum in the syringe.
- Withdraw the needle without removing it from under the skin and try a slightly different angle or direction.
- When blood appears in the syringe, hold it still and gently pull back on the plunger to obtain the maximum amount of blood available.
- Pulling back on the plunger too much will cause the heart to collapse.
- If blood stops flowing, rotate the needle or pull it out slightly.

Blood Collection from the Posterior Vena Cava:

- Open the abdominal cavity of anesthetized mouse by making a V-cut through the skin and abdominal wall 1 cm caudal to the rib cage.
- Shift the intestines over to the left and push the liver forward.
- Locate the widest part of the posterior vena cava (between the kidneys).

- Use a 23-25 gauge needle and a 1 ml syringe.
- Carefully insert the needle into the vein and draw blood slowly until the vessel wall collapses.
- Pause to allow the vein to refill and then repeat three or four times or until no more blood is available.

Blood Collection from the Axillary Vessels:

- Lay the anesthetized mouse on its back.
- Stretch out a forelimb and pin the front foot.
- Make a deep incision in the axilla (armpit) at the side of the thorax.
- Hold the skin at the posterior part of the incision using forceps to create a cupped area.
- Incise the blood vessels in the area with a scalpel or straight edge razor and collect blood as it pools.
- It may be important to consider that tissue fluids will contaminate the blood sample.

Blood Collection from the Orbital Sinus:

- Quickly remove the eyeball from the socket with a pair of tissue forceps.
- Hold the mouse in the palm of your hand over a collection tube.
- Massage the body of the mouse with your hand by squeezing the rear half, then the middle, then the head of the mouse so that you are milking the blood toward the eye.
- When done properly, large drops of blood will flow from the orbital sinus.

VIVA QUESTIONS:

Q.-1. Define blood.

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Q.-2. Explain various types of veins present in mice.

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Q.-3. What is the need of blood collection.

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Experiment No. 7

OBJECT:

Study of common laboratory techniques: Serum and plasma separation.

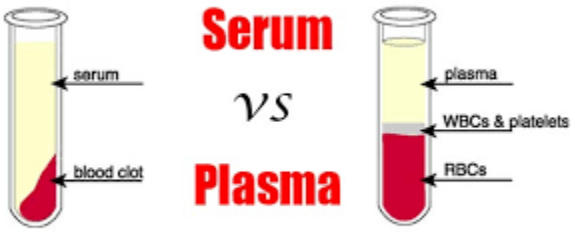
REFERENCE:

https://www.proimmune.com/ecommerce/pdf_files/PR31.pdf

THEORY:

Blood plasma is the liquid component of blood, in which the blood cells are suspended. It makes up about 60% of total blood volume. It is composed of mostly water (90% by volume) and contains dissolved proteins, glucose, clotting factors, mineral ions, hormones and carbon dioxide. Plasma is the supernatant fluid obtained when anti-coagulated blood has been centrifuged. The blood is mixed with an appropriate amount of anticoagulant like heparin, oxalate or ethylene diamine tetra acetic acid (EDTA). This preparation should be mixed immediately and thoroughly to avoid clotting.

Serum is the liquid fraction of whole blood that is collected after the blood is allowed to clot. Serum is clearer than plasma because of fewer proteins. The clot is removed by centrifugation and the resulting supernatant, designated serum, is carefully removed using a Pasteur pipette. Proteins are sometimes considered as interfering substances in some tests as they react with the reagent and thereby yield inaccurate results. Serum is the preferred specimen in clinical testing as the interference that may be caused by a plasma specimen because of the presence of an anticoagulant, is eliminated.



Serum = Plasma – Clotting Factors

Blood Plasma Preparation

Materials and Equipment

Human blood sample.

