LAB MANUAL

Ex Pharm Software

BUREAU FOR HEALTH & EDUCATION STATUS UPLIFTMENT

(Constitutionally Entitled As Health-Education, Bureau)

www.heb-nic.in

Copyright Notice

Documentation version 4.0

Trademarks

Trade Mark No. : 2967042

Licensing:

Experiments List	
1	Study of muscle relaxant activity with the help of "rota rod apparatus".
2	Study of cns depressents & stimulants using "actophotometer".
3	Study of analgesic activity with the help of "tail flick apparatus".
4	Study of antihistaminic drugs with the help of histamine chamber (mast cell stabilization method).
5	Study of analgesic activity with the help of "hot plate apparatus".
6	Study of drugs acting on cns using "elevated plus maze".
7	Study of anticonvulsant activity using "electro covulsiometer".
8	Experiment on effects of various drugs on rabbit's eye.
9	To study analgesic activity by writhing test.
10	To study PTZ induced convulsions in mice.
11	Effect of different drugs on frog's heart.
12	Effect of agonist & antagonist and bioassay of histamine on the ileum of guinea pig.
13	Experiments of rat blood sugar.
14	3 Modules - To record the dose response curve and to determine the pD2 value for acetylcholine (on frog rectus abdominis muscle), serotonin (on rat fundus strip) and histamine (on guinea pig ileum).
15	4 Modules - Bioassay of acetylcholine (on frog rectus abdominis muscle), oxytocin (on rat uterine horn) and serotonin (on rat fundus strip), acetylcholine (on rat colon)- by matching, interpolation, 3 point and 4 point method.
16	Study of diuretic activity using metabolic cage.
17	Study of anti-inflammatory activity using carrageenan induced paw oedema method.
18	Rabbit pyrogen test.
19	3 Modules - Effects of drugs on the dog BP and heart rate.
20	Effects of drugs on the ciliary motility of frog oesophagus (gastro intestinal tract).
21	Study of anti ulcer activity - using pylorus ligation method.
22	Study of stereotype and anti-catatonic activity of drugs on mice.
23	Evaluation of effect of acetylcholine (spasmogens) using rabbit jejunum.
24	Evaluation of anti psychotic drugs using cook's pole climbing apparatus.
25	Evaluation of sedative drugs using cook's pole climbing apparatus.
26	Acute skin irritation test (draize test).
27	Acute eye irritation test (draize test).
28	Effect of saline purgatives on frog intestine.
29	4 Modules - Amphibian nerve muscle experiments.
30	Study of effect of hepatic microsomal enzyme inducer on the phenobarbitone sleeping time in mice.
31	Determination of pA2 value of prazosin using rat anococcygeus muscle (by schilds plot method).
Supportive Resources	
1	Study of different laboratory animals used in pharmacology.
2	Study of different physiological salt solutions used in pharmacology.
3	Different routes of drug administration in laboratory animals.
4	Commonly used blood withdrawal techniques in laboratory animals.
5	Different methods of anaesthesia and euthanasia in laboratory animals.

Experiment-1

Aim/Object: To study muscle relaxant activity with the help of "rota rod apparatus"

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Introduction and Principle: Reduction of motor co-ordination, CNS depression and skeletal muscle relaxation lead to decrease in the fall off time and decrease in number of free ridings of animal balancing on the rotarod. Thus lesser fall off time and less number of free ridings indicate that the administered drug has CNS depressant or muscle relaxant activity that either lead to decrease in the motor co-ordination or decrease in the gripping power.

Equipment:

Rotarod apparatus has a horizontal grooved rod rotating at a fixed speed. The mice are made to balance on this rod. Dependent upon their motor co-ordination, Central nervous activity and grip strength the animal either stay on the rotating rod for specific time and after that fall down on the platform of each compartment. The floor of each compartment has sensors that deactivate the timers and the exact fall off time for each rat is displayed on the respective display.

Instructions/Procedure:

1. *Animals are divided into two groups.*
2. *Administer one group with the drug to be tested and other with vehicle by intraperitoneal route.*
3. *Click/Pick (Load) rodents to put them on the rod of the rotarod apparatus.*
4. *Start the rotarod apparatus at the predefined speed of instrument (25 rotations/60 sec).*
5. *Observe the animals for fall off, When the animal fall off, respective time will be displayed on the timer, record the fall off time of each animal.*

OBSERVATION TABLE:

Group Selected	Readings	Mouse No.1	Mouse No.2	Mouse No.3	Average
Vehicle Treated	Fall off time (Sec)				
	Free Ridings				
Diazepam Treated	Fall off time (Sec)				
	Free Ridings				

Student's Name: _____

INFERENCE:

Group Selected	Readings	Mouse No.1	Mouse No.2	Mouse No.3	Average
Vehicle Treated	Fall off time (Sec)	52	50	55	52.33
	Free Ridings	1	0	2	1
Diazepam Treated	Fall off time (Sec)	42	39	45	42
	Free Ridings	2	0	2	1.33

Student's Name: _____

*Observation table after completion of the experiment can be downloaded with respective student's name.

Result/Conclusion:

The observed reduction in fall off time and free riding shows that Diazepam at 1 mg/kg IP dose produces decrease in motor co-ordination and decrease in muscle strength.

Experiment-2

Aim/Object: Study of cns depressents & stimulants using "actophotometer".

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Introduction and Principle: The drug acting on central nervous system as stimulants or suppressants affects the locomotor activity in experimental animals. Such changes in the motor activity can be easily determined by using simple techniques in which the animal treated with drugs is kept in an open compartment having square marking on its floor. The lines of such squares crossed by the animal are counted by the observer. The number on lines crossed correlates with the locomotor activity. Alternatively, the locomotor activity can be determined using actophotmeter or more sensitively by using automated video tracking system in addition to the horizontal movement the vertical movement(rearing -standing standing on hindlegs and exploration of surroundings) is also considered as a parameter associated with locomotor activity.

Equipment:

Actophotometer has a central chamber with arrangement of light sources and photocells at the base of two opposite walls. The light of each source is focused on a photocell. Any Interruption in the path of light activates the photocells and this is counted as a measure of horizontal locomotor activity of the mice kept in the chamber.

Instructions/Procedure:

1. *Animals are divided into two groups (6 animals in each).*
2. *Administer one group with the drug to be tested and other with vehicle by oral route.*
3. *Put one animal at a time in the Actophotometer*
4. *Start the instrument*
5. *Count the locomotor activity for specific time.*
6. *Repeat the procedure at the interval of 30 minutes.*
7. *Record the observations.*
8. *The mice in test group are injected Diazepam (1 Mg /kg Oral) and locomotor activity is measured twice as stated above.*

OBSERVATION TABLE:

Control Group		**Drug Treated Group**		**Percentage Decrease**	
Locomotor Index	**Rearings**	**Locomotor Index**	**Rearings**	**Locomotor Index**	**Rearings**

Student's Name: _____

INFERENCE:

Control Group		**Drug Treated Group**		**Percentage Decrease**	
Locomotor Index	**Rearings**	**Locomotor Index**	**Rearings**	**Locomotor Index**	**Rearings**
163	4	76	2	46.62576687116564%	50%
161	4	67	2	41.61490683229814%	50%
154	3	59	1	38.311688311688314%	33.33333333333333%
159	4	77	2	48.42767295597484%	50%
158	4	77	1	48.734177215189874%	25%
159	4	77	2	48.42767295597484%	50%

Student's Name: _____

*Observation table after completion of the experiment can be downloaded with respective student's name.

Result/Conclusion:

The observed reduction in Locomotor Index shows that the given test drug (Diazepam) exerts inhibition of the locomotor activity

Experiment-3

Aim/Object: Study of analgesic activity with the help of "tail flick apparatus".

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Introduction and Principle: The tail flick test is a test of the pain response in animals, similar to the hot plate test. It is used in basic pain research and to measure the effectiveness of analgesics, by observing the reaction to heat. It was first described by D'Amour and Smith in 1941.
Analgesic drug increases pain threshold (ability to tolerate a painful stimulus). This effect can be estimated in animal models of analgesia. To induce pain, following type of stimuli can be used:

- Radiant heat projected on the tail, paw or shaved skin
- Cold stimulus applied through a cold plate
- Mechanical pressure on tail or paw applied using a clamp
- Electrical stimulus on both pulp
- Chemical stimulus (formalin)

The time taken by the animal to reveal pain sensation through vocalization or paw licking or effort to escape the painful stimulus (withdrawal of the body part on which pain is inflicted-paw or tail) is determined. The analgesics increase the time taken by the treated animal to reveal pain sensation.

Equipment:

Tail Flick Analgesiometer

Instructions/Procedure:

- *The interface shows two groups of mice (containing six mice in each group) which have been randomly selected and allotted to two groups. Select the groups to be treated with the study drug and the vehicle.*
- *Administer the respective treatment to individual animals by intraperitoneal route.*
- *Click the mouse to put the selected mouse on the Tail flick Apparatus.*
- *Record the response time at which the mouse move the tail.*

- *The mice in test group are injected Morphine (10 Mg /kg Oral) and the mice in control group are administered with the vehicle in the same volume. The response time are recorded after 1 hour of drug administration.*

Step to be followed:

Step-1:

Select the animal to be treated with vehicle or drug dependent upon its group and administer the respective treatments.

Step-2:

After 1 hour of the treatments, Load the mice on the Tail-flick Analgesiometer.

OBSERVATION TABLE:

Response Time		
Vehicle Treated	**Drug Treated**	
		AVERAGE

Student's Name: _____

INFERENCE:

Response Time		
Vehicle Treated	**Drug Treated**	
8	16	
14	14	
10	10	
9	9	
13	13	
13	13	
11.166666666666666	11.166666666666666	**AVERAGE**

Student's Name: _____

*Observation table after completion of the experiment can be downloaded with respective student's name.

Result/Conclusion:

Increase in the average response time in Drug treated mice shows that the pain threshold is increased in the Drug treated mice.

Experiment-4

Aim/Object: Study of antihistaminic drugs with the help of histamine chamber (mast cell stabilization method).

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Introduction and Principle: Mast cells are crucial in allergic diseases because they protect the body from the antigen. Proinflammatory mediators (such histamine and eicosanoid) are secreted when IgE antibodies produced in response to antigen attach to mast cells. This practical is based on this concept. This experiment is basically used to evaluate activity of antihistaminic drugs on animals because antihistaminic drugs inhibit the inflammatory effects of histamine.

Equipment:

Histamine chamber

Instructions/Procedure:

1. *Animals are divided into two group.*
2. *Administer one with "Antihistaminic Drug" and the other with the "Normal Saline" by the suitable route.*
3. *Put animals in the "Histamine Chamber."*
4. *Provide animals the exposure of "Histamine".*
5. *Record the observations.*

OBSERVATION TABLE:

GROUP SELECTED	ALLERGIC RESPONSE (YES/NO)
VEHICLE TREATED	
DRUG TREATED	

Student's Name: _____

INFERENCE:

GROUP SELECTED	ALLERGIC RESPONSE (YES/NO)
VEHICLE TREATED	Yes
DRUG TREATED	No

Student's Name: _____

*Observation table after completion of the experiment can be downloaded with respective student's name.

Result/Conclusion:

On exposure to Histamine, Saline treated animal (Guinea Pig) shows allergic Response, whereas Antihistaminic (Antiallergic) drug treated animal (Guinea Pig) do not show allergic Response.

Experiment-5

Aim/Object: Study of analgesic activity with the help of "hot plate apparatus".

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Introduction and Principle: Analgesic drug increase pain threshold (ability to tolerate a painful stimulus). The effect can be evaluated in animal models of analgesia. To induce pain, following type of stimuli can be used:

- Radiant heat projected on the tail, paw or shaved skin
- Cold stimulus applied through a cold plate
- Mechanical pressure on tail of paw applied using a clamp
- Chemical stimulus (formalin)

The time taken by the animal to reveal pain sensation through vocalization or paw licking of effort to escape the painful stimulus (withdrawal of the body part on which pain is inflicted-paw or tail) is determined. The analgesics incrase the time taken by the treated animal to reveal pain sanction.

Equipment:

Eddy's hot-plate is a device used to give heat stimulus to the paws of animal. A metal plate at the top of hot plate apparatus can be heated in the controlled manner and maintained at the required temperature for the duration of an experiment. Generally, in testing of the analgesic activity of drug in animal, 55*C temperature is used as a thermal stimulus.

Instructions/Procedure:

1. *Animals are divided into two groups (6 animals in each)*

2. *Administer one group with the drug (Pentazocine 10mg/kg) to be tested and other with vehicle by intraperitoneal route.*
3. *After 60 minutes put the mice on the Hot Plate maintained in 55*C.*
4. *Record the response time at which the mouse licks its force paws or jumps.*

OBSERVATION TABLE:

Mice	Vehicle Treated	Drug Treated
Mice 1		
Mice 2		
Mice 3		
Mice 4		
Mice 5		
Mice 6		
Average		

Student's Name: _____

INFERENCE:

Mice	Vehicle Treated	Drug Treated
Mice 1	17	27
Mice 2	21	26
Mice 3	19	29
Mice 4	18	30
Mice 5	20	26
Mice 6	17	28
Average	18.66	27.66

Student's Name: _____

*Observation table after completion of the experiment can be downloaded with respective student's name.

Result/Conclusion:

Animals treated with analgesic drug shows increase in time to show "Paw Licking Response", which reflects analgesic activity of drug.

Precautions:

1. Ensure to take animals out at cut off time of 17 seconds.
2. Heat may harm to rodents so take utmost care and ensure to take out the rodents from hot plate timely.

Experiment-6

Aim/Object: Study of drugs acting on cns using "elevated plus maze".

Reference: “Ex Pharm” Software by “Bureau for Health & Education Status Upliftment”, assessed at: https://heb-nic.in/Ex-Pharm/login.php

Introduction and Principle: The elevated plus maze task is a simple method to assess anxiety-like behaviors in rodents. This version describes the procedure used in mice. However, the protocol may also be applied to rats, considering a proportionally larger apparatus (arms : 10x50 cm; height 55 cm). Briefly, the test is performed on a plus-shaped apparatus with two open and two closed arms. The animal is allowed to freely explore the maze while the duration and frequency of entries into open and closed aims is recorded. The task is based on an approach-avoidance conflict, meaning that the animal is faced with a struggle between a propensity to explore a novel environment and an unconditioned fear of high and open spaces. Consequently, an anxiety-like state is characterized by increased open arm avoidance, compared to control animals. On account of being a very popular test, there can be considerable variations in the procedures applied across different laboratories. Here we provide a working protocol that has been able to detect both anxiogenic and anxiolyitic drug effects under the specified conditions.

The elevated plus maze (EPM) is an model of anxiety 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 (a preference to remain near to, or touching, vertical surfaces).

Equipment:

The EPM apparatus consists of a "+"-shaped maze elevated above the floor with two oppositely positioned closed aims, two oppositely positioned open arms, and a center area.

Instructions/Procedure:

Instructions:

1. *Divide animals in 2 Groups of 6 animals each.*

2. *Vehicle treated group - Administer normal saline*
3. *Drug Treated Group - Administer test drug*
4. *Allow the animals to explore elevated plus maze*
5. *Evaluate the time spent by each animal in open arm and close arm*
6. *Enter the time in observation table*
7. *Compare the time spent in open and closed arm by vehicle treated and drug treated animals.*

Procedure:

1. *Divide animals in 2 Groups of 6 animals each*
2. *Vehicle treated group - Administer normal saline*
3. *Drug Treated Group - Administer test drug*
4. *Allow the animals to explore elevated plus maze*
5. *Evaluate the time spent by each animal in open arm and close arm*
6. *Enter the Time in observation table*
7. *Compare the time spent in open and closed arm by vehicle treated and drug treated animals*

OBSERVATION TABLE:

	Vehicle Treated		**Drug Treated**	
Mice & Treatment	**Time spent in Close Arms (Second)**	**Time spent in Open Arms (Second)**	**Time spent in Open Arms (Second)**	**Time spent in Close Arms (Second)**
Mice 1				
Mice 2				
Mice 3				
Mice 4				
Mice 5				
Mice 6				

Student's Name: _____

INFERENCE:

	Vehicle Treated		**Drug Treated**	
Mice & Treatment	**Time spent in Close Arms (Second)**	**Time spent in Open Arms (Second)**	**Time spent in Open Arms (Second)**	**Time spent in Close Arms (Second)**

Mice 1	**34**	**18**	**45**	**7**
Mice 2	**32**	**20**	**43**	**9**
Mice 3	**34**	**18**	**45**	**7**
Mice 4	**31**	**21**	**44**	**8**
Mice 5	**32**	**20**	**46**	**6**
Mice 6	**34**	**18**	**43**	**9**

Student's Name: _____

*Observation table after completion of the experiment can be downloaded with respective student's name.

Result/Conclusion:

The drug has shown anti anxiety activity as the time spent in open arm by animal has increased.

Experiment-7

Aim/Object: Study of anticonvulsant activity using "electro covulsiometer".

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Introduction and Principle: Electrical shock given through the electrodes applied on the ear pinna results into a burst of exitatory neurotransmitters from the brain. This activate the brain activity during grand-mal epilepsy. Prior treatment of animals with the drugs, reduces the exited activity of brain**.**

Equipment:

Electroconvulsiometer is used to deliver the electric shock of required intensity to the subject for required duration. This instrument is used to evaluate the anticonvulsant effect of pharmacological agents against electro shock induced convulsions in experimental animals. An electrical stimulus with an intensity that induced characteristic convulsion is applied to the animals through the electrode placed on ear pinna. The duration of tonic and clonic seizures are measured. The drug to be tested is administered to separate group of animals and its effect on such duration on such convulsions is measured. Anticonvulsant pharmacological agents reduce the duration of seizures induced by electrical shocks.

Instructions/Procedure:

1. *Animals are divided into two groups (6 animals in each)*
2. *Administer one group with the drug (Phenytoin 25 mg/kg) to be tested and other with vehicle by intraperitoneal route.*
3. *After 30 minutes, attach electrodes of the convulsiometer on the ears of the mouse.*
4. *Give shock of 30mA intensity for 0.2 seconds duration and measure tonic seizures, clonic seizures and stupor. Also report the survival/ death of animal.*

5. *Determine average duration determine whether treatment with phenytoin reduces duration of these stages of epilepsy.*

OBSERVATION TABLE:

Treatment	**Time (Sec) of different Phases of Seizure**			**Recovery/Death**
	Tonic	**Clonic**	**Stupor**	
Vehicle Treated				
Vehicle Treated				
Vehicle Treated				
Vehicle Treated				
Vehicle Treated				
Vehicle Treated				
Average				
Drug (Phenytoin) Treated				
Drug (Phenytoin) Treated				
Drug (Phenytoin) Treated				
Drug (Phenytoin) Treated				
Drug (Phenytoin) Treated				
Drug (Phenytoin) Treated				
Average				

Student's Name: _____

INFERENCE:

Treatment	**Time (Sec) of different Phases of Seizure**			**Recovery/Death**
	Tonic	**Clonic**	**Stupor**	
Vehicle Treated	16	7	22	Recovery
Vehicle Treated	14	10	24	Recovery
Vehicle Treated	18	8	20	Recovery
Vehicle Treated	20	16	13	Recovery
Vehicle Treated	13	11	20	Recovery
Vehicle Treated	14	9	20	Recovery
Average	15.8333	10.6666	19.8333	

Drug (Phenytoin) Treated	11	7	18	Recovery
Drug (Phenytoin) Treated	11	6	13	Recovery
Drug (Phenytoin) Treated	10	8	14	Recovery
Drug (Phenytoin) Treated	13	8	15	Recovery
Drug (Phenytoin) Treated	14	6	18	Recovery
Drug (Phenytoin) Treated	12	6	17	Recovery
Average	11.8333	6.8333	15.8333	

Student's Name: _____

*Observation table after completion of the experiment can be downloaded with respective student's name.

Result/Conclusion:

Treatment of mice with 25 mg/kg Phenytoin decreases the duration of clonic and tonic convulsions and also reduces the duration of stupor. The efficacy of phenytoin in the maximal electroshock induced convulsions is shown.

Experiment-8

Aim/Object: Experiment on effects of various drugs on rabbit's eye

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Aim of the experiment

To determine the effects of a given drugs on the size of the pupil, light reflex and intraocular tension of the animal eye.

Overview:

The Iris is composed of two types of muscle fibers, 1) circular and 2) radial. The circular fibers are supplied by parasympathetic nerve fibers and the radial ones are innervated by sympathetic nerve fibers. The stimulation of sympathetic and parasympathetic nerves leads to mydriasis and miosis respectively.

Materials required:

1. Rabbits,
2. Rabbit holder
3. Measuring scale,
4. Droppers,
5. Torch,
6. Cotton wool.

Solutions:

1. Normal saline
2. Physostigmine 0.5%
3. Atropine sulphate 1.0%
4. Ephedrine 0.5%
5. Adrenaline hydrochloride 0.1%
6. Lignocaine hydrochloride 1.0%

Procedure:

1. Measure the diameter of both the pupils.

2. Note the intraocular tension (Low, Normal or High).

3. Check out light and corneal reflexes.

4. Note down the readings.

5. Keep one eye (either right or left eye) as control and the other as test.

6. Apply saline in the control eye and a drug in the test eye.

7. Take the readings (diameter of pupils, intraocular tension and reflexes)

Directions:

Instill drug one by one and observe their effects on the rabbit eye. Tabulate the data and draw conclusions.

OBSERVATION TABLE:

S. No.	Drug	Intraocular Tension	Light Reflex	Corneal Reflex	Pupil Size
1					
2					
3					
4					
5					

Student's Name: _____

INFERENCE:

S. No.	Drug	Intraocular Tension	Light Reflex	Corneal Reflex	Pupil Size
1	Epinephrine	Decreased	Normal / Same as saline response	Animal blinks the eyelid on touching cotton	Increased
2	Atropine	No effect / Same as saline response	Normal / Same as saline response	Animal blinks the eyelid on touching cotton	Increased
3	Ephedrine	No effect / Same as saline response	Normal / Same as saline response	Animal blinks the eyelid on touching cotton	Increased
4	Physostigmine	Decreased	Normal / Same as saline response	Animal blinks the eyelid on touching cotton	Reduced

5	Lignocaine	No effect / Same as saline response	Normal / Same as saline response	Animal do not blinks the eyelid on touching cotton	No effect / Same as saline response

Student's Name: _____

*Observation table after completion of the experiment can be downloaded with respective student's name.

Result/Conclusion:

Epinephrine, Atropine & Ephedrine shown mydriatic effect, Physostigmine shown miotic effect & Lignocaine shown local anesthetic effect.

Experiment-9

Aim/Object: To study analgesic activity by writhing test.

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Introduction and Principle: Acetic acid induces as inflammatory response in the abdominal cavity, with subsequent activation of nociceptors. When animals are inraperitoneally injected with acetic acid, a painful reaction and acute inflammation emerge in the peritoneal area.

Analgesic activity of the peripheral analgesics (test compound) is inferred from a decrease in the frequency of writhigs.

Instructions/Procedure:

1. *Divide animals in 2 Groups of 6 animals each.*
2. *Vehicle treated group - Administer normal saline*
3. *Drug Treated Group - Administer test drug*
4. *Count the frequency of writing in animals*
5. *Enter the number of writhings in observation table*
6. *Compare the writhings in vehicle treated and drug treated animals.*

OBSERVATION TABLE:

Mice	Vehicle Treated	Drug Treated
	Number of Writhing	Number of Writhing
Mice 1		
Mice 2		
Mice 3		
Mice 4		
Mice 5		
Mice 6		

Student's Name: _____

INFERENCE:

Mice	Vehicle Treated	Drug Treated

	Number of Writhing	Number of Writhing
Mice 1	2	1
Mice 2	2	1
Mice 3	2	2
Mice 4	2	1
Mice 5	2	1
Mice 6	2	1

Student's Name: _____

*Observation table after completion of the experiment can be downloaded with respective student's name.

Result/Conclusion:

The drug has shown analgesic activity as the writhings frequency is decreased in drug treated animals.

Experiment-10

Aim/Object: To study PTZ induced convulsions in mice

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Introduction and Principle: Seizures occur due to insufficient inhibitory action (e.g., GABA) or extreme excitation (e.g. ghutamate). Pentylenetetrazol causes generalized clonic movements by antagonizing the inhibitory GAB Anergic transmission which leads to tonic characterized by flexion of limbs and epilepsy.

This experiment is performed to study the activity of anti-co avulsant/antiepileptic drug against PTZ (Pentylenetetrazole) induced convulsions.

Instructions/Procedure:

1. *Weight and number the animals.*
2. *Divide them into two groups (Control and Test) each comprising of at least 6 mice.*
3. *Control group will receive normal saline and test group receive diazepam.*
4. *After 30 min. inject PTZ (Pentylenetetrazole) to both groups and keep the animals for 1 hour under observation and observe the seizures.*
5. *Count the number of animals showing seizures in Control Group (Normal Saline + PTZ) & Test Group (Diazepam+PTZ) and record in observation table.*

OBSERVATION TABLE:

Number of Animal Shown Convulsions	
Control Group (Normal Saline+PTZ)	**Test Group (Diazepam+PTZ)**

Student's Name: _____

INFERENCE:

Number of Animal Shown Convulsions	
Control Group (Normal Saline+PTZ)	**Test Group (Diazepam+PTZ)**

6	**2**

Student's Name: _____

*Observation table after completion of the experiment can be downloaded with respective student's name.

Result/Conclusion:

PTZ (Pentylenetetrazole) included convulsions whereases anticonvulsant drug (Diazepam) shown reduction in convulsions.

Experiment-11

Aim/Object: Effect of different drugs on frog's heart

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Aim of the experiment:

To study the effects of Drugs on the Isolated Heart of Frog

Overview:

Many drugs act on the heart. Adrenergic and cholinergic drugs produce opposite effects. These drugs act through respective receptors. Some drugs act directly on the heart. This experiment demonstrates the effects of a few drugs (agonists, antagonists, calcium and potassium) on the isolated heart of frog.

Materials Required:

1. Starling's heart lever
2. Stand
3. A kymograph with drum and smoked paper

Solutions:

S.No.	Name of Drug	Dose(mcg)	Concentration
1.	Potassium Chloride	2000	10 mg/ml
2.	Epinephrine(Adrenaline)	2	10 mcg/ml
3.	Norepinephrine(Noradrenaline)	2	10 mcg/ml
4.	Isoprenaline	2	10 mcg/ml
5.	Propranolol	200	1 mg/ml
6.	Acetylcholine	2	10 mg/ml
8.	Calcium Chloride	2000	10 mg/ml
9.	Atropine sulphate	20	100 mg/ml

Volume of above solutions to be injected = 0.2 ml; mcg = micrograms

Instructions/Procedure:

1. *Isolation of frog heart is done as per the routine procedure.*
2. *Inject the drugs and observe the following parameters*

(a) Heart rate
(b) Tone
(c) Force of contraction

3 Tabulate the data.

OBSERVATION TABLE:

Treatment	Time (Sec) of different Phases of Seizure			Recovery/Death
	Tonic	Clonic	Stupor	
Vehicle Treated				
Vehicle Treated				
Vehicle Treated				
Vehicle Treated				
Vehicle Treated				
Vehicle Treated				
Average				
Drug (Phenytoin) Treated				
Drug (Phenytoin) Treated				
Drug (Phenytoin) Treated				
Drug (Phenytoin) Treated				
Drug (Phenytoin) Treated				
Drug (Phenytoin) Treated				
Average				

Student's Name: _____

INFERENCE:

Treatment	Time (Sec) of different Phases of Seizure			Recovery/Death
	Tonic	Clonic	Stupor	
Vehicle Treated	16	7	22	Recovery
Vehicle Treated	14	10	24	Recovery
Vehicle Treated	18	8	20	Recovery
Vehicle Treated	20	16	13	Recovery
Vehicle Treated	13	11	20	Recovery
Vehicle Treated	14	9	20	Recovery

Average	15.8333	10.6666	19.8333	
Drug (Phenytoin) Treated	11	7	18	Recovery
Drug (Phenytoin) Treated	11	6	13	Recovery
Drug (Phenytoin) Treated	10	8	14	Recovery
Drug (Phenytoin) Treated	13	8	15	Recovery
Drug (Phenytoin) Treated	14	6	18	Recovery
Drug (Phenytoin) Treated	12	6	17	Recovery
Average	11.8333	6.8333	15.8333	

Student's Name: _____

*Observation table after completion of the experiment can be downloaded with respective student's name.

Result/Conclusion:

Treatment of mice with 25 mg/kg Phenytoin decreases the duration of clonic and tonic convulsions and also reduces the duration of stupor. The efficacy of phenytoin in the maximal electroshock induced convulsions is shown.

Experiment-12

Aim/Object: Effect of agonist & antagonist and bioassay of histamine on the ileum of guinea pig

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Practice 1 - Dose Dependent Response in Guinea Pig ileum

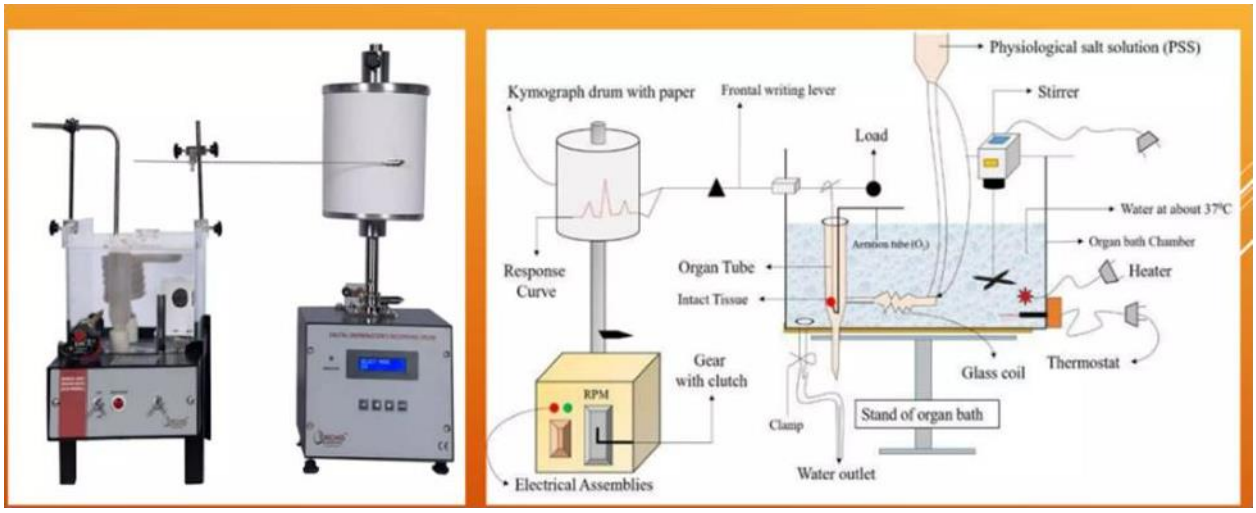
Experimental Pharmacology:

Bioassay of Histamine on the guinea-pig ileum praparation

Bioassay of Histamine using guinea-pig ileum by Matching Assay

Equipment:

Organ Bath & Kymograph Drum Assembly – Setup



Instructions/Procedure:

1. *Choose the dose and press inject button. Clicking the buttons + and - will double or halve the dose. The dose can be manually entered by clicking into the dose box.*
2. *Once the dose response curve is obtained, click the button 'Matching Assay' in Do box.*
3. *You will be asked to enter the dose of standard curve. Then a panel with the following buttons appears: 1. Standard 2. Double-Standard 3. Half-Standard 4.Unknown*
4. *Clicking buttons 1-3 will inject the respective dose of the standard. No.1 will inject the full dose of the standard selected by the student, no. 2 will double the dose and no. 3 will injects half the dose of standard. Before pressing unknown button, the dose of unknown in ml has to*

be selected. This can be done at the Dose selection box. Click the unknown button to inject the selected dose of unknown.

5. *After matching, press 'Calculate' button and enter the volume of unknown needed to match. The concentration of Histamine in the unknown solution is calculated and displayed.*

Note- Graph can be downloaded with the help of save graph option.

Result/Conclusion:

Bioassay of test sample was performed and concentration of test was calculated.

Practice 2 - To study effect of agonist and antagonist on guinea pig ileum

Introduction and Principle:

What is agonist: Drug which activates Receptor.

What is antagonist: Drug which blocks the Receptor

Agonist drugs for guinea pig ileum: Acetylcholine & Histamine

Antagonist drugs for guinea pig ileum:

- Atropine (Antagonise Acetylcholine)
- Mepyramine (Antagonise Histamine)

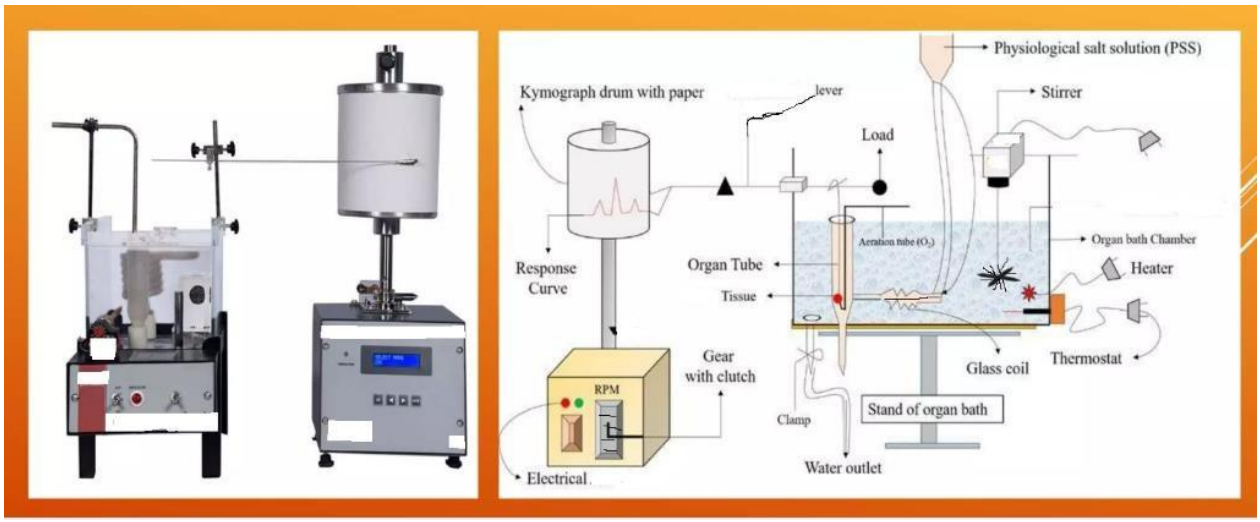
Atropine at a specific concentration almost totally blocks the response to acetylcholine but has no effect on the histamine-induced contraction.

Mepyramine at a specific concentration almost totally blocks the response to exogenously applied histamine but has no effect on the acetylcholine-induced contraction. Please note that at higher concentrations mepyramine does also antagonise muscarinic cholinoceptors.

Note that if the same dose of either of the agonists is repeated, the size of the contraction varies slightly from experiment to experiment.

Equipment:

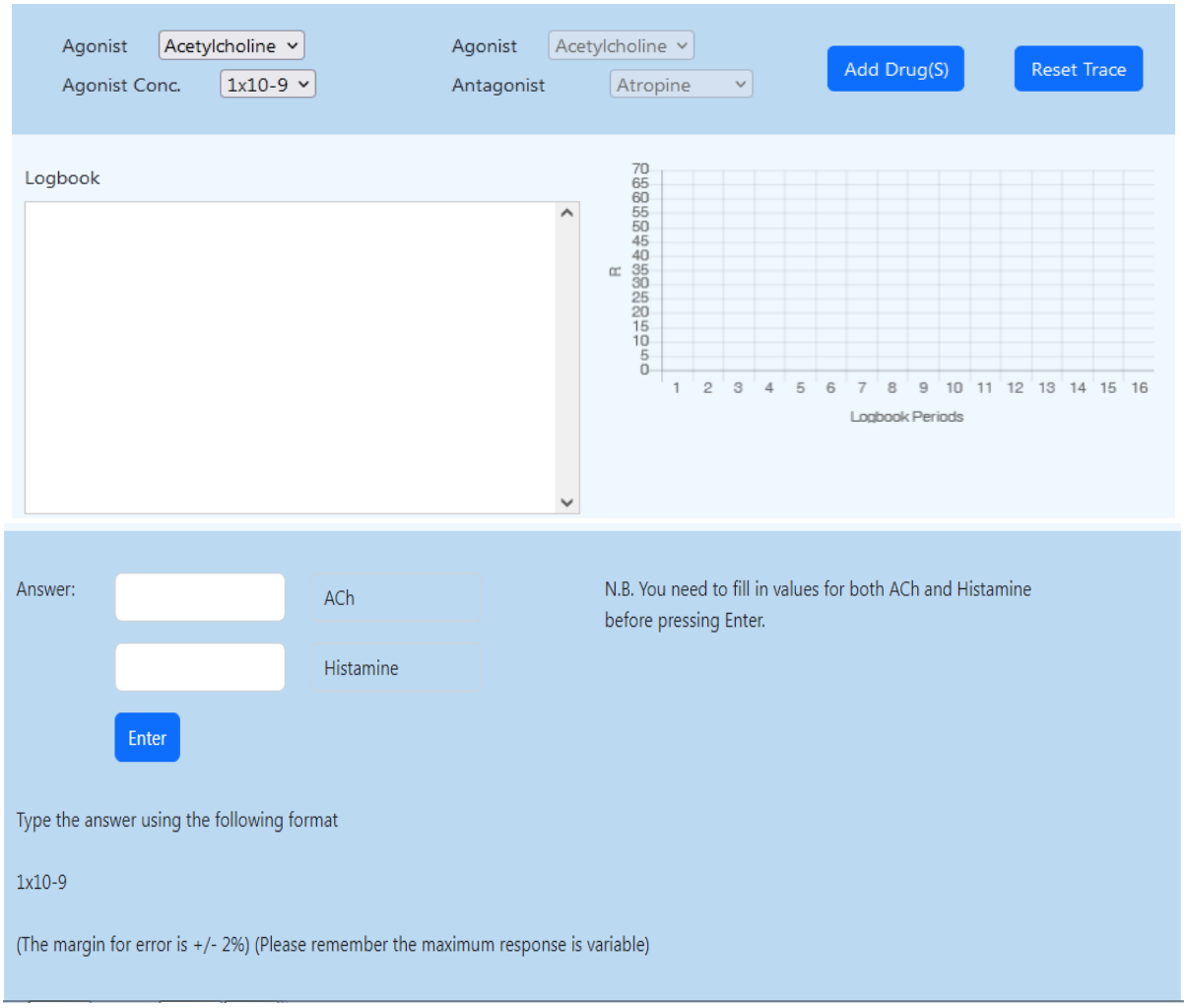
Organ / Tissue Bath Assembly



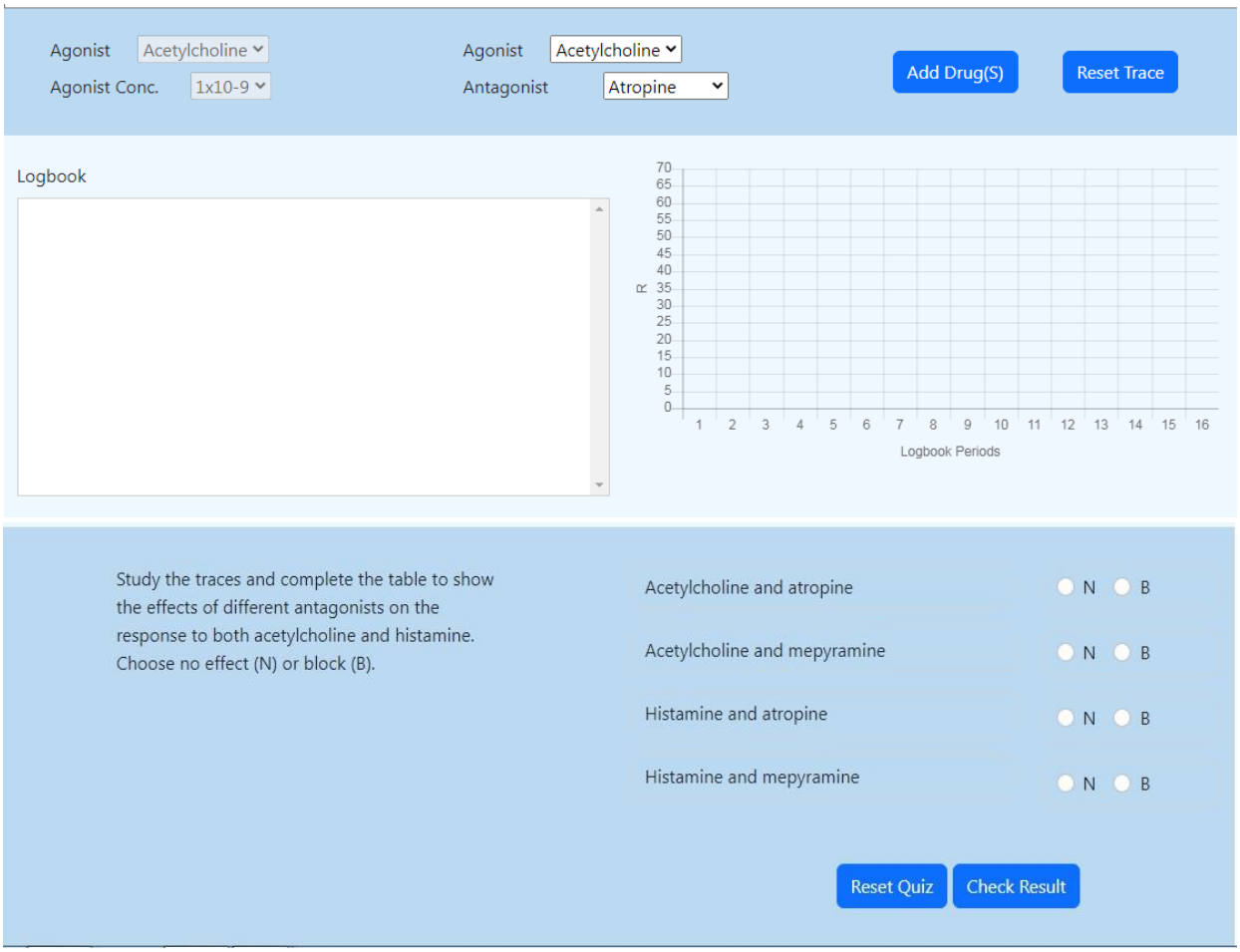
Instructions/Procedure:

1. *Arrange organ bath and rotating drum assembly as per standard protocol*
2. *Dissect guinea pig & Isolate guinea pig ileum as per standard protocol*
3. *Mount the ileum in organ bath assembly*
4. *Apply agonist (Acetylcholine & Histamine) and record the response.*
5. *Determine a 'standard dose' (one which produces approximately 75% of the maximum response) for acetylcholine and histamine. Select a low concentration to begin with and increase the dose in a geometric manner.*
6. *Apply antagonist Atropine (Antagonise Acetylcholine) & Mepyramine (Antagonise Histamine) and record the response.*
7. *Test the action of the antagonists atropine and mepyramine against exogenously applied acetylcholine and histamine. The concentrations of ACh and histamine are pre-selected as those which produce a sub-maximal (75%) contraction as determined in 'Part 1' (i.e. acetylcholine 8x10-8 M; histamine 8x10-7 M). In each experiment antagonists are added to the bathing fluid and left for 5 minutes before the agonist is added.*

Part 1: Add agonists to the bath. Determine a 'standard dose' (one which produces approximately 75% of the maximum response) for acetylcholine and histamine. Select a low concentration to begin with and increase the dose in a geometric manner.



Part 2: Action of antagonists. Test the action of the antagonists atropine and mepyramine against exogenously applied acetylcholine and histamine. The concentrations of ACh and histamine are pre-selected as those which produce a sub-maximal (75%) contraction as determined in 'Part 1' (i.e. acetylcholine 8x10-8 M; histamine 8x10-7 M). In each experiment antagonists are added to the bathing fluid and left for 5 minutes before the agonist is added.



Note- To find out the finding of part 2, 'Check Result' option can be used.

Inference:

Atropine at a specific concentration almost totally blocks the response to acetylcholine but has no effect on the histamine-induced contraction.

Mepyramine at a specific concentration almost totally blocks the response to exogenously applied histamine but has no effect on the acetylcholine-induced contraction. Please note that at higher concentrations mepyramine does also antagonise muscarinic cholinoceptors.

Note that if the same dose of either of the agonists is repeated, the size of the contraction varies slightly from experiment to experiment.

Result/Conclusion

Role of agonist and antagonist was evaluated.

Experiment-13

Aim/Object: Experiments of rat blood sugar

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Objective: Demonstration of the effect of insulin on the healthy rat and on the rat with insulin-dependent diabetes mellitus (produced by administering aloxan, a substance which destroys the cells from the Langerhans islets of the pancreas).

Introduction and Principle: Insulin is a polypeptidic hormone when is synthesized by the cells from the Langerhans islets of the pancreas.

The main metabolic action of this hormone is to lower the level of the glucose in the whole blood, by increasing the transfer of glucose across the plasmatic membrane of target cells, where it enhances:

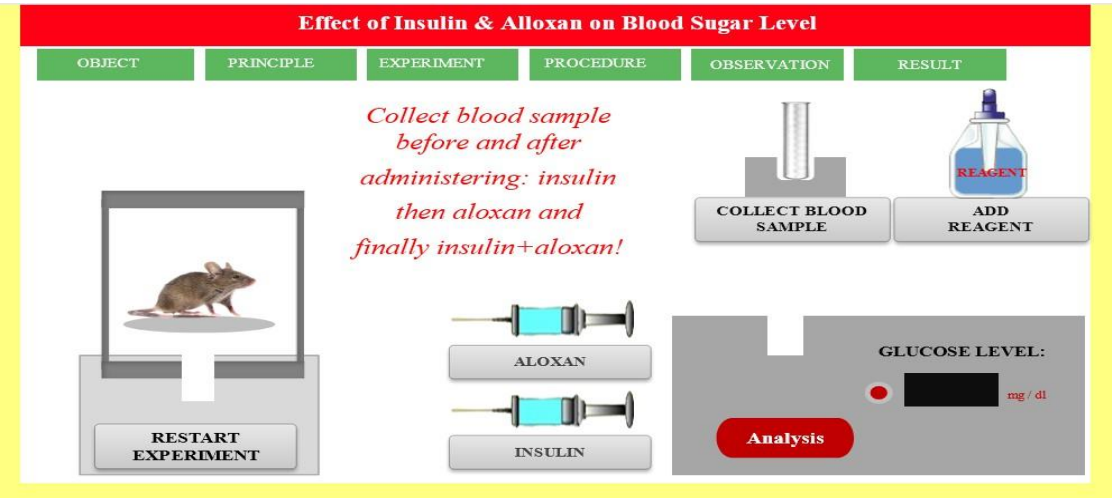
- Glycolysis;
- Inclusion of glucose in the glycogen molecule (in the hepatic and muscular tissues).
- Transformation of glucose in lipids and proteins.

Diabetes mellitus is a metabolic disorder which may be classified into types I and II:

- Insulin-dependent diabetes mellitus (Type I), which is caused by the insufficient synthesis of insulin in the endocrine pancreas;
- Non-insulin-dependent diabetes mellitus (Type II), which is characterized by a sufficient synthesis of insulin, without a suitable response from the target cells.

Collect a blood sample from the normal rat and then another blood sample from the rat with diabetes mellitus, before and after insulin administration. Measure the blood glucose.

EXPERIMENT:



Instructions/Procedure:

1. *Click on the 'Collect a Blood Sample' button;*
2. *Click the 'ADD A REAGENT' button;*
3. *Click the 'ANALYSE' button;*
4. *The value of the blood glucose is recorded;*
5. *Click the 'RESTART EXPERIMENT' buttons;*
6. *Insulin is given by clicking on the arrow which indicates this substance and action from point 1,2,3,4 and 5 are repeated;*
7. *Aloxan is given by clicking on the arrow which indicates this substance and actions from point 1,2,3,4 and 5 are repeated;*
8. *Alaxan and insulin given by clicking the corresponding arrows and actions from points 1,2,3,4 and 5 are repeated.*

OBSERVATION TABLE:

Input Blood Sugar Value in Observation Box

Normal (Control) Analysis	
Aloxan Analysis	
Insulin Analysis	
Aloxan+Insulin Analysis	

Student's Name: _____

INFERENCE:

Normal (Control) Analysis	86
Aloxan Analysis	129
Insulin Analysis	76
Aloxan+Insulin Analysis	96

Student's Name: _____

*Observation table after completion of the experiment can be downloaded with respective student's name.

Result/Conclusion:

Alloxan increased blood sugar level (by destructing beta cells of pancreas) whereas insulin reverses (reduces) the increased blood sugar level (Increased due to alloxan injection)

Experiment-14

Aim/Object: 3 Modules - To record the dose response curve and to determine the pD2 value for acetylcholine (on frog rectus abdominis muscle), serotonin (on rat fundus strip) and histamine (on guinea pig ileum)

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Practical 1- To record the dose response curve and to determine and pD2 value for acetylcholine on frog rectus abdominis muscle.

Objectives-

1. To record the dose response curve for acetylcholine on isolated frog rectus abdominis muscle
2. To determine the pD2 value for acetylcholine on frog rectus abdominis muscle

Introduction and Principle: The negative logarithm to base 10 of the EC50 of an agonist is called pEC50 or pD2 (old term). It indicates the potency of an agonist but not its efficacy, the higher pD2=small EC50, reflects higher potency of the agonist.

Equipment:

Instructions/Procedure:

1. *The capacity of the organ tube is 20mL.*
2. *Start with the minimum possible dose (like 0.002 mL).*
3. *When the first measurable response (a response that can measured e.g. at least 5mm height, at least 10mg force) is achieved, repeat the same dose and see whether you get a reproducible*

response. This proves the reproducibility of the response, proper acclimatisation of the tissue to in-vitro conditions.

4. *Record the response of geometrically increasing doses till the maximum response is achieved (subsequent doses give equal responses)*
5. *Once the maximum response is achieved, do not repeatedly expose the tissue to maximal dose or higher doses. Such repeated exposures may affect the responsiveness of the tissue.*
6. *Plot a Graph between Log Concentration and Percentage Response.*
7. *pD2 Value can be finded out by tracing the point in log DRC at which 50% response of maximum response is shown.*

OBSERVATION TABLE:

Determine the pD2 value of acetylcholine

a. Observation table with findings

S.No.	Conc of Ach in Microgram/ml (In Applicator)	Amount Added in Organ Bath		Conc of Ach in Microgram/ml (In Organ Bath (Organ Bath Contains 20 ml Solution)	Response (In mm)	Conc of Ach (Micromole/L) Mol. Wt of Ach. Salt: 181.66	Conc of Ach (Micromole/ml)	Log conc	%Response
		In ml	In Microgram						
1	50	0.002	0.1	0.005	0	0.000275999	2.75999E-07	-6.55909	0
2	50	0.04	2	0.1	3	0.000551998	5.51998E-07	-6.25806	5.3571429
3	50	0.08	4	0.2	9	0.001103996	1.104E-06	-5.95703	16.071429
4	50	0.16	8	0.4	17	0.002207993	2.20799E-06	-5.656	30.357143
5	50	0.32	16	0.8	26	0.004415986	4.41599E-06	-5.35497	46.428571
6	50	0.8	40	2	39	0.011039965	1.104E-05	-4.95703	69.642857
7	50	1.6	80	4	51	0.022079929	2.20799E-05	-4.656	91.071429
8	50	3.2	160	8	56	0.044159859	4.41599E-05	-4.35497	100
9	50	6.4	320	16	56 (Celing Response)	0.088319717	8.83197E-05	-4.05394	100

- pD2 Value can be finded out by tracing the point in log DRC at which 50% response of maximum response is shown.

- pD2 = -log [EC50]

- pD2 value of Acetylcholine is 5.3

Student's Name: _____

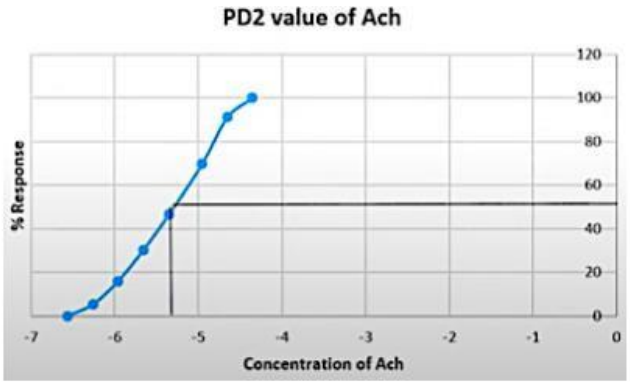
b. Observation table for entering Data and student name

S.No.	Conc of Ach in Microgram/ml (In Applicator)	Amount Added in Organ Bath		Conc of Ach in Microgram/ml (In Organ Bath (Organ Bath Contains 20 ml Solution)	Response (In mm)	Conc of Ach (Micromole/L) Mol. Wt of Ach. Salt: 181.66	Conc of Ach (Micromole/ml)	Log conc	%Response
		In ml	In Microgram						
1									
2									
3									
4									
5									
6									
7									
8									
9									

Student's Name: _____

*Observation table after completion of the experiment can be downloaded with respective student's name.

c. Graph



- pD2 Value can be finded out by tracing the point in log DRC at which 50% response of maximum response is shown.
- pD2 = -log [EC50]
- pD2 value of Acetylcholine is 5.3

Note- Graph can be downloaded with the help of save graph option.

Result/Conclusion: By the experiment we got the pD2 Value of Acetylcholine Sample i.e: 5.3

Practical 2- To record the dose response curve and to determine and pD2 value for Serotonin on rat stomach strip (fundus part).

Objectives-

1. To record the dose response curve for Serotonin on isolated Rat stomach (fundus part) strip.
2. To determine the pD2 value for Serotonin on Rat stomach (fundus part) strip

Introduction and Principle: The negative logarithm to base 10 of the EC50 of an agonist is called pEC50 or pD2 (old term). It indicates the potency of an agonist but not its efficacy, the higher pD2=small EC50, reflects higher potency of the agonist.

Equipment:

Instructions/Procedure:

1. *The capacity of the organ tube is 20mL.*
2. *Start with the minimum possible dose (like 0.002 mL).*
3. *When the first measurable response (a response that can measured e.g. at least 5mm height, at least 10mg force) is achieved, repeat the same dose and see whether you get a reproducible response. This proves the reproducibility of the response, proper acclimatisation of the tissue to in-vitro conditions.*
4. *Record the response of geometrically increasing doses till the maximum response is achieved (subsequent doses give equal responses)*
5. *Once the maximum response is achieved, do not repeatedly expose the tissue to maximal dose or higher doses. Such repeated exposures may affect the responsiveness of the tissue.*
6. *Plot a Graph between Log Concentration and Percentage Response.*

7. *pD2 Value can be finded out by tracing the point in log DRC at which 50% response of maximum response is shown.*

OBSERVATION TABLE:

Determine the pD2 value of serotonin

a. Observation table with findings

S. No.	Conc. of Serotonin in Microgram/ ml (In Applicator)	Amount Added in Organ Bath		Conc. of Serotonin in Microgram/ml (In Organ Bath (Organ Bath Contains 20 ml Solution)	Response (In mm)	Conc. of Serotonin (Micromole/ L) Mol. Wt of Serotonin Salt: 212.68	Conc. of Serotonin (Micromole/ ml)	Log conc.	%Response
		In ml	In Microgram						
1	50	0.002	0.1	0.005	3	2.35095E-05	2.35095E-08	-7.628756647	5.660377358
2	50	0.004	0.2	0.01	9	4.7019E-05	4.7019E-08	-7.327726652	16.98113208
3	50	0.008	0.4	0.02	17	9.4038E-05	9.4038E-08	-7.026696656	32.0754717
4	50	0.016	0.8	0.04	26	0.000188076	1.88076E-07	-6.72566666	49.05660377
5	50	0.032	1.6	0.08	31	0.000376152	3.76152E-07	-6.424636665	58.49056604
6	50	0.064	0.32	0.16	37	0.000752304	7.52304E-07	-6.123606669	69.81132075
7	50	0.16	8	0.04	46	0.00188076	1.88076E-06	-5.72566666	86.79245283
8	50	0.32	16	0.8	50	0.00376152	3.76152E-06	-5.424636665	94.33962264
9	50	0.64	32	1.6	53	0.007523039	7.52304E-06	-5.123606669	100
10	50	1.28	64	3.2	53 (Celing Response)	0.007523039	7.52304E-06	-5.123606669	100

- pD2 Value can be finded out by tracing the point in log DRC at which 50% response of maximum response is shown.
- pD2 = -log [EC50]
- Interpolated: -6.7
- pD2 value of Serotonin: 6.7

Student's Name: _____

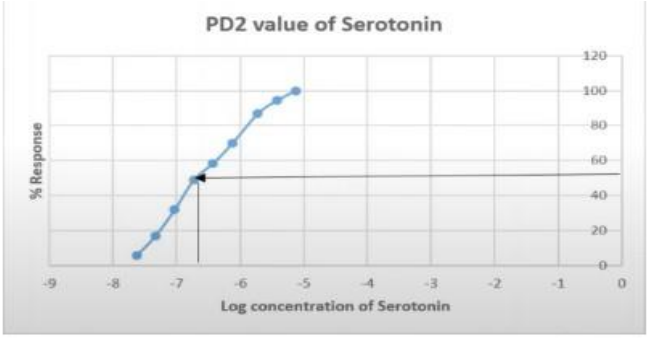
b. Observation table for entering Data and student name

S.No.	Conc of Ach in Microgram/ml (In Applicator)	Amount Added in Organ Bath		Conc of Ach in Microgram/ml (In Organ Bath (Organ Bath Contains 20 ml Solution)	Response (In mm)	Conc of Ach (Micromole/L) Mol. Wt of Ach. Salt: 181.66	Conc of Ach (Micromole/ml)	Log conc	%Response
		In ml	In Microgram						
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									

Student's Name: _____

*Observation table after completion of the experiment can be downloaded with respective student's name.

c. Graph



- pD2 Value can be finded out by tracing the point in log DRC at which 50% response of maximum response is shown.
- pD2 = -log [EC50]
- Interpolated : -6.7
- pD2 value of Serotonin : 6.7

Note- Graph can be downloaded with the help of save graph option.

Result/Conclusion:

By the experiment we got the pD2 Value of Serotonin Sample i.e: 6.7

Practical 3- To record the dose response curve and to determine and pD2 value for Histamine on Guinea Pig Illeum.

Objectives-

1. To record the dose response curve for Histamine on isolated Guinea Pig Ileum.
2. To determine the pD2 value for Histamine on Guinea Pig Ileum

Introduction and Principle: The negative logarithm to base 10 of the EC50 of an agonist is called pEC50 or pD2 (old term). It indicates the potency of an agonist but not its efficacy, the higher pD2=small EC50, reflects higher potency of the agonist.

Equipment:

Instructions/Procedure:

1. *The capacity of the organ tube is 20mL.*
2. *Start with the minimum possible dose (like 0.002 mL).*
3. *When the first measurable response (a response that can measured e.g. at least 5mm height, at least 10mg force) is achieved, repeat the same dose and see whether you get a reproducible response. This proves the reproducibility of the response, proper acclimatisation of the tissue to in-vitro conditions.*
4. *Record the response of geometrically increasing doses till the maximum response is achieved (subsequent doses give equal responses)*
5. *Once the maximum response is achieved, do not repeatedly expose the tissue to maximal dose or higher doses. Such repeated exposures may affect the responsiveness of the tissue.*
6. *Plot a Graph between Log Concentration and Percentage Response.*

7. *pD2 Value can be finded out by tracing the point in log DRC at which 50% response of maximum response is shown.*

OBSERVATION TABLE:

Determine the pD2 value of histamine

a. Observation table with findings

S. No.	Conc. of Histamine in Microgram/ml (In Applicator)	Amount Added in Organ Bath		Conc. of Histamine in Microgram/ml (In Organ Bath (Organ Bath Contains 20 ml Solution)	Response (In mm)	Conc. of Histamine (Micromole/L) Mol. Wt of Serotonin Salt: 212.68	Conc. of Histamine (Micromole/ml)	Log conc.	%Response
		In ml	In Microgram						
1	50	0.002	0.1	0.005	2	2.71651E-05	2.71651E-08	-7.56599	3.773585
2	50	0.004	0.2	0.01	3	5.43301E-05	5.43301E-08	-7.26496	5.660377
3	50	0.008	0.4	0.02	8	0.00010866	1.0866-07	-6.96393	15.09434
4	50	0.016	0.8	0.04	14	0.00021732	2.1732E-07	-6.6629	26.41509
5	50	0.032	1.6	0.08	20	0.000434641	4.34641E-07	-6.36187	37.73585
6	50	0.16	8	0.4	52	0.002173204	2.1732E-06	-5.6629	98.11321
7	50	0.32	16	0.8	53	0.004346409	4.34641E-06	-5.36187	100
8	50	0.64	32	1.6	53 (Ceiling Response)	0.008692818	8.69282E-06	-5.06084	100

- pD2 Value can be finded out by tracing the point in log DRC at which 50% response of maximum response is shown.

- pD2 = -log [EC50]

- Interpolated : -6.2

- pD2 value of Histamine : 6.2

Student's Name: _____

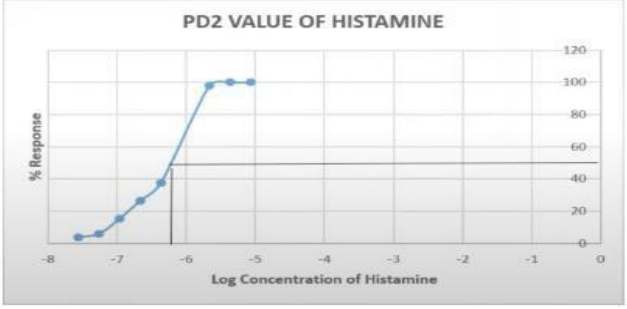
b. Observation table for entering Data and student name

S. No.	Conc. of Histamine in Microgram/ml (In Applicator)	Amount Added in Organ Bath		Conc. of Histamine in Microgram/ml (In Organ Bath (Organ Bath Contains 20 ml Solution)	Response (In mm)	Conc. of Histamine (Micromole/L) Mol. Wt of Serotonin Salt: 212.68	Conc. of Histamine (Micromole/ml)	Log conc.	%Response
		In ml	In Microgram						

Student's Name: _____

*Observation table after completion of the experiment can be downloaded with respective student's name.

c. Graph



- pD2 Value can be finded out by tracing the point in log DRC at which 50% response of maximum response is shown.
- pD2 = -log [EC50]
- Interpolated : -6.2
- pD2 value of Histamine : 6.2

Note- Graph can be downloaded with the help of save graph option.

Result/Conclusion:

By the experiment we got the pD2 Value of Histamine Sample i.e: 6.2

Experiment-15

Aim/Object: 4 Modules - Bioassay of acetylcholine (on frog rectus abdominis muscle), oxytocin (on rat uterine horn) and serotonin (on rat fundus strip),Bioassay of acetylcholine (on rat ileum/colon) - by matching, interpolation, 3 point and 4 point method.

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Practical 1- Bioassay of Acetylcholine on the isolated rectus abdominis muscle of frog

Directions:

You are given a Acetylcholine solution of unknown concentration. Find out the concentration of Acetylcholine using a isolated rectus abdominis muscle of frog preparation. Obtain a Dose-Response curve and carry out bioassay

Equipment:

Instructions/Procedure:

Instruction for Matching Methods:

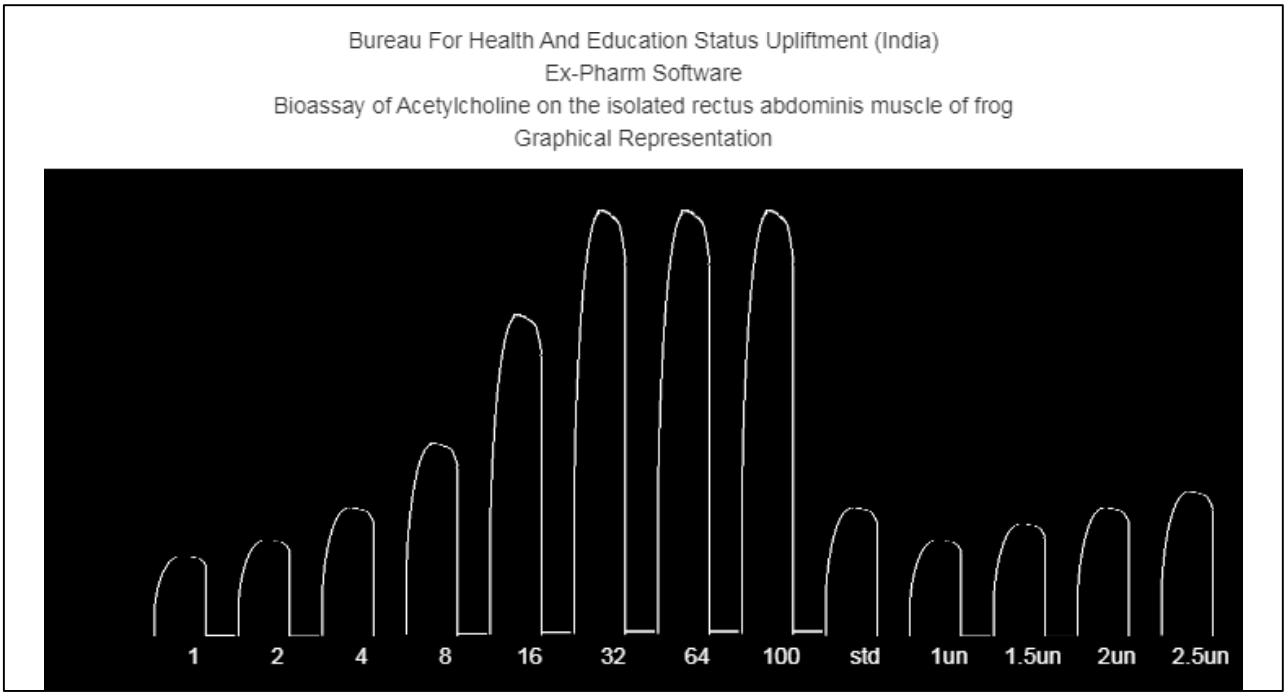
Bioassay of Acetylcholine using isolated rectus abdominis muscle of frog by Matching Method

Procedure:

1. *Choose the dose and press inject button. Clicking the buttons + and - will double or halve the dose. The dose can be manually entered by clicking into the dose box.*
2. *Once the dose response curve is obtained, click the button 'Matching Assay' in Do box.*

3. *You will be asked to enter the dose of standard curve. Then a panel with the following buttons appears: 1. Standard 2. Double-Standard 3.Half-Standard 4. Unknown*
4. *Clicking buttons 1-3 will inject the respective dose of the standard. No.1 will inject the full dose of the standard selected by the student, no. 2 will double the dose and no. 3 will injects half the dose of standard.*
5. *Before pressing unknown button, the dose of unknown in ml has to be selected. This can be done at the Dose selection box. Click the unknown button to inject the selected dose of unknown.*
6. After matching, press 'Calculate' button and enter the volume of unknown needed to match. The concentration of Acetylcholine in the unknown solution is calculated displayed.

Result-



Note- Graph can be downloaded with the help of 'save graph' option.

- After completion of experiment, concentration of Acetylcholine of unknown solution is:4 / 2 = 2 mcg/ml

Practical 2- Bioassay of oxytocin using rat uterine horn by by Interpolation method

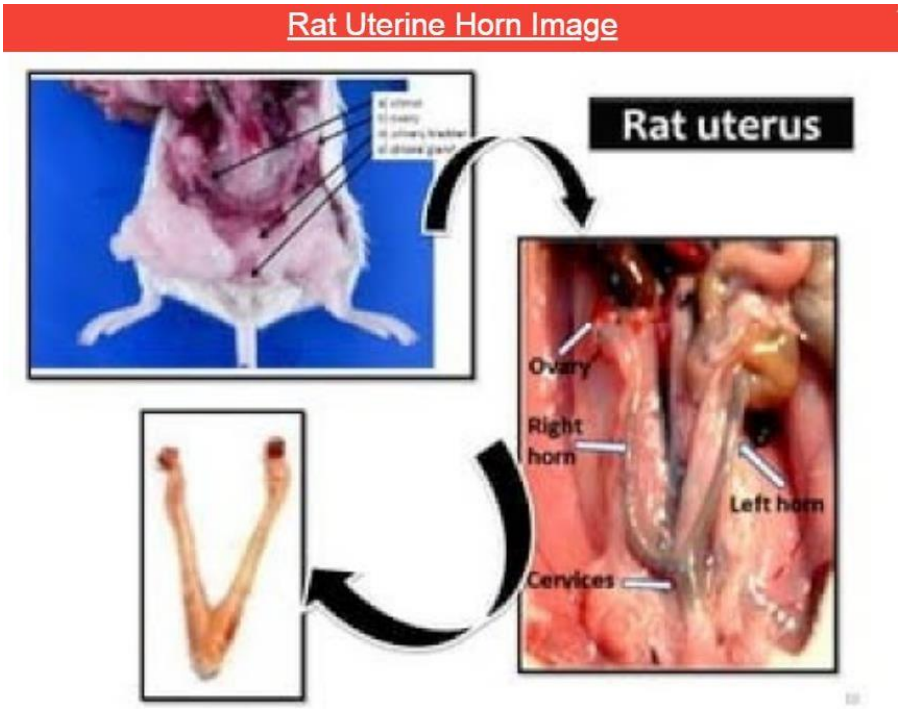
Requirements:

Animal: Female rat (120-150 g)

Solutions: Oxytocin, stilboestrol, De Jalon

Apparatus: Dissection tray, Gimbal lever, Sherington's revolving drum, Kymograph, reservoir, stand, forceps, petri plate, scissors, pipettes, thread, surgical needle, plasticin.

Rat Uterine Horn Image



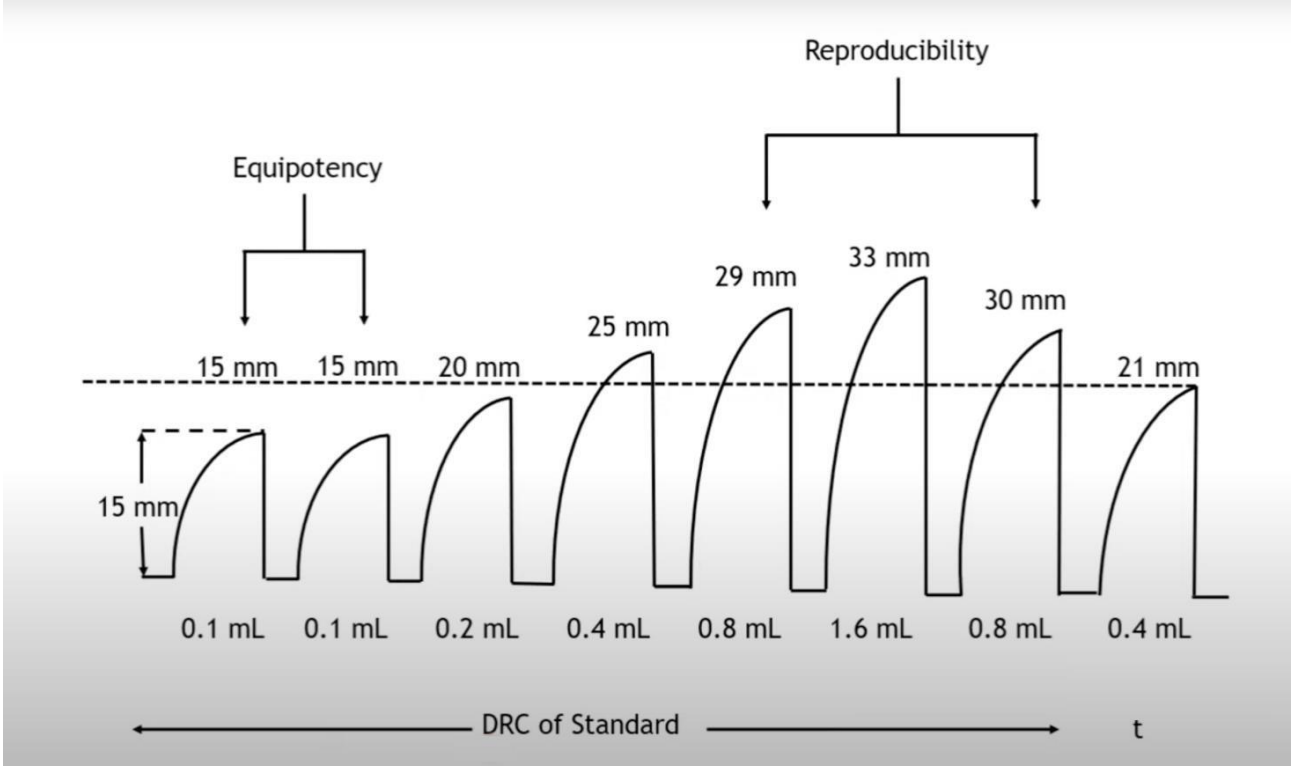
Instructions/Procedure:

Procedure for Interpolation Methods:

1. *Examine the vaginal smear under microscope to know about the proper stage of ostrus cycle.*
2. *If the rat is not in frank ostrus, inject 0.1 mg/kg of stilboestrol and wait for 24 hrs. (Vaginal smear is prepared by taking a drop of the vaginal wash and putting on the glass slide).*
3. *Sacrifice the animal and cut open the pelvic region to expose both the horns of uterus.*
4. *Separate the horns gently from the surrounding fatty material. and transfer them into dish containing De Jalon's solution.*
5. *Record the DRC of Standard.*
6. *Repeat the first dose (0.1 mL) till the height of the response obtained is same to ensure the stabilization of tissue preparation.*
7. *Elicit the responses of Oxytocin in increasing doses in geometric progression (0.2, 0.4, 0.8, 1.6, 3.2 mL) till you get the maximum response with high concentration.*
8. *Record the response of a certain volume of test solution.*
9. *Note that the volume selected should lie in between the volumes used for recording response for standard solution.*
10. *After recording all the responses stop the kymograph, properly label the tracing.*
11. *Measure the heights of responses (in mm) and tabulate the result.*
12. *Plot the graph of log of concentration of Oxytocin against % response.*
13. *Find out the concentration of test solution from the graph.*

14. *Calculate the concentration it in terms of micrograms/ mL.*

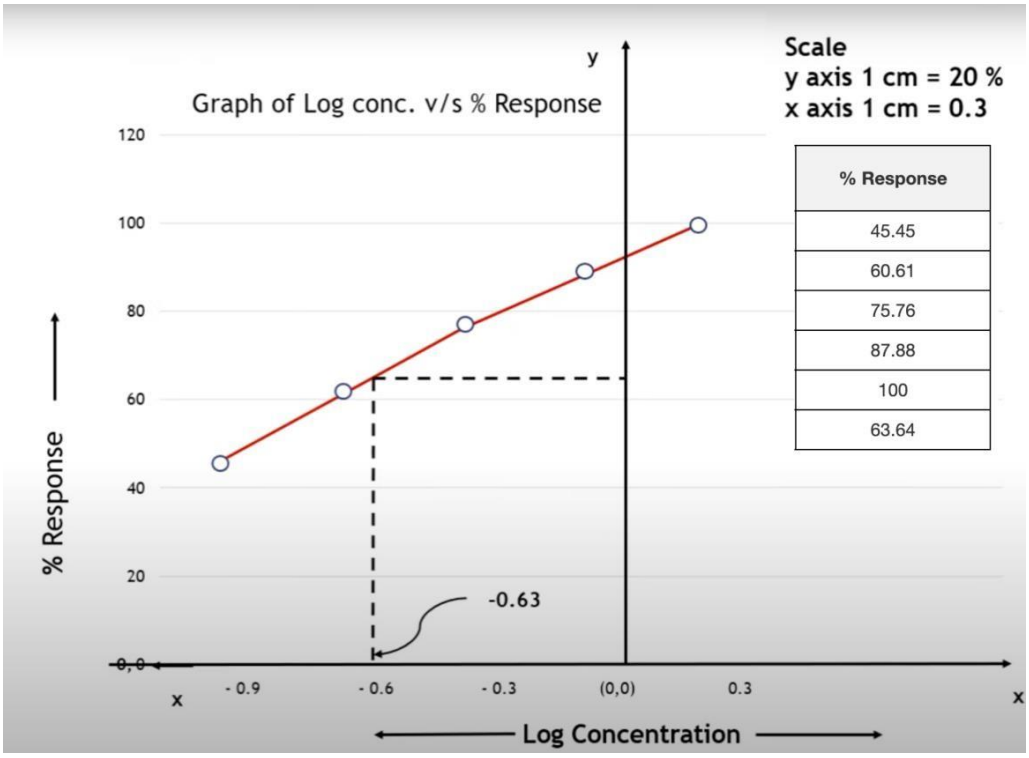
Observation-



Conc. of Standard Oxytocin = 1 µg/mL

Sample	Dose (mL)	Conc. (µg)	Log conc.	Height of contraction (mm)	% Res.
Standard (Oxytocin)	0.1	0.1	-1	15	45.45
	0.2	0.2	-0.6989	20	60.61
	0.4	0.4	-0.3979	25	75.76
	0.8	0.8	-0.097	29	87.88
	1.6	1.6	0.2041	33	100
Test	0.4	-	-0.63	21	63.64

Note- observation table can be downloaded by save/download option.



From graph

Log conc. = -0.63

Antilog (-0.63) = 0.23

0.4 mL test sample contains = 0.23 µg Oxytocin

Thus 1 mL test sample contains (0.23 x 1)/0.4 = 0.58 µg/mL

Result- Concentration of test = 0.58 µg/mL

Practical 3- Bioassay of serotonin using rat fundus strip by three-point bioassay

Requirements-

Animal/tissue	: Rat Fundus Strip Muscle (Rat)
Instrument	: thermo statically controlled organbath, chymograph
Lever	: Frontal writing
Magnification	: 7-10x
Tenin/load	: Up to 1 gm
Air	: O_2/Carbogen
Temperature	: 32-37°C
Drug	: Serotonin

Precautions:

1. Clean the organ bath before starting the experiment specially inner organ bath (chances of presence of previous drug used)

2. Balance the writing lever horizontal with the help of load
3. Prepare PSS for the experiment, while taking exact quantity of chemicals (1% variability is acceptable)
4. Add the calcium chloride at the end of PSS preparation (to avoid any precipitation: PSS should be clear) D
5. Try to minimize the handling of tissue (especially at the middle part)
6. Always use the finger to hold the tissue instead of forceps
7. Maintain the dose cycle properly (tissue sensitivity depend on this cycle).

Instructions/Procedure:

1. *The dose-response curves (DRC) of the standard and the test are carried out until submaximal responses are obtained for both.*
2. *Two doses of the standard s_1 and s_2 which lie between 25% and 75% are taken.*
3. *Then a test response (t) is selected such that the response lies between those shown by s_1 and s_2 (i.e. $H_1 < T < H_2$).*
4. *After selecting the doses, responses of the standard & test are recorded by the Latin sequence method in order to reduce errors.*
5. *Nine responses of the 3 doses selected are recorded and the average of the responses of each dose is calculated.*

Latin sequence design

$$\begin{pmatrix} s_1 & t & s_2 \\ t & s_2 & s_1 \\ s_2 & s_1 & t \end{pmatrix}$$

6. *Substitute the values in the formula to obtain the concentration of the test solution:*

Conc. of test *= conc. of std x (s_1/t) x antilog$_{10}$ $(((T - H_1) / (H_2 - H_1)) \times \log_{10}(S_2/s_1))$*

s_1 and s_2 = lower and higher dose of standard

t = dose of test

T = average height of test

H_1 and H_2 = average heights of lower and higher dose of standard

OBSERVATION TABLE:

Sample	Dose (ml)	Conc. (µg)	Height of conc. (mm)	Mean height
S1	0.2	2	7	H1=7
S1			8	
S1			6	
T	0.4	To be calculated by below mentioned formula	10	T=9.67
T			9	
T			10	
S2	0.4	4	14	H2=13
S2			12	
S2			13	

Note- Substitute the values in the formula to obtain the concentration of the test solution:

Conc. of test = conc. of std x (s1/t) x antilog10 (((T - H1) / (H2 - H1)) x log10(S2/s1))

s1 and s2 = lower and higher dose of standard

t = dose of test

T = average height of test

H1 and H2 = average heights of lower and higher dose of standard

Result/Conclusion:

Concentration of test = 6.8µg/ml

Practical 4- Bioassay of acetylcholine using rat ileum/colon by four-point bioassay

Requirements-

Animal & tissue: Rat (150-200g), Overnight fasted, Ileum.

Physiological solution: Tyrode solution

Drugs: Acetylcholine (100µg/ml), Ach test solution, Fixing solution

Instruments: Student organ bath, Aerator, Insulin syringe to inject drugs in small fractions, Dissecting board and various dissecting instruments, Simple straw lever and stand, Pipette, Stop watch

Miscellaneous: Kymograph paper, plasticin, clips, and thread.

Instructions/Procedure:

1. *Sacrifice the rat by a blow on the head and carotid bleeding.*
2. *Cut open the abdomen expose the stomach.*
3. *Identify and isolate the ileum, put it in the dish containing tyrode solution.*
4. *Clean and cut 2-3 cm ileum piece and then Mount in organ bath with proper specification.*
5. *The dose response curves (DRC) of the standard and the test are carried out until submaximal response are obtained for both (Base line 30 sec. & Contact time 60 sec. & 3 Washing of 60 sec. each)*
6. *Select two standard doses (s1 & s2) in ratio of 1:2 & two test dose (tl & t2), t1 shall fall between s1 & s2.*
7. *Employing the latin sequence design the responses are recorded in a random fashion as:*

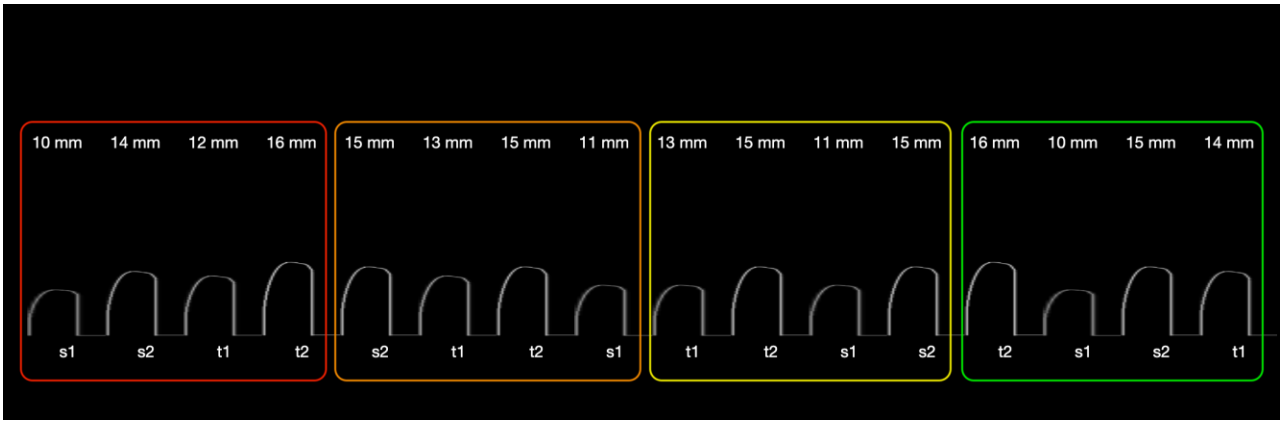
   s1, s2, t1, t2

   s2, t1, t2, s1

   t1, t2, s1, s2

   t2, s1, s2, t1
8. *Concentration of test* = $(n_1 \div t_1) \times Antilog\{((T_2-S_2)+(T_1-S_1)) \div ((T_2-T_1)+(S_2-S_1)) \times Log\ (n_2 \div n_1)\} \times C_s$

OBSERVATION TABLE:



Observation

Conc. of Standard Ach = 100 µg/mL

Dose	I	II	III	IV	Mean
S_1	10	11	11	10	10.5
S_2	14	15	15	15	14.75
T_1	12	13	13	14	13
T_2	16	15	15	16	15.5

Formula for 4 point bioassay

Concentration of test = $(n_1 \div t_1) \times \text{Antilog}\{((T_2-S_2)+(T_1-S_1)) \div ((T_2-T_1)+(S_2-S_1)) \times \text{Log}\,(n_2 \div n_1)\} \times C_s$

Where,
n_1 & n_2 = dose in ml of s_1 & s_2
t_1 = Dose in ml of test
C_s = Concentration of Standard
S_1 & S_2 = Average Response Height of low and high dose of standard
T_1 & T_2 = Average Response Height of low and high dose of test

From the above table

n_1 n = 0.1,$_2$= 0.2
t_1 t = 0.4,$_2$= 0.8
S_1= 10.5, S_2= 14.75
T_1 T = 13,$_2$= 15.5

Result/Conclusion:

Concentration of Test = 34.82 µg/mL

Note- observation table can be downloaded through save/download option.

Experiment-16

Aim/Object: Study of diuretic activity using metabolic cage

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Introduction and Principle: Diuretics are the compounds which increase the flow of urine. Normal urine output in rats is very small (1-2 ml/rat/day). Hence, to get the measurable quantity the animals are first hydrated. The urine output is increased after administration of diuretics like urea and furosemide. Increase in volume of urine is measured with the help of measuring cylinder and compared with the normal urine output.

Equipment:

The metabolic cage is designed to allow measurement of fluid intake, and to separate and collect feces and urine for numerous qualitative and quantitative determinations. In addition, the metabolic cage permits observation of the animal, feces, and urine at all times.

Instructions/Procedure:

1. *Divide animals into two groups (6 animals in each)*
2. *Administer one group with the drug (Furosemide 10mg/kg) to be tested and other with vehicle by oral route.*
3. *After that place the animals in metabolic cages.*
4. *Record the total volume of urine collected after 5 hr.*
5. *Compare the urine output between vehicle treated and drug treated group*

OBSERVATION TABLE:

Vehicle Treated	Drug Treated	

		Average

Student's Name: _____

INFERENCE:

Vehicle Treated	Drug Treated	
2.8	10.2	
3	10.4	
3.2	10.9	
3	10	
2.9	10.6	
3.1	11	
3	10.5166	Average

Student's Name: _____

*Observation table after completion of the experiment can be downloaded with respective student's name.

Result/Conclusion:

Increase in the average urine output in Furosemide (diuretic drug) treated animal shows that urine level is increased in the Furosemide treated animal.

Experiment-17

Aim/Object: Study of anti-inflammatory activity using carrageenan induced paw oedema method

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Introduction and Principle:

Inflammation is a protective response to injury. It occurs in three phases:

(a) The first phase being oedema and swelling with accompanying pain. These effects are produced as a result of the dilation and increased permeability of the blood vessels (veins) due to the release of certain mediators such as histamine, serotonin and kinins etc.

(b) In the second phase, leukocytes migrate to this area and mopping up operations starts.

(c) Second phase is followed by repair, which is ushered in by the proliferation of fibroblasts and synthesis of connective tissue.

The ability of a compound to reduce the local oedema induced in rat paw by various irritants is the most widely used test to screen new non-steroidal anti-inflammatory drugs. Many compounds like formalin, carrageenan, Kaolin, yeast and dextran have been used as irritants to produce oedema.

Equipment:

Plethysmometer is an equipment used to measure paw volume of rat while preforming anti-inflammatory activity.

Instructions/Procedure:

Procedure**:**

Twelve healthy male albino rats weighing 100-200 gms will be selected and made into two groups of six animals each. All the animals will be kept on fasting for 18 hours. The hind paw of the rats

will be marked at the level of tibio tarsal junction of hind leg, so that while measuring the volume, the dipping will be done to the same level. 0.1 ml of 1% Carrageenan will be administered to the rats into the plantar surface of the right hind limb to induce paw oedema. The volume will be measured immediately and after 3 hours using plethysmometer. One group serve as control, 0.3 ml of Normal saline will be given orally. Another group will receive the test drug, Acetyl salicylic acid 300 mg/Kg. After 30 minutes of the administration of drug, the change in the paw volume was compared with the control animals. The percentage of oedema compared to the control by the test drug.

Instructions**:**

1. *Take 12 animals*
2. *Inject carragenan in paw of animals, wait for specified time.*
3. *Divide animals into two groups (6 animals in each)*
4. *Administer one group with the drug (Acetyl salicylic acid 300mg/kg) to be tested and other with vehicle by oral route, wait for 30 minutes.*
5. *After that evaluate paw volume.*
6. *Record the paw volume change.*
7. *Compare the paw volume change in between vehicle treated and drug treated group.*
8. *Animals are divided into two groups.*
9. *Administer one group with the drug to be tested and other with vehicle by intraperitoneal route.*
10. *Click "LOAD" button.*
11. *Click "START" button. Adjust the speed of instrument (25 rotations/60 sec).*
12. *When the animal fall off respective time will be displayed on the timer.*

OBSERVATION TABLE:

Vehicle Treated - Paw Volume (ml)	**Drug Treated - Paw Volume (ml)**	
		Average

Student's Name: _____

INFERENCE:

Vehicle Treated - Paw Volume (ml)	**Drug Treated - Paw Volume (ml)**	
0.78	0.51	

0.75	0.53	
0.74	0.49	
0.77	0.55	
0.73	0.58	
0.74	0.56	
0.7516	0.5366	Average

Student's Name: _____

*Observation table after completion of the experiment can be downloaded with respective student's name.

Result/Conclusion:

Decreased average paw volume in drug (Acetyl salicylic acid) treated animals show anti-inflammatory activity of drug (Acetyl salicylic acid).

Experiment-18

Aim/Object: Rabbit pyrogen test

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Introduction and Principle: The Rabbit Pyrogen Test in an in vivo test to detect pyrogens qualitatively. Rabbits have a similar pyrogen tolerance to humans, so by observing a change in body temperature in rabbits it is possible to make a determination of the presence of pyrogens. This method can detect non-bacterial endotoxin pyrogens as well as bacterial endotoxins.

Instructions/Procedure:

1. *Animals are divided into two groups (3 animals in each)*
2. *Administer one group with the substance to be tested and other with vehicle by intravenous route.*
3. *Temperature of each animal is recorded with tele-thermometer.*

OBSERVATION TABLE:

Group Selected	Readings	Rabbit No. 1	Rabbit No. 2	Rabbit No. 3	Total
Vehicle Treated	Temperature Noted				
Test Substance Treated (Pyrogen added)	Temperature Noted				
	Temperature Increased				

Student's Name: _____

INFERENCE:

Group Selected	Readings	Rabbit No. 1	Rabbit No. 2	Rabbit No. 3	Total
Vehicle Treated	Temperature Noted	38.21	38.5	38.89	115.60

Test Substance Treated (Pyrogen added)	Temperature Noted	38.8	39.1	39.5	117.40
	Temperature Increased	0.59	0.60	0.61	1.80

Student's Name: _____

*Observation table after completion of the experiment can be downloaded with respective student's name.

Result/Conclusion:

As rise in body temp of each rabbit is more than 0.6, so it shows that the test sample is not pyrogen free.

Precautions:

1. Rabbits must be healthy and mature

2. New Zealand or Belgian Whites used mostly

3. Either sex may be used but males are preferred

4. Must be individually housed between 20 and 23°c

5. Their temperature should not vary more than ± 3° c.

6. Atmosphere should be free from disturbances which likely to excite them

7. Equipment and material used in test (glassware, syringes, needles etc) must be made free from

8. Pyrogens by heating at 250° c for not less then 30 minutes or any other method.

9. Retaining boxes (should be comfortable for rabbits as much as possible)

10. Thermometers or thermistor probe (should be inserted in standardized position in rectum, precision of ± 0.1° C)

Experiment-19

Aim/Object: 3 Modules - Effects of drugs on the dog BP and heart rate

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Practical 1- Effects of drugs on the dog BP and Heart Rate

Theory:

Drugs & Pharmacological Actions

Drug name (Dose in mg/kg)	Pharmacological Action
Epinephrine (Adrenaline) Dose : 2 Range : 1 – 3 Mg/kg Body Weight	It stimulates the alpha and beta adrenergic receptors. Conventional doses will increase the BP followed by a short fall before reaching the basal level (biphasic response due to alpha and beta receptor responses). The heart rate decreases due to vagal reflex.
Norepinephrine (Noradrenaline) Dose : 3 Range : 2 - 5 Mg/kg Body Weight	It stimulates mainly the alpha and beta1 receptors. The heart rate is generally reduced due to vagal reflex in response to increased BP.
Isoprenaline Dose : 3 Range : 2 - 5 Mg/kg Body Weight	It is a potent, non- selective beta adrenergic stimulant. It increases the systolic BP, but decreases the diastolic BP. Because the decrease is more pronounced than the increase, the mean arterial pressure typically falls.
Acetylcholine Dose : 5 Range : 2 - 10 Mg/kg Body Weight	Acetylcholine (ACh) leads to a sharp a fall in BP which returns to basal level quickly.
Histamine Dose : 3 Range : 2 - 5 Mg/kg Body Weight	Acts on H1 and H2 receptors to produce a fall in BP Stimulation of H1 produces a rapid onset short lived decrease in BP whereas H2 stimulation leads to a fall characterized by slower onset and longer duration.
Ephedrine Dose : 100 Range :100-200 Mg/kg Body Weight	It acts on both alpha and beta receptors and in addition enhances the release of norepinephrine from sympathetic neurons. It increases the BP and heart rate.
Phentolamine Dose : 1000 Mg/kg Body Weight	This drug, an alpha blocker, reduces BP

Propranolol Dose : 1000 Mg/kg Body Weight	It is a beta blocker which reduces BP and heart rate.
Atropine Dose : 750 Range : 500-1000 Mg/kg Body Weight	This drug is a muscarinic cholinergic antagonist. It competitively antagonizes ACh. .
Cimetidine Dose : 5000 Mg/kg Body Weight	It is a H2 blocker, which also partially blocks the effect of histamine on BP.

Adrenaline:

It is a sympathomimetic catecholamine which produces effect through alpha beta 1 and beta 2 receptors. As a result of beta 1 receptors stimulation there is sudden increase in heart rate and force of contraction & increase B.P. Immediately due to reflex inhibition there is slight decrease in B.P., but reaches to the periphery where alpha1 receptor stimulation produces vasoconstruction ad hence, further rise in blood pressure is observed. A notch is seen due to sudden changes in the response. Adrenaline action is slowly terminated by reuptake & by monoamine oxidase (MAO) and catechol-o-methyl transfers (COMT) enzymes. When the concentration of adrenaline is reduced beta receptor action predominates and as a result slight fall in blood pressure (secondary fall) is seen. Dale's vasomotor reversal.

Dale (1914) found that when adrenaline is given after administration of ergot alkaloid, it produces fall in blood pressure instead of rise. This unusual phenomenon was described as vasomotor reversal.

Noradrenaline (NA)

It is a sympathomimetic catecholamine having predominate alpha receptor action. The effect of NA differs from that the heart rate is not increased. On the contrary it may be decreased slightly. Only rise in blood pressure is seen and the action of arch is rapidly terminated by cholinesterase enzyme and hence, the response of Ach gets quickly recovered. Heart rate does not change in response to ach.

Histamine :

It is a naturally occurring autacoids which causes generalized vasodilation through H1 and H2 receptors. When histamine is administered in the dog there is marked fall in blood pressure. The recovery from histamine is rapid as compared to isoprenaline but slower as compared to acetylcholine.

Adrenaline produce rise in blood pressure through alpha adrenoceptor. When alpha adrenoceptor are blocked by any alpha-blockers (like phentolamine, tolaxoiine, phenoxybenzamie or ergot alkaloids), the beta2-adrenoceptor action of adrenaline predominates and fall in blood pressure is seen. This fall in blood pressure is known as Dale's vasomotor reversal.

Nicotinic receptor action of acetylcholine. The Nicotinic receptor action of Ach can be demonstrated by blocking all muscarinic receptor using atropine like drugs and by using high dose

of Ach. Nicotinic receptors are found in (1) skeleton muscles and (2) autonomic ganglia. Since the muscarinic receptors are blocked, autonomic ganglia are stimulated by

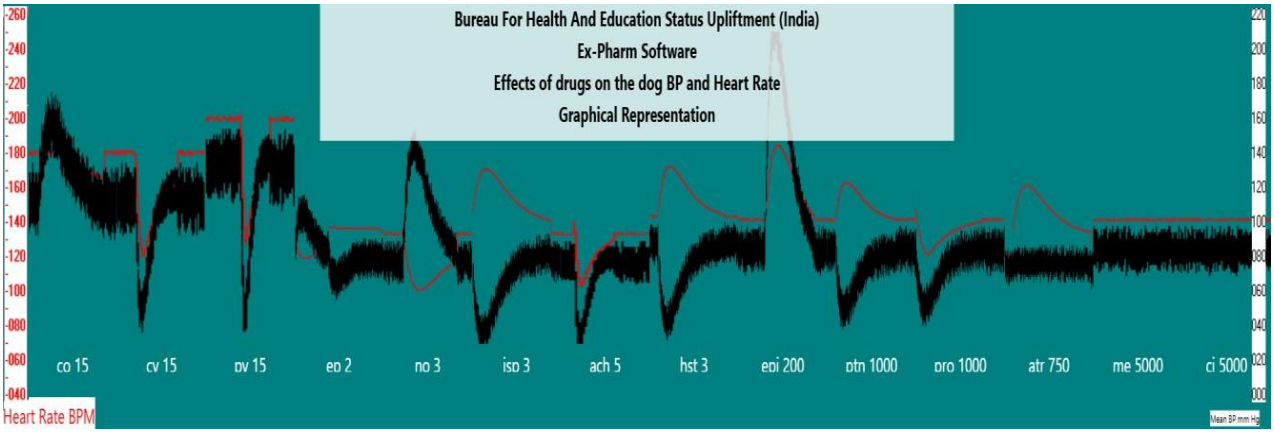
Ach as a result of which there is rise in blood pressure. This is nicotinic receptor action o Ach.

The response of isoprenaline can be blocked by beta blocker propranolol. The response of noradrenaline is blocked by phentoamine-the alpha-blocker. The response of histamine is blocked by mepyramine and metiamide, the H1 and H2 receptor antagonists respectively.

Instructions/Procedure:

1. *Set the assembly*
2. *Select the drug*
3. *Record the response graph of Individual drugs*
4. *Save the graph*
5. *Enter findings in observation table*
6. *Download observation table.*

Graph:



Carotid Oclusion (CO)
Central Vagus (CV)
Peripheral Vagus (PV)
Epinephrine (ep)
Noreinephrine (no)
Isoprenaline (isp)
Acetylecholine (ach)
Histamine (hst)
Ephedrine (epi)
Phentolamine (ptn)
Propranolol (pro)
Atropine (atr)
Mepyramine (me)
Cimetidine (ci)

OBSERVATION TABLE:

S.No.	Drug Name/Dose	Pharmacological Response
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		

Student's Name: _____

INFERENCE:

S.No.	Drug name (Dose in mg/kg)	Pharmacological Response
1	Epinephrine (Adrenaline) Dose : 2 Range : 1 – 3 Mg/kg Body Weight	It stimulates the alpha and beta adrenergic receptors. Conventional doses will increase the BP followed by a short fall before reaching the basal level (biphasic response due to alpha and beta receptor responses). The heart rate decreases due to vagal reflex.

2	Norepinephrine (Noradrenaline) Dose : 3 Range : 2 - 5 Mg/kg Body Weight	It stimulates mainly the alpha and beta1 receptors. The heart rate is generally reduced due to vagal reflex in response to increased BP.
3	Isoprenaline Dose : 3 Range : 2 - 5 Mg/kg Body Weight	It is a potent, non- selective beta adrenergic stimulant. It increases the systolic BP , but decreases the diastolic BP. Because the decrease is more pronounced than the increase, the mean arterial pressure typically falls.
4	Acetylcholine Dose : 5 Range : 2 - 10 Mg/kg Body Weight	Acetylcholine (ACh) leads to a sharp a fall in BP which returns to basal level quickly.
5	Histamine Dose : 3 Range : 2 - 5 Mg/kg Body Weight	Acts on H1 and H2 receptors to produce a fall in BP Stimulation of H1 produces a rapid onset short lived decrease in BP whereas H2 stimulation leads to a fall characterized by slower onset and longer duration.
6	Ephedrine Dose : 100 Range :100-200 Mg/kg Body Weight	It acts on both alpha and beta receptors and in addition enhances the release of norepinephrine from sympathetic neurons. It increases the BP and heart rate.
7	Phentolamine Dose : 1000 Mg/kg Body Weight	This drug, an alpha blocker, reduces BP
8	Propranolol Dose : 1000 Mg/kgBody Weight	It is a beta blocker which reduces BP and heart rate.
9	Atropine Dose : 750 Range : 500-1000 Mg/kg Body Weight	This drug is a muscarinic cholinergic antagonist. It competitively antagonizes ACh.
10	Cimetidine Dose : 5000 Mg/kg Body Weight	It is a H2 blocker, which also partially blocks the effect of histamine on BP.

Student's Name: _____

*Observation table after completion of the experiment can be downloaded with respective student's name.

Result/Conclusion:

Different drugs shown response as per their pharmacological receptors dependent action. Results of effect of each drug is tabulated below.

Note- Graph can be downloaded through 'Save graph' option.

Practical 2- Reversal action of adrenaline on blood pressure

Steps for doing the curve:

1. Inject 3ug/ml Adrenaline
2. Inject 3ug/ml Noradrenaline
3. Inject 1000ug/ml Phentolamine

4. Inject 3ug/ml Noradrenaline
5. Inject 3ug/ml Adrenaline
6. Note the graph patterns after each injection.

Note- Graph can be downloaded through 'Save graph' option.

Result/Conclusion: Reversal action of adrenaline on blood pressure was recorded.

Practical 3- Reversal action of acetylcholine on blood pressure

Steps for doing the curve:

1. Inject 5 ug/ml Acetylcholine
2. Inject 2000 ug/ml Atropine
3. Inject 5 ug/ml Acetylcholine
4. Inject 150 ug/ml Acetylcholine
5. Note the graph patterns after each injection.

Note- Graph can be downloaded through 'Save graph' option.

Result/Conclusion: Reversal action of acetylcholine on blood pressure was recorded.

Experiment-20

Aim/Object: Effects of drugs on the ciliary motility of frog oesophagus (gastro intestinal tract)

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Practical 1: Virtual Practice - for Acetylcholine

Object:

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

Procedure:

Pith a frog. Slit open the oesophagus from the buccal cavity to the stomach. Wipe the blood gently using a cotton swab dipped in Frog's Ringer solution, proceeding from cephalic to caudal end. Moisten the surface with Ringer solution. Place two pins at a distance of 2-3 cm. Place one seed on the groove near the pin at cephalic end. Start the stopwatch and observe the time taken for the seed to reach the pin at the caudal end. Take 2 such readings and calculate the average.

Instructions/Procedure:

- *The inerface shows two groups of frog (containing six frog in each group) which have been allotted to two groups. Select the groups to be treated with the test drug (Acetylcholine) and the vehicle.*
- *Administer the respective treatment to individual animals and then put poppy seed at intestine part of frog.*
- *Click the frog to observe the selected frog.*
- *Record the response time at which the seed move.*
- *Record the response time at which the seed move at the caudal end of frog intestine.*
- *The frog in test group are injected Acetylcholine and the frog in control group are administered with the normal saline.*

OBSERVATION TABLE:

Response time		
Vehicle Treated	**Drug Treated**	

		Average

Student's Name: _____

INFERENCE:

Response time		
Vehicle Treated	**Drug Treated**	
28	17	
29	18	
32	17	
30	12	
31	20	
33	19	
30.5	17.1666	Average

Student's Name: _____

*Observation table after completion of the experiment can be downloaded with respective student's name.

Result/Conclusion:

The drug (acetylcholine) shown increased motility (movement).

Practical 2: Virtual Practice - for Atropine

Object:

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

Procedure:

Pith a frog. Slit open the oesophagus from the buccal cavity to the stomach. Wipe the blood gently using a cotton swab dipped in Frog's Ringer solution, proceeding from cephalic to caudal end. Moisten the surface with Ringer solution. Place two pins at a distance of 2-3 cm. Place one seed on the groove near the pin at cephalic end. Start the stopwatch and observe the time taken for the seed to reach the pin at the caudal end. Take 2 such readings and calculate the average.

Instructions/Procedure:

1. *The inerface shows two groups of frog (containing six frog in each group) which have been allotted to two groups. Select the groups to be treated with the test drug (Atropine) and the vehicle.*
2. *Administer the respective treatment to individual animals and then put poppy seed at intestine part of frog.*

3. *Click the frog to observe the selected frog.*
4. *Record the response time at which the seed move.*
5. *Record the response time at which the seed move at the caudal end of frog intestine.*
6. *The frog in test group are injected atropine and the frog in control group are administered with the normal saline.*

OBSERVATION TABLE:

Response time		
Vehicle Treated	**Drug Treated**	
		Average

Student's Name: _____

INFERENCE:

Response time		
Vehicle Treated	**Drug Treated**	
28	33	
31	38	
33	38	
32	37	
30	33	
29	35	
30.5	35.66	Average

Student's Name: _____

*Observation table after completion of the experiment can be downloaded with respective student's name.

Result/Conclusion:

The drug (Atropine) shown decreased motility (movement).

Experiment-21

Aim/Object: Study of anti ulcer activity - using pylorus ligation method

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Introduction and Principle: Peptic ulcer is one of the most prevalent gastrointestinal disorders. The aim of the present study is to demonstrate the antiulcer activity of drugs using pylorus ligand (SHAY) rat model. This was first demonstrated by Shay in 1945. Ligation of rat pylorus results gastric acid accumulation in the fore-stomach leads to acute gastric ulcers. This procedure is used to screen the drugs for their anti-secretary and antiulcer activity.

Instructions/Procedure:

1. *Rats fasted for 24 hrs prior to pyloric ligation.*
2. *Randomly divided into 2 groups of 3 animals each.*
3. *Drugs administered once for 2 days and 30 mins prior to ligation*
4. *Rats anesthetized with ether.*
5. *Pyloric ligation procedure done.*
6. *Rats placed in separate cages and allowed to recover.*
7. *19 hrs after pyloric ligation, animals sacrificed by decapitation.*
8. *Abdomen opened and stomach dissected out.*
9. *Contents of the stomach collected in a centrifuge tube.*
10. *Stomach opened along greater curvature and ulcers observed under 10x magnification.*

OBSERVATION TABLE:

Group Selected	Mouse No.1 (ulcer count)	Mouse No.2 (ulcer count)	Mouse No.3 (ulcer count)	Average
Vehicle Treated				
Omeprazole Treated				

Student's Name: _____

INFERENCE:

Group Selected	Mouse No.1 (ulcer count)	Mouse No.2 (ulcer count)	Mouse No.3 (ulcer count)	Average

Vehicle Treated	3	2	2	2.3333
Omeprazole Treated	1	1	1	1

Student's Name: _____

*Observation table after completion of the experiment can be downloaded with respective student's name.

Result/Conclusion:

Higher number of Ulcer count in vehicle treated group than drug treated and reflects the antiulcer activity of drug (omeprazole).

Experiment-22

Aim/Object: Study of stereotype and anti-catatonic activity of drugs on mice.

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Introduction and Principle:

This experiment presents a quantitative, observational method for the assessment of rodent stereotyped and anti catatonic behavior which consists of motor responses that are repetitive, invariant, and seemingly without purpose or goal. The most classic behavioral pattern that is characteristic of stereotypy and anti catatonic behaviour is that elicited by high doses of CNS stimulants, in rodents, although it can also occur in response to other drugs or neurotoxic treatments affecting the basal ganglia.

Measurement of stereotypy, can be adapted to sampling many forms of spontaneous behaviors, including rearing, grooming & jumping responses of behavioral checklists and scoring the same.

Instructions/Procedure:

1. *Animals are divided into two groups*
2. *Administer one group with the stereotypy OR anti- catatonic behaviour inducer to be tested and other with vehicle by oral route.*
3. *Evaluate one animal at a time*
4. *Start the Experiment*
5. *Count the jumping activity for specific duration.*
6. *Record the observations.*

OBSERVATION TABLE:

Control Group	**Drug Treated Group**
No. of Jumpings	**No. of Jumpings**

Student's Name: _____

INFERENCE:

Control Group	**Drug Treated Group**
No. of Jumpings	**No. of Jumpings**
1	5

Student's Name: _____

*Observation table after completion of the experiment can be downloaded with respective student's name.

Result/Conclusion:

The mice in test (Stereotypy OR Anti-Catatonic Behaviour Inducer Treated) group show more number of Jumping activity as compare to control (Vehicle treated) group.

Experiment-23

Aim/Object: Evaluation of effect of acetylcholine (spasmogens) using rabbit jejunum

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Objectives- To record the dose response curve for acetylcholine on isolated rabbit jejunum

Instructions/Procedure:

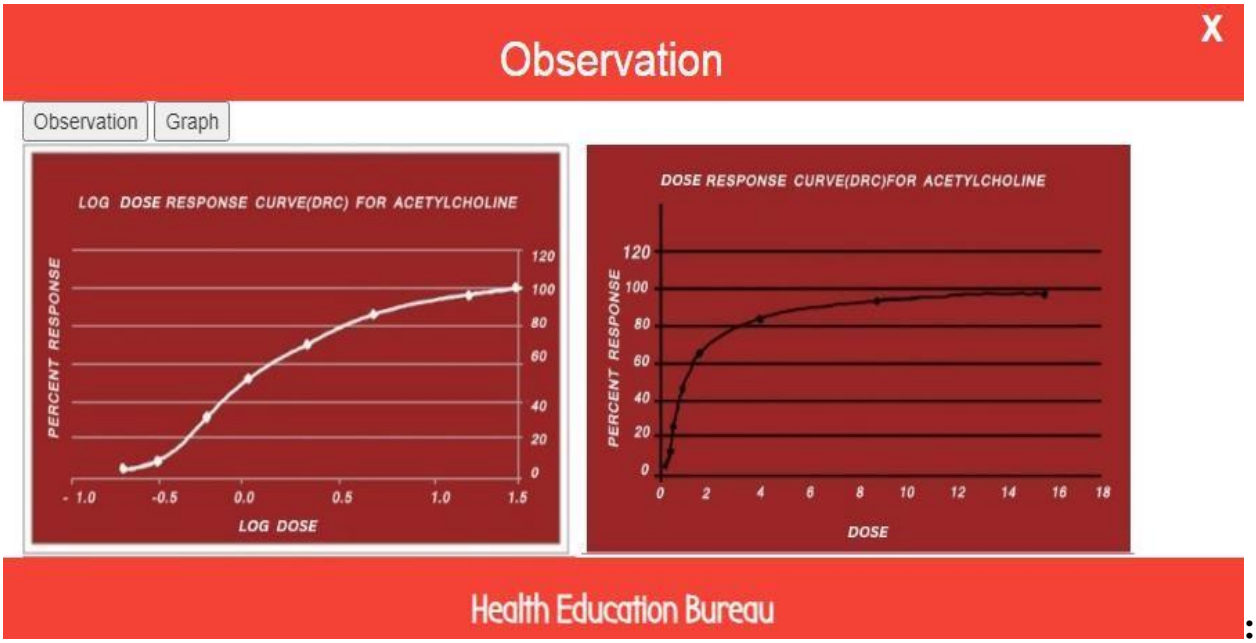
1. *Start with the minimum possible dose (like 0.05 mL of 20 ug/mL).*
2. *Each subsequent dose should be double (or should be increased in a geometric proportion, i.e. the quotient of two subsequent doses should be constant).*
3. *When the first measurable response (a response that can measured e.g. at least 5mm height, at least 10mg force) is achieved, repeat the same dose and see whether you get a reproducible response. This proves the reproducibility of the response proper acclimatization of the tissue to in-vitro conditions.*
4. *Record the response of geometrically increasing doses till the maximum response is achieved (subsequent doses give equal responses).*
5. *Ideal doses are 20 ug/mL (0.05 to 0.08 mL), 50 ug/mL (0.05 to 0.08 mL), 100 ug/mL (0.8 mL), 200 ug/mL (0.8 mL), 400 ug/mL (0.8 mL).*
6. *Once the maximum response is achieved, do not repeatedly expose the tissue to maximal dose or higher doses. Such repeated exposures may effect the responsiveness of the tissue.*
7. *The capacity of the organ tube is 20mL.*

OBSERVATION TABLE:

S.No.	**Conc. of ACh (micro gram/ml)**	**Amount added to organ**		**Conc. in organ bath**	
		In ml	In micro gram	(micro gram/ml)	Log Conc.

Student's Name: _____

INFERENCE:



:

Result/Conclusion: Evaluation of the effect of acetylcholine (spasmogen) using rabbit jejunum was observed and recorded.

Experiment-24

Aim/Object: Evaluation of anti psychotic drugs using cook's pole climbing apparatus

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Introduction and Principle:

Animals treated with Antipsychotic drugs do not show conditioned avoidance response

Equipment:

Pole Climbing Apparatus

- Pole climbing apparatus, has a grid floor that acts as a source of shock. In the centre of the roof there is a wooden pole.

- The apparatus also contains one buzzer

Instructions/Procedure:

1. *Animal were kept on grid floor of Pole Climbing Apparatus*
2. *Training of animals is conducted in the pole climbing apparatus, which has a floor that acts as a source of shock. In the centre of the roof there is a wooden pole. The animals are trained as follows.*
    - *Buzzer is pressed (Conditioned Stimulus)*
    - *After 20 Seconds Shock (Unconditioned Stimulus) of 20v delivered to the floor grid.*
    - *The animals were trained to climb the pole to avoid shock.*
    - *This was repeated until the animals learned to climb the pole soon after hearing the buzzer even without receiving the shock.*
    - *Such animals, which climb the pole within 1 to 5s after pressing the buzzer, were chosen for this study.*
3. *Antipsychotic drug administered*
4. *Testing of rats were conducted after giving antipsychotic drugs*

- *Animals do not climb on pressing the buzzer*
- *Animals climbs on passing current on grid floor.*

OBSERVATION TABLE:

	Response on Buzzer	**Response on Current**
Control Group		
Anti-Psychotic Drug Group		

Student's Name: _____

INFERENCE:

	Response on Buzzer	**Response on Current**
Control Group	Positive	Positive
Anti-Psychotic Drug Group	No-Response	Positive

Student's Name: _____

*Observation table after completion of the experiment can be downloaded with respective student's name.

Result/Conclusion:

Animals treated with Antipsychotic drugs do not show conditioned avoidance response (Response to buzzer).

Experiment-25

Aim/Object: Evaluation of Sedative Drugs Using Cook's Pole climbing Apparatus.

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Introduction and Principle:

Animals treated with Sedatives/Hypnotics drugs do not show conditioned and unconditioned both responses.

Equipment:

Pole Climbing Apparatus

- Pole climbing apparatus, has a grid floor that acts as a source of shock. In the centre of the roof there is a wooden pole.

- The apparatus also contains one buzzer

Instructions/Procedure:

1. *Animal were kept on grid floor of Pole Climbing Apparatus*
2. *Training of animals is conducted in the pole climbing apparatus, which has a floor that acts as a source of shock. In the centre of the roof there is a wooden pole. The animals are trained as follows.*
    - *Buzzer is pressed (Conditioned Stimulus)*
    - *After 20 Seconds Shock (Unconditioned Stimulus) of 20v delivered to the floor grid.*
    - *The animals were trained to climb the pole to avoid shock.*
    - *This was repeated until the animals learned to climb the pole soon after hearing the buzzer even without receiving the shock.*

- *Such animals, which climb the pole within 1 to 5s after pressing the buzzer, were chosen for this study.*

3. *Sedative drug administered*
4. *Testing of rats were conducted after giving Sedative drugs*
    - *Animals do not climb on pressing the buzzer*
    - *Animals do not climbs on passing current on grid floor.*

OBSERVATION TABLE:

	Response on Buzzer	**Response on Current**
Control Group		
Sedative Drug Group		

Student's Name: _____

INFERENCE:

	Response on Buzzer	**Response on Current**
Control Group	Positive	Positive
Sedative Drug Group	No Response	No Response

Student's Name: _____

*Observation table after completion of the experiment can be downloaded with respective student's name.

Result/Conclusion:

Animals treated with Sedative/Hypnotic drugs do not show conditioned avoidance response (Response to buzzer) and also Unconditioned response (Response to mild current).

Experiment-26

Aim/Object: Acute Skin Irritation Test (Draize Test)

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Introduction and Principle: This test work on the principal of skin irritation/toxicity (erythema and edema formation).

Instructions/Procedure:

1. *12 Rabbits are taken and divide in to 2 groups, 6 in test group and 6 in control group.*
2. *A known amount of test substance is introduced under a one square inch patch.*
3. *The patch is applied to skin of 6 albino rabbits of test group (with abraded skin).*
4. *The patch (without test substance) is applied to skin of 6 albino rabbits of control group (with abraded skin).*
5. *The patch is secured in place with adhesive tape and the entire trunk of the animal is wrapped with an impervious material for a 24hours period.*
6. *After 24 hours the patches are removed and resulting reaction is evaluated for erythema and edema formation and response is categorized (Mild/Moderate/Sever) on below mentioned basis.*

Category	Draize	Code Skin Reaction
Non Toxic	Non Toxic	If no erythema and edema is seen.
Mild Toxic	Mild	Well defined erythema and slight edema (edges of area well defined by definite raising)
Moderate Toxic	MOD	Moderate to severe erythema and moderate edema (area raised approximately 1 mm)
Severe Toxic	SEV	Severe erythema (injuries in depth) and severe edema (raised more than the 1 mm and extending beyond area of exposure)

*The reaction is again evaluated at the end of 72 hours, if after 72 hours no reaction is seen, then the sample is termed as nontoxic but if after 72 hours' mild/moderate/sever reaction is seen then the sample is termed as toxic.

OBSERVATION TABLE:

	Day 1	**Day 3**	**Result**

Control Group			
Test Group			

Student's Name: _____

INFERENCE:

	Day 1	**Day 3**	**Result**
Control Group	No Reaction	No Reaction	Non-Toxic
Test Group	Mild/Moderate/Sever Reaction	Mild/Moderate/Sever Reaction	Toxic

Student's Name: _____

*Observation table after completion of the experiment can be downloaded with respective student's name.

Result/Conclusion:

In test group the sample shown higher irritation reaction as compared to control group, so it can be concluded that the test sample is toxic.

Experiment-27

Aim/Object: Acute eye irritation test (draize test)

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Introduction and Principle: This test work on the principal of eye irritation/toxicity (erythema and edema formation).

Instructions/Procedure:

1. *6 Rabbits are taken.*
2. *A known amount of test substance is introduced in one eye of all 6 animals, whereas second eye is considered as control.*
3. *Following Response is evaluated after 24, 48 and 72 hrs.*
    - *Ulceration*
    - *Opacity of Cornea*
    - *Inflammation of Iris*
    - *Inflammation of conjunctiva*

OBSERVATION TABLE:

	Day 1 (Response)	**Day 2 (Response)**	**Day 3 (Response)**	**Result**
Control Eye				
Test Eye				

Student's Name: _____

INFERENCE:

	Day 1 (Response)	**Day 2 (Response)**	**Day 3 (Response)**	**Result**
Control Eye	**No Reaction**	**No Reaction**	**No Reaction**	**Non-Toxic**
Test Eye	- Ulceration - Opacity of Cornea - Inflammation of Iris - Inflammation of conjunctiva	- Ulceration - Opacity of Cornea - Inflammation of Iris - Inflammation of conjunctiva	- Ulceration - Opacity of Cornea - Inflammation of Iris - Inflammation of conjunctiva	**Toxic**

Student's Name: _____

*Observation table after completion of the experiment can be downloaded with respective student's name.

Result/Conclusion: The sample shown reaction on 24, 48 and 72 Hrs. in (4 out of 6 rabbits) so sample is toxic.

Experiment-28

Aim/Object: Effect of saline purgatives on frog intestine

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Introduction and Principle: Saline purgatives are the salts comprising of highly charged ions and do not crosses cell membrane freely. They remain inside the lumen and retain water through osmotic forces. They increase the volume of the contents of the bowel, stretch the colon and produces normal stimulus for contraction of the muscle that leads to defecation. The aim of the present study is to examine the effect of saline purgative on frog intestine.

Instructions/Procedure:

1. *Pith the frog and place it on a dissecting board.*
2. *Expose the abdominal cavity and carefully trace the small intestine.*
3. *Make the small intestine into three compartments by tying threads of different colours in such a way that no fluid can move from one compartment to the other.*
4. *Inject 0.2 ml of each hypotonic solution into first compartment, 0.2 ml of hypertonic solution to second compartment and 0.2 ml of isotonic solution into third compartment.*
5. *Wait for 20 minutes and the observations are to be recorded.*

OBSERVATION TABLE:

Hypotonic solution causes the fluid to move from lumen into circulation by process osmosis thereby shrinks the intestine. Hypertonic solution (saline purgative) moves the fluid from cells into the lumen and swells the intestine and isotonic solution did not show any fluid movement across the intestinal membrane.

Solution	Compartment	Effect
Hypotonic Solution (0.2 Ml of 0.9% Sodium Chloride)	First	Shrinking
Hypertonic solution – Saline Purgative (0.2 Ml of 27% Magnesium Sulfate)	Second	Swelling
Isotonic solution (0.2 Ml of Frog Ringer)	Third	No change

Student's Name: _____

*Observation table after completion of the experiment can be downloaded with respective student's name.

Result/Conclusion: Hypertonic solution (saline purgative) moves the fluid from cells into the lumen and swells the intestine and shows purgative effect.

Experiment-29

Aim/Object: 4 Modules - Amphibian nerve muscle experiments

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Practical-1 Recording of simple muscle twitch

Overview- A single brief electric shock of adequate stimulus applied to the nerve of a skeletal muscle gives rise to a brief contraction of muscle followed by relaxation called simple muscle twitch.

Materials required

1. Nerve Muscle Preparation
2. Assembly
3. A kymograph with drum and smoked paper

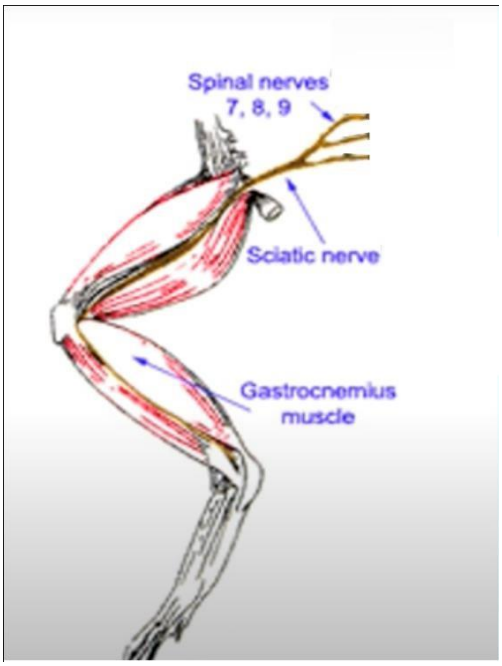
Theory:

S.No.	Name	Time
1.	LP- Latent period	0.015sec
2.	CP- Contraction Period	0.055sec
3.	RP - Relaxation period	0.06sec
4.	Time tracing	100 Hz frequency each wave = 0.01 sec

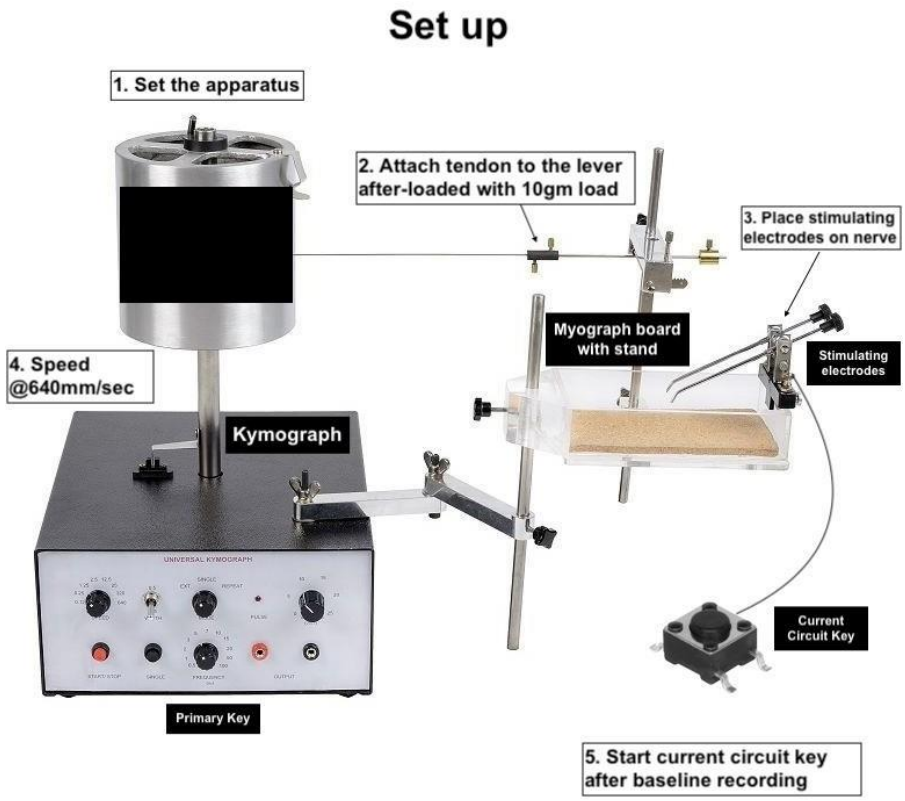
Components of simple muscle twitch:

1. Latent period- period from the point of stimulus to point of onset of contraction Duration of latent period depends upon:
    a. Conduction of impulse along the nerve
    b. Neuromuscular junction
    c. Excitation contraction coupling
    d. Stretching of series elastic elements (tendons
    e. Inertia of recording lever
2. Contraction period- period between onset of contraction to the point that corresponds to the peak of contraction
3. Relaxation period- period from the peak of contraction to the end of relaxation.

Nerve Muscle Preparation:



Equipment:



Instructions/Procedure:

1. *Start current circuit*
2. *Record the observations*

OBSERVATION TABLE:

S. No.	Name	Value
1.	LP- Latent period	
2.	CP- Contraction Period	
3.	RP - Relaxation period	

INFERENCE/RESULT:

S. No.	Name	Value
1.	LP- Latent period	0.015sec
2.	CP- Contraction Period	0.055sec
3.	RP - Relaxation period	0.06sec

Precaution:

1. Avoid unnecessary handling of tissue.
2. Don't let nerve muscle preparation dry up (add ringer solution at regular intervals).
3. Drum should be at fast speed.
4. Keep short circuit key closed (To avoid accidental passage of current to tissue).

Practical-2 Effect of temperature on simple muscle twitch.

Overview- The changes in temperature of ringer solution causes the change in muscle contraction. With the change in temperature there is a change in amplitude and duration of different periods of contractions (latent, contraction and relaxation period). The changes are mainly due to change in rate of conduction velocity in the nerve, enzymatic and chemical activation of muscles and a change in viscosity of the muscles.

Materials required:

1. Nerve Muscle Preparation
2. Assembly

3. Normal ringer solution
4. Cold ringer solution (10°C)
5. Warm ringer solution (40°C)
6. A kymograph with drum and smoked paper

Procedure:

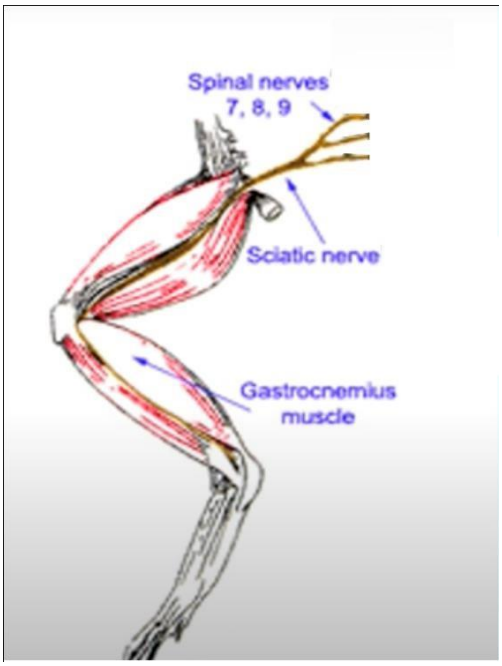
1. Dissect the frog and make ready nerve muscle preparation
2. Mount the nerve-muscle preparation for recording of simple muscle curve
3. Record a simple muscle curve on the revolving drum with the ringer solution in the muscle trough at room temperature
4. Replace the ringer solution with the warm ringer solution and wait for 1-3 min. to allow the muscle to warm up
5. Using the same baseline, same point of stimulation and same strength of stimulus, record the effect of warm ringer on a simple muscle curve.
6. Replace the warm ringer solution with a normal ringer solution and wait for some time for the preparation to come back to normal temperature.
7. Pour cold ringer solution to muscle trough and wait for 2-3 min. to allow the muscle to cool.
8. Record the effect of the cold ringer solution.
9. Record the graphical observations in a tabular form.

Observations:

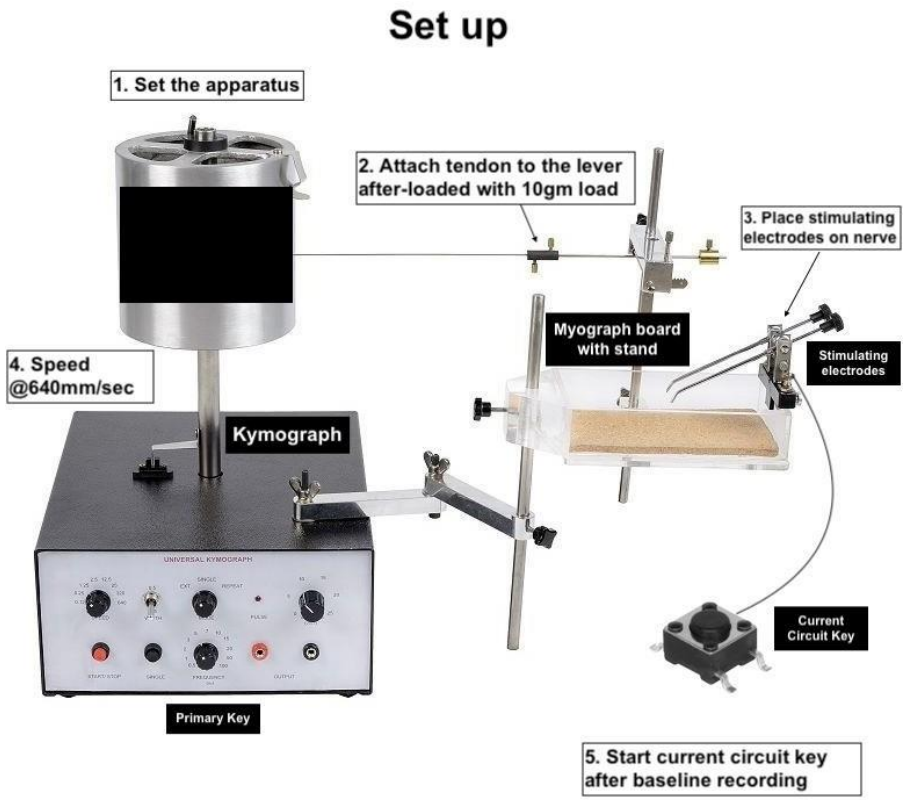
Compare the recording of the three muscle curves recorded at three different temperatures of ringer solution. Study the height and slope of contraction and duration of latent period, contraction period and relaxation period of each curve.

Temperature	Amplitude	Rate of Contraction	LP- Latent period	CP- Contraction Period	RP - Relaxation period
Warm	↑	↑	↓	↓	↓
Cold	↓	↓	↑	↑	↑

Nerve Muscle Preparation:



Equipment:



Instructions/Procedure:

1. *Select solution*
2. *Start current circuit*
3. *Record the observations*

OBSERVATION TABLE:

Temperature	Amplitude	Rate of Contraction	LP-Latent Period	CP-Contraction Period	RP-Relaxation Period
Warm					
Cold					

INFERENCE/RESULT:

Temperature	Amplitude	Rate of Contraction	LP-Latent Period	CP-Contraction Period	RP-Relaxation Period
Warm	↑	↑	↓	↓	↓
Cold	↓	↓	↑	↑	↑

Precaution:

1. Temperature of the hot ringer solution should not exceed 42°C. Exceeding above will denature the protein. This phenomenon is called heat rigor.
2. The temperature of the cold solution should not be less than 4°C because at this temperature muscle proteins will be coagulated. This prevents muscle contraction
3. The effect of the warm ringer solution should be recorded before recording the effect of cold ringer solution because cold ringer solution inhibits all enzymatic and metabolic activities of the muscles. It may cause difficulty to revive muscle.

Practical-3 Effect of repeated stimuli on simple muscle twitch

Overview- A single brief electric shock of adequate stimulus applied to the nerve of a skeletal muscle gives rise to a brief contraction of muscle followed by relaxation called simple muscle twitch.

Materials required:

1. Nerve Muscle Preparation
2. Assembly

3. A kymograph with drum and smoked paper

Procedure:

1. Arrange the setup of nerve-muscle preparation

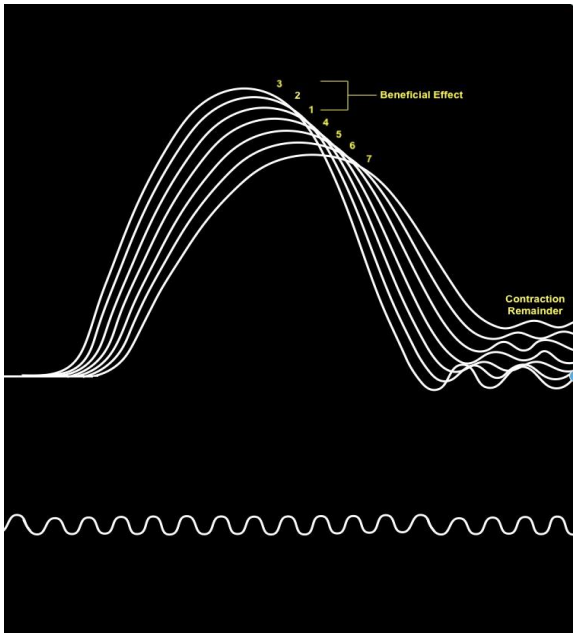
2. Give the stimuli and record the response on kymograph

3. Repeatedly give stimuli of same strength several times and record the response on kymograph

Principle:

In first few curves height will increase (due to beneficial effect) but then height will gradually decrease on giving repeated stimuli of same strength, due to fatigue (decreased working capacity of muscle cell on repeated contraction). The duration of all the phases increase gradually and then later on the baseline also comes little upwards This phenomena of baseline to come upwards is called as contraction remainder.

Graph:



Observations:

Same Strength Stimuli – Number	Effect on Height
1	Increase
2	Increase
3	Increase
4	Decrease
5	Decrease
6	Decrease

7	Decrease

Explanation:

Q.1 What is the cause of fatigue?

Ans:1 The cause of fatigue may be on repeated stimuli. On repeated several stimuli or on repeated contraction, there may be decreased nutrient and oxygen supply there may be depletion of acetylcholine stores and there may be accumulation of the metabolites. so all these may be causes of Fatigue

Q:2 Fatigue is reversible or irreversible?

Ans:2 Fatigue is a reversible phenomena. On giving some rest the fatigue goes winds off. Why, because on giving some rest there is supply of oxygen. The supply of nutrients acetylcholine will get stored in the vesicles and there will be removal of the stored metabolites like lactic acid potassium ions and all that. so fatigue is a reversible phenomenon.

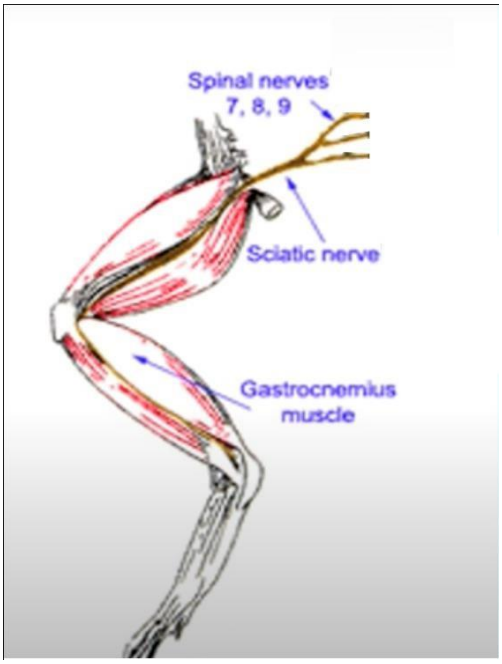
Q:3 What is the cause for increase height of curves in the beginning (beneficial effect)?

Ans:3 That is because of beneficial effect. In beneficial effect there is increased force of contraction or in other words there is increased height of contraction for few contractions. Why, because gradually later on contractions are getting benefit from the previous contraction. Now what is benefit from the previous contraction? In previous contraction there is release of calcium ions so that calcium ions will take some time to get entry into the sarcoplasmic reticulum, because calcium ion come rapidly out of that. But go little slowly into the sarcoplasmic reticulum. So more and more calcium ion will get accumulate will with the repeated contraction. So now because more calcium ions are available now because of that there will be more forceful contraction of the muscle because of more number of actin myosin fibers will get involved next because of repeated contraction. There is increased temperature. There is increased enzymatic activity. There is increased metabolic activity in the cell so that will also do forceful contraction or increase height of contraction. Next cause may be because of increased temperature. there is decreased viscosity around the muscle fibres, so because of that there will be decreased resistance around the muscle, or inside the muscle, so now the actin myosin fibre will contract more vigorously, more forcefully, so that is also cause for the increase force of contraction, or in other words beneficial effects, so one is more release of calcium ions, more temperature, more enzymatic activity, more metabolic activity, more temperature will lead to decreased resistance. Decreased viscosity, decreased resistance that will lead to increase of force of contraction of the actin and myosin filaments.

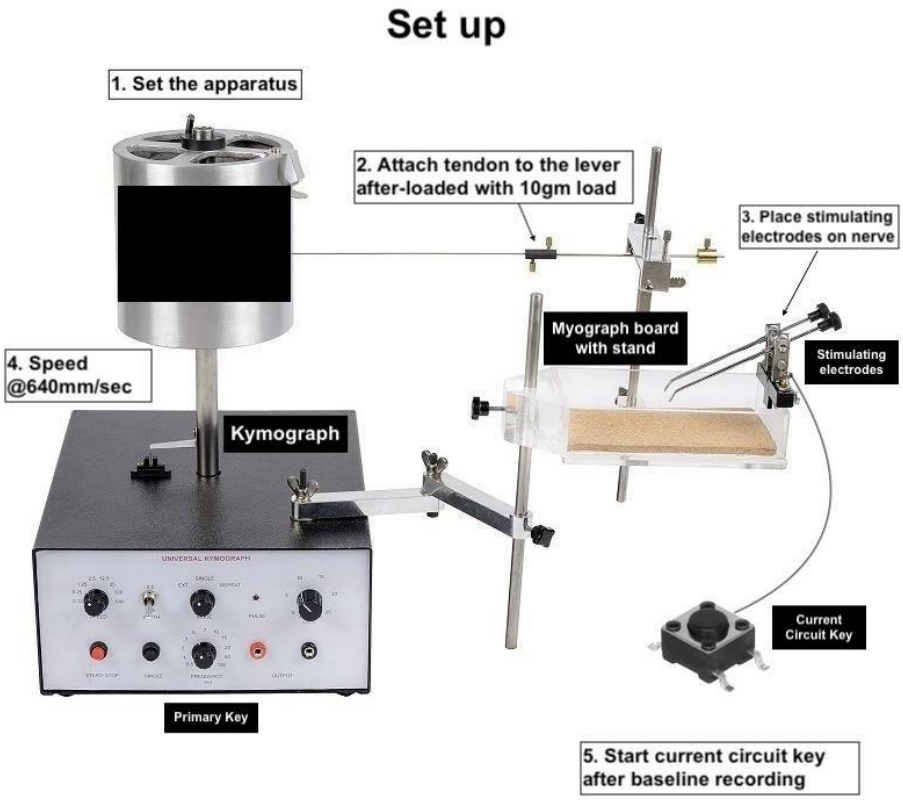
Q:4 Why baseline is shifted upwards with repeated contractions (Contraction Reminder)?

Ans: 4 The baseline comes Up with repeated contractions. This phenomena is also known as contraction remainder with repeated contraction on. when the fatigue sets in the muscle is not able to relax completely, so here, there is incomplete relaxation of the muscle because we know that for relaxation we need ATP's for detachment of the acting, and myosin, we need ATP's now, because during fatigue there is decrease supply of the ATP's. So that will lead to incomplete relaxation of the

muscle. That will lead to the contraction remainder or upward shifting of the baseline of the curves.
Nerve Muscle Preparation:



Equipment:



Instructions/Procedure:

1. *Arrange the setup of nerve-muscle preparation*
2. *Give the stimuli and record the response on kymograph*
3. *Repeatedly give stimuli of same strength several times and record the response on kymograph*

OBSERVATION TABLE:

Same Strength Stimuli – Number	Effect on Height
1	
2	
3	
4	
5	
6	
7	

INFERENCE/RESULT:

Same Strength Stimuli – Number	Effect on Height
1	Increase
2	Increase
3	Increase
4	Decrease
5	Decrease
6	Decrease
7	Decrease

Precaution:

1. Avoid unnecessary handling of tissue
2. Don't let nerve muscle preparation dry up (add ringer solution at regular intervals)
3. Drum should be at fast speed
4. Keep short circuit key closed (To avoid accidental passage of current to tissue)

Practical-4 Determination of conduction velocity of sciatic nerve

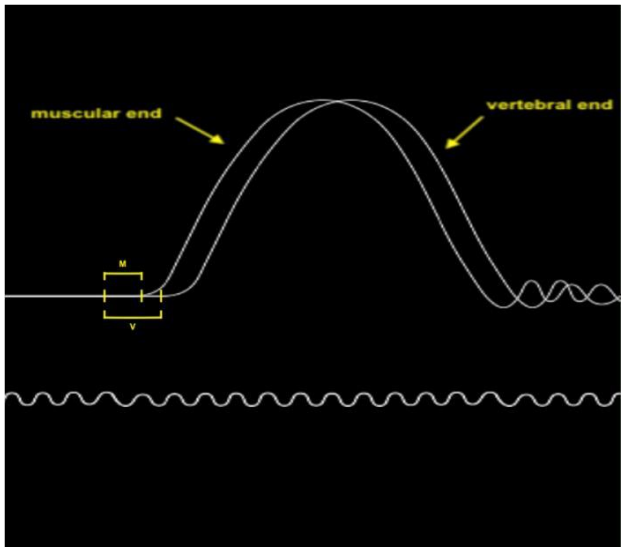
Overview:

In this experiment the stimuli are given in the nerve itself but the location is different first one is at the vertebral end second one is at the muscle end.

One graph which is obtained when the stimulus is being given at the muscle end and another graph is obtained when stimuli is being given at the vertebral end.

We will measure the time gap between two graphs. There is difference in the latent period in the graphs when stimuli is given at muscular end and when given at vertebral end. If we take difference of these two latent periods we can count the difference of the time. This difference is equal to the half of the cycle it means this is equal to 0.005 seconds In the setup, the distance between vertebral and muscular end is 10 centimetres. So velocity will be Velocity (Conduction Velocity) = Distance (10 Centimetre) / Time (0.005 Seconds) = 20 Meters per Second

Graph:



Materials required:

1. Nerve Muscle Preparation
2. Assembly
3. Normal ringer solution
4. A kymograph with drum and smoked paper

Procedure:

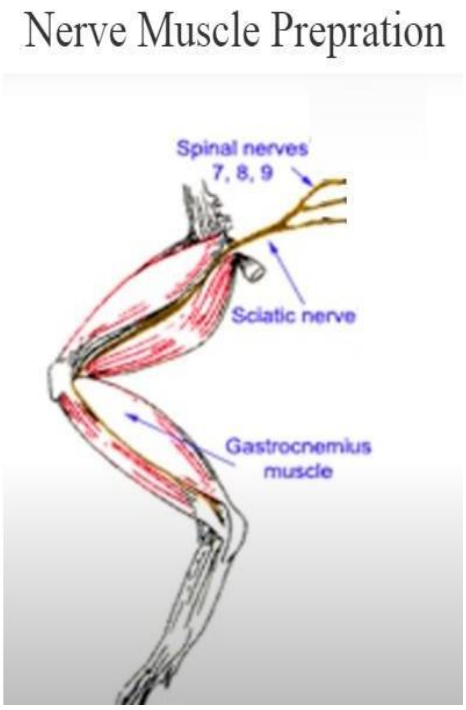
1. Set up the assembly
2. Give Impulse at vertebral end
3. Record the graph

4. Give Impulse at muscular end
5. Record the graph
6. Observe the time difference between the graphs.
7. Divide the distance travelled in both cases by time difference.

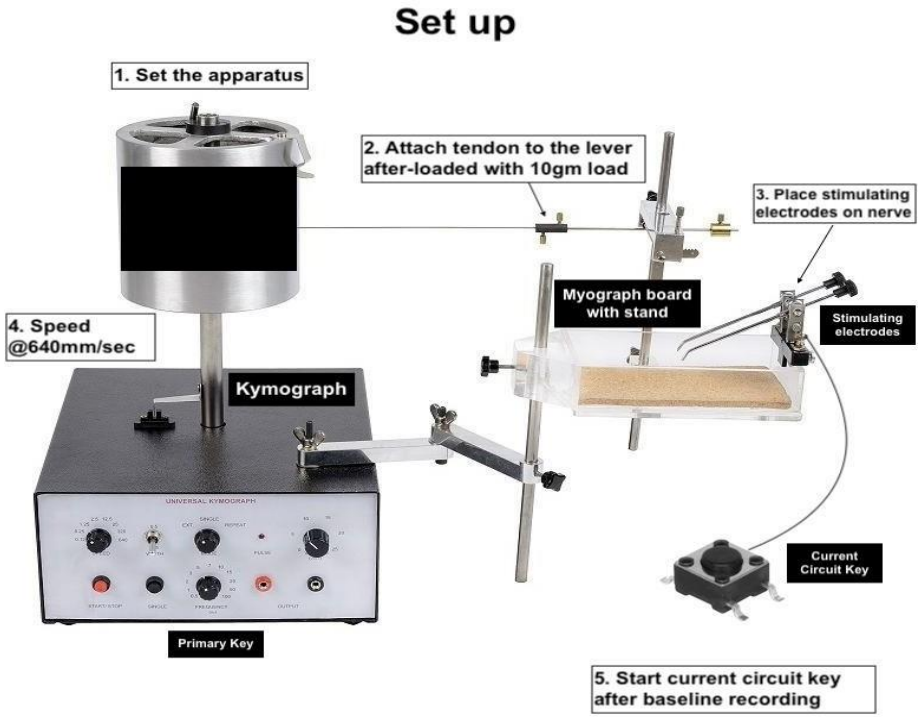
Observations:

Sr. No.	Distance Travelled (Distance between vertebral end and muscular end)	Time Taken (Time difference between the latent phases of both cases of stimuli)	Velocity (Distance Travelled/Time Taken)
1	10 Centimetre	0.005 Seconds	20 Meters Per Second

Nerve Muscle Preparation:



Equipment:



Instructions/Procedure:

1. *Set up the assembly*
2. *Give Impulse at vertebral end*
3. *Record the graph*
4. *Give Impulse at muscular end*
5. *Record the graph*
6. *Observe the time difference between the graphs.*
7. *Divide the distance travelled in both cases by time difference.*

OBSERVATION TABLE:

Sr. No.	Distance Travelled (Distance between vertebral end and muscular end)	Time Taken (Time difference between the latent phases of both cases of stimuli)	Velocity (Distance Travelled/Time Taken)
1.			

INFERENCE/RESULT:

Sr. No.	Distance Travelled (Distance between vertebral end and muscular end)	Time Taken (Time difference between the latent phases of both cases of stimuli)	Velocity (Distance Travelled/Time Taken)
1.	10 Centimetre	0.005 Seconds	20 Meters Per Second

Precaution:

1. Avoid unnecessary handling of tissue
2. Don't let nerve muscle preparation dry up (add ringer solution at regular intervals)
3. Drum should be at fast speed
4. Keep short circuit key closed (To avoid accidental passage of current to tissue)

Experiment-30

Aim/Object: Study of effect of hepatic microsomal enzyme inducer on the phenobarbitone sleeping time in mice

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Introduction and Principle: The microsomal enzyme inducer drugs induce hepatic microsomal oxidative enzyme systems to enhance the metabolism of other drugs, as a result, in the presence of an enzyme-inducer, the duration of action of the second drug will be reduced.

This has significant clinical relevance because when more than one drug is administered at a time one drug may modify the action of another through the microsomal enzyme-inducing property.

Instructions/Procedure:

1. *Weight and number the animals.*
2. *Divide them into two groups, each comprising of at least 6 mice.*
3. *To the first group administer distilled water, for 5 days*
4. *To second group inject microsomal enzyme inducer (Pheobarbital-45 Mg/Kg) drug in specified dose once daily for 5 days.*
5. *On the 5th day, inject phenobarbitone 50 Mg/Kg in specified dose to both the groups.*
6. *Note the onset and duration of sleep due to phenobarbitone in both the groups.*

OBSERVATION TABLE:

	Distilled water + Phenobarbitone Treated	**Microsomal Enzyme Inducer (Phenobarbital) drug + Phenobarbitone Treated**
Mice & Treatment	**Duration of sleeping time in minutes**	**Duration of sleeping time in minutes**
Mice-1		
Mice-2		
Mice-3		
Mice-4		
Mice-5		
Mice-6		

Student's Name: _____

INFERENCE:

	Distilled water + Phenobarbitone Treated	**Microsomal Enzyme Inducer (Phenobarbital) drug + Phenobarbitone Treated**
Mice & Treatment	**Duration of sleeping time in minutes**	**Duration of sleeping time in minutes**
Mice-1	25	15
Mice-2	24	17
Mice-3	26	16
Mice-4	25	17
Mice-5	25	16
Mice-6	24	16

Student's Name: _____

*Observation table after completion of the experiment can be downloaded with respective student's name.

Result/Conclusion:

The animals pretreated with microsomal enzyme inducer (phenobarbital) drug sleep for a shorter duration of the time as compared to animals treated with distilled water due to reduction in duration of action as a result mirosomal enzyme induction.

Experiment-31

Aim/Object: Determination of pA2 value of prazosin using rat anococcygeus muscle (by schilds plot method).

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Introduction and Principle: pA2 is the principle method for studying the antagonist action for a selected agonist which is defined as the negative logarithm to base 10 of the antagonist concentration (molar units) corresponding to a dose-ratio of 2 (i.e. the concentration that produces a 2-fold shift in the agonist concentration-response curve). This method is developed by Schild in 1957.

Requirement:

Animal/Tissue	:	Anococcygeus Muscle (Rat)
PSS	:	Krebs'
Instrument	:	thermo statically controlled organbath, chymograph
Lever	:	Frontal Writing
Magnification	:	7-10x
Tenin/Load	:	up to 1 gm
Air	:	O_2/Carbogen
Temperature	:	32-37^oC
Drug	:	Prazosin [M.wt:383.4g/mol]
		Norepinephrine [M.wt:169.18 g/mol]

Instructions/Procedure:

1. *Apply different doses and concentrations of agonist (Norepinephrine) in increasing manner with proper washout cycles (0.1ml x10-9, 0.2 ml x10-9, 0.4ml x 10-9, 0.8 ml x 10-9, 0.1 ml x 10-8, 0.2 ml x 10-8) till maximum (ceiling) response is achieved.*
2. *From observed responses find out the concentration at which 25% to 75% response of maximum response is achieved and that concentration of agonist will be called as "2R". In this case the "2R" concentration is 0.8 x 10-9*
3. *Apply different doses and concentrations of antagonist (0.2 x 10-10, 0.4 x 10-10, 0.3 x 10-10) with 2R concentration (0.8 x 10-9) of agonist, to find out the concentration of antagonist (Prazosin) at which the response of agonist is reduced to half (50%).*

4. *The concentration of antagonist at which the response of agonist is reduced to half (50%) will be called as PA2 Value, in this case it is 0.3 x 10-10.*
5. *To find out the PA2 value a graph can also be plotted between % Response to 2R and -log (Antagonist), the graph is also called as Schilds plot.*

Observation: After completion of analysis of all agonist and antagonist doses, graph will be generated.

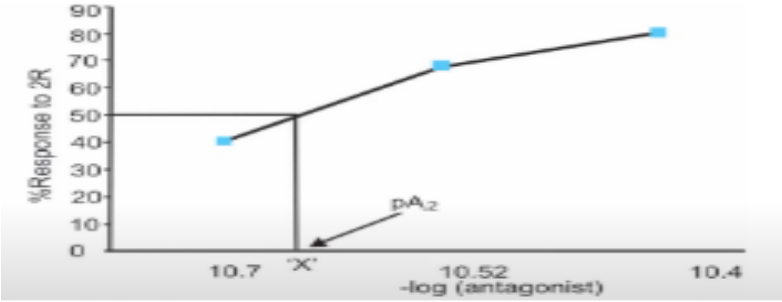


Figure: Represents the graph plot of % response to 2R versus -log (antagonist) and determination of pA2 at the X-'axis'

Hint: After plotting the %response versus - log (antagonist), pA2 value is directly extrapolated through the graph at which its % response remains 50%.

Result: The PA2 value of prazosin against Norepinephrine using rat anococcygeus muscle (by Schilds plot method) is 0.3 X 10^{-10}

Precautions before starting Experiment:

1. Clean the organ bath before starting the experiment especially inner organ bath (chances of presence of previous drug used).
2. Balance the writing lever horizontal with the help of load.
3. Prepare PSS for the experiment, while taking exact quantity of chemicals (1% variability is acceptable)
4. Add the calcium chloride at the end of PSS preparation (to avoid any precipitation: PSS should be clear).
5. Try to minimize the handling of tissue (especially at the middle part)
6. Always use the finger to hold the tissue instead of forceps.
7. Maintain the dose cycle properly (tissue sensitivity depend on this cycle).

Supportive Resources-1

Aim/Object: Study of different laboratory animal used in pharmacology.

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Theory:

Experimental Animals Used in Pharmacology (RABBIT)

Scientific Name	**Oryctolagus Cuniculus**
Body Temperature	**38.7-39.1 °C**
Respiration Rate	**55 Per Minute**
Pulse Rate	**135 Per Minute**
Blood Volume	**45-70 ml/kg**
Preferred Humidity	**40-50 %**
Room Temperature	**15.5-18.5 °C**
Mating Age	**6-9 Months**
Gestation Periods	**28-31 Days**
Breading Life	**3 Years**
Life Span	**4-5 Years**
Water Consumption	**200-300 ml/Day**
Body Weight	**2000-3000 gm**

Feed	Lucerne Grass, Carrot, Bengal gram, Wheat Bran
Common Disease	T.B, RTI, Intestinal infection, Ear Cancer
USE	Pyrogen Testing, Anti Diabetic Study. Bioassay of Insulin, Capillary Permeability Study. Embryo Toxic Study
Advantages	1. Docile Animal 2. It has a huge caecum and a long appendix 3. Enzyme atropine esterase is present in rabbit liver and plasma so it can tolerate large doses of atropine.

Experimental Animals Used in Pharmacology (GUINEA PIG)

Scientific Name	**Cavia Porcellus**
Body Temperature	**37.6-38.9 °C**
Respiration Rate	**60-110 Per Minute**
Pulse Rate	**150 Per Minute**
Blood Volume	**65-90 ml/kg**
Prefer Humidity	**45%**
Room Temperature	**18.5-27**
Life Span	**2 Years**
Mating Age	**12-20 Weeks**
Estrus Cycle	**15-19 Days**
Gestation Periods	**59- 72 Days**
Water Consumption	**250-350 ml/day**
Body Weight	**200-1000 gm**

Feed	Crushed Oats, Carrot, Cabbage, Protein Food		
Common Disease	Abscesses in Lymph Gland, Respiratory Tract Infection.		
USE	1. Evaluation of bronchodilator (Anti-Asthmatic) 2. Sensitization Study like Egg Albumin and Horse Scrum. 3. Study of local anesthetics 4. Bioassay of Digitalis. Histamines, and acetyl choline 5. Screening of spasmodic and antispasmodic compounds. 6. Study of Vitamin C metabolism 7. Study of anti TB Drugs 8. Isolation preparation of Ileum. tracheal chain	Advantages	1. Docile Animal 2. Highly susceptible for TB and Anaphylaxis 3. Highly Sensitive to Penicillin and Histamine

Experimental Animals Used in Pharmacology (RAT)

Scientific Name	***Rattus norvegