B. PHARM SEMESTER – IV

PRACTICALE JOURNAL

SUBJECT: PHARMACOLOGY I – BP408P

AS PER PHARMAY COUNCIL OF INDIA (PCI) SYLLABUS

PREPARED BY:

DR.NAITIK D. TRIVEDI,

M. PHARM, PH. D.,

LECTURER (GOVERNMENT AIDED)

A. R. COLLEGE OF PHARMACY & G. H. PATEL INSTITUTE OF PHARMACY

VALLABH VIDYANAGAR, ANAND, GUJARAT

MOBILE: +91 – 9924567864

E-MAIL: mastermindnaitik@gmail.com

WEB SITE: https://drnaitiktrivedi.com

https://sites.google.com/site/drnaitiktrivedi/

&

DR. UPAMA N. TRIVEDI,

M. PHARM, PH. D

ASSOCIATE PROFESSOR & HoD,

INDUBHAI PATEL COLLEGE OF PHARMACY AND RESEARCH CENTRE, DHARMAJ, GUJARAT

E-mail: ups.aasthu@gmail.com

CERTIFICATE

Class:_____ **Year:**_____

This is to certify that Mr./Miss. _____

of B. PHARM SEMESTER – IV at _____

Enrollment No. _____ & Roll No. _____ has satisfactory completed his/her _____ out of _____ experiments/practical of the subject Pharmacology I – BP402P for the academic year 20 __ to 20 __.

Signed by:

_____ _____ _____

Head of Department **External Examiner** **Subject Teacher**

Date of certified:

INDEX

15.	**14.**	Study of anxiolytic activity of drugs using rats/mice.		**69 - 81**	
16.	**15.**	Study of local aneshetics by different methods		**82 - 90**	

All the practical is as per the PCI syllabus. This are just for the reference purpose.

EXPERIMENT NO.: 1 **DATE:**

AIM: GENERAL INTRODUCTION OF PHARMACOLOGY AND EXPERIMENTAL PHARMACOLOGY

DEFINITIONS:

1. **PHARMACOLOGY:** The word pharmacology is made of two parts, pharmacon (drug) and logus (discourse or study). Pharmacology means study of drugs, their pharmacodynamics, pharmacokinetics and toxicities.
2. **CLINICAL PHARMACOLOGY:** The branch concerned with the scientific studies on the effects of drug treatment in human being.
3. **PHARMACOKINETICS:** It is study of absorption, distribution, metabolism and excretion of drugs. i.e study of what body does to the drug.
4. **PHARMACODYNEMICS:** It is study of mechanism action and site of action of the drugs i.e it is study of what drug does to the body.
5. **ABSORPTION:** Drug goes from site of administration to systemic circulation or blood.
6. **DISTRIBUTION:** Drug goes from systemic circulation to various compartments like fat, muscles, tissue, organ etc.
7. **METABOLISM:** Conversion of drug in to excretion form.
8. **ELIMINATION OR EXCRETION:** Removal of drug from the body.
9. **BIOAVAILABILITY:** Fraction of an administered dose of unchanged drug that reaches the systemic circulation
10. **DRUG:** It is the active ingredient which is useful for diagnosis, treatment, mitigation and prevention of any disease or disorder in human beings or animals.
11. **MEDICINE:** The substances used to deliver drug in stable and acceptable form and it consist lubricant, binder, sweetener like other additives constituents with active ingredients.
12. **PHARMACOEPIDEMIOLOGY:** Study of effects of drugs in large numbers of people.
13. **PHARMACOGENOMICS:** Application of genomic technologies to new drug discovery and further characterization of older drugs.
14. **NEUROPHARMACOLOGY:** Effects of medication on central and peripheral nervous system functioning.
15. **PSYCHOPHARMACOLOGY:** Effects of medication on the psyche; observing changed behaviors of the body and mind, and how molecular events are manifest in a measurable behavioral form.

16. **PHARMACOGENETICS:** Clinical testing of genetic variation that gives rise to differing response to drugs.
17. **THEORETICAL PHARMACOLOGY:** Study of metrics in pharmacology.
18. **POSOLOGY:** How medicines are dosed. It also depends upon various factors like age, climate, weight, sex, and so on.
19. **PHARMACOGNOSY:** A branch of pharmacology dealing especially with the composition, use, and development of medicinal substances of biological origin and especially medicinal substances obtained from plants.
20. **PHARMACOVIGILANCE (PV)**: It is defined as the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other drug-related problem.
21. **SIDE EFFECTS:** A secondary but predictable effects, typically undesirable effect of a drug or medical treatment.
22. **ADVERSE EFFECTS:** A secondary but unpredictable effects, typically undesirable effect of a drug or medical treatment.
23. **TOXIC EFFECTS:** Harmful effects of the drug which is related to dose (Excess).

OBJECTIVES OF EXPERIMENTAL PHARMACOLOGY

1. To screen drug substance for their biological activities.
2. To study the toxicity of drugs.
3. To study mechanism of action and site of action of the drug.

Experimental Pharmacology involves:

a) Preclinical Experiments:

- Which consist of animal studies for deciding the safety, efficacy, pharmacokinetics and pharmacodynamics of a new drug or a new drug formulation.
- The purpose of pre-clinical study is to develop adequate data to decide that it is reasonably safe to proceed with human trials of the drug.
- Experiments are generally performed on rodent like mouse, rat, guinea pig, hamster, rabbit.
- After successful result, experiments are performed on larger animals like cat, dog, monkey.
- As the evaluation progresses unfavorable compounds get rejected at each step.
- So, that only few out of thousands reach the stage when administration to man is considered.

b) Clinical Experiments:

These follow preclinical studies. In clinical pharmacology, efficacy, safety, and pharmacokinetics of a drug substance is determined through its use in healthy human volunteers and patient populations under controlled conditions. Only those drugs which are found safe and effective in preclinical (animal) studies are further investigated in such studies.

Phases of Clinical Trial

1. **Phase I** **:** First in man → safety
2. **Phase II** **:** First in patient →dose, dosage form
3. **Phase III** **:** Efficacy, ADRs
4. Post marketing surveillance or **Phase IV** : Evaluation in the real clinical setting

1. **Phase I:**

- **Objectives**
  1. To assess a safe & tolerated dose
  2. To see if pharmacokinetics differ much from animal to man
  3. To see if kinetics show proper absorption, bioavailability
  4. To detect effects unrelated to the expected action
  5. To detect any predictable toxicity

– **Inclusion criteria**
  - Healthy volunteers : Uniformity of subjects: age, sex, nutritional status [Informed consent a must]
  - Exception: Patients only for toxic drugs Eg AntiHIV, Anticancer

– **Exclusion criteria**
  - Women of child bearing age, children

– **Methods:**
  - First in Man : Small number of healthy volunteers
  - First in a small group of 20 to 25
  - Start with a dose of about 1/10 to 1/5 tolerated animal dose
  - Slowly increase the dose to find a safe tolerated dose
  - If safe → in a larger group of up to about 50 – 75
  - No blinding
  - Performed by clinical pharmacologists
  - Centre has emergency care & facility for kinetics study
  - Performed in a single centre
  - Takes 3 – 6 months [70% success rate]

2. **Phase II**

- First in patient [different from healthy volunteer]
- Early phase [20 – 200 patients with relevant disease]
  - Therapeutic benefits & ADRs evaluated
  - Establish a dose range to be used in late phase
  - Single blind [Only patient knows] comparison with standard drug
- Late phase [50 – 500]
  - Double blind

- Compared with a placebo or standard drug
- Outcomes
  - Assesses efficacy against a defined therapeutic endpoint
  - Detailed P.kinetic & P.dynamic data
  - Establishes a dose & a dosage form for future trials
- Takes 6 months to 2 years [35% success rate]

3. Phase III:

- Large scale, Randomised, Controlled trials
- Target population: 250 – 1000 patients
- Performed by Clinicians in the hospital
- Minimises errors of phases I and II
- Methods
  - Multicentric → Ensures geographic & ethnic variations
  - Diff patient subgroups Eg pediatric, geriatric, renal impaired
  - Randomised allocation of test drug /placebo / standard drug
  - Double blinded:
  - Cross over design
  - Vigilant recording of all adverse drug reactions
  - Rigorous statistical evaluation of all clinical data
- Takes a long time: up to 5 years [25% success]
- Cross Over Design

Group	Week 1	Week2	Week3
I	Standard	Placebo	Test
II	Placebo	Test	Standard
III	Test	Standard	Placebo

* A wash out period of a week between two weeks of therapy

4. Phase IV or Post marketing Surveillance

- No fixed duration / patient population
- Starts immediately after marketing
- Report all ADRs
- Helps to detect
  - Rare ADRs
  - Drug interactions

* Also new uses for drugs [Sometimes called Phase V]

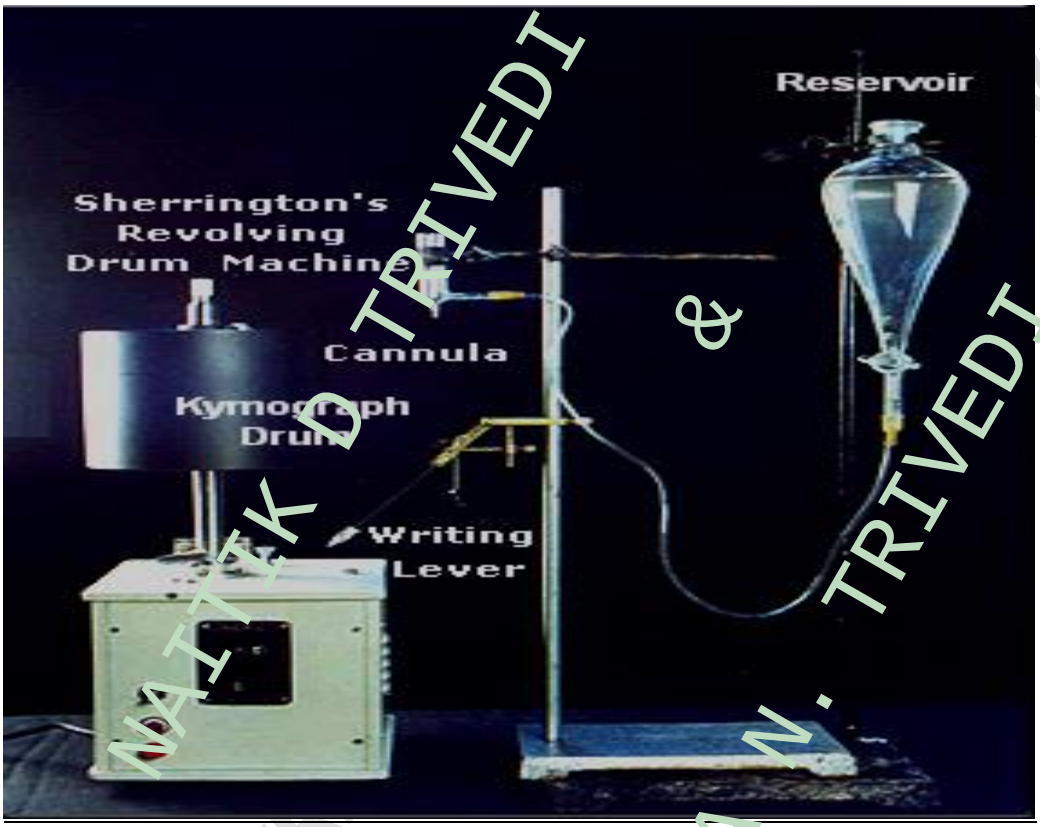
TEACHER'S SIGNATURE

EXPERIMENT NO.: 2 **DATE:**

AIM: INSTRUMENTS USE IN EXPERIMENTAL PHARMACOLOGY

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

Equipment 1: Equipment used for isolated and perfused frog heart

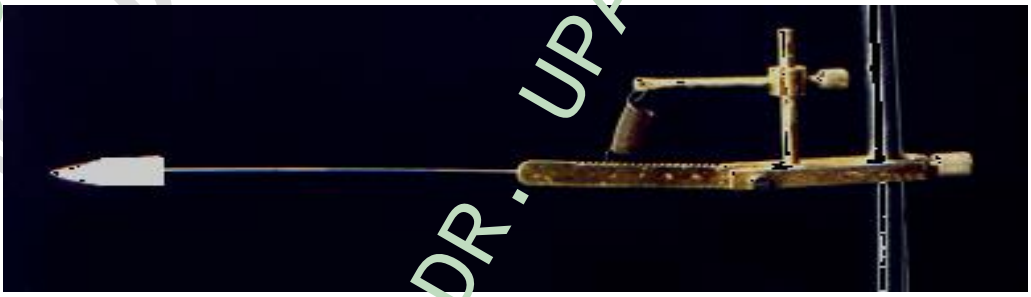


1. **Reservoir:** an ideal reservoir has an arrangement to deliver the physiological solution at a fixed rate and with constant pressure.
   For practical purpose even glass separators can be used as reservoirs.
2. **Margate bottle:** it consists of an aspirator bottle fitted with tight stopper perforated by a glass tube reaching nearly the bottom of the bottle. It is used as a steady pressure had since the pressure always corresponds to the lower level of the glass tube no matter how much liquid there is above this level in the bottle.
3. **Writing Levers:**
   Levers are meant for recording and magnifying the responses of isolated tissues to drugs. The levers are attached to the isolated tissues and are used to record various types of contractions in them.
   Levers: Side way writing, Frontal Writing, Sterling's, Broodie's Universal, Gimbal, Auxotonic.

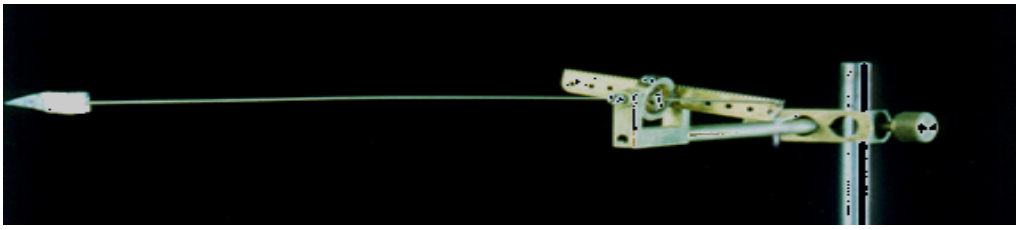
a. **Frontal Writing levers:** this is used for recording of isotonic contraction of the isolated tissues. In this lever the writing end (stylus) can freely rotate around its axie. This minimizes the friction between the stylus and the kymograph. With frontal writing lever, the contraction of the isolated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

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

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

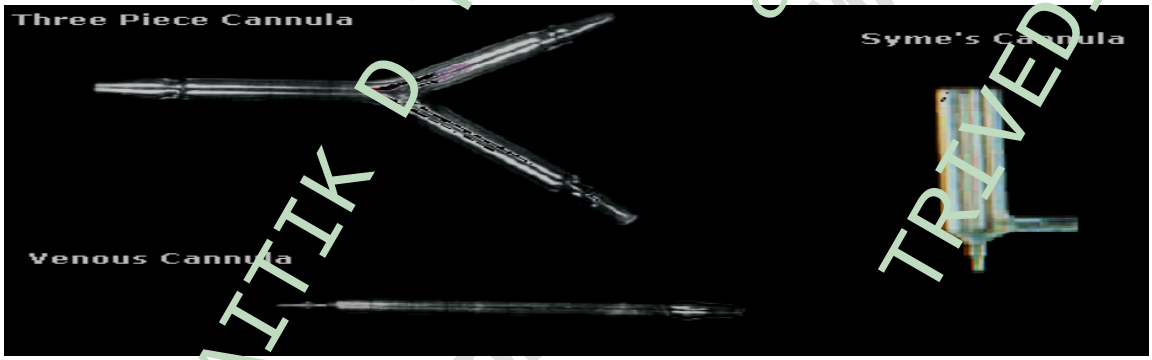


d. **Gimble lever:** The friction between the writing end and the kymograph is minimum in the Gimble lever because the pressure of stylus on the kymograph depends on gravity.



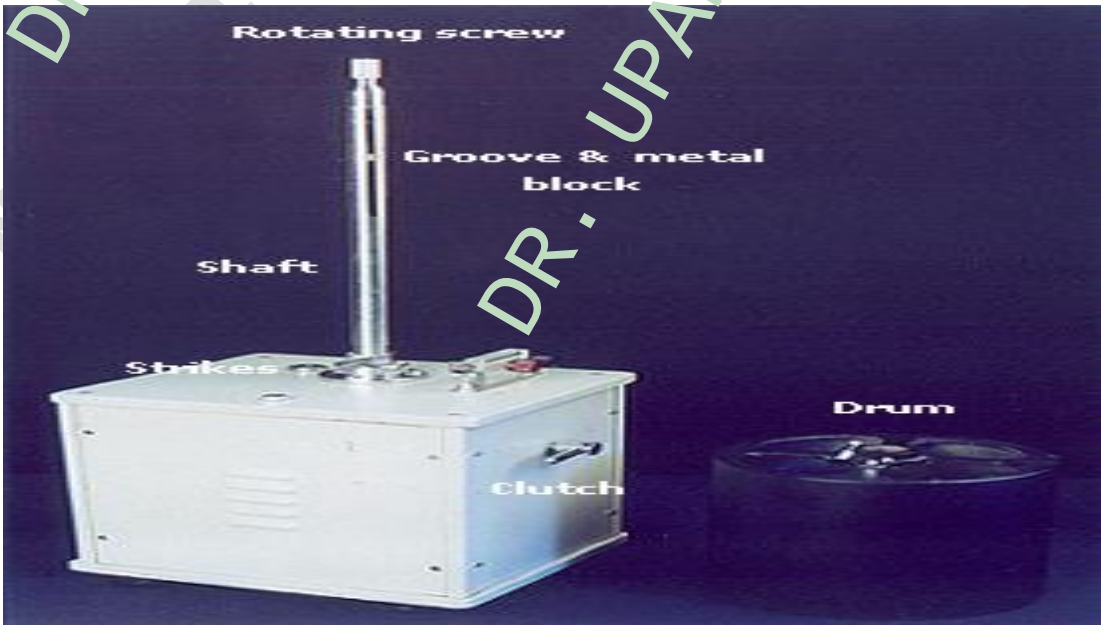
e. **Paton's Auxotonic lever:** it is designed in such a way that the load on the tissue goes on increasing as tissue contracts.

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



5. Sherrington Recording Drum and Drum Cylinder:

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



a) Base hoofs (legs) with adjustable **leveling screws** to keep drum horizontal if surface of the table is uneven.

b) Side hoof to turn the drum on its side so that shaft becomes horizontal.

c) Gear rod arrangement with fast, slow and neutral gears and clutch (starter). The gear rod is attached to a cone wheel which has 4 pulley grooves. Desirable speed of drum can be obtained by changing gear position and shaft drum pulley connections.

d) Contact screw on the surface. A wire can be fixed from main plug to convey the current through base and

e) Contact foil with a contact screw mounted on an insulated material on the superior surface of the base. Second wire can be connected here.

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

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

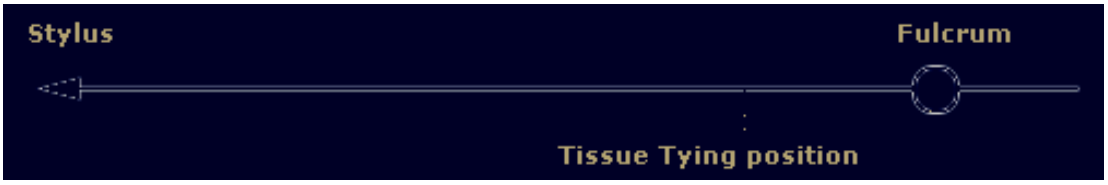
CONTRACTIONS:

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

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

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

   Eg.: electrically stimulated muscle twitches.



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

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

6. Rotating Drum:

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

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

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

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

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

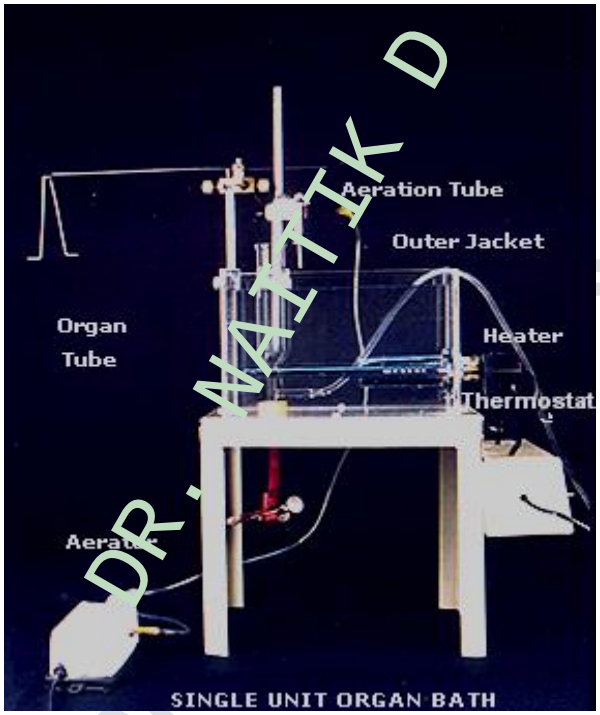
resion in two liters of sprite. After passing the paper through the solution, it is drained and then allowed to try.

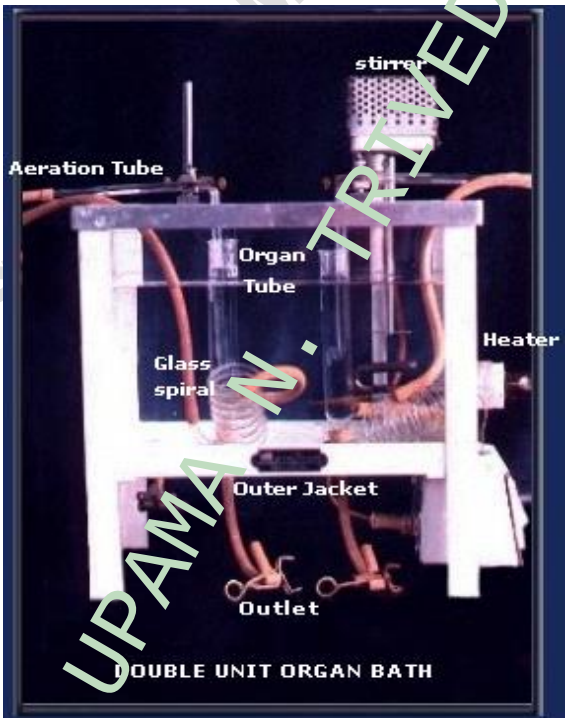
c) Recording of responses on drum cylinder without smoking (Sketch-pen tip):

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

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

Equipment 2: Equipments used for isolated tissue preparations.





STUDENT'S ORGAN BATH:

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

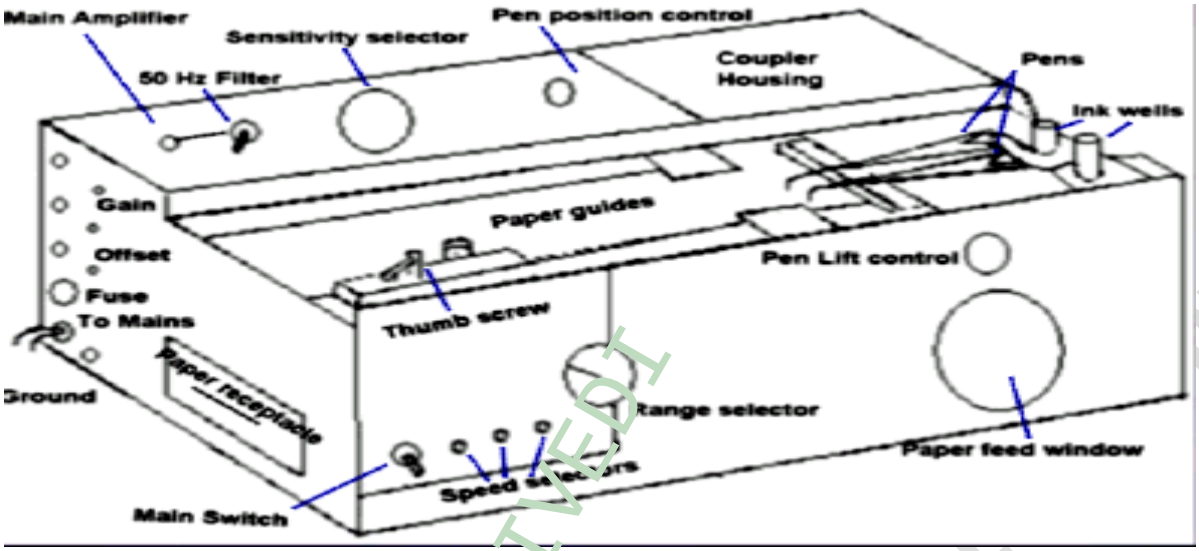
3) **Glass coil:** it is also called as preheating coil. This is of about double the capacity of organ tube. The glass spiral is connected in the midway between and organ tube. It holds the physiological salt solution at 37ºC, which then enters the organ tube. Thus it avoids fluctuations in the temperature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

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

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

PHYSIOGRAPH AND POLYGRAPH:

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



Most of the recorders consist of following three components:

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

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

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

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

(ii) DAMPING,

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

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

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

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

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

(2) Main Amplifier has 3 controls:

(i) 50 Hz filter ON/OFF. When it is "ON" it filter 50 Hz artifacts.

(ii) Sensitivity selector for selecting the sensitivity of the amplifier ranging from 50 v to500 v in 4 steps and from 1 mv to 100 mv in 7 steps.

(iii) Base line control for adjusting position of pen.

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

Different types of couplers are available for recording various parameters.

(i) **Strain Gauge Coupler :** This coupler with the help of strain gauge transducer, plethysmography, spirometry , experiments of frog sciaticgastrocnemius preparation (simple muscle curve, successive stimuli, tetanus, fatigue, isometric contractions etc.), experiments on frog, rabbit or rat heart, isolated tissue (ileum, uterus, vas-deferens, anococcygeus etc.) Springs of different tensile strength are available with the transducer.

(ii) **Biopotential Coupler : It** is a useful for recording of E.C.G. (Electrocardiogram), E.E.G. (Electroencephalogram), E.M.G. (Electromyogram), E.O.G. (Electro-oculogram), Sensory and motor nerve conduction velocities in humans.

(iii) **Electrocardiogram (EKG) Coupler: It** is used for recording clinical ECG. It consists of a knob to select various leads.

(iv) **Pulse-Respiration coupler:** It is used for recording arterial pulse with a photoelectric pulse transducer and respiratory movements with a respiration belt transducer.

(v) **Temperature Coupler: This** is used for recording surface or rectal temp.

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

(1) Connect the respective transducer. Put the chart papers in proper position. Fill the inkwell and check the free flow of ink from pen. Select the chart speed.

(2) Never put the instrument "ON" without connecting the transducer.

(3) Put the instrument's main switch and sensitivity (and not the coupler)to the "ON" position at least for "15 minutes".

(4) Adjust the position of pen (stylet) with the help of baseline knob as required. The knob of sensitivity usually kept at 200V position. However, it may be changed as required (500V or 1mV in isotonic transducer0.

(5) Put the "Coupler" position to the "ON" position.

(6) Adjust the balance in other words readjust the original place of the pen with the help of "Balance".

(7) To change the baseline, put of the balance and then change the baseline and then balance.

(8) After adjusting the baseline with the balance hang the weight (1gm) on the transducer. See the deflection of pen and it should be 10 mm if not, adjust with the help of "Gain".

(9) Finer adjustment of sensitivity is done by "Gain" and it should be used in rare circumstances only.

(10) When you tie the tissue it is advisable to switch off the "Balance".

(11) Never try to adjust "Damp" or "Offset".

(12) In case of any problem please ask the concerned teacher.

(13) Add the different concentration of drugs and record the readings.

- **AT THE END OF THE EXPERIMENT:**

(A) Remove ink from the inkwells and clean with the help of water. Flush the capillary outlet and capillary.

(B) Clean the pen-writer.

(C) Cover the instrument.

(D) Put the pen writer in the locker safely.

(E) Transducers may be left connected to the physiograph but it must be kept in the box provided.

(F) Never try to stretch the spring of the transducer it may damage the transducer.

D. SOME OTHER INSTRUMENTS USED FOR EXPERIMENTAL PHARMACOLOGY ON ANIMAL

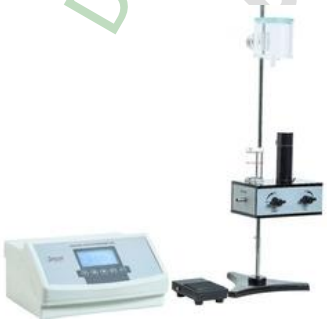
1. Rota rod

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

Rotarod test is widely used to evaluate drug effects on motor coordination, balance and motor learning in rodents. The principle of this test is that rats or mice are first trained to walk on a rod rotating at a certain speed. Once the animals have learned this, the effect of a test-compound on their motor performance is evaluated. Animals experiencing impaired motor coordination are unable to cope with the rotating rod and will drop off when the rotation speed exceeds their motor coordination capacity. When the animal drops from rod safely into its own lane, the time latency to fall is automatically recorded. Rotarod is controlled by an advanced microprocessor which provides precise timing control and accurate speed regulation. Rotation can be electronically set at a constant speed (2 to 60 rpm) using a dial on the front panel.

2. Digital Plethysmometer

"For screening of inflammation or oedema in mouse or rat"



The Digital Plethysmometer is a micro controlled volume meter, specially designed for accurate measurement of the rat / mouse paw swelling. The Digital Plethysmometer is a highly useful tool to evaluate the inflammatory response experimentally induced in small rodents and to screen potential anti-inflammatory or anti-oedema properties of pharmacological substance. The water displacement produced by the immersion of the animal paw in the measuring tube is reflected in to the second tube, inducing the volume displacement. This displacement value is recorded. The control unit can be zeroed between successive readings by pressing the reset key, so as to avoid the error arising due to adherence of water to the paw of the animal.

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

3. The Digital Tele-Thermometer

"For measuring the temperature of laboratory animals"

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

4. Pole climb apparatus

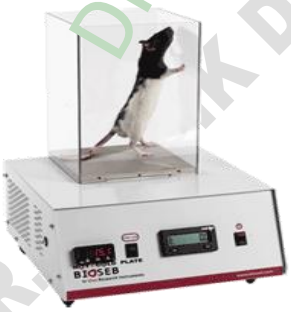
For the study of antipsychotic drugs

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

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

5. Hot Plate Apparatus

For testing heat induced pain in mice & rat



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

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

6. Photoactometer

To study spontaneous motor activity in open field

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

7. Tail Flick Analgesia Meter

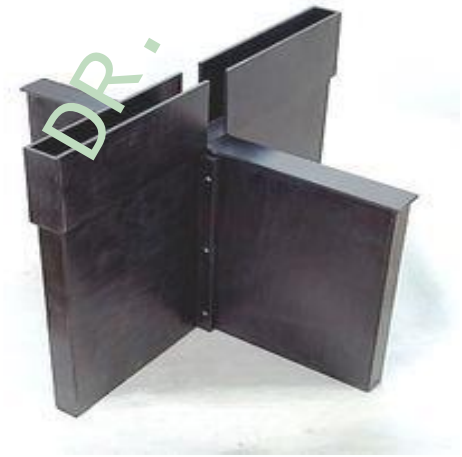
For measuring pain sensitivity in mice & rats

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

Cut off time is 10-12 sec.

8. Maze apparatus

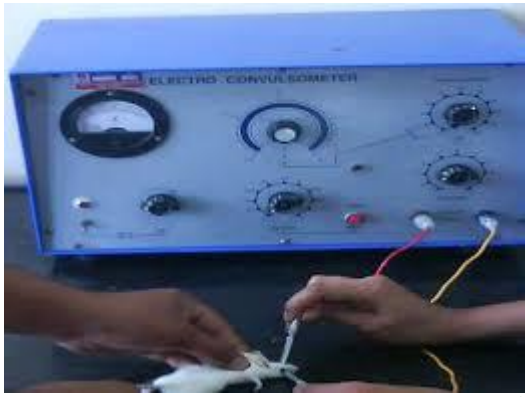
For measuring anxiolytic activity



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

9. Elector Convulsometer

For measuring the anti-epileptic activity



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

Eye/ear electrode are used.

10. Histamine Chamber

For measuring anti asthmatic/histaminic activity

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

E. AIM: TO STUDY THE PHYSIOLOGICAL SALT SOLUTION.

Constituents	Frog's ringer soln.	Mammalian ringer soln.	Tyrode soln.	Krebs Henseleit salt solution	Ringer locke solution	De-jalon solution
NaCl	6.5g	9.2g	8.0g	6.90g	9.15g	9.00g
KCl	1.4g	0.42g	0.2g	0.35g	0.42g	0.42g
MgCl2	0.3g	----	0.1g	0.11g	----	---
MgSO4.7H2O	----	----	---	---	---	---
NaH2PO4.2H 2O	0.1g	----	0.05g	0.14g	----	----
KH2PO4	----	----	---	----	----	----
Glucose	2.0g	----	1.0g	2.0g	1.00g	0.50g
NaHCO3	0.2g	0.2g	0.1g	2.10g	0.15g	0.50g
Sodium lactate	-----	----	----	----	3.10g	----
CaCl2	0.12g	0.24g	0.2g	0.28g	0.24g	0.06g

PURPOSE OF INGREDIENTS:

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

Potassium Chloride: It maintain ionic balance of the preparation

Calcium Chloride: It maintain contractility of the tissue

Sodium bicarbonate: It maintain the alkaline pH of the solution

Glucose: It act as an energy source

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

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

Note:

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

TEACHER'S SIGNATURE

EXPERIMENT NO.: 3 **DATE:**

AIM: INTRODUCTION OF ANIMAL USED FOR EXPERIMENTAL PHARMACOLOGY

THEORY:

The use of animals in research and education dates back to the period when humans started to look for ways to prevent and cure ailments. Most of present day's drug discoveries were possible because of the use of animals in research.

Animal experimentation is the term used to explain the use of animals in experimentation in education, training and research. The terms animal testing, animal experimentation, animal research, in vivo testing and vivisection are often used interchangeably although they carry different meanings. "Vivisection", a term preferred by those who oppose the use of animals in research, means cutting into or dissecting a living animal. Researchers prefer to use the term 'animal experimentation'.

About 50-100 million animals ranging from zebra fish to non-human primates are used for experimentation every year. While experiments of vertebrates are regulated in most countries, those on invertebrates are not, and hence their accurate usage statistics are lacking.

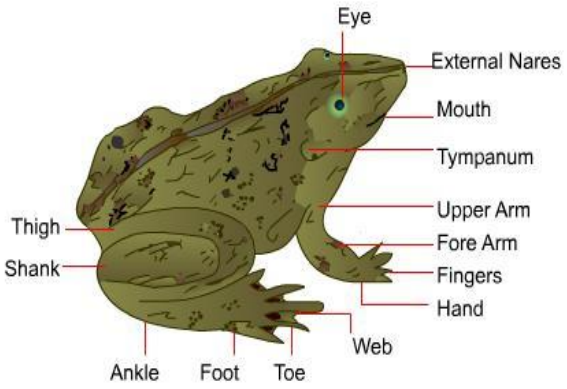
The number of animal used in teaching varies from 1% to 10% of total animals used. Most of the animals used are small rodents. These are mostly used for fundamental biological research and breeding purposes. Most animals are used in only one procedure and are less commonly reused. These laboratory animals are obtained from various sources in different countries. The sources also vary based on species required. These experimental animals are mostly obtained through breeders. However, at some places these animals might be caught from the wild areas or from auctions. In India, animals have been mostly provided by small time traders, until a few years ago, after the CPCSEA guidelines were modified and affected, which bans the procurement of animals from unauthorized sources.

The greatest drug discoveries in the 19 th and 20 th centuries were possible due to the use of animals. Over the last century, every Nobel Prize for medical research has been dependent on animal research. The first Nobel prize in 1901 in medicine was for serum therapy and research involving use of horses. The latest 2012 Nobel laureates in physiology or medicine also worked on animals. There is a strong relationship between rapid progress in experiments on animals and progress in clinical medicine. In the 1880s, Behring used horses for production of diphtheria antitoxin and the development of a vaccine against diphtheria and tetanus leading to the first Noble prize in physiology or medicine in 1901. Insulin was first isolated from dogs in 1922 and it revolutionized the treatment of diabetes. In the 1970s, antibiotic treatment and vaccines for leprosy were developed using armadillos. Domagk introduced antibacterial activity of prontosil in 1939 by experiments on chicken.

In India, The prevention of cruelty to animals (PCA) Act 1960 was amended in 1982.. For this purpose, the Government has formulated "Breeding of and experiments on animals (Control and Supervision) Rules, 1998" as amended during 2001 and 2006, to regulate the experimentation on animals. The CPCSEA provides guidelines for performing experiments on animals and maintenance of animal house. The registration of animal house is mandatory with CPCSEA and is to be renewed every 3 years. There are now some preconditions for renewal by CPCSEA. Besides the rules and procedures laid down by the CPCSEA, the Indian National Science academy (INSA) and Indian Council of Medical Research have also formulated certain guidelines for care and use of animals in scientific research as well as in medical colleges.

Year	Law/Regulation
1960	Prevention of Cruelty to Animals (PCA) Act 1960, amended 1982
1964	Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)
1972	Wild life protection act
1992	Indian National Science Academy (INSA) "Guidelines for care and use of animals in scientific research", revised 2001
1998	"Breeding of and Experiments on Animals (Control and Supervision) Rules, 1998", amended 2001, 2006
2001	Indian Council of Medical Research (ICMR) "Guidelines for use of Laboratory animals in Medical Colleges"
2009	MCI amendment-Recommends to use alternatives to replace animal experiments
2012	Ministry of Health & Family Welfare bans use of animals in educational institutes
2012	University Grants Commission (UGC) "Guidelines for discontinuation of dissection and animal experimentation in zoology/life sciences in a phased manner"

1. FROG: (Adult Weight 50-100gm)



Biological Source: Rana Tigrina

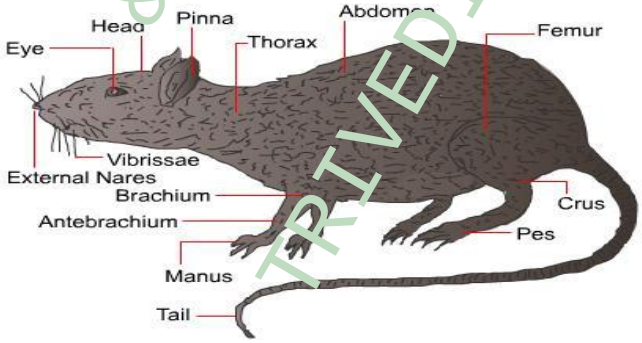
Common Strain Used: Rana esculenta, Rana pipiens and Rana temporaria.

Specific Characteristics: Frog is a cold blooded amphibian. It has three chambers in its heart, two auricles and one ventricle.

Used in Experimental Pharmacology:

- Study of isolated tissue like rectus, abdominis muscle, heart, sciatic nerve preparation etc.
- To study the effect of drug acting on central nervous system, neuromuscular junction and heart.
- Whole frog is also used in screening of certain drugs like anesthetics.

2. RAT: (Adult Weight 200-250gm)



Biological Name: Rattus Norvegicus.

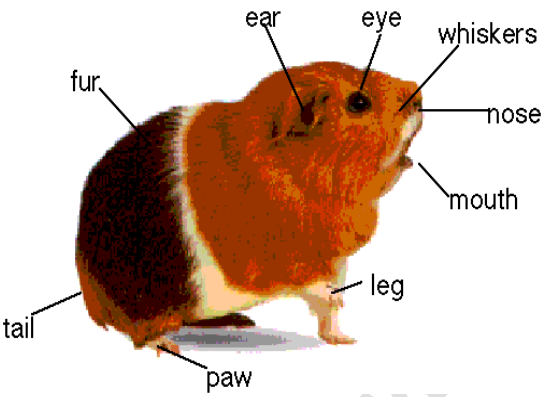
Common Strain Used: Albino rats of wistar strain, Sprague-Dawley, Wistar Kyoto, Lewis, and Porton.

Specific Characteristics: Rat is a warm blooded rodent. It can't vomit and does not possess the vomiting center. It has no tonsil and gallbladder in its body. Hence it can't be used in screening of the drugs having activities on vomiting center, or gall bladder. Rat is omnivorous animal. It shows resistance to the effects of cardiac glycosides.

Uses in experimental pharmacology:

- Psychopharmacological Studies.
- Study of analgesics and anticonvulsants
- Bioassay of various hormones such as insulin, oxytocin, vasopressin etc.
- Study of estrus cycle, mating behaviour and lactation.
- Studies on isolated tissue preparations like uterus, stomach, vasdeferens, anoccoccygeus muscle, fundus strip, aortic strip, heart rate etc.
- Chronic study on blood pressure.
- Gastric acid secretion studies.
- Study of hepatotoxic and antihepatotoxic compound.
- Acute and chronic toxicity studies.
- Study on mast cells using peritoneal fluid and mesenteric attachments.

3. GUINEA PIG: (Adult Weight 400-600gm)



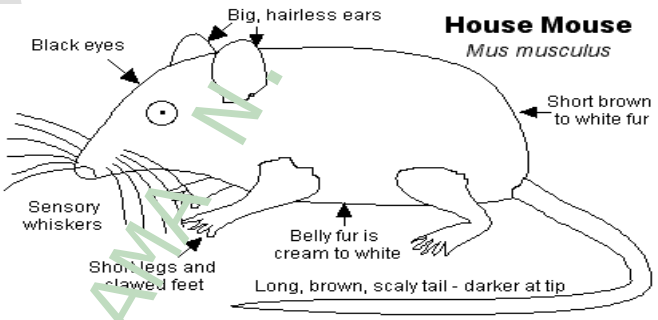
Biological name: Cavia Procellus.

Specific Characteristics: It is a docile animal. It is susceptible to tuberculosis and anaphylaxis. It is highly sensitive to histamine and penicillin. It required exogenous ascorbic acid in diet. Guinea pig is a warm blooded rodent. Its name itself has become synonymous to an experimental animal.

Use in Experimental pharmacology:

- Evaluation of bronchodilators.
- Anaphylactic and immunological studies.
- Study of histamine and anti histamines.
- Bioassay of digitalis.
- Evaluation of local anesthetics.
- Hearing experiments because of sensitive cochlea.
- Study in isolated tissue specially, ileum, tracheal chain, vas deferens, taenia coli, hearts etc.
- Study of tuberculosis and ascorbic acid metabolism.

4. MOUSE: (Adult Weight 20-25gm)



Biological name: mus musculus.

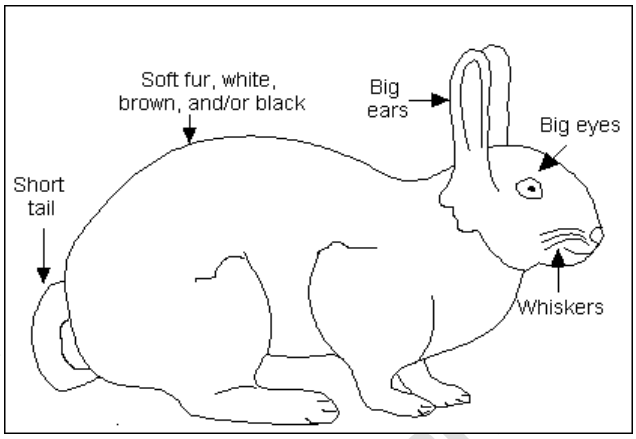
Common Strain used: Laca, balb-c and Swiss albino.

Specific characteristics: Mouse is most wide used animal in different toxicity studies. It is a warm blooded rodent. Mice are very sensitive to the sedative effects of hexobarbitone. They are smallest, cheap and easy to handle.

Used in Experimental Pharmacology:

- Bioassay of Insulin.
- Toxicological and teratogenic study.
- Screening of analgesic and anticonvulsants.
- Screening of chemotherapeutics agents.
- Study related to genetic and cancer research.
- Study of Drugs acting on central nervous system.

5. RABBIT: (Adult Weight 1.5-3kg)



Biological Name: Oryctolagus cuniculus.

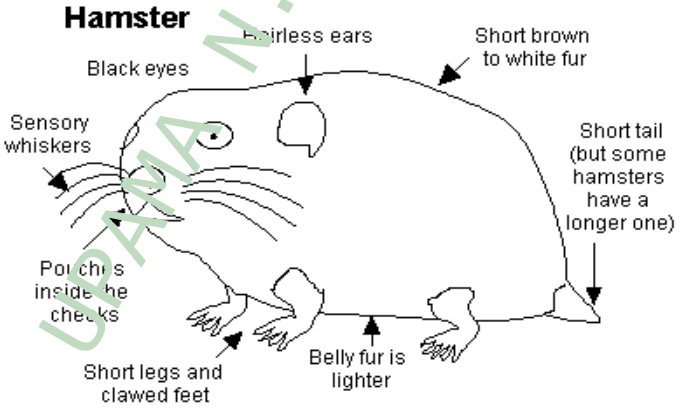
Strains Used: New Zealand White, Himalayan Black.

Specific Characteristics: it is docile animal with large ears. Usually New Zealand white rabbits are used. Rabbit is a warmed blooded mammalian animal. Some strains of rabbit are resistance to effective of atropine because they have higher concentration of atropinase enzyme in their blood. In this species coitus it induces secretion of leutenising hormone (LH) in females, which leads to ovulation. Hormone progesterone is known to block such ovulation.

Use in Experimental Pharmacology:

- Pyrogen testing.
- Bioassay of anti-diabetics and sex hormones.
- Irritancy tests.
- Study of drug used in glaucoma.
- Screening of agents affecting capillary permeability.
- Pharmacokinetics studies.

6. HAMSTER:



Biological Name. Mesocriceius Auratus and Cricetulus Griseus

Specific Characteristics: they have short body with short legs and tail. The skin is loose and covered with dense short soft fur. The cheeks pouches are prominent and extend upto the shoulder region.

Use in Experimental Pharmacology:

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

TEACHER'S SIGNATURE

EXPERIMENT NO.: 4 **DATE:**

AIM: MAINTENANCE OF LABORATORY ANIMALS AS PER CPCSEA GUIDELINES.

Source: http://cpcsea.nic.in

The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)

The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) is a statutory Committee, which is established under Chapter 4, Section 15(1) of the Prevention of Cruelty to Animals Act 1960.

India is one of the pioneering countries to institute Prevention of Cruelty to Animals Act in 1960 whereas such Act was instituted in France in 1963 and in USA in 1966. The detailed rules for experimentation on animals were first enacted by the Ministry of Agriculture in 1968 and were implemented by a Committee set up in pursuance of Section 15(1) of the PCA Act, 1960.

However, the Committee was later wound up in 1977. After a hiatus of 13 years, a recommendation to reconstitute Committee for the purpose of control and supervision of experiments on animals (CPCSEA) was received from Animal Welfare Board of India (AWBI). After due consideration of the recommendation of AWBI, CPCSEA was set up by this Ministry on 8th February, 1991.

The Committee was, subsequently, reconstituted on 23rd February, 1996 with 15 Members and one Member Secretary. Since then, this Ministry has reconstituted the CPCSEA routinely. The present Committee was constituted on 30th August, 2012 under the chairmanship of Additional Secretary, MoEF with 17 Members. At present, Shri Hem Pande, Additional Secretary, MoEF & CC is the Chairman of the Committee.

The main functions of CPCSEA are:

1. Registration of establishments conducting animal experimentation or breeding of animals for this purpose.
2. Selection and assignment of nominees for the Institutional Animal Ethics Committees of the registered establishments.
3. Approval of Animal House Facilities on the basis of reports of inspections conducted by CPCSEA.
4. Permission for conducting experiments involving use of animals.
5. Recommendation for import of animals for use in experiments.
6. Action against establishments in case of established violation of any legal norm/stipulation.
7. Conduct of Training Programmes for the Nominees of CPCSEA.
8. Conduct / Support of Conference / workshop on Animal Ethics.

MAINTENANCE OF LABORATORY ANIMALS AS PER CPCSEA GUIDELINE:

- The goal of these guidelines is to promote the human care of animal used in biomedical and behavioural research and testing.
- To avoid unnecessary pain before, during and after experiment.
- To provide guideline for:
  - Housing, care, breeding and maintenance
  - Source of experimental animals
  - Acceptable experimental procedures for anesthesia and euthanasia

It is essential to take care of below mention points regarding the experimentation on animal as per CPCSEA guideline:

ANIMAL PROCUREMENT

1. QUARANTINE
2. STABILIZATION AND
3. SEPARATION

1. QUARANTINE:

- Separation of newly received animals from those already in the facility until the health and possibly the microbial status of newly received animal have been determine.
- A minimum duration of quarantine for small animal-1 week and for larger animal-6 week.

2. STABILIZATION:

- Physiologic, psychological and nutritional stabilization should be given before their use.
- Duration of stabilization will depend on type and duration of animal transportation, and species of animal.

3. SEPARATION:

- Physical separation of animal by species is recommended to prevent interspecies disease transmission and to eliminate anxiety and possible physiological and behavioural changes due to interspecies conflict.
- Housing different species in separate room.
- It shall be acceptable to house different species in the same room, e.g. two species have a similar pathogen status and are behaviourally compatible.

SURVELLANCE, DIAGNOSIS, TREATMENT AND CONTROL OF DISEASE:

- All animal should be observed for signs of illness, injury, or abnormal behaviour by animal house staff.
- Animals that show signs of a contagious disease should be isolated from healthy animals in the colony.

ANIMAL CARE AND TECHNICAL PERSONNEL:

- Animal care require technical and husbandry support.
- Institution should employ people trained in laboratory animal or provide for both formal and on the job training to ensure effective implementation of the program.

PERSONAL HYGIENE:

- Animal care staff maintain a high standard of personal cleanliness.
- Clothing suitable for use in the animal facility should be supplied and laundered by the institution.
- It acceptable to use disposable gear such as gloves, masks, head covers, coats, coveralls and shoe covers.
- Person should change clothing as often as is necessary to maintain personal hygiene.
- Personnel should not be permitted to eat, drink, smoke or apply cosmetic in animal rooms.

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:

- 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.
- Provision should be made for observation of the animal at appropriate intervals.

PHYSICAL RELATIONSHIP OF ANIMAL FACILITIES OF LABORATORIES:

- Animal shall be housed in an isolated building located as far away from human habitations as possible and not exposed to dust, smoke, noise, wild rodent, insects and birds.
- This separation can be accomplished by having the animal quarters in a separate building, wing, floor or room.
- The animal room should occupy about 50-60% of the total constructed area and the remaining area should be utilized for service such as stores, washing, office and staff, machine rooms, quarantine and corridors.
- Since animals are very sensitive to environmental changes, sharp fluctuations in temperature, humidity, light, sound and ventilation should be avoided.

FUNCTIONAL AREAS:

- Ensure separation of species or isolation of individual projects when necessary.
- Receiving and storage areas for food, bedding.

- Space for administration, supervision, and direction of the facility.
- Showers, sinks, lockers and toilets for personnel.
- Washing and sterilization equipment and supplies.
- For holding soiled and cleaned equipment.
- For repairing cages and equipment.
- To store wastes prior to incineration or removal.

PHYSICAL FACILITIES:

i. BUILDING MATERIALS

Moisture-proof, fire-resistant, seamless materials are most desirable for interior surfaces including vermin and pest resistance.

ii. CORRIDOR:

Wide enough to facilitate the movement of personnel as well as equipments and should be kept clean.

iii. UTILITIES:

Water lines, drain pipes and electrical connection.

iv. ANIMAL ROOM DOORS:

Rust, vermin and dust proof. it properly within their frames and provided with an observation window.

v. FLOORS:

Smooth, moisture proof, non-absorbent, skid-proof, resistant to wear, acid, solvents, adverse effects of detergents and disinfectants. Capable of supporting racks, equipment and stored items without becoming gouged, cracked, or pitted.

vi. DRAINS:

Floor drains are not essential in all rooms used exclusively for housing rodents.

vii. WALLS & CEILINGS:

Free of cracks, unsealed utility penetrations, or imperfect junction with doors, ceilings, floors and corners.

viii. STORAGE AREAS:

Separate storage areas should be designed for feed, bedding, cages and materials not in use.

ix. FACILITIES FOR SANITIZING EQUIPMET AND SUPPLIES:

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

x. EXPERIMENTAL AREA:

It should be carried out in a separate area from the place where animals are housed.

ENVIRONMENT:

i. TEMPERATURE AND HUMIDITY CONTROL:

- Air conditioning
- Temperature with in the range of 64.4-84.0 F
- Relative humidity- 30-70% throughout the year
- For large animal comfortable zone-18-37°°c

ii. POWER & LIGHTING:

- The electrical system should be safe and provide appropriate lighting and a sufficient no. of power outlets.
- A time control light system should be used.

iii. NOISE CONTROL:

- Noise free environment

ANIMAL HUSBANDRY CAGING & HOUSING SYSTEM:

- Adequate ventilation
- Meet the biological need of animal
- Keep the animal dry and clean
- Facilitate research while maintaining good health of the animals
- Cages made of steel or painted steel
- Feeding and watering devices should be easily accessible for filing, changing, cleaning and servicing.

FOOD:

- It should be fed palatable, non-contaminated and nutritionally adequate food.
- Feeders should allow easy access to food while avoiding contamination by urine and faeces.
- Food should be available in a mounts sufficient to ensure normal growth in immature animals and maintenance of normal body weight, reproduction and lactations in adults.
- Areas in which diets are stored should be kept clean and enclosed to prevent entry of insects or other animals.
- Diet should be free from heavy metals

BEDDING:

- Absorbent, free of toxic chemicals or other substances that could injure animals or personnel, and of a type not readily eaten by animals.
- It should be removed and replaced with fresh materials as often as necessary to keep animal clean and dry.
- It should be easily stored

- Uncontaminated
- Non-toxic
- Non-malodorous
- Disposable by incineration

WATER:

- Fresh
- Potable
- Uncontaminated

WATER DISPOSAL:

- The most preferred method of waste disposal is incineration.
- 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.

PEST CONTROL:

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

EMERGENCY, WEEKEND AND HOLIDAY CARE:

- Animal should be cared for by qualified personnel every day, including weekends and holidays, to safeguards their well- being including emergency veterinary care.

RECORD KEEPING:

- Animal house plans
- Animal house staff record
- 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 no. of animals used
- Death record
- Clinical record of sick animals training record of staff involved in animal activities ¬ Water analysis report

STANDARD OPERATING PROCEDURES (SOPs)/ GUIDELINES:

- Maintain SOPs describing procedures/ methods adapted with regard to animal husbandry, maintenance, breeding, animal house microbial analysis and experimentation record.
- SOPs should contain following items-

- Name of author
- Title of SOP
- Date of preparation
- Reference of previous SOP on the same subject and date
- Location and distribution of SOPs with sign of each recipient
- Objectives
- Detailed information of the instruments used in relation with animals
- Normal value of all parameters.

TRANSPORT OF LABORATORY ANIMALS:

- The main considerations for transport of animals are the mode of transport, the containers, the animal density in cages, food and water during transit, protection from transit infection, injuries and stress.

ANAESTHESIA:

- Sedatives, analgesics and anaesthetics should be used to control pain or distress under experiment. Before use actual anaesthetics the animals is prepared for anaesthesia by overnight fasting and using pre-anaesthetics.
- Local or general anaesthetics may be used depending on type of surgical procedure.

DISPOSAL:

- The transgenic and knockout animal should be first enthanized and then disposed off as prescribed elsewhere in the guidelines.
- A record of disposal and the manner of disposal should be kept as a matter of routine.

EXPERIMENT NO.: 5 **DATE:**

AIM: A. INTRODUCTION OF COMMON LABORATORY TECHNIQUES USED FOR BLOOD WITHDRAWAL, SERUM AND PLASMA SEPARATION FROM EXPERIMENTAL ANIMALS

Blood sample from experimental animals are frequently required for study of effects of drugs on biochemical parameters and for the study of pharmacokinetics of drugs in the experimental animals.

The sampling procedures for blood collections are of two types:

A) Non-terminal Blood collection:

In this type, blood is collected from the conscious or unconscious experimental animals through a single or multiple withdrawals. Animal are not sacrificed after non-terminal blood collection.

a) Lateral Tail Vein or Ventral/Dorsal Artery:

- Can be used in both rats and mice by cannulating the blood vessel or by nicking it superficially perpendicular to the tail.
- Obtainable volume: Mouse - small to medium [50-100 ul]
  : Rat – medium [0.2-0.4 ml]
- Procedure is carried out in the conscious mice or rat. The tail is dipped in warm water (about 50-60^{o}C) or xylol is applied to the tail to increase circulation through tail vein. The needle (25-27 gauge, 0.5 to 1 length) is inserted, bevel up in the distal portion of tail vein. The blood is slowly aspirated avoiding the collapse of vein.
- Sample collection using a needle minimizes contamination of the sample, but is more difficult to perform in the mouse.
- Sample collection by nicking the vessel is easily performed in both species, but produces a sample of variable quality that may be contaminated with tissue and skin products.
- Sample quality decreases with prolonged bleeding times and tail stroking.
- Repeated collection possible.
- Relatively non-traumatic.
- Routinely done without anesthesia, although effective restraint is required.
- In most cases warming the tail with the aid of a heat lamp or warm compresses will increase obtainable blood volume.

- Arterial sampling produces larger volumes and is faster, but special care must be taken to ensure adequate hemostasis.
- Piercing the tail vein with a needle is also a way to collect a very small blood sample.

b) Mandibular Vein/Artery:

- Can be used in both rats and mice by piercing the mandibular vein or artery with a needle [20G] or stylet.
- Obtainable volume: medium to large [100-200 ul, mouse; 0.4-0.5 ml rat]
- Sample quality is good.
- The procedure is customarily done on an unanesthetized animal, but effective restraint is required.
- Arterial sampling produces large volumes very rapidly.
- Venous sampling produces medium volumes more slowly.
- Ensure that gentle pressure is applied for approximately 30 seconds post-collection to ensure hemostasis.

c) Saphenous/Lateral Tarsal:

- Can be used in both rats and mice by piercing the saphenous vein with a needle [23-25G: mouse, 21-23G: rat].
- Obtainable blood volumes: small to medium [mouse: 100 ul; rat: 0.4 ml]
- Repeat sampling is possible.
- Variable sample quality.
- The procedure is customarily done on an unanesthetized animal, but effective restraint is required.
- Can be more time-consuming than other methods due to time required for site preparation.
- After training, it requires more practice than tail or retro-orbital sampling to reliably withdraw more than a minimal amount of blood. Prolonged restraint and site preparation time can result in increased animal distress when handling an unanesthetized animal.
- Temporary favoring of the limb may be noted following the procedure.
- Care must be taken to ensure adequate hemostasis following the procedure.

d) Retro-orbital:

*Note: Due to the increased risk of complications associated with this procedure, the CPCSEA recommends that other routes of blood collection be considered prior to use of this method. The mandibular technique permits an equivalent volume of blood to be collected in a rapid manner with less risk or complications.

- Individuals performing the procedure must be certified by Animal Ethical Committee (AEC).
- Can be used in mice by penetrating the retro-orbital sinus with a glass capillary tube [0.5 mm in diameters] or via the retro-orbital plexus in rats with a capillary tube.
- Must be performed by a skilled operator.
- Follow-up required 24-48 hours after blood collection. If complications such as squinting or bulging of the eye are noted, an animal health report must be completed.
- Obtainable volume: medium to large
- Collection is limited to once per eye.
- In the hands of an unskilled operator, retro-orbital sampling has a greater potential than other blood collection routes to result in the following complications:
  - Hematoma and excessive pressure on the eye resulting from retro-orbital hemorrhage
  - Corneal ulceration, keratitis, rupture of the eyeball or micro-ophthalmia caused by pressing on the eye to stem persistent bleeding or from a hematoma
  - Damage to the optic nerve and other intra-orbital structures leading to vision deficits or blindness
  - Fracture of the bones of the orbit and neural damage by the pipette; loss of vitreous humour due to penetration of the eyeball
- Skilled personnel can conduct retro-orbital bleeding in unanesthetized mice. Anesthesia is recommended for retro-orbital blood collection in mice and is required during the training of personnel.
- In rats, the presence of a venous plexus rather than a sinus can lead to greater orbital tissue damage than in the mouse. General anesthesia must be used unless scientific justification is provided and approved by the CPCSEA. In addition, a topical ophthalmic anesthetic, e.g. proparacaine or tetracaine, is recommended prior to the procedure. Retro-orbital bleeding performed in rats by a trained practitioner

represents more than “minimal or transient pain or distress” and therefore should be considered a Category 2 procedure.

- Care must be taken to ensure adequate hemostasis following the procedure.

B) Terminal/Post-Mortem blood collection: In this type, large volume of blood is collected in single or multiple withdrawals from the anesthetized experimental animals. Animal is generally sacrificed during or after such blood collection.

Blood withdrawal by cardiac puncture or axillary cut down are considered terminal procedures and must be performed only after ensuring that the animal is under surgical anesthesia. The post-mortem collection from the aorta is performed immediately after euthanasia.

a) Cardiac Puncture

- Can be used in both rats and mice by penetrating the heart.
- Must be performed by a skilled operator.
- Obtainable volume: medium to large.
- Animal must be euthanized immediately after blood collection.

b) Axillary cut down

- Can be used in both rats and mice.
- Axillary vessels are cut with a scalpel blade or scissors and the pooled blood is collected via capillary tube.
- Obtainable volume: medium to large.
- Animal must be euthanized immediately after blood collection prior to recovery from anesthesia.

c) Pre-mortem collection from the aorta or vena cava

- Can be used in both rats and mice as a pre-mortem procedure on anesthetized animals.
- Blood is collected using a needle.
- Animal must be euthanized immediately after blood collection prior to recovery from anesthesia.
- Obtainable volume: medium to large.

d) Post-mortem collection from the aorta

- Can be used in both rats and mice as a post-mortem procedure in a euthanized animal.
- Must be done rapidly after euthanasia to ensure blood flow.

- Aorta is cut and the blood pools in the pleural cavity.
- Blood is collected in a mini capillary tube. The tube must be held continuously in a horizontal position during the blood draw.
- Obtainable volume: medium to large

Summary of Blood Sampling Techniques

Route	Anesthesia Required		Speed		Sample Quality		Repeat Samples		Relative Obtainable Volume *(approximations)*		Potential for Complications	
	Mouse	Rat	Mouse	Rat	Mouse	Rat	Mouse	Rat	Mouse	Rat	Mouse	Rat
Tail Vein	No	No	Med	Med	Fair	Good	Yes	Yes	Small (50 ul)	Small *(.2 mls)*	Low	Low
Tail Artery	No	No	Fast	Fast	Good	Very Good	Yes	Very Good	Medium *(100 ul)*	Medium *(.4 mls)*	Low	Low
Retro-orbital	No	Yes	Fast	Med	Very Good	Good	Alternate eyes	Alternate eyes	Med.-Large *(200 ul)*	Med.-Large *(.5 mls)*	Moderate-High	Moderate-High
Saphenous	No	No	Med.	Med	Good	Good	Yes	Yes	Small-Med. *(100 ul)*	Small-Med. *(.4 mls)*	Low	Low
Mandibular Vein	No	No	Slow-Med.	Slow-Med.	Fair-Good	Fair-Good	Yes	Yes	Small-Med. *(100 ul)*	Small-Med. *(.4 mls)*	Moderate	Moderate
Mandibular Artery	No	No	Very Fast	Very Fast	Very Good	Very Good	Yes	Yes	Large *(200 ul)*	Large *(.5 mls)*	Moderate	Moderate

Blood Collection Limits

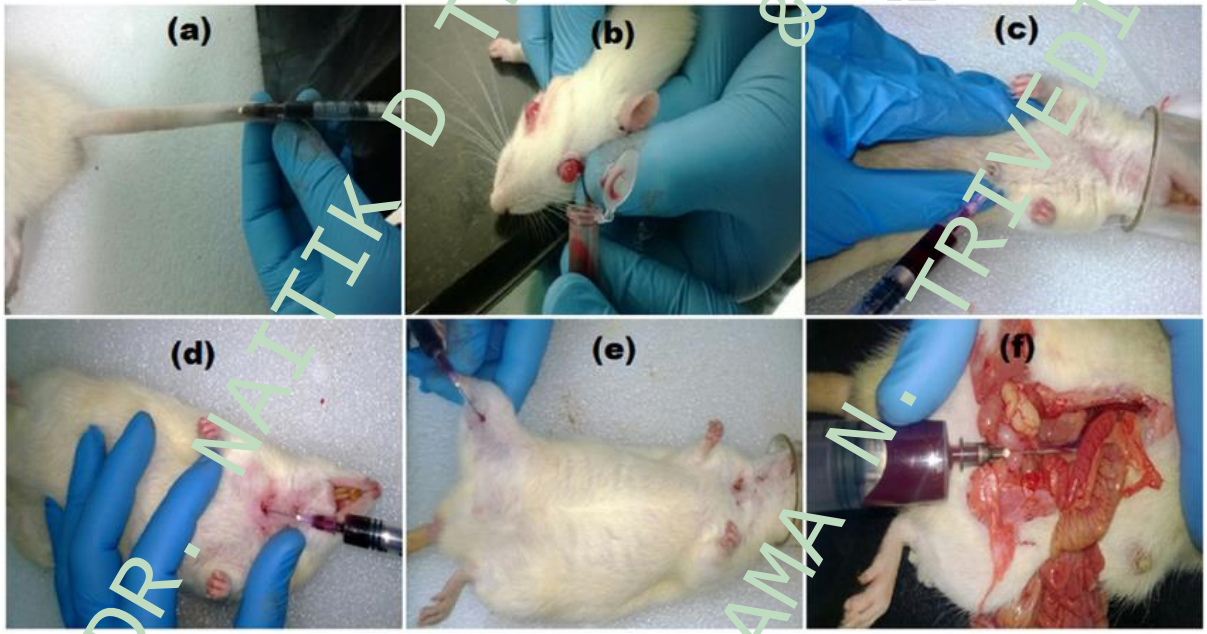
The AEC limits one time survival blood collection to 15% of an animal's blood volume in most circumstances. Serial blood sampling limit vary by species, strain, and frequency of blood collection as outlined in Tables 1 and 2. The AEC may require monitoring for anemia (using assays such as hematocrit and/or serum protein levels) when repeated collections or collection of larger volumes are required. Blood collected for diagnostics or other veterinary procedures must be considered when evaluating total volume available for experimental use. In all cases blood collection volumes should be limited to the minimum volume that will allow for successful experimentation or diagnostics.

Table 1:

Species	Blood Volume Mean (ml/kg)	Blood Volume Range (ml/kg)	Blood Volume (average)		
Mouse (25 g average Wt.)	58.6	55-80	7.5%	10%	15%
Rat (250 g)	64	58-70	1.2 ml	1.6 ml	2.4 ml
Rabbit (4 kg)	56	44-70	17 ml	22 ml	34 ml
Nonhuman primate (NHP; 8 kg)	56	55-75	34 ml	45 ml	67 ml

Table 2:

Single sampling		Multiple sampling	
% Circulatory blood volume removed	**Approximate recovery period**	**% Circulatory blood volume removed (cumulative volume)**	**Approximate recovery Period**
7.5%	1 week	7.5%	1 week
10%	2 weeks	10-15%	2 weeks
10-15%	4 weeks	20%	3 weeks



Rat blood sampling sites: (a) Lateral tail vein, (b) Retro-orbital sinus, (c) Cardiac puncture, (d) Jugular vein, (e) Saphenous (lateral tarsal) vein, and (f) Inferior vena cava.

B. ANAESTHETICS AND EUTHANASIA USED FOR ANIMAL STUDIES.

THEORY:

A fundamental responsibility of individuals that use animals in research, teaching or testing is to anticipate and eliminate or minimize any potential that procedures may cause animal pain, distress, or discomfort.

Although animals that are in pain may not behave like humans, (e.g., pain in animals may be accompanied by immobility and silence, in contrast to the groans and cries of human patients), it is assumed that procedures that cause pain in humans cause pain in animals.

The presence of pain in animals can be recognized by alterations in animal behavior (e.g., reduced activity, reduced grooming, hunched- up posture, altered gait, changes in temperament, vocalizations, reduced food and water intake, reduced urinary and fecal output), and in physiological variables, (e.g., reduced depth of respiration, increased heart rate, and reduced hydration status).

Animal pain, distress, and discomfort can produce a range of undesirable physiological changes, which may radically alter measured responses to experimental stimuli, as well as the rate of recovery from surgical procedures, hence, its avoidance and alleviation are in the best interest of both the animal and researcher. Reducing post-procedural/post-operative pain, distress, and discomfort is accomplished by good nursing care, (e.g., keeping the animal warm, clean, dry and well padded), and by the administration of analgesic drugs.

In addition to the avoidance and alleviation of pain and discomfort, adequate post-procedural /postoperative animal care also includes efforts to prevent and/or treat post-anesthetic complications, (e.g., aspiration, hypostatic pneumonia, cardiovascular and respiratory depression, dehydration, and infection). The prevention or minimization of animal pain, distress, or discomfort by the proper use of tranquilizers, anesthetics, and analgesics is scientifically and ethically essential to the humane care, use, and treatment of research animals.

1. **ANAESTHESIA:**

   The word anaesthesia has been derived from Greek word that means “without perception of insensibility”. Anesthesia is the act of providing sensation-free relief from pain or pain-producing procedures. Anesthesia must be performed by a person with knowledge of and familiarity with the drugs to be used in the animal species under consideration.

   There are numerous anesthetics available for use in rodents. Some of the more popular agents include:

- Chloralose
- Urethane
- Barbiturates
- Paraldehyde
- Magnesium Sulphate
- Ketamine
- Tribromoethanol

CHLORALOSE:

- It is a compound of chloral and glucose prepared by heating equal parts of anhydrous glucose and charcoal, when both α- chloralose (active form) and β-chloralose (in active form) are formed.
- It is prepared as one percent solution by boiling in 0.9% NaCl or in distilled water, and administered intravenously or intraperitoneally at a temperature of 30-400C before the chloralose comes out of solution.
- Disadvantages: It is suitable only for acute experiments, usually in dogs and cats, inducing surgical anaesthesia for 3-4 hours or longer.
- Advantages: 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. Reflexes are not depressed but may be slightly exaggerated including responses to bilateral carotid occlusion.

URETHANE (Ethyl Carbamate):

- It is readily soluble in water giving a neutral solution. Usually 25% solution in water is used.
- Disadvantages: It is suitable only for acute experiments since it has delayed toxic effect on liver, and may also cause agranulocytosis and pulmonary adenomata.
- Mice develop an exceptionally high incidence of lung tumours regardless of the route of administration.

BARBITURATES:

- Barbiturates interfere with nerve impulse transmission both in the central nervous system and in the ganglia producing depression of cardiovascular and spinal cord reflexes.
- In rabbits pedal reflex (leg retraction) is lost first, then pupillary and finally palpebral reflex.

- Pentobarbital Pentobarbital is a barbiturate and, historically, the most commonly used anesthetic in rodents.
- Advantages: At recommended doses, it causes minimal cardiovascular depression. It is also relatively long acting and can provide approximately 45 minutes of surgical anesthesia. Disadvantages:- Pentobarbital is a potent inducer of the hepatic microsomal enzyme system. Causes pronounced respiratory depression as well as hypothermia, particularly when repeated doses are given.
- Phenobarbitone sodium Phenobarbitone sodium and barbitone sodium are used for prolonged experiments.
- Tiopentone sodium Thiopentone sodium (pentothal) is used for surgical operations of short duration. It produces rapid induction with minimum excitation.

PARALDEHYDE:

- Advantages: 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.
- Disadvantages: Under its influence the basal blood pressure as well as the response to vasopressor and depressor drugs are low. Bilateral carotid occlusion produces poor pressor response or even a depressor response.

MAGNESIUM SULPHATE:

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

TRIBROMOETHANOL

- Advantages: In most rodents, tribromoethanol produces good surgical anesthesia, with good skeletal muscle relaxation and only a moderate degree of respiratory depression. It is relatively inexpensive and not a controlled agent.
- Disadvantages:- It is a potential for causing peritonitis. When exposed to either light or temperatures >400C, tribromoethanol degrades into two byproducts: hydrobromic acid and dibromoacetaldehyde. Both of these compounds are highly irritating when administered IP and result in peritonitis and visceral adhesions which may be fatal.

KETAMINE HYDROCHLORIDE:

- Ketamine hydrochloride, a dissociative anesthetic, disrupts pain transmission and suppresses spinal cord activity with some action at opioid receptors. Visceral pain is not abolished with dissociative anesthetics and there is poor muscle relaxation and analgesia.
- Disadvantages: Ketamine is a poor anesthetic when used alone, but is more often combined with other agents. When combined with other drugs, it is usually administered IP. Ketamine is acidic, can be irritating, and cause muscle necrosis when administrated IM. Ketamine-induced nerve damage can cause selfmutilation in rodents. Ketamine is a controlled substance. Store in a locked cabinet and maintain a log of its use.

2. **EUTHANASIA:**

The term euthanasia is derived from the Greek terms eu meaning good and thanatos meaning death. The act of inducing humane death in an animal by a method that induces rapid loss of consciousness and death with a minimum of pain, discomfort or distress. Methods of euthanasia fall into two broad categories:

A. Chemical methods:

i. Inhalant agents:

Eg.: ether, halothane, methoxyflurane, isoflurane, enflurane, chloroform, nitrogen, nitrous oxide, carbon di oxide, carbon monoxide, argon, hydrogen cyanide.

ii. **Injectable agents**:

Eg.: barbiturates, chloral hydrate, ethanol, ketamine, magnesium sulphate, potassium chloride, neuromuscular blocking agents.

B. **Physical methods**:

Eg.: Penetrating Captive Bolt, Euthanasia by a Blow to the Head, Gunshot, Cervical Dislocatiod, Decapitation, Electrocution, Microwave Irradiation, Thoracic (Cardiopulmonary, Cardiac) Compression, Kill Traps, Maceration, Adjunctive Methods, Exsanguination, Stunning, Pithing

Introduction about some Common Methods of Euthanasia

Inhalation of anesthesia gas – acceptable with conditions for rodents and other small animals (< 7 kg). Typically used as part of a two-step process with a secondary physical method of euthanasia such as decapitation or cervical dislocation.

Inhalation of CO2 - acceptable with conditions, including the special considerations listed below.

Immersion agents – e.g. MS 222/Tricaine. Acceptable for aquatic species, usually in connection with a secondary physical method.

Cervical Dislocation – acceptable for small birds, mice and immature rats. Requires training and should be performed under anesthesia unless specifically approved by the CPCSEA.

Decapitation – acceptable for rodents and small rabbits. Requires training; anesthesia recommended unless approved by the CPCSEA. Guillotines must be sharpened and adjusted frequently to ensure proper performance.

Injectable barbiturate agents – e.g. sodium pentobarbital, Euthasol®, Eutha 6®, Fatal Plus® - acceptable for most species.

Exsanguination/Cardiac Perfusion – acceptable with conditions; animals must be anesthetized.

Special Considerations for the use of carbon dioxide gas as a euthanasia agent:

The following additional guidelines must be followed when using CO2:

- CO2 must be delivered from compressed gas canister only. Gas should be delivered using a gradual fill method- a displacement rate from 10% to 30% of the chamber volume per minute is recommended. Use of a flowmeter is strongly recommended.
- Use of dry ice to deliver CO2 gas is unacceptable.
- High concentrations of CO2 have been determined to cause pain and distress. The practice of immersion, where conscious animals are placed directly into a container filled with 100% CO2, is unacceptable.
- Use of the rodents' home cage is recommended as it minimizes stress in the animals.
- Chambers used for CO2 euthanasia must not be overcrowded. Overcrowding in this situation is defined as less than one half the normal housing space normally required for the animals.

- If animals are removed from their home cages prior to euthanasia, the container used to transport animals between the housing area and procedure room/euthanasia chamber must not be overcrowded.
- Male mice from different cages should not be mixed in transport cages or the euthanasia chamber to prevent distress and/or fighting.
- Detailed instructions for the use of CO2 euthanasia are posted in all vivarium procedure rooms and can be reviewed on the CPCSEA website.

TEACHER'S SIGNATURE

EXPERIMENT NO.: 6 **DATE:**

AIM: STUDY OF DIFFERENT ROUTES OF DRUGS ADMINISTRATION IN MICE/RATS

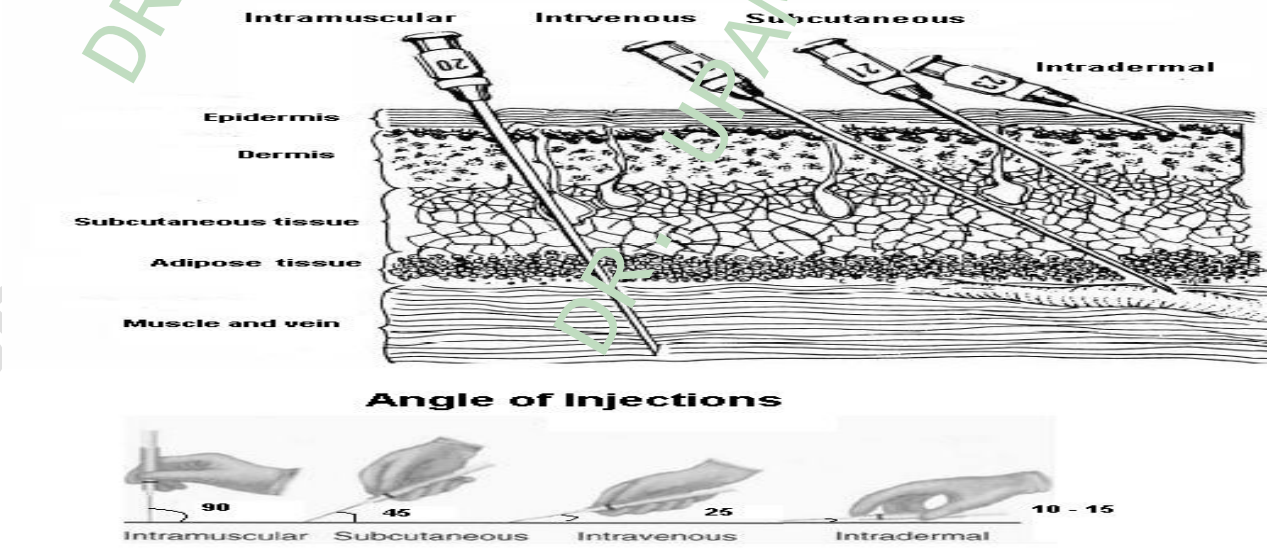
Drugs substance can be administrated to the experimental animals by different routes of administration as

Gastrointestinal

- Oral (per os) - through the mouth - Care to be taken. The administered material should not enter the respiratory tract. Accidental entry of the material in respiratory tract is traced by appearance of material in nasal cavity and violent striving by the animal.
- Gavage - into the stomach via a tube or gavage needle
- Rectal (per rectum) - into the rectum via the anus
- NPO (nil per os) - nothing by mouth. Usually prescribed prior to general anesthesia.

Parenteral

- Intravenous (IV) - directly into the venous bloodstream
- Intraperitoneal (IP) - into the abdominal cavity
- Subcutaneous (SC) - under the skin
- Intramuscular (IM) - into a muscle
- Intradermal (ID) - into or between layers of skin
- Intrathecal (IT) - into the subarachnoid space of the spinal cord
- Intracranial (IC) - into the substance of the brain



The route selected for drug administration is governed by the nature of the agent being administered, the animal, the purpose of administration, and other factors. The techniques for

each route vary from species to species, but all require a general understanding of local anatomy at the injection site.

The investigator should know the physiological properties of the substance to be injected because considerable tissue damage and discomfort can be caused by irritating vehicles or drugs. For example, the rabbit foot pad should not be used as an injection site; sodium pentobarbital should be administered only intravenously or intraperitoneally, not subcutaneously or intramuscularly, because of its irritating properties.

NEEDLE SIZES AND RECOMMENDED INJECTION VOLUMES

SPECIES	Intravenous	Intraperitoneal	Intramuscular	Subcutaneous
Mouse	Lateral tail vein; 0.2 ml; ~ 25 ga	2-3 ml; ~ 25 ga	**NR** Quadriceps/posterior thigh; 0.05 ml; ~ 25 ga	Scruff; 2-3 ml; ~20 ga
Rat	Lateral tail vein; 0.5 ml; ~ 23 ga	5-10 ml; ~ 21 ga	**NR** Quadriceps/posterior thigh; 0.3 ml; ~23-25 ga	Scruff; 5-10 ml; ~ 20 ga
Hamster	Femoral / jugular vein (cut down); 0.3 ml; ~ 25 ga	3-4 ml; ~21 ga	**NR** Quadriceps/posterior thigh; 0.1 ml; ~ 25 ga	Scruff; 3-4 ml; ~ 20 ga
Guinea Pig	Ear vein, saphenous vein; 0.5 ml; ~ 23 ga	10-15 ml; ~ 21 ga	Quadriceps/posterior thigh; 0.3 ml; ~ 21 ga	Scruff; 5-10 ml; ~ 20 ga
Rabbit	Marginal ear vein; 1-5 ml (slowly); ~21 ga	50-100 ml; ~ 20 ga	Quadriceps/posterior thigh, lumbar muscles; 0.5-1 ml; ~ 20 ga	Scruff, flank; 30-50 ml; ~ 20 ga
Cat	Cephalic vein, 2-5 ml (slowly); ~21 ga	50-100 ml; ~ 20 ga	Quadriceps/posterior thigh; 1 ml; ~ 20 ga	Scruff, back; 50-100 ml; ~20 ga
Dog	Cephalic vein; 10-15 ml (slowly); ~ 21 ga	100-200 ml; ~ 18 ga	Quadriceps/posterior thigh; 2-5 ml; ~ 20 ga	Scruff, back; 100-200 ml; ~ 20 ga
Primate (Squirrel/Owl monkey, galago)	Femoral vein; 0.5-1 ml (slowly); ~ 21 ga	10-15 ml; ~ 21 ga	Quadriceps/posterior thigh; 0.3-0.5 ml; ~ 21 ga	Scruff, 5-10 ml,~ 20 ga
Primate* (Rhesus, Cyno, Snow)	Cephalic, recurrent tarsal, or jugular veins; 5-10 ml (slowly); ~ 20 ga	25-50 ml; ~ 20 ga	Quadriceps/ posterior thigh, triceps; 1-3 ml; ~ 20 ga	Scruff; 10-30 ml; ~ 20 ga
Primate* (Baboon)	Cephalic, recurrent tarsal, and jugular veins; 10-20 ml (slowly); ~ 20 ga	50-100 ml; ~ 18 ga	Quadriceps/ posterior thigh, triceps; 1-3 ml; ~ 20 ga	Scruff, 10-30 ml per site; 60-100 total; ~ 20 ga

* Must be chemically restrained

NR = Not recommended. Requires extreme care.

IV INJECTION SITES

SITE	SPECIES
Jugular vein	Cat, sheep, dog, goat, rabbit, horse, cow
Cephalic vein (Fore limb)	Dog, cat, large primates
Saphenous vein (Hind limb)	Monkey, dog, guinea pig (difficult)
Tail vein	Rat, mouse
Marginal ear vein	Rabbit, pig
Alar vein (Wing vein)	Bird
Femoral vein	Monkey, cat

TEACHER'S SIGNATURE

EXPERIMENT NO.: 7 **DATE:**

AIM: TO STUDY OF EFFECT OF HEPATIC MICROSOMAL ENZYME INDUCERS ON THE PHENOBARBITONE SLEEPING TIME IN MICE.

REQUIREMENTS:

- Mice (20-25g),
- Syringe,
- Needle (22-24 Gauge),
- Stop watch

DRUGS:

- Pentobarbitone sodium (10 mg/ml)
- Diazepam (1 mg/kg)
- Saline (0.9% NaCl)

THEORY:

- Sedatives produce the calming effects and anxiolytic effects leading to drowsiness and reduce anxiety.
- In therapeutic dose sedative are anxiolytic but in larger doses it produce hypnosis means sleep and some of them may also produce anesthesia.
- Phenobarbital, also known as phenobarbitone or phenobarb, is a medication recommended by the World Health Organization for the treatment of certain types of epilepsy in developing countries.
- Phenobarbione is a cytochrome P450 inducer, and is used to reduce the toxicity of some drugs.
- Sedation and hypnosis are the principal side effects (occasionally, they are also the intended effects) of phenobarbitone.

PROCEDURE:

- First selected mice dived in to three groups and each group consist three mice
- First group receive Saline (0.1 ml, i.p)
- Second group receive Pentobarbitone sodium (40 mg/kg, i.p)
- Third group receive Diazepam (5 mg/kg, i.p)
- Volume of drug injected should not exceed 0.5 ml in mice.
- The time of the onset of action is loss of righting reflex in mice i.e animal fails to uphold it normal position or falls asleep (hypnosis) is record for each animal.

- The animals are placed on their back leaving sufficient space in between two animals.
- Next, the time of recovery from sleep is recorded.
- It is the time from the loss of righting reflex and the time when animal turns to recover its normal posture.

OBSERVATION TABLE:

Groups	Drugs	Dose	Animals	Time of Onset	Time of recovery
I	Saline		1	-	-
			2	-	-
			3	-	-
II	Pentobarbitone	40 mg/kg	4	10	140
			5	8	130
			6	14	150
III	Diazepam	5 mg/kg	7	25	90
			8	30	90
			9	31	110

RESULTS:

- Pentobarbitone group shows loss of righting reflex means onset of action quick than diazepam than saline.
- The time of recovery from sleep is increase in pentobarbitone than diazepam than saline.

DISCUSSION:

- Barbiturates, benzodiazepine etc., induce sleep in human and animals by depressing central nervous system.
- They are called sedative and hypnotics.

TEACHER'S SIGNATURE

EXPERIMENT NO.: 8 **DATE:**

AIM: TO STUDY THE EFFECT OF DRUGS ON CILIARY MOTILITY OF FROG OESOPHAGUS

INTRODUCTION:

- Cholinergic drugs causes contraction of cilia leading to increased movements.
- Anticholinergics drugs causes paralysis of cilia leading to decreased movements.

OBJECTIVE:

- To find out the action of certain drugs on the ciliary motility of frog oesophagus.

MATERIALS:

- Frog.
- Poppy seeds.
- Frog wooden board.
- Stop watch
- Drugs & Solution:

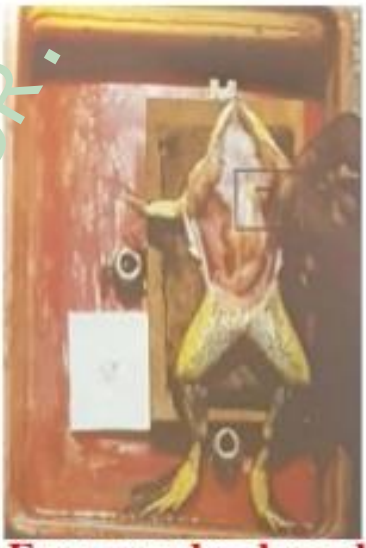
  A) Acetylcholine 10%: Cholinergic agonist.

  B) Physostigmine 10%: Anti-choline esterase inhibitor.

  C) Atropine 0.1%: Non-selective muscarinic agonist, or Cholinergic antagonist.

  D) Frog Ringer: Nutrient solution to keep the tissue viable.

  E) d-Tubocurarine: Muscle relaxant



Frog wooden board

Poppy seeds

METHOD:

Frog's oesophagus:

- The preparation was made by passing one blade of a pair of scissors into the mouth of the frog so as to cut off the head, leaving the floor of the mouth and lower jaw intact.
- The spinal cord was destroyed and the frog pinned on a cork mat/wooden board, dorsal surface uppermost. The skin of the back was divided down the midline, and the posterior body wall was removed by cuts parallel to the midline for 3 cm.
- The oesophagus was thus exposed and was opened dorsally from the buccal cavity to the stomach.
- It was laid out so that its inner surface was nearly horizontal, using one or two pins.
- The cork mat with the frog was then placed in a perspex chamber and covered with a perspex lid.
- A piece of cotton-wool on which hot water was poured to keep the air moist was placed on each side of the frog.
- There was a small slot in the lid at right angles to the midline of the oesophagus, and small particles, the movement of which was to be observed, were dropped on to the oesophagus through the slot.
- We used poppy seeds, selected by passing them through a sieve of mesh 40, and then retaining those which would not pass through a mesh 60.
- A line marked on the perspex lid indicated the distance to be travelled by the particles.
- We used a distance of 8 mm.

Recording ciliary movement:

- The preparation was irrigated at intervals of 5-20 min. with diluted Locke's solution (10 vol. solution to 14 vol. distilled water).
- Readings consisted in taking the time for 10 seeds to travel the 8 mm. distance, from which the mean time was calculated, and from this the distance travelled in 100 sec. was further calculated. When determinations of the rate of transport had thus been made for a period of 30-40 min., the oesophagus was irrigated with a solution of the drug in the diluted Locke's solution.
- The irrigation was repeated at intervals between readings as before. The effect of the drug having been observed, irrigation was then resumed with diluted Locke's solution only, and a return of the rate of transport to about the initial value occurred.
- In some experiments the oesophageal mucous membrane was removed from the frog, and observations were made when no possibility remained of a circulation through the membrane.

RESULT:

Eserine sulphate:

An experiment illustrating the effect of eserine sulphate in concentration of 10^{-4} (Figure 1) in which the transport of poppy seeds is shown as ordinate. By observations during 35 min. it was established that the rate was about 7 mm. per 100 sec. During this period the mucous membrane was washed with diluted Locke's solution every 5 min. Eserine was then applied, and 5 min. later the rate had risen to about 14 mm. per 100 sec., where it remained for the 30 min. during which the membrane was regularly washed with eserine solution. At the end of the 30 min. period the membrane was once more washed with diluted Locke solution. The rate of transport soon returned to about the initial value. A series of experiments was performed with different eserine concentrations, and the changes in the rate of ciliary movement were expressed as a percentage of the initial rate. In each experiment the initial rate was taken as the mean of all rates observed before the application of eserine, and the rate after applying eserine was taken as the mean rate during the whole period of application. In all experiments the removal of eserine resulted in a return to the initial rate. The results are given in Table I, in which it will be seen that, as the concentration of eserine rose, the mean effect rose up to a maximum at 10^{-4}, beyond which it declined; 4 x 10^{-4} eserine did not increase the rate of ciliary movement but depressed it.

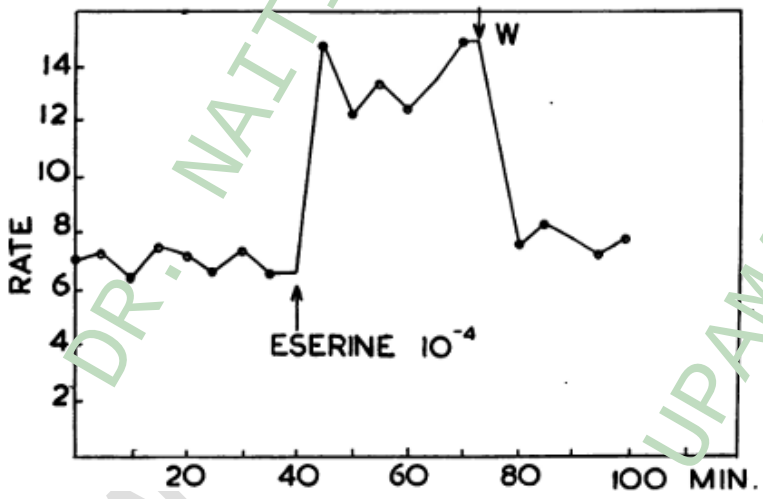


FIG. 1.—Action of 10^{-4} eserine on ciliary movement in the oesophagus of the pithed frog. Ordinate is rate in mm./30 sec. Each point is the mean of 10 observations. At W membrane washed with diluted Locke solution.

EFFECT OF ESERINE ON CILIARY MOVEMENT IN THE FROG OESOPHAGUS

(Each figure is taken from a separate experiment)

	Percentage change in rate at concentrations indicated			
	10^{-5}	10^{-4}	2×10^{-4}	4×10^{-4} eserine
	+55	+145	+78.5	−20.5
	+40	+79.5	+14.5	+38
	+24	+83	+27.5	−43
	+35.5	+81.8	+53	−16.8
	+52	+87	+19.6	−13.2
Mean ..	+41.3	+95.2	+38.6	−11.1

Acetylcholine:

The application of acetylcholine, like that of the lower concentrations of eserine, caused an increase in the rate of ciliary movement. The only concentration used was 10^{-5}, and that produced increases in five experiments of 79, 25, 44, 54, and 56 per cent respectively, the mean increase being about 50 per cent. In three of the experiments it was observed that the removal of the acetylcholine was followed by a transient slowing of the rate to much less than the initial rate. The effect is illustrated in Fig. 2; in view of the earlier work on the auricles it suggests that in the presence of applied acetylcholine the natural production of acetylcholine was inhibited, and was only resumed at the initial rate sometime after the applied acetylcholine had been removed.

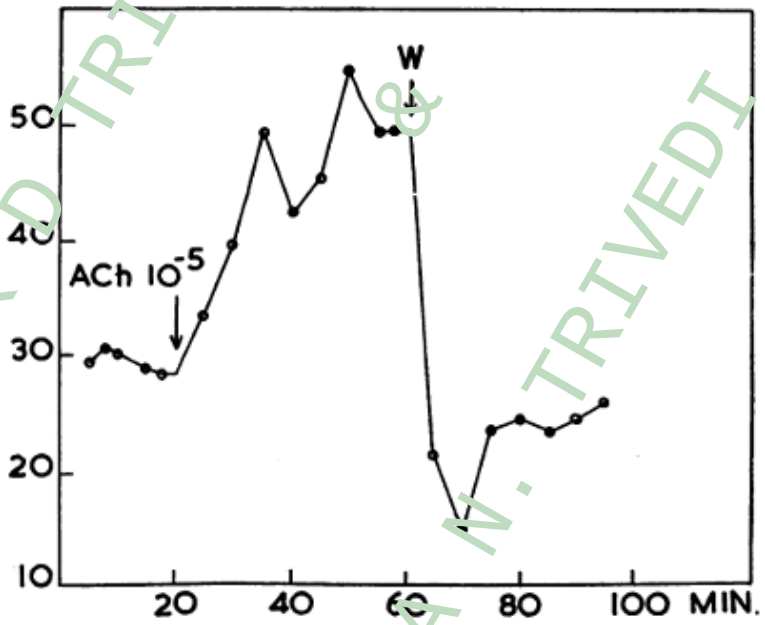


FIG. 2.—Action of 10^{-5} acetylcholine on ciliary movement in the oesophagus of the pithed frog. Ordinate is rate in mm./100 sec. Note the drop in rate after the acetylcholine had been removed by washing at W; the rate fell to less than the initial value and then slowly recovered.

Atropine sulphate:

Atropine in a concentration of 10^{-6} was found to inhibit ciliary movement. In three experiments the rate was diminished to 18, 64, and 40 per cent of its original value, and when the atropine was removed the rate was rapidly restored.

d-Tubocurarine:

d-Tubocurarine in a concentration of 10^{-6} was also found to inhibit ciliary movement to the same extent as atropine. The effect was seen not only in the oesophagus in situ, but also in the isolated preparation.

SOURCE: CILIARY MOVEMENT AND ACETYLCHOLINE BY PAMELA KORDIK, E. BULBRING, AND J. H. BURN, from the Department of Pharmacology, Oxford University (Received October 2, 1951), Brit. J. Pharmacol. (1952), 7, 67.

EXPERIMENT NO.: 9 **DATE:**

AIM: TO STUDY THE EFFECTS OF VARIOUS DRUGS ON RABBIT EYE

REQUIREMENTS:

Rabbits, Eye Droppers, rabbit holder,

DRUG :

Acetylcholine, carbachol, physostigmine, atropine, ephedrine, lignocaine

THEORY:

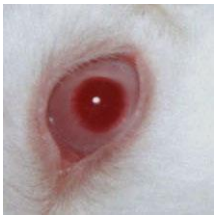
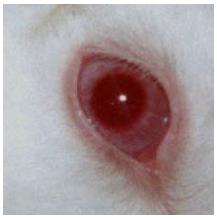
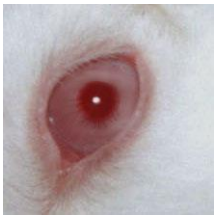
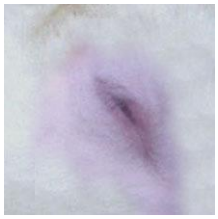
- Iris contains two types of smooth muscles.
- Sphincter pupillae and dilator pupillae (Radial muscles).
- Contraction of sphincter pupillae constricts pupil produce meiosis and contraction of radial muscles produces dilation of pupils known as mydriasis.
- Additionally eye contains ciliary muscles that are involved in adjustment of lens for distance and near vision.

PROCEDURE:

Keep the rabbit in a rabbit holder in such a way that the head will be protruding outside. Consider its right eye as control eye (in each case 2-3 drops of normal saline are instilled in this eye) and left eye as the Test eye (in each case 2-3 drops of normal saline are instilled in this eye).

Testing of reflexes:

1) **Corneal touch reflexes:** Can be studied by touching the cornea of eye with a cotton pledget or a piece of paper and observing whether the rabbit blinking the eyelids or not. Check in both control eye and test eyes.
2) **Light reflexes:** it is studied by focusing a torch on the eye and observing whether the pupil is constricted in response to the light or not. Check in both control eye and test eye.
3) **Effects of drug on diameter of pupils:** the dilation or constriction of pupil after adding the drug solution is observed and compared with the diameter of pupil in the both eye.

Normal Eye Pupil Dilate Constricted Pupil Blinking of eye

OBSERVATION TABLE:

Sr.No	Drug Solution	Pupil Size	Light Reflex	Touch Reflex
1	Saline	No Change	Present	Present
2	Ephedrine	Increase	Present	Present
3	Carbachol	Decrease	Present	Present
4	Physostigmine	Decrease	Present	Present
5	Atropine	Increase	Absent	Present
6	Lignocaine	No Change	Present	Absent

DISCUSSION:

- Acetylcholine/Carbachol binds to muscarinic receptors (M3) of sphinctor muscles in the iris due to which the spinctor muscle contract and pupils size is reduced causing meiosis.
- Physostigmine is a reversible cholinesterase inhibitor drug which inhibits destruction of acetylcholine. This result in the increase in concentration of acetylcholine which binds to the muscarinic receptors in sphincter muscles and causes meiosis.
- Ephedrine binds to alpha receptors (α1) in radial muscles and constricting of redial muscles dilate the pupil and pupil size get increased causing mydriasis.
- Atropine is a competitive antagonist of acetylcholine at muscarinic receptors. Atropine binds to muscarinic receptors and inhibits action of acetylcholine on these receptors in the spinctor muscles. This causes paralysis of the cilliary muscles and resultant increase in pupil size. Due to paralysis of cilliary muscles, pupil does not show light reflexes.
- Lignocain/Cocain is a local anesthetics agent hence corneal reflexes are loss if Lignocain/Cocain is instilled. There is no any changes in size of the pupil but touch reflex shows negative result means touch reflex absent in lignocaine treated eye. But when we focus light in to eye pupil size get increase and decrease so it gives positive result with light reflex.

TEACHER'S SIGNATURE

EXPERIMENT NO.: 10 **DATE:**

AIM: TO STUDY THE EFFECTS OF SKELETAL MUSCLE RELAXANTS USING ROTA-ROD APPARATUS.

Apparatus: Rota rod

Drug: Diazepam

Animal: Mice

Principle:

Generally, anxiolytics are known as minor tranquilizers and neuroleptics or antipsychotics known as major tranquilizers. Minor tranquilizers or anti-anxiety agents like benzodiazepines produce specifically the skeletal muscle relaxation. The site of activity is CNS. Disturbance in maintenance of tone and posture is the 1st sign of centrally mediated skeletal muscle relaxation. A mouse when allow to stay on a slow rotating rod fails to stay on the rod maintaining its posture, when a skeletal muscle relaxant is given. This property is utilized in the rotarod test.

Theory:

Skeletal muscle relaxants are drugs that are used to relax and reduce tension in muscles. They are more simply referred to as muscle relaxants.

Some work in the brain or spinal cord to block or dampen down excessively stimulated nerve pathways. These are called centrally acting muscle relaxants and examples include baclofen, methocarbamol, and tizanidine.

Others act directly on muscle fibers and are classified as peripherally acting muscle relaxants. Examples include dantrolene and the different types of botulinum toxin. Although dantrolene acts directly on the muscle itself, it also appears to indirectly act on the central nervous system and can cause drowsiness.

Cannabis extract also has muscle relaxing properties and is thought to act both centrally and peripherally.

Procedure

In this practical fall of time is being recorded when the mice falls from the rod.

Turn on the rotarod apparatus and perform the below phases

a) **Training phase:**
   - It consists of 3 trials at 20 rpm constant speed.
   - All trials being performed 10 minutes of intervals.
   - Note the fall in time from rod for mice and take mean of obtained value.

b) **Test phase:**
   - Now, inject the test drug (Diazepam 2mg/Kg).
   - After 30 minutes place the mice on rotarod.

- Note the fall in time from rod for mice and take mean of obtained value.

Dose calculation:

Mice weight 30 G = 30 X 10^{-3}Kg

Dose of diazepam is 2 mg/Kg

1 Kg animal required ---------- 2 mg dose

30 X 10^{-3}Kg animal required ---------- (?) = 60 X 10^{-3} mg

Stoke solution = 0.2 mg/mL

0.2 mg drug required -------- 1 mL dose

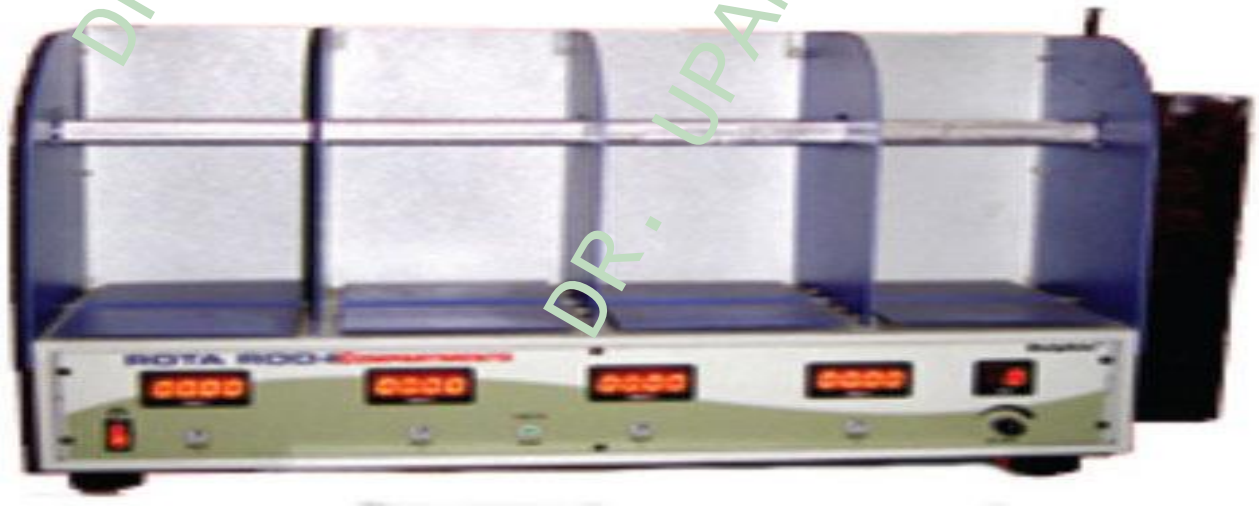
60 X 10^{-3}mg drug required ------- (?) = 0.3 mL dose

OBSERVATIONS:

S.NO	Body weight (GM)	Drug treatment dose	Volume of drug injected (mL)	Fall of time (in sec)		% decrease in activity
				Before drug	After drug	
1.	40	Diazepam 2mg/kg (i.p)	0.40	305	68	77.7
2.	34		0.34	266	78	70.67
3.	30		0.30	209	55	73.68
4.	30		0.30	321	103	67.91

DISCUSSION:

Motor co-ordination in mice is found to be decrease when administered the drug diazepam. Hence we can conclude that the diazepam have skeletal relaxant property.



TEACHER'S SIGNATURE

EXPERIMENT NO.: 11 **DATE:**

AIM: TO STUDY THE EFFECT OF DRUGS ON LOCOMOTOR ACTIVITY USING ACTOPHOTOMETER.

Drug: Diazepam 2 mg/Kg (i.p), Stoke solution – 0.2 mg/mL

Animal: Mice (20 – 25 G)

Equipment: Actophotometer

Principle:

Most of the CNS acting drugs influence the locomotor activity in man and animal. The CNS depressant drugs such as barbiturates and alcohol reduces the motor activity while the stimulant such as caffeine and amphetamines increases the 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 photo cell 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 & mice may be used for testing in this equipment.

PROCEDURE:

1. Weigh the animals (20-25 g mice) & number them.
2. Turn on the equipment (check & make sure that all the photo cells are working for accurate recording) and placed individually each mouse in the activity cage for 10 minutes. Note the basal activity score of all the animals (6).
3. Inject the drug chlorpromazine hydrochloride (Dose: 3 mg/kg, ip; make a stock solution containing 0.3 mg/ml of the drug & inject 1 ml/100 g body wt of mouse), and after 30 mins re-test each mouse for activity scores for 10 mins. Note the difference in the activity, before & after chlorpromazine.
4. Calculate percent decrease in motor activity.

Dose calculation:

Mice weight 30 G = 30 X 10^{-3}Kg Dose of diazepam is 2 mg/Kg

1 Kg animal required ---------- 2 mg dose

30 X 10^{-3}Kg animal required ---------- (?) = 60 X 10^{-3} mg

Stoke solution = 0.2 mg/mL

0.2 mg drug required -------- 1 mL dose

60 X 10^{-3}mg drug required ------- (?) = 0.3 mL dose

OBSERVATIONS:

S.NO	Body weight (GM)	Drug treatment dose	Volume of drug injected (mL)	Locomotor activity(Scores) in 10 min		% decrease in activity
				Before drug	After drug	
1.	40	Diazepam 2mg/kg (i.p)	0.40	717	201	71.96
2.	34		0.34	787	194	75.34
3.	30		0.30	696	298	55.91
4.	30		0.30	780	156	80

CONCLUSION:

Reduction in the motor activity indicates CNS depressant property of the drug.

Increase in the motor activity indicates CNS stimulant property of the drug.

OTHER DRUGS:

- CNS depressants:
  - Chlorpromazine hydrochloride (3 mg/kg, ip in case of both rat & mice)
  - Fluoxetine (10 mg/kg, ip in case of rat)
  - Imipramine (10-20 mg/kg, ip in case both mice & rat)
  - Phenobarbitone sodium (10 mg/kg, ip in case of both rat & mice)
  - Alcohol (0.5-2 g, ip, po in case of both mouse & rat)
- CNS stimulants:
  - Caffeine (8-10 mg/kg, ip in case of mice & 30 mg/kg, ip in case of rat)
  - Amphetamine (1.5 mg/kg, ip in case of mice & 3-5 mg/kg, sc, ip in case of rat)

TEACHER'S SIGNATURE

EXPERIMENT NO.: 12 **DATE:**

AIM: TO STUDY THE ANTI CONVULSIVE OR ANTIEPILEPTIC ACTIVITY OF DRUG USING MAXIMUM ELECTROCONVULSIVE SHOCK SEIZURE (M. E. S) AND CHEMICAL INDUCE (PENTYLENETETRAZOL - PTZ) CONVULSIONS METHODS.

Requirement: Electro convulsiometer, Electrode (Eye or Ear), Stop watch.

Animal: Rat or Mice

Drugs: Pentylenetetrazol (Leptazole 80 mg/kg),
Phenytoin (100 mg/kg),
Trimethadione (40 mg/kg),
Saline.

Principle:

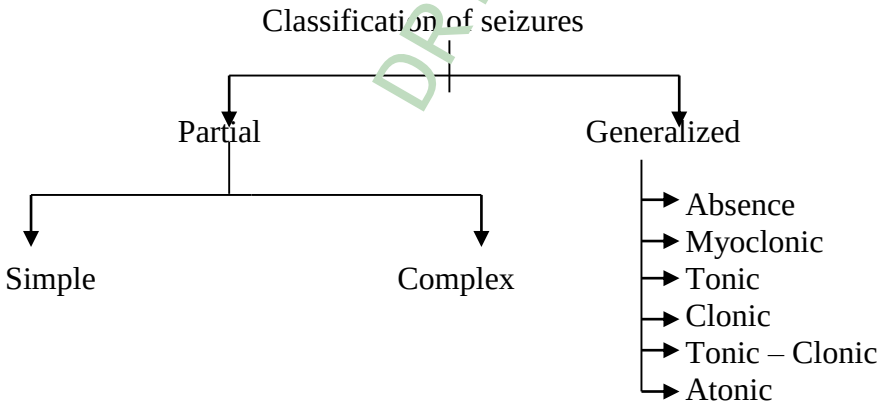
The convulsion in rat and mice can be induced by giving high voltage current near the brain or by suitable CNS stimulants (Eg. Pentylenetetrazol). The screening of antiepileptic agents can be done by experimentally induced convulsion (Seizures) and their prevention by drug under test.

Theory:

Epilepsy: "These are group of disorders of the CNS characterized by paroxysmal cerebral dysrythmia manifesting as brief episodes of the loss of consciousness with or without characteristic body movement, sensory or psychiatric phenomenon."

- Epilepsy has a focal origin in the brain.
- Epilepsy is derived from the greek word meaning "to seize upon" or "taking hold of"

Seizures: These are sudden alterations in behaviour or motor function caused by an electrical discharge from the brain.

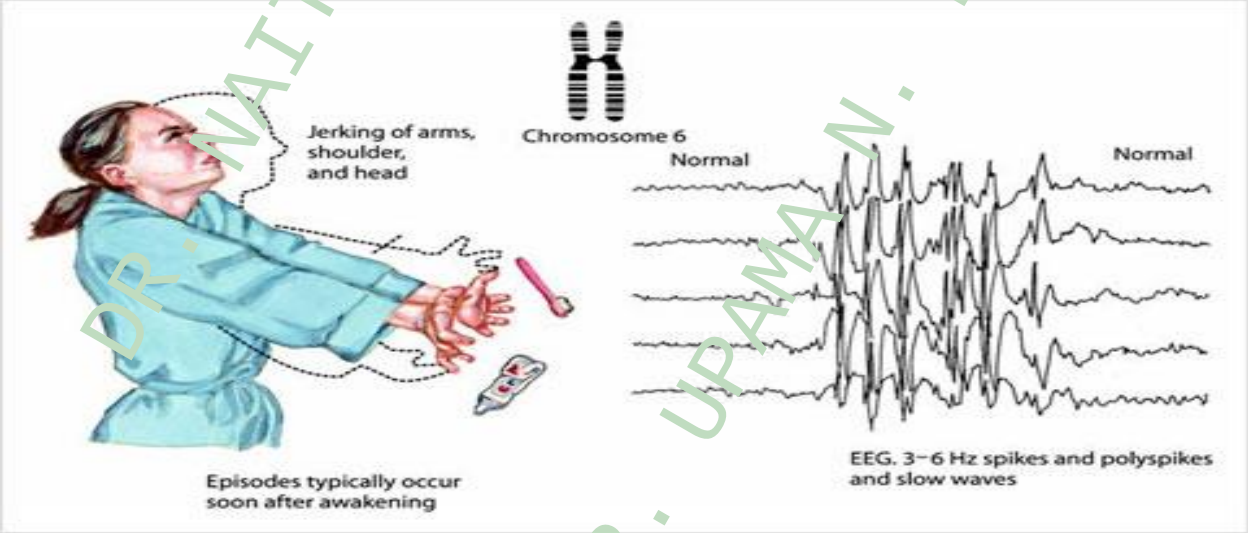


1. **Partial seizures:** Seizure arises from specific area of one side of brain.
   Partial seizures are subdivided between simple and complex partial seizures, which are distinguished by the presence or absence of impairment of consciousness.

- Simple partial seizures are defined as seizures without impairment of consciousness
- Complex partial seizures are defined as seizures with impairment of consciousness

2. **Generalized:** throughout all areas of both sides of brain
   - **Absence seizure (petit mal):** patients seem to lose concentration, stare and fluttering of eyelids fro a while, mistaken for day dreaming, in children.

- **Clonic (myoclonic):** Alternate contraction and relaxation, jerking.



- **Tonic :** Muscular contraction
- **Atonic :** Relaxation, flaccid paralysis

- **Tonic- clonic (grandmal).** strong contraction of musculature- Resp. stops,salivation often occur- tonic phase lasts for I min-followed by violent, jerks upto 2-4 min.

➢ **Drug used to treat epilepsy are classify as**:

1. Barbiturates	Phenobarbitone, Mephobarbitone
2. Deoxybarbiturate	Primidone
3. Hydantoin	Phenytoin
4. Iminostilbenes	Carbamazepines
5. Succinimides	Ethosuximide
6. Aliphatic carboxylic acid	Valproic acid
7. Benzodiazepines	Clonazepam, Diazepam
8. Newer agents	Lamotrigine, Gabapentin, Vigabatrin
9. Miscellaneous	Phenacetamide, Acetazolamide

Procedure:

a) Maximum Electro convulsive seizure (MES):

- The rat weight 150-250 gm or mice weight 20-40 gm are used in the experiments.
- The animals are first tested by giving maximum current 150mA in rat and 80mA in mice for 0.2 sec.
- Those animals which shows characteristics course of convulsion are selected.
- Then the selected rat or mice of either sex are randomly divided in to two groups as control and test. Each group consist six animals.
- The control group is administered with saline solution and the test group is administered with Phenytoin (100 mg/kg).

- Then gives produce the convulsion by giving maximum electroconvulsive shock in mice 80mA and in rat 150 mA for 0.2 sec through the electrode place on ear pinna.
- Record the reading for Clonic convulsion, Straub tail, Tonic convulsions, Stupor and Recovery for control and test groups of animal.

b) Chemical methods:

- The animals are injected with Leptazole (80 mg/kg, i.p).
- Those animals which shows characteristics course of convulsion are selected.
- Then the selected rat or mice of either sex are randomly divided in to two groups as control and test. Each group consist six animals.
- The control group is administered with saline solution and the test group is administered with Phenytoin (25 mg/kg, i.p).
- The Leptazole (80 mg/kg, i.p) administered and the time taken for the convulsion to start is note.
- Picrotoxin (6-7 mg/kg) may also be used instead of Leptazole to produce convulsion.

.

Observation and Results:

1) **Reading of control group animal is:**
   - Clonic convulsion : 13 sec.
   - Straub tail : present.
   - Tonic conculsions : 0.8 sec
   - Stupor : 120 sec
   - Recovery
2) **Reading of test group animal is:**
   - Clonic convulsion : 0.6 sec.
   - Straub tail : absent
   - Tonic conclusions : 0.4 sec
   - Stupor : 50 sec
   - Recovery

Observation table:

Effect of Phenytoin on the electrically induced convulsion in mice

Sr. no	Treatment	Time (in Sec) of different phase of seizure			
		Tonic	Clonic	Stupor	Recovery/ death
1	Saline (0.2 ml)	13	08	120	Recovery
2	Saline (0.2 ml)	12	06	125	Recovery
3	Saline (0.2 ml)	10	07	110	Recovery
4	Saline (0.2 ml)	13	09	130	Recovery
5	Saline (0.2 ml)	14	08	124	Recovery
6	Saline (0.2 ml)	12	06	120	Recovery
7	Phenytoin (100mg/kg)	06	03	50	Recovery
8	Phenytoin (100mg/kg)	05	04	45	Recovery
9	Phenytoin (100mg/kg)	07	03	55	Recovery
10	Phenytoin (100mg/kg)	06	05	60	Recovery
11	Phenytoin (100mg/kg)	05	03	62	Recovery
12	Phenytoin (100mg/kg)	06	02	65	Recovery

Discussion:

- Epilepsy is synchronous discharge of impulses from brain characterized by ora (noice), cry tonic and clonic convulsion. There is spontaneous occurrence of brief episodes associated with disturbance in consciousness and excessive ECG spike.
- It is characterize that a drug showing prevention against electrically induced convulsion are effective in Grand-mal epilepsy in human beings and those drugs which prevent only chemically induced convulsion are effective therapeutically in petit mal epilepsy.

TEACHER'S SIGNATURE

EXPERIMENT NO.: 13. A **DATE:**

AIM: TO STUDY OF STEREOTYPE BEHAVIOUR IN RAT/MICE OR TO STUDY THE TAMING EFFECTS OF CHLORPROMAZINE IN RATS AND MICE OR TO STUDY THE EFFECTS OF CHLORPROMAZINE ON APOMORPHINE INDUCED COMPULSIVE BEHAVIOUR

REQUIREMENTS:

- Rats: 150-200gm
- Mice: 20-40gm
- Syringe and needles
- Clean beaker (250 ml for mice, 1000 ml for rats)

DRUGS:

- Apomorphine:
  - Dose: 2.5 mg/kg (i.p)
  - Stock solution:0.25 mg/mL,
  - Inject: 1 mL/100 GM body weight of animal
- Chlorpromazine (0.3 mg/mL)
  - Dose: 3 mg/kg (i.p)
  - Stock solution: 0.3 mg/mL,
  - Inject: 1 mL/100 GM body weight of animal

PRINCIPLE:

Compulsive behavior is defined as purposeless activity exhibited by the animal. This purposeless activity is supposed to be identical to the behavioral disorder seen in schizophrenic patient who also shows repetitive purposeless activity. This behavioral abnormality in schizophrenia is due to the excessive neuronal activity of dopamine receptor agonist, through its dopaminergic activity inducer compulsive stereotyped in rat and mice. The stereotyped behavior induces repetitive standing (rearing), continuous sniffing (touching the nose to the wall of the container) and licking to the wall of the container. These behaviors can be easily observed and subjectively scored also.

THEORY:

Psychosis: "means out of touch with reality or unable to separate the real form and unreal form"

Schizophrenia: "It is well describe by false perception"

- Psychosis or schizophrenia is arising due to increase the dopamine level.

- There are mainly two types of dopamine receptors D1 and D2.
- In psychosis D2 receptors are mainly involved and which increases the dopamine level in brain.

PROCEDURE:

- Weigh the animal divide it in to two groups
- Each group consist three animals.
- One group act as control groups and they all receive saline
- Second group act as test group they all receive chlorpromazine (3 mg/kg).
- After 30 minutes inject apomorphine (2.5 mg/kg) in to the entire animal
- Place them individually in to separate beakers and observe the intensity of compulsive behaviour like as:

  Rearing: Repetitive standing

  Sniffing: Touching of nose to the wall of the container

  Liking: Licking the wall of container
- Note the onset of responses at 15, 30 and 60 min after giving the apomorphine injection.

Gives the score according to severity like as:

1 – Presence of response 2 – Moderate response 3 – Sever response

OBSERVATION TABLE:

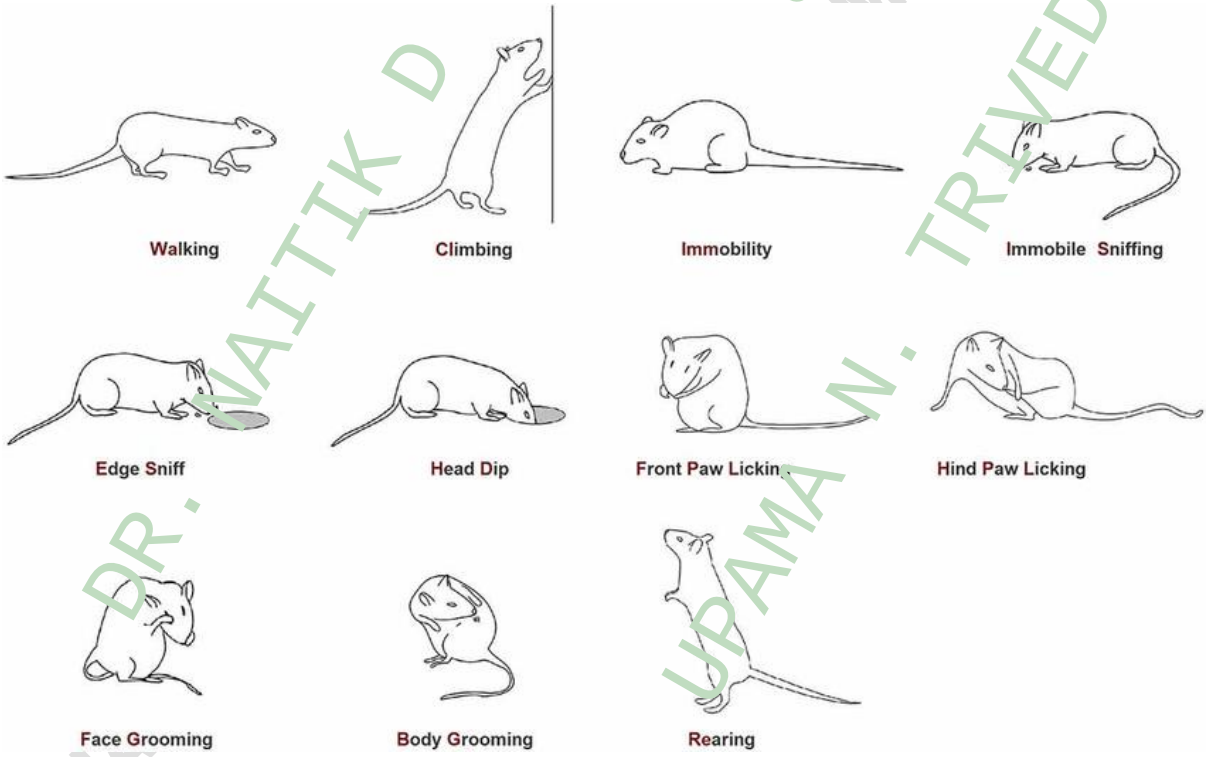
Effects	**Time in minutes**	**Score for Group-1 Animals (Saline + Apomorphine)**			**Score for Group-2 Animals (Chlorpromazine + Apomorphine)**		
		1	**2**	**3**	**1**	**2**	**3**
Rearing	**15 min**	2	2	2	1	1	1
	30 min	3	3	3	1	2	2
	60 min	2	2	1	1	1	1
Sniffing	**15 min**	3	3	3	1	3	2
	30 min	3	3	2	2	2	2
	60 min	2	2	2	1	1	1
Licking	**15 min**	3	2	2	1	2	2
	30 min	3	3	3	1	2	2
	60 min	1	1	1	1	1	2
Total Score		**22**	**21**	**19**	**10**	**15**	**15**

DISCUSSION:

- The rearing, sniffing and licking behavior of animal is known as compulsive behavior of animal which is identical to schizophrenia and psychosis in human.
- In the psychosis the level of dopamine gets increased and anti-psychosis drug decreases the level of dopamine is known as taming effects.
- Apomorphine increases the dopamine level while chlorpromazine decreases the dopamine level.

RESULTS:

Total score of Group-2 animals is decreased than the group-1 animals means chlorpromazine decrease the total score of Group-2 animals as compare to Group-1 animal so it gives the taming effects against the Apomorphine induced compulsive behavior.



TEACHER'S SIGNATURE

EXPERIMENT NO.: 13. B **DATE:**

AIM: TO STUDY ANTI-CATATONIC ACTIVITY OF DRUGS ON RATS/MICE OR TO STUDY THE DRUG INDUCED (HALOPERIDOL) CATATONIA IN RATS/MICE OR TO STUDY THE ANTI-PARKINSONISM DRUGS IN RATS/MICE

REQUIREMENTS:

Animal: Rat

Drug: Heloperidol (1 mg/Kg, Stoke solution 1 mg/mL)

Equipments: Two wooden blocks – one is 3 cm long and other is 9 cm long.

PRINCIPLE:

Phenothiazine and butyrophenone types of antipsychotic drugs are known to produce extrapyramidal side effects in man. These effects such as akinesia, rigidity, tremors are called Parkinson like because in Parkinson disease the major clinical symptoms include difficulty to move and change posture and tremors. These effects of antipsychotic drugs are due to excessive blockage of dopamine receptors in the extrapyramidal motor system.Therefore phenothiazines are commonly used to produce Parkinson like extrapyramidal symptoms in laboratory animal and to study anti- Parkinsonism drugs like levodopa, atropine, and scopolamine.

PROCEDURE:

Divide the animal in to two groups.

Group – I received haloperidol while Group - II were inject with levodopa than after 30 minutes it receive haloperidol.

Observed severity of catatonia response as follow:

Stages	Description	Score
Stage – I	Rat moves normally when placed on table	0
Stage – II	Rat moves only when touched or pushed	1
Stage – III	Rat placed on the table with front paws set alternatively on a 3 cm long block fail to correct the posture in 10 seconds	0.5 (for each paw – total score 1)
Stage – IV	Rat placed on the table with front paws set alternatively on a 9 cm long block fail to correct the posture in 10 seconds	1 (for each paw – total score 2)

OBSERVATION TABLE:

Animal group	No. of animals	Body weight	Drug treatment	Volume injected in mL	Cumulative Score of all stages
Control	1	250	Haloperidol (1 mg/Kg, i.p)	0.25	3
	2	320		0.32	3
	3	302		0.3	2.5
	4	340		0.34	2
	5	278		0.27	2.5
Test	1	260	Levodopa (15 mg/Kg, i.p)	0.26	1
	2	334		0.33	1
	3	315		0.31	0.5
	4	325		0.32	0.5
	5	345		0.34	0.5

DISCUSSION:

Anticatatonic drugs like levodopa, atropine and scopolamine treatment half an hour before haloperidol were reduced the catatonic score/intensity.



TEACHER'S SIGNATURE

EXPERIMENT NO.: 14 **DATE:**

AIM: TO STUDY THE ANXIOLYTIC ACTIVITY OF DRUGS USING RATS/MICE.

INTRODUCTION:

- Anxiety is chronic condition characterized by an excessive and persistent sense of apprehension with physical symptoms such as sweating, palpitations, and feelings of stress. Anxiety disorders have biological and environmental causes.
- Anxiety is a commonly experienced emotion. It is an uncomfortable feeling of apprehension or fear coupled with sensations of physical arousal.
- If anxiety is excessive or interferes with functioning, it is considered a pathologic anxiety disorder.
- Anxiety is different from fear due to its cognitive component (i.e. fear of the future)
- Anxiety can be helpful and adaptive (e.g. anxiety about giving lectures!)
- An anxiety disorder may be a primary disorder or it may occur secondary to medical causes or substances (e.g., medications or illicit substances); it may occur as a response to acute stressors or it may be associated with another psychiatric disorder.

Types of Anxiety Disorders:

1) Generalized Anxiety Disorder
2) Panic Disorder
3) Phobic Disorders
   - Agoraphobia, Acrophobia,Aquaphobia
4) Obsessive-Compulsive Disorder
5) Post-Traumatic Stress Disorder

MODELS FOR ANTIANXIETY ACTIVITY FOR RATS/MICE

1. **ANTI-ANXIETY TEST (LIGHT-DARK MODEL):**

Purpose and Rationale

- Crawley and Goodwin (1980) Crawley (1981) described a simple behavior model in mice to detect compounds with anxiolytic effects. Mice and rats tend to explore a novel environment.
- In a two chambered system, where the animals can freely move between a brightly open field and a dark corner.
- They show more crossings between the two chambers and more locomotor activity after treatment with anxiolytics.
- The numbers of crossings between the light and dark sites are recorded.

Procedure:

- The testing apparatus consists of a light and a dark chamber divided by a photocell-equipped zone.
- A animal cage, 44 × 21 × 21 cm, is darkened with black spray over one-third of its surface.
- A partition containing a 13 cm long × 5 cm high opening separates the dark one third from the bright two thirds of the cage.
- An electronic system using four sets of photocells across the partition automatically counts movements through the partition and clocks the time spent in the light and dark compartments.
- Naive male mice or rats are placed into the cage.
- The animals are treated 30 min before the experiment with the test drugs or the vehicle ntraperitoneally and are then observed for 10 min.
- Groups of 6–8 animals are used for each dose.

Evaluation:

- Dose-response curves are obtained and the number of crossings through the partition between the light and the dark chamber are compared with total activity counts during the 10 min.

2. ANTICIPATORY ANXIETY IN MICE:

Purpose and Rationale:

- When group-housed mice are removed one by one from their home cage, the last mice removed have always higher rectal temperatures than those removed first .
- This phenomenon is interpreted as being caused by anticipatory fear for an aversive event.
- This test is thought to be a model of anticipatory anxiety.

Procedure:

- Groups of 18 male albino Swiss mice weighing 25–30 g are used.
- Test drugs or standard (diazepam) or solvent are administered orally in various doses to groups of 18 mice prior to the test.
- Thirty min later, the first 3 mice are removed from the cage and the rectal temperature measured by inserting a silicone lubricated thermistor probe (2 mm diameter) for 2.5 cm into the rectum.
- The average temperature of these 3 mice is taken as basal value.
- thereafter body temperature is determined in the remaining three animals.
- The difference of the mean value of these mice and the basal values is calculated as increase. Vehicle treated test groups display increases of 1.1 to 1.3 °C.

Evaluation:

- The mean increase values of treated groups ±SEM are compared by ANOVA statistics with the controls.

3. SOCIAL INTERACTION IN RATS:

Purpose and Rationale:

- In an unfamiliar and brightly lit environment, the normal social interaction of rats (e.g. sniffing, nipping, grooming) is suppressed.
- Anxiolytics counteract this suppression.

Procedure:

- Male Sprague-Dawley rats (225–275 g body weight) are housed in groups of 5 animals.
- The apparatus used for the detection of changes in social behavior and exploratory behavior consists of a open-topped box (51 × 51 cm and 20 cm high) with 17 × 17 cm marked areas on the floor.
- One hour prior to the test, two naive rats from separate housing cages are treated with the test compound orally. They are placed in to the box and their behavior is observed over a 10-min period by remote video recording.
- Two types of behavior can be noted:
    - Social interaction between the animals is determined by timing the sniffing of partner, crawling under or climbing over the partner, genital investigation of partner.
    - Exploratory motion is measured as the number of crossings of the lines marked on the floor of the test box.
- Six pairs are used for each dose.

Evaluation:

- The values of treated partners are compared with the data from 6 pairs of untreated animals using single factor analysis of variance followed by Dunnett'st-test.

4. ELEVATED PLUS MAZE TEST:

Purpose and Rationale:

- Out of many possibilities to modify maze tests e.g. water maze, the Y-mace, the radial maze and the elevated plus maze have found acceptance in many laboratories.
- The test has been proposed for selective identification of anxiolytic and anxiogenic drugs.

- Anxiolytic compounds, by decreasing anxiety, increase the open arm exploration time; anxiogenic compounds have the opposite effect.

Procedure

- The plus-maze consists of two open arms, 50 × 10 × 40 cm, and two enclosed arms, 50 × 10 × 40 cm, with an open roof, arranged so that the two open arms are opposite to each other.
- The maze is elevated to a height of 50 cm. The rats (200–250 g body weight) are housed in pairs for 10 days prior to testing in the apparatus.
- During this time the rats are handled by the investigator on alternate days to reduce stress.
- Groups consist of 6 rats for each dose.
- Thirty min after i.p. administration of the test drug or the standard, the rat is placed in the center of the maze, facing one of the enclosed arms.
- During a 5 min test period the following measures are taken:
- the number of entries into and time spent in the open and enclosed arms.
- the total number of arm entries.

Evaluation

- Motor activity and open arm exploratory time are registered. The values of treated groups are expressed as percentage of controls. Benzodiazepines and valproate decrease motor activity and increase open arm exploratory time

5. WATER MAZE TEST:

Purpose and Rationale:

- Spatial learning of rats can be tested in a water maze

Procedure:

- The water maze consists of a circular tank with 100 cm diameter and a wall 20 cm above the water level.
- A circular platform (9 cm diameter) is hidden 2 cm below the water level.
- The water is made opaque using titanium dioxide suspension and is kept at about 23 °C during the experiment.
- Training takes place on three consecutive days, with the rats receiving 4 consecutive trials per day with an trial interval of 6–10 min.
- Latency to find the platform is measured as the time of placement of the rat in the water to the time it finds the platform.
- If the animal fails to find the platform in any trial within 3 min it is placed on it for 10 s.

Evaluation:

- The platform is removed and the time spent in the target quadrant (the quadrant in the center of which the platform has been located) and the number of annulus crossings across the actual location where the platform has been located in the first 60 s of exposure are measured.
- The time to the first annulus crossing is taken as a measure of performance of trial.
- Buspirone as well as benzodiazepines increase the latency to find the platform in the training period and impair the number and the time of annulus crossings.

6. STAIRCASE TEST:

Purpose and Rationale:

- When introduced into a novel environment, rodents experience a conflict between anxiety and exploratory behavior manifested by increased vigilance and behavioral activity.
- In the staircase paradigm, step-climbing is purported to reflect exploratory or locomotor activity, while rearing behavior is an index of anxiety state.
- The number of rearings and steps climbed are recorded in a 5 min period.
- The dissociation of these parameters is considered to be characteristic for anxiolytic drugs.

Procedure:

- For experiments with mice the staircase is composed of five identical steps 2.5 cm high, 10 cm wide and 7.5 cm deep.
- male mice with a weight between 18 and 24 g are used. Each animal is used only once.
- The drug or the standard is administered orally 1 h or 30 min subcutaneously before the test. The animal is placed on the floor of the box with its back to the staircase.
- The number of steps climbed and the number of rears are counted over a 3-min period.

Evaluation:

- Twelve mice are used for the untreated control groupnm and for the group receiving the standard.
- The average number of steps and rearings of the control group is taken as 100%.
- The values of treated animals are expressed as percentage of the controls.

7. CORK GNAWING TEST IN THE RAT:

Purpose and Rationale:

- Cork gnawing behavior in the rat has been proposed as a screening method for buspirone-like anxiolytics.

Procedure:

- Adult male Evans rats serve as subjects.
- They are housed 4 per cage on a regular light/dark cycle with free access to food and water
- For the test session one animal is placed in a stainless steel cage with wire mesh bottom.
- A session consists of placing the subject in the test cage with a cork stopper weighing between 2–3 g for 30 min.
- Initially, the amount gnawed is relatively high and variable within and between subjects. After 30 training sessions, the amount is low and stabilized.
- The test compounds are injected 30 min before the test and food is withdrawn.
- The average cork loss during the previous control days is taken as baseline and the amount after drug treatment is expressed as percentage of baseline.
- Buspirone-related compounds as well as benzodiazepines and meprobamate show a dose dependent increase of cork gnawing.

Evaluation:

- Each cork is weighed to the nearest 0.01 g before and after the session.

8. SCHEDULE INDUCED POLYDIPSIA IN RATS:

Purpose and Rationale:

- Food deprived rats exposed to a procedure in which food is delivered intermittently will drink large amounts of water if given the opportunity to do so.
- This behavioral phenomenon is termed schedule-induced.

Procedure:

- Male Wistar rats weighing 180–250 g are individually housed at a 12 h/12 h light/dark cycle for a 1 week acclimation period with free access to food and water.
- Then they are placed on a restricted diet which maintains 80% of their free feeding body weight.
- To induce polydipsia, rats are placed in test chambers housed in sound attenuated boxes where a pellet dispenser automatically dispenses two 45 mg pellets on a fixed time 60-s feeding schedule over a 150 min test session.
- Water is available at all times in the test chambers.

- After 4 weeks exposure to the 60s feeding schedule, approximately 80% of the rats meet the pre-determined criterion for water consumption (greater than 60 ml water per session) and are considered to have polydipsic behavior.

Evaluation:

- The experimental data comparing the effects of chronic administration of compounds on schedule-induced polydipsia are analyzed with the Mann Whitney U-test.

9. FOUR PLATE TEST IN MICE:

Purpose and Rationale

- The four plate test in mice has been used for the rapid screening of minor tranquilizers.

Procedure:

- The test box has the shape of a rectangle (25 × 18 × 16 cm).
- The floor is covered with 4 identical rectangular metal plates (8 × 11 cm) separated from one another by a gap of 4 mm.
- The plates are connected to a source of continuous current which applies to 2 adjacent plates a mild electrical shock of 0.35 mA for 0.5 s. This evokes a clear flight reaction of the animals.
- Adult male Swiss albino mice, weighing 17 to 23 g, are randomly divided into different groups.
- Thirty min before the test the animals are injected intraperitoneally with the test drug or the vehicle.
- At the beginning of the test, the mouse is gently dropped on to a plate and is allowed to explore the enclosure for 15 s.
- After this, every time the animal crosses from one plate to another, the experimenter electrifies the whole floor for 0.5 s, which evokes a clear flight-reaction of the mouse.

Evaluation:

- The number of times the apparatus is electrified is counted each minute for 10 min.
- The delivery of shocks decreases dramatically the motor activity.
- The number of shocks received during the first min is taken as parameter. This number is increased by minor tranquilizers, such as benzodiazepines.

10. FOOT SHOCK INDUCED FREEZING BEHAVIOR IN RATS:

Purpose and Rationale:

- Footshock-induced freezing behavior in rats has been proposed as a model for anxiolytics.

Procedure:

- Male Sprague-Dawley rats with a weight between 200 and 350 g are used.
- The animals receive a single i.p. injection of the test compound or the vehicle 30 min prior to being placed in a standard conditioning chamber (e.g., Coulborn Instruments) for a 6.5 min session.
- 2 and 2.5 min after the start of the session, a footshock (0.5 mA, 0.5 s) is delivered through the grid floor of the chamber. Following exclusive behaviors are observed:
- Freezing: immobility with rigid body posture
- Sedated posture: sitting or sleeping
- Small exploratory movements: movements involving the torso or front paws only, vertical movements of the head, or sniffing.
- Locomotion: activity involving hind paws, grooming or rearing. Frequency of rearing is also counted. All behaviors are monitored for the entire 6.5 min session.

Evaluation:

- Duration of foot-shock induced freezing after the second shock is taken as the critical parameter.
- Time spent in freezing posture after administration of test compounds is compared with the controls.

11. mCPP INDUCED ANXIETY IN RATS:

PURPOSE AND RATIONALE

- The metabolite of the antidepressant drug trazodone 1- (3-chlorphenyl) piperazine (=mCPP), classified as 5- HT1C agonist or 5-HT1B/2C agonist has been shown to be anxiogenic .
- Antagonism against these symptoms has been proposed as a screening model for anxiolytic drugs.

Procedure:

- Male Sprague Dawley rats (220–250 g) are housed in groups of 6 under a 12 h light/dark cycle with free access to food and water.

mCPP - induced locomotion:

- Rats are used.

- They are dosed either orally 1 h, or i.p. 30 min before the locomotion test with test compound or vehicle, and injected 20 min before the test with 7 mg/kg mCPP i.p. or saline in groups of four.
- At 0 h they are each placed in automated locomotor activity cages.
- locomotion is recorded by means of alternately breaking two photocell beams traversing opposite ends of the box 3.9 cm above floor level.

mCPP-induced hypophagia:

- Rats are individually housed on day 1 and on day 3 they are deprived of food.
- Twenty-three hours later, they are orally treated with the test drug or vehicle.
- Forty min later, they are given 5 mg/kg mCPP or saline i.p.
- After a further 20 min, weighted amounts of their normal food pellets are placed in their food hoppers and the amount remaining after 1 h is measured.

Evaluation:

- The effect of the test compound on mCPP-induced hypolocomotion is determined by one-way ANOVA and Newman-Keuls test. The dose producing 50% disinhibition of mCPP is also estimated.
- Feeding test data are subjected to one-way ANOVA and Dunnett's test.

12. ACOUSTIC STARTLE RESPONSE IN RATS:

Purpose and Rationale:

- The acoustic startle reflex is a relatively simple behavior that occurs naturally in mammals.
- It consists of a series of rapid movements beginning at the head and contraction and extension of major muscle groups .
- Startle response can be used to determine sites and mechanisms of drug action

Procedure:

- Male Wistar rats weighing about 200 g are used.
- Acoustic startle reflexes are measured in a specially build apparatus, e.g., Coulborn Instruments Acoustic Response Test System.
- The animals are individually placed in 8 × 8 × 16 cm open air cages that restrict locomotion but do not immobilize the animal.
- Sound-attenuating acoustic chamber used when sound is produced physical movement is measured.

- Data are recorded automatically by an interfaced microcomputer. Pre-tests are performed with all animals to obtain control values.
- The animals are treated 2 h prior the experiment with test drugs or vehicle given orally or subcutaneously.

Evaluation:

- The results are given as percentage of the change, related to the values obtained in the pre-test and assessed by a one-way ANOVA, followed by Dunnett's test when appropriate.

13. UNCONDITIONED CONFLICT PROCEDURE (VOGEL TEST):

Purpose and Rationale:

- Described a simple and reliable conflict procedure for testing anti-anxiety agents.
- Thirsty, naive rats were administered shocks while licking water.

Procedure:

- The apparatus is a clear Plexiglas box (black in color) ,The entire apparatus has a stainless-steel grid floor.
- A water bottle with a metal drinking tube is fitted to the outside of the small compartment, so that the tube extended into the box at a height 3 cm above the grid.
- Rats lick in constant rate of 7 licks per sec. A drinkometer circuit is connected between the drinking tube and the grid floor of the apparatus, so that the rat completes the circuit whenever it licks the tube.
- Shock is administered to the feet of the animal.
- Thirty min after i.p inj, the rat is placed in the apparatus and allowed to find the drinking tube and to complete 20 licks before shock is administered.
- The rat controls shock duration by withdrawing from the tube.
- A 3-min timer is automatically started after the termination of the first shock. During the 3-min period, shocks are delivered following each twentieth lick.
- The number of shocks delivered during the 3-min session is recorded for each animal.

Evaluation:

- The number of shocks received after treatment is compared with untreated animals. Benzodiazepines increase dose-dependent the number of shocks. Barbiturates at low doses active in this test.

14. NOVELTY-SUPPRESSED FEEDING:

Purpose and Rationale:

- Placing a hungry rat into an unfamiliar environment with access to food results in a suppression of feeding behavior relative to the condition when the test environmentis familiar.
- This effect has been termed hyponeophagia and occurs because of the novelty of the test environment. The avoidance of novel foods is termed food neophobia.
- Both hyponeophagia and food neophobia have been assumed to measure anxiety by eliciting a conflict situation arising from a fear of the novel setting and foods, and the drive to eat.

Procedure:

- The testing apparatus consists of individual Plexiglas open fields, 76 × 76 × 46 cm.
- Thirty Purina lab chow pellets are placed in a pile directly in the center of the open field. Animals are handled for 3 weeks prior the behavioral testing. Forty-eight hours prior to testing.
- all food is removed from the home cage, although water is still available. 1 One h prior to testing, animals receive an intraperitoneal injection of test drugs or vehicle.
- At the time of testing, the animals are placed into individual open fields containing the food, and the latency to begin eating is measured..
- If the animal has not eaten within 720 s, the test is terminated and the animal is assigned a latency score of 720 s.

Evaluation:

- An anxiolytic effect is defined as a significant decrease in mean latency to begin eating compared with vehicle controls.

15. SHOCK PROBE CONFLICT PROCEDURE:

Purpose and Rationale:

- Rats being placed in a novel test environment containing a probe.
- Number of times that the animal makes physical contact with it, is reduced when the probe is electrified.
- Rats treated with anxiolytics continue to touch the electrified probe.

Procedure:

- Apparatus: The test environment consists of a Plexiglas chamber, measuring 40 × 40 × 40 cm, and having a metal grid floor.

- Whenever the animal touches both wires simultaneously with some part of its body, a DC current flows through the animal.
- Sixty min after treatment with saline or test substance, the animal is placed in a back corner of the testbox facing away from the probe.
- The number of responses the animal makes during the subsequent 5-min episode is counted.

Evaluation:

- Dose-response curves can be established for various drugs at different shock intensities.
- The Mann-Whitney U-test is used to evaluate differences between experimental conditions.
- To control whether a drug treatment increases responding above the saline control level, an one-tail t-test is used.

16. ULTRASOUND INDUCED DEFENSIVE BEHAVIOR IN RATS

Purpose and Rationale:

- Production of ultra-sonic calls in the 20–27 kHz range are used.
- Rats display specific defence behavior as a part of their natural survival strategy.

Procedure:

- The apparatus consists of a circular open field 75 cm in diameter, 46 cm high walls, with a video camera suspended above.
- Locomotor behaviors are recorded and analyzed using a computer automated tracking system capable of rapid movements. This allows the ultrasound induced change in locomotor behavior to be quantified in maximum speed, average speed and distance traveled by the animals.
- Animals are placed in the test arena 20 min after intraperitoneal injection of drug or vehicle and locomotor activity is measured.
- After 2 min they are exposed to a 1-min, 20 kHz, square wave ultrasound tone followed by a further 2 min without sound. This procedure is repeated for each intensity with a 1-min interval.
- Locomotor activity values are then calculated for the maximum speed, average speed and total distance traveled through out the 5-min test period and expressed as a series of 15–20-s .

Evaluation:

- Maximum speed is analyzed using a two-way ANOVA.

- Significant interactions between treatment and time are followed by one-way ANOVAs for individual time points with post-hoc Duncan's new multiple range test.

17. ANXIETY/DEFENSE TEST BATTERY IN RATS:

Purpose and Rationale

- It is the procedures designed to assess the defensive reactions of rats to a natural predator, the cat.
- The primary measures, taken both during and after cat presentation, include movement arrest and risk assessment and the inhibition of non-defensive behaviors .

Procedure:

- The test apparatus for both the proxemics/activity and eat/drink procedures consists of two parallel subject chambers (53 × 20 × 25 cm).
- Subject movements are monitored by five photocells mounted at equal distances
- The initial study assesses the effects of cat exposure onproxemics/activity, followed 7 days later by analysis of eat/drink behavior during and after cat exposure. Both procedures are carried out under dim red light.

Proxemic/activity testing:

- Rats are individually placed in each compartment of the test apparatus. Following a 5-min pre-cat period, the cat is introduced to the cat compartment for 5 min. Following removal of the cat,,behavior is recorded for a further 15 min post-cat period,

Eat/drink testing:

- Rats are individually given 2 g of finely crushed chocolate cereal on the 2 days after the proxemic/activity test, to familiarize them with this highly preferred food.
- In order to induce a mild water deprivation, water bottles are removed, 24 h prior to eat/drink testing.
- Same as proxemic / activity testing butMeasures of eating frequency and duration, and drinking frequency are taken for the cat and post-cat periods.

Evaluation:

- The data are analyzed by analysis of variance (ANOVA).

EXPERIMENT NO.: 15 **DATE:**

AIM: TO STUDY THE LOCAL ANESTHETICS BY DIFFERENT METHODS

INTRODUCTION:

- Local anesthesia is any technique to induce the absence of sensation in a specific part of the body, generally for the aim of inducing local analgesia, that is, local insensitivity to pain, although other local senses may be affected as well.
- They are used in various techniques of local anesthesia such as:
  - Topical anesthesia (surface)
  - Topical administration of cream, gel, ointment, liquid, or spray of anaesthetic dissolved in DMSO or other solvents/carriers for deeper absorption
  - Infiltration
  - Brachial plexus block
  - Epidural (extradural) block
  - Spinal anesthesia (subarachnoid block)
  - Iontophoresis

PROPERTIES OF IDEAL ANAESTHETICS AGENTS:

- It should not irritate the tissue to which it is applied.
- It should not make any long-lasting changes on nerve structure.
- Its systemic toxicity should be minimal.
- It must be effective regardless of whether it is injected into tissue or applied locally on mucous membranes.
- The time of onset of anesthesia should be minimal.
- Duration of action must be sufficiently long to allow the procedure to be completed but not so long as to necessitate extended recovery.
- It should have enough potency to administer full anesthesia without supplementing additional concentrated solutions that are potentially damaging.
- It should not produce allergic reaction.
- It should be stable in solution and should spontaneously undergo biotransformation in the body.
- It should be sterile or capable of being sterilized by heat without deterioration.

SOME SCREENING METHODS FOR LOCAL ANAESTHETICS FOR PRECLINICAL STUDY:

1. CONDUCTION ANAESTHESIA

A. CONDUCTION ANESTHESIA IN THE SCIATIC NERVE OF THE FROG

Procedure

- Frogs (*Rana temporaria*) of either sex are used and are kept at 4 °C. The frog is decapitated with a pair of scissors. The skin is incised in the thigh region at both sides and the sciatic nerves are carefully exposed in the thigh, avoiding any stretching and injury of the nerve.
- The frog is suspended on a vertical board. Small pieces of white cotton are soaked with different concentrations of the test preparations (between 0.05% and 1%) or the standard and placed gently around the sciatic nerve for 1 min.
- Then the cotton swab is removed and the frog is placed with its extremities into a bath with 0.65% NaCl solution. This allows testing for duration and reversibility of the local anesthetic effect.
- One side is used for the test preparation and the other for the standard (e.g., 0.25% butanilicaine). Every 3 min the frog is removed from the bath and the toes of the legs or the ankle joint are pinched three times with a small forceps.
- The reflex contraction is abolished when conduction anesthesia is effective. The stimuli are repeated every 3 min until anesthesia vanishes. Two to 5 frogs are used for each concentration.

Evaluation

- Time of onset and duration of anesthesia are recorded for each concentration. Time-response and dose-response curves can be established.

B. CONDUCTION ANESTHESIA IN THE SCIATIC NERVE OF THE RAT

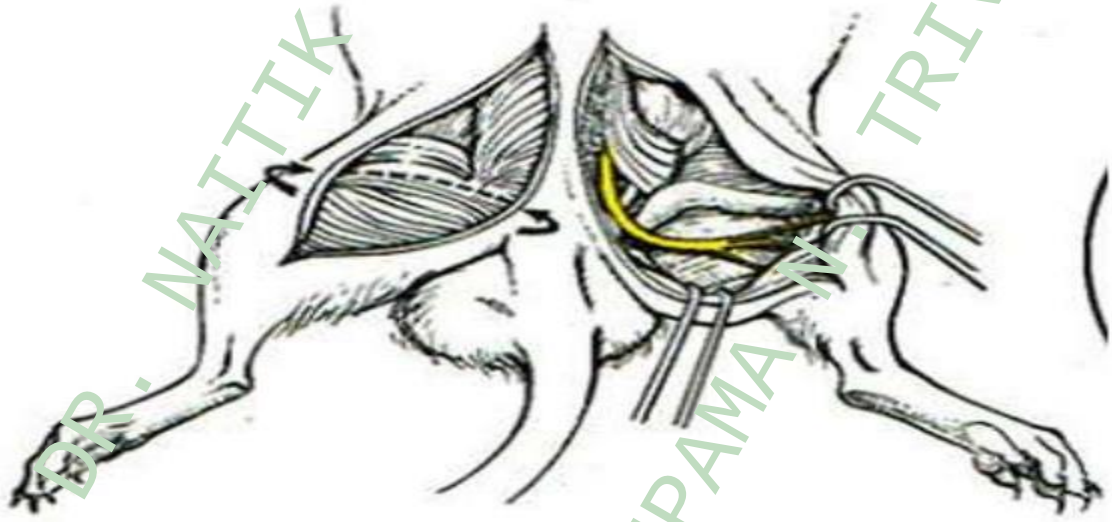
Procedure:

- Male Wistar or Sprague Dawley rats weighing 125 to 175 g are used. The animal is suspended in a prone position by grasping the base of the tail and thoracic cage.
- A hind limb is extended to its full length and the depression for needle insertion is located by palpation with the left index finger. The site of injection is the area under the skin at the junction of the biceps femoris and the gluteus maximus muscles.
- The sciatic nerve is blocked in the midthigh region with 0.2 ml of the drug solution administered by a 24- to 25-gauge needle attached to a 0.25 ml tuberculin syringe.

- Usually a 1% solution of the test drug in 0.9% NaCl is used as a test solution. The other leg is used for a control drug (e.g., procaine or lidocaine).
- Immediately after the injection, repeated checks of the digit of the foot and the walking behavior are performed.
- In the normal foot, the digits are wide apart, while in the blocked leg the digits of the foot are close together. Also the successful block is evidenced by dragging of the leg and an inability of the animal to use the leg in walking up the inclined wire mesh cover of the cage.
- After the time of block for each leg is noted, each animal is examined every 5 to 10 min in order to note the time of recovery.

Evaluation

- From the data, averages for onset and duration of action are calculated, plus the frequency of blocks are noted. Using various doses of test compound and standard, dose-response curves can be established and potency ratios calculated.



C. CONDUCTION ANESTHESIA ON THE MOUSE TAIL

Procedure

- Groups of 10 mice (NMRI-strain) of both sexes with a weight between 18 and 22 g are used for each dose. Before administration of the test compound or the standard the normal reaction time is determined.
- The animal is placed into a small cage with an opening for the tail at the rear wall. The tail is hold gently by the investigator. By opening of a shutter, a light beam exerting radiant heat is directed to the proximal third of the tail.

- After about 6 s, the reaction of the animal is observed by the investigator. The mouse tries to pull the tail away and turns the head. The shutter is closed with a switch when the investigator notices this reaction.
- Mice with a reaction time of more than 6 s are not used in the test. The test compounds and the standard are injected in a volume of 0.1 ml on both sides in the area of the tail root.
- The animals are submitted to the radiant heat again after 10 min. The area of heating is about 1.5 cm distal to the injection site. For each individual animal the reaction time is noted.

Evaluation

- There are two possibilities for evaluation:
  i. The average values of reaction time after each time interval are calculated and compared with the pretest value by analysis of significance.
  ii. At each time interval only those animals which show a reaction time twice as high or higher as the pretest value are regarded as positive. Percentages of positive animals are counted for each time interval and each dose and *ED*50 values are calculated according to LITCHFIELD and WILCOXON.

D. RETROBULBAR BLOCK IN DOGS

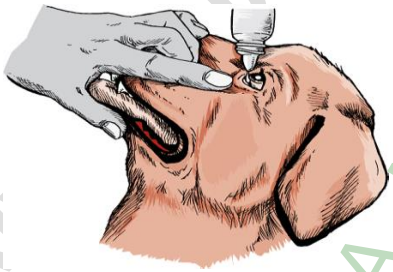
Procedure.

- Young female mongrel dogs weighing 13–15 kg are used. Twenty-four h before the test, 0.25% eserine (Physostigmine) ointment is placed in each conjunctival sac of the dog. Pentobarbital (25 mg/kg) is administered intravenously, then repeated with 10 mg/kg at hourly intervals, thus maintaining the animal in light anesthesia (corneal reflex present).
- Ten min after induction, the dog is put into 30-degree head-down position, and 20 ml of 0.05% tetracaine is forced into the epidural space through the interarcuate ligament. Horner's syndrome occurs within 5 min.
- A 150-watt surgical lamp is now focused upon the eye from 1 meter distance. 15 min later, a retrobulbar block is performed: The sclera is seized with an ophthalmic forceps and the eyeball is pulled downward and medially; a 23-gauge needle is then introduced through the superior rectus muscle, tangentially to the globe.

- It is immobilized as soon as a click indicates penetration of the retrobulbar space; correct placement is confirmed by free motion of the needle tip and protrusion-rotation of the eyeball upon injection of 1 ml of air.
- After aspiration, 2 ml of the tested anesthetic is then injected at a rate of 0.5 ml per second. The pupil dilates and reaches its maximal diameter (6 mm) within a few minutes.
- This apparent diameter is estimated with a 2-cm long ruler calibrated in millimeters, whose center is gently applied to the corneal center.
- The pupil is measured every 15 s for 5 min, then every 5 min until reappearance of maximal miosis (pinpoint and asymmetrical), a precise endpoint which generally coincides with corneal reflex and lacrimation.

Evaluation

- Drug latency (in min) and duration (in 5-min units) are averaged for both eyes of each animal, and the mean and standard deviation then calculated for all test animals. Analysis of variance is performed to find significant differences between various local anesthetics.



2. INFILTRATION ANESTHESIA

Procedure

- Adult guinea pigs of either sex weighing 250–300 g are chosen. On the day preceding the experiment the hair on the back is clipped and two areas of 4–5 cm diameter are shaved.
- This produces a certain amount of irritation which disappears overnight. The sensitivity of the skin is greatest in the midline and slightly more so in the front than in the back area. For this reason each concentration of a local anesthetic must be tested in both areas.
- Six tests using three guinea pigs can be performed simultaneously. The doses of local anesthetics are always injected intracutaneously in 0.1 ml saline. Three guinea pigs receive one dose in the front area and another dose in the back area; the size of the wheal is marked with ink.

- One side is used for the test preparation, the other side for the standard (e.g., 1% butanilicaine).
- The reaction to pin prick is tested 5 min after injection in the following way. After observing the animal's normal reaction to a prick applied outside the wheal, six pricks are applied inside the wheal and the number of pricks is counted to which the guinea pig fails to react.
- The pricks are applied at intervals of about 3–5 s. Six pricks are applied every 5 min for 30 min. Having completed the test on 3 guinea pigs, the same solutions are injected into 3 other guinea pigs, but the solution which was used for the front is now used for the back area and vice versa.

Evaluation

- The number of times the prick fails to elicit a response during the 30 min period is added up, and the sum, out of possible 36, gives an indication of the degree of anesthesia.
- Using various doses, dose-response curves can be established. For time-response curves, the prick tests are repeated every 10 min. Half-life times are calculated as the time, when after complete anesthesia 3 out of 6 pricks elicit again a response.

3. SURFACE ANESTHESIA

A. SURFACE ANESTHESIA ON THE CORNEA OF RABBITS

Procedure

- Albino rabbits of either sex weighing 2.5–3 kg are placed into rabbit holding cages. The upper and lower eyelashes are carefully clipped.
- The conjunctival sac of one eye is held open, thus forming a pocket. From a 1 ml syringe with a 22-gauge needle, 0.5 ml of a solution of the anesthetic is applied into the conjunctival sac for 30 s.
- Then the procedure is repeated, so that 1.0 ml is applied within 1 min. One ml of the standard (0.1% solution of tetracaine hydrochloride) is applied to the other eye.
- Effective local anesthetics extinguish the corneal reflex (blinking) elicited by any touch of the cornea. For quantitative purposes, the irritation with a bristle according to von Frey (1894, 1896, 1922) has been recommended.
- An equine hair bending at a load of 230 mg is attached perpendicularly to a glass rod.
- Within 25 s, the cornea is touched 100 times. The summation of many stimuli applied this way gives better results than a single touch with a glass rod. The test is started 5 min after application of the drug and repeated every 5 min until anesthesia vanishes and blinking

occurs again. The time between disappearance and reappearance of the corneal reflex is registered.

Evaluation

- Using the time of loss of the corneal reflex as parameter after application of different doses, dose-response curves can be established and potency ratios versus the standard calculated.

B. SUPPRESSION OF SNEEZING REFLEX IN RABBITS

Procedure

- Groups of male rabbits weighing 3 kg are used. Using a cotton tampon, the test solution is applied to the mucous membrane of one nostril.
- The solution of a standard local anesthetic is administered to the nasal mucosa of the other nostril. After 2 min the mucous membrane is stimulated by a fine pencil. Loss of the sneezing reflex is regarded as sign of complete anesthesia.
- The stimulation is repeated after 3, 6, 10 and 15 min and continued every 5 min until the sneezing reflex reappears. Various concentrations of test compound and standard are applied.

Evaluation

- Using the loss of the sneezing reflex as parameter after application of different doses, dose-response curves can be established and potency ratios versus the standard calculated. Furthermore, the duration of activity can be evaluated.

4. EPIDURAL ANESTHESIA:

EPIDURAL ANESTHESIA IN GUINEA PIGS:

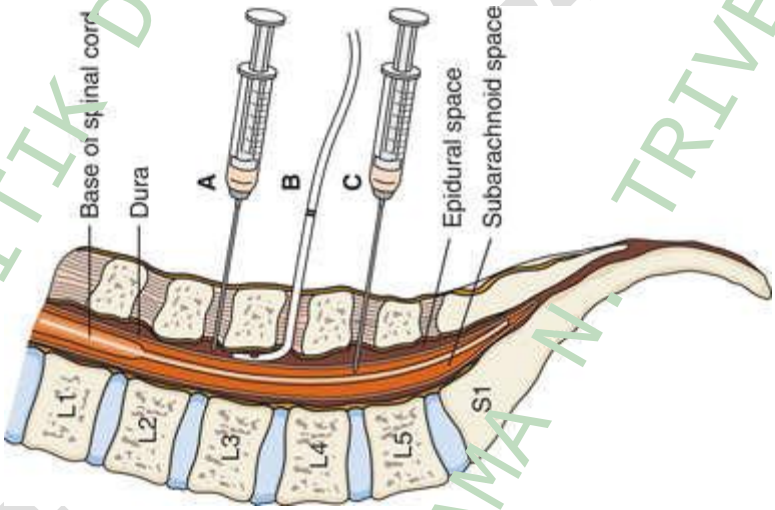
Procedure

- Male guinea pigs weighing 300–500 g are anesthetized by means of an intraperitoneal injection of an aqueous solution of chloral hydrate 42.5 g/l; ethanol 90 g/l; propylene glycol 428 g/l; sodium pentobarbitone 9.75 g/l; and magnesium chloride 21 g/l. A skin incision is made from the level of the lumbosacral fossa and approximately 1.5 cm down in order to expose the sacral area in the mid-line.
- With the vertebral column flexed, the lumbosacral intervertebral ligament is carefully incised. Through this small opening a polyethylene catheter (PE 10) is inserted maximally 1.5 cm along the roof of the vertebral canal to the L4–L5 region.

- The catheter is sutured to the overlying lumbar fascia which is then closed. The catheter is tunneled under the skin and exteriorized through an incision in the neck region.
- After fixation of the catheter to the fascia of the neck muscles and suturations of the incisions, the catheter is filled with saline and sealed.
- After a recovery period of at least 1 day, 0.1 ml of 2.0% lidocaine is injected over a period of 1 min, and the motor and sensory blocks are assessed.
- The injection of lidocaine which results in a bilateral, reversible blockade indicates a successful preparation. A minimum of 8 animals are used in the further experiments for each test solution.

Evaluation

- Mean time to onset of block and mean duration of block are calculated from number of legs blocked.



5. INTRATHECAL (SPINAL) ANESTHESIA:

SPINAL ANESTHESIA IN RATS

Procedure

- Male Sprague-Dawley rats weighing 50–75 g are used. The rat is held firmly by the pelvic girdle. A 30-gauge needle is attached to a 25-µl Hamilton syringe is inserted into the tissue on one side of the L5 or L6 spinous process at an angle of about 20°.
- The needle is advanced to the groove between the spinous and transverse processes and then moved forward the intervertebral space at an angle of about 10°. About 0.5 cm of the needle is then in the vertebral column.

- Correct placement of the needle is indicated by an arching of the tail. Drugs are dissolved in saline or water and administered in a volume of 5 µl.
- Antinociception is determined in a modification of tail flick assay in rats by placing the tail of the rat under a focused radiant heat source.

Evaluation

- The degree of antinociception is defined as the percentage of maximum possible effect. This percentage is determined for each dose at each time measured allowing to calculate *ED*50 values.