Vacutainer tubes containing anticoagulant (e.g. BD Vacutainer plastic EDTA tube, 10 ml, lavender top)

Serological pipettes of appropriate volumes (sterile)

Centrifuge tubes

Cryovials

Benchtop centrifuge (NOT refrigerated) with swing-out rotor and appropriate carriers

Procedure:

Draw blood into vacutainer tube(s) containing 1.8 mg K2EDTA per ml blood (may vary depending on manufacturer). Be sure to draw the full volume to ensure the correct blood-to-anticoagulant ratio.

Invert vacutainer tubes carefully 10 times to mix blood and anticoagulant and store at room temperature until centrifugation.

Samples should undergo centrifugation immediately. This should be carried out for a minimum of 10 minutes at 1000-2000 rpm at room temperature. Do not use brake to stop centrifuge.

This will give three layers: (from top to bottom) plasma, leucocytes (buffy coat) and erythrocytes.

Carefully aspirate the supernatant (plasma) at room temperature and pool in a centrifuge tube. Take care not to disrupt the cell layer or transfer any cells.

Inspect plasma for turbidity. Turbid samples should be centrifuged and aspirated again to remove remaining insoluble matter.

Aliquot plasma into cryo vials and store at –80°C. Ensure that the cryovials are adequately labeled with the relevant information, including details of additives present in the blood.

Blood Serum Preparation

Materials and Equipment

- Human blood sample
- Vacutainer tubes (containing either no additive or a clot activator)

1. Clot activator and silica gel:

- e.g. BD Vacutainer Plus plastic serum tube, 10 ml, red top
- e.g. BD Vacutainer Plus plastic serum tube (transport tube), 10 ml, mottled red/grey top

2. No additive:

- e.g. BD Vacutainer Plus tube with clear BD Hemogard closure, 3 ml, clear top
- Serological pipettes of appropriate volumes (sterile)
- Centrifuge tubes
- Cryovials
- Benchtop centrifuge (NOT refrigerated) with swing-out rotor and appropriate carriers

Procedure:

Draw whole blood into vacutainer tube(s) containing no anticoagulant. Draw approximately 2½ times the volume needed for use e.g. 10 ml blood for 4 ml serum.

Incubate in an upright position at room temperature for 30-45 min (no longer than 60 min) to allow clotting. If using a clot-activator tube, invert carefully 5-6 times to mix clot activator and blood before incubation.

Centrifuge for 15 min at manufacturers recommended speed (usually 1000-2000 rpm). Do not use brake to stop centrifuge.

Carefully aspirate the supernatant (serum) at room temperature and pool into a centrifuge tube, taking care not to disturb the cell layer or transfer any cells. Use a clean pipette for each tube.

Inspect serum for turbidity. Turbid samples should be centrifuged and aspirated again to remove remaining insoluble matter.

Aliquot into cryovials and store at –80 °C. Ensure that the cryovials are adequately labeled with the relevant information, including details of additives present in the blood.

VIVA QUESTIONS:

Q.-1. Define blood with its component?

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Q.-2. What is the plasma and serum?

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Q.-3. Explain supernatant.

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Experiment No. 8

OBJECT:

To study the effect of hepatic microsomal enzyme induction on the duration of action of pentobarbital sodium.

REFFERENCE:

"Hand Book of Experimental Pharmacology" by S.K. Kulkarni, Ninth Edition 2007, Published by Vallabh Prakashan, New Delhi, Page No. 114-115.

REQUIREMENTS:

Animal- Mice 20-25 gram, Drugs- Phenobarbitone sodium dose- 50 mg/kg ip and Pentobarbitone sodium dose- 4.5 mg/ml of the drug and inject 1 ml/100 gram of body weight of animal.

THEORY:

The drugs which induce hepatic microsomal oxidative enzyme system enhance the metabolism of other drugs. As result in the presence of an enzyme- inducer the duration of action of second drug will be reduced. This has significant clinical relevance because when more then one drug is administered as the same time one drug may modify the action of another through the microsomal enzyme inducing property.

The common drugs which induce hepatic microsomal enzyme system are phenobarbitone and meprobamate. Co-administration of any drug with either of these drugs may effect the deposition of second drug and therefore, the desired pharmacological effects.

PROCEDURE:

1. Weigh and number the animals. Divide them into two groups, each comprising of at least 6 mice.
2. To the first group inject phenobarbitone once daily for 5 days. To the second group inject distilled water, similarly, for 5 days.
3. One hour after the last dose of phenobarbitone on the 5^{th} day, inject pentobarbitone to both the groups.
4. Note the onset and duration of sleep due to pentobarbital in both the groups.

OBSERVATION:

S. No.	Body wt. (g)	Treatment	Dose (mg/kg, ip)	Onset of action (min)	Duration of action (min)	% reduction in sleeptime

Phenobarbital is injected once-a-day for 5 days and one hour after the last dose the animals are injected with pentobarbital.

RESULTS AND DISCUSSION:

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VIVA QUESTIONS

Q.-1. Discuss mechanism of action of barbiturates.

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Q.-2. Write note on sedative and hypnotic action of barbiturates.

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Q.-3. Give adverse effects of phenobarbitone.

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Experiment No. 9

OBJECT:

To study the mydriatic and mitotic effect of topically applied atropine on rabbit eye. (Demo)

REFERENCE:

Kulkarni S.K "Hand book of Experimental Pharmacology" , Vallabh Prakashan Ninths edition Page No. 153

REQUIREMENTS:

Rabbits (2-5kg)**,** Atropine (1%w/v), physostigmine (1% w/v)**,** Rabbit holder, Torch light

THEORY:

Local action of a large number of drugs in an eye can be achieved without systemic effect by the application of drugs as eye drops, or eye ointments. Most of these drugs belong to antimicrobial, autonomic or local anaesthetic groups. The eyes are supplied both by sympathetic and parasympathetic nerves. The superior palpebral muscle and the dilator pupillae (radial fibres) of the iris have sympathetic supply. The sphincter pupillae (circular fibres) of the iris has parasympathetic supply which exercises dominant control. The ciliary muscle is also supplied by the parasympathetic nerves and when it contracts, the ciliary body is moved inwards and forwards. Because of this the lens bulges forward and the eye is accommodated for near vision. The opposite effect is produced by the relaxation of ciliary muscle resulting in paralysis of accommodation (cycloplegia). When the pupil dilates, the iris folds back near the opening of the canal of Schlemn and the drainage of aqueous humour is decreased thereby increasing in intraocular pressure. Constriction of the pupil, by opposite action, will increase drainage and reduce intraocular pressure.

Topically applied drugs can affect the eye by changing conjuctival congestion, pupillary size, light reflex, corneal sensitivity and intraocular pressure. However, the effect of drugs on pupillary size, light reflex and corneal reflex can be easily studied by the students themselves. The pupillary size can be measured by placing a transparent plastic scale in front of the eye but as close as possible. Light reflex is elicited by directing the light of a torch towards the pupil. The sensitivity of the cornea is tested by gently touching the cornea with a fine cotton swab stick from the side and not from the front of the eye. This elicits corneal reflex which manifests as blinking of the eyelids.

Physostigmine is a reversible cholinesterase inhibitor which increases the endogenous acetylcholine and stimulates the circular muscles or iris to produce pupillary constriction without any effect on light reflex and corneal reflex. Atropine, an antimuscarinic agent, blocks the effect of endogenously released acetylcholine on the circular muscles of the iris and the muscles of the ciliary bed to produce mydriasis and spasm of accommodation leading to cycloplegia but without producing loss of corneal reflex.

PROCEDURE:

1. Place the rabbit in the rabbit box keeping the head outside.
2. Observe size of the pupil in both the eyes.
3. Examine the effect of light reflex by holding the torch in front of eye moving light beam to and fro.
4. Examine the corneal reflex by touching a side of the cornea with a cotton piece.
5. Install a few drops of atropine solution in the conjunctiva (4-6 times) over a period of 8-10 minutes in the right eye of the rabbit. The left eye of the rabbit would serves as control. Install normal saline in the left eye.
6. Record the pupillary size, light reflex and corneal reflex after ten minutes of drug installation and tabulate the observations.
7. Repeat the experiment with physostigmine and ephedrine.

OBSERVATION:

Drug	Pupillary size (mm) (Constriction/ Dilataion)	Light reflex (Present/ Absent)	Corneal reflex (Present/ Absent)
Saline			
Physostigmine			
Ephedrine			
Atropine			

RESULT AND DISCUSSION:

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VIVA QUESTIONS

Q.-1. What is the mydriatic and mitotic effect of drug?

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Q.-2. Write the name of topically applied drug.

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Q.-3. Write the name of topically applied local anaesthetics.

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Q.-4. Why Rabbit is used for experimental animals.

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Experiment No. 10

OBJECT:

To study the muscle relaxant property of diazepam in mice using rota-rod apparatus.

REFERENCE:

"Hand Book of Experimental Pharmacology" by S.K. Kulkarni, Ninth Edition 2007, Published by Vallabh Prakashan, New Delhi, Page No. 122-123.

REQUIREMENTS:

Animal- mice 20-25 g, drug- diazepam dose- 4 mg/kg ip, equipment- Rota-rod apparatus.

THEORY:

One of the important pharmacological actions of antianxiety agents of benzodiazepine class of drugs is muscle relaxing property. The skeletal muscle relaxation together with taming or calming effect these agents reduces anxiety and tension. The loss of muscle-grip is an indication of muscle relaxation. This effect can be easily studied in animals using inclined plane or rotating rods. The difference in the fall off time from the rotating rod between the control and diazepam-treated animal is taken as an index of muscle relaxation. The angle of the slope of the inclined plane or the rate of rotation of the rod should be adjusted such that a normal mouse can stay on the plane or on the rod for an appreciable period (3-5 min) of time.

PROCEDURE:

1. Weigh the animals and number them.
2. Turn on the rota-rod. Select an appropriate speed (20-25 rpm is ideal).
3. Place the animal one by one the rotating rod. (If the rod is divided into several compartments, one can place more than one mouse at a time).
4. Note down the fall off time when the mouse falls from the rotating rod.
5. A normal (untreated) mouse generally falls off within 3-5 minutes.
6. Inject diazepam to all the animals. After 30 min repeat the experiment as done in step 3.
7. Note the fall off time.
8. Compare the fall off time of animals before and after diazepam treatment.

OBSERVATIONS:

S.No.	Body Wt. g	Treatment	Dose Mg/kg	Fall off time (sec) Before drug After drug	% decrease in time

RESULTS AND DISCUSSION:

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VIVA QUESTIONS

Q.-1. Define centrally acting muscle relaxant with examples.

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Q.-2. How diazepam produce skeletal muscle relaxation effect.

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Q.-3. Discuss adverse effects of benzodiazepines.

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Q.-4. Give difference between neuromuscular blockers and central muscle relaxant.

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Experiment No. 11

OBJECT:

To study the CNS stimulants by evaluation of locomotor activity of mice using actophotometer.

REFERENCE:

"Hand Book of Experimental Pharmacology" by S.K. Kulkarni, Ninth Edition 2007, Published by Vallabh Prakashan, New Delhi, Page No. 117-119.

REQUIREMENTS:

Animal- mice 20-25 g, drugs- Amphetamine dose- 1.5 mg/kg ip, Caffeine dose- 30 mg/kg ip, equipment- Actophotometer.

THEORY:

Most of the central nervous system acting drugs influence the locomotor activities in man and animals. The CNS depressant drugs such as barbiturates and alcohol reduce the motor activity while the stimulants such as caffeine and amphetamines increase the motor activity. In other words, the locomotor activity can be an index of wakefulness (alertness) of mental activity.

The locomotor activity (horizontal activity) can be easily measured using an actophotometer, which operates on photoelectric cells which are connected in circuit with a counter. When the beam of light falling on the photocell is cut off by the animal, a count is recorded. An actophotometer could have either circular or square arena in which the animal moves. Both rats and mice may be used for testing in this equipment.

PROCEDURE:

1. Weigh the animals and number them.
2. Turn on the equipment (check and make sure that all the photocells are working for accurate recording) and place individually each mouse in the activity cage for 10 min.
3. Note the basal activity score of all the animals.
4. Inject amphetamine/ caffeine and after 30 min re-set each mouse for activity scores for 10 min.
5. Note the difference in the activity, before and after drug.
6. Calculate per cent decrease in motor activity.

OBSERVATIONS:

S.No.	Body wt.(g)	Treatment	Dose mg/kg	Locomotor activity in 10 min Before After treatment	% change in activity

RESULTS AND DISCUSSION:

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VIVA QUESTIONS

Q.-1. Define CNS stimulants and enumerate them.

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Q.-2. How CNS stimulants affects locomotor activity.

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Q.-3. Write short note on nicotine as CNS stimulant.

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Q.-4. Discuss uses of different CNS stimulants.

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Experiment No. 12

OBJECT:

To study the anticonvulsant property of diazepam against pentylenetetrazol-induced clonic convulsions in mice.

REFFERENCE:

"Hand Book of Experimental Pharmacology" by S.K. Kulkarni, Ninth Edition 2007, Published by Vallabh Prakashan, New Delhi, Page No. 133-134.

REQUIREMENTS:

Animal- Mice 20-25 gram, Drugs- Pentylenetetrazol dose- 80 mg/kg, ip & Diazepam dose- 4 mg/kg, ip.

THEORY:

Pentylenetetrazol is a central nervous system stimulant. It produces jerky type of clonic convulsions in rats and mice. The convulsive effect of this drug is considered to be analogus to petil mal type of convulsions in man. Recently pentylenetetrazol has been reported to act through GABA-benzodiazepine receptor mechanisms in the brain. It is widely used as a tool in experimental pharmacology to study convulsions and anticonvulsant action of drugs.

PROCEDURE:

1. Weigh and number the animals. Divide the animals into two groups each comprising of 5 animals.
2. One group is used for studying the effects of pentylenetetrazol alone (control) and the other for studying the protective effect of diazepam.
3. Inject pentylenetetrazol to control animals and note the onset of action (indicated by Straub's tail, jerky movements of whole body and convulsions) and severity of convulsions due to the drug.
4. Inject diazepam to second group. After 30 min inject pentylenetetrazol to these animals which have received diazepam. Note onset and severity of convulsions (step 2).
5. Note either delay or complete abolition of convulsions in mice treated with diazepam.

OBSERVATIONS:

S. No.	Body Wt. (g)	Treat-ment	Dose (mg/kg)	Convulsions Onset (Sec)	Convulsions Nature & sever-ity	Convulsions Death/Recovery

RESULTS AND DISCUSSION:

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VIVA QUESTIONS

Q.-1. Define epilepsy and seizures.

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Q.-2. Enumerate different types of epilepsy.

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Q.-3. Discuss anticonvulsant effect of diazepam.

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Q.-4. Discuss tonic- clonic type of epilepsy.

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Experiment No. 13

OBJECT:

To study the effect of local anaesthetics lignocaine on rabbit eye. (Demo)

REFERENCE:

Kulkarni S.K "Hand book of Experimental Pharmacology" , Vallabh Prakashan Ninths edition Page No. 129

REQUIREMENTS:

Rabbits (2-5kg), lignocaine (1%w/v), Rabbit holder, wick of cotton

THEORY:

Local anaesthetics are those drugs which cause reversible loss of the nerve conduction and hence loss of sensory perception of pain when applied locally. Ideal local anaesthetics have the following characteristics.

1. They should not cause irritation.
2. Onset of action should be quick.
3. Duration of action should be sufficient.
4. They should be free from systemic toxicity.
5. They should be stable.

Local anaesthetics produce their effect by inhibiting the permeability of sodium ions and hence prevent depolarization. They displace calcium ions from their binding site. Thus they reduce the rate and rise of action potential and block the conduction of impulses Lignocaine is used as a surface, infiltration, nerve block and spinal anaesthesia. This experiment can detect activity of lignocaine as surface anaesthetic agent. Absence of corneal reflex is indicative of local anaesthetic activity.

PROCEDURE:

1. Place the rabbit in the rabbit box keeping the head outside.
2. Cut off the eye lashes of the rabbit at least 24 hours before starting experiment since eye lashes interfere with corneal reflex.
3. Examine the corneal reflex by touching a side of the cornea with a wick of cotton.
4. To test corneal reflex in the animal from its side and then touch the cornea with wick of cotton. If the hand is brought from front, the animal visualizes the hands and closes the eye.
5. Install a few drops of lignocaine solution in the right eye (2-3 drops). The left eye of the rabbit would serves as control and install normal saline.
6. Examine the corneal reflex after every one minute.
7. Report the onset and duration of action of lignocaine. The loss of corneal reflex is the indicative of the onset of action of the drug. Positive corneal reflex is the indication of the recovery of corneal sensation.
8. Repeat the experiment with other local anaesthetics (lidocaine, xylocaine).

OBSERVATION:

RESULT AND DISCUSSION:

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VIVA QUESTIONS

Q.-1. What is the Ideal characteristic of local anaesthetics?

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Q.-2. Write the name of topically applied local anaesthetics.

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Q.-3. Write the mechanisn of action of local anaesthetics.

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Experiment No. 14

OBJECT:

To study the anxiolytic (antianxiety) effect of diazepam in mice using elevated plus-maze apparatus.

REFFERENCE:

"Hand Book of Experimental Pharmacology" by S.K. Kulkarni, Ninth Edition 2007, Published by Vallabh Prakashan, New Delhi, Page No. 135-137.

REQUIREMENTS:

Animal- Mice 20-25 gram, Drugs- Diazepam dose- 2 mg/kg, ip, Equipment- Plus-maze consisted of two open arms (16X5 cm) and two enclosed arms (16X5X12 cm) with an open roof and is elevated to a height of 25 cm.

THEORY:

Elevated plus-maze is the simplest apparatus to study anxiolytic response of almost all type of antianxiety agents. Exposure of the animals to novel maze alley evokes an approach-avoidance conflict which is stronger in open arm as compared to enclosed arm. Rodents (rats and mice) have aversion for high and open space and prefer enclosed arm and, therefore, spend greater amount of time in enclosed arm. When animals enter open arm, they freeze, become immobile, defecate and show fear-like movemonts. The plasma cortisol level is also reported to be increased, as a true reflection of anxiety.

PROCEDURE:

1. Weigh and number the animals. Divide them into two groups each consisting of 4-5 mice.
2. One group is used as control and other for drug (diazepam) treatment.
3. Place the animals individually in the centre of the maze, head facing towards open arm and start the stop watch and note following parameters for five minutes:
    - First preference of mouse to open or enclosed arm.
    - Number of entries in open and enclosed arms (An arm entry defined as the entry of four paws into the arm).
    - Average time each animal spends in each arm (average time = total duration in the arm/ number of entries).

4. Inject diazepam to the test group. After 30 min place the animals individually in the centre of the maze and note all parameters as described under step 2.
5. Compare the preference of the animal to open/enclosed arm, average time spent in open arm and number of entries in open arm in each group.

OBSERVATIONS:

S. No.	Treatment (mg/kg, ip)	n	% Preference to Open arm	Open arm	
				Number of entries	Average time spent

RESULTS AND DISCUSSION:

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VIVA QUESTIONS

Q.-1. Discuss mechanism of action of diazepam.

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Q.-2. Define sedation and hypnotic action of drugs.

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Q.-3. Define anxiety and enumerate antianxiety drugs.

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Q.-4. Give difference between barbiturates and benzodiazepines.

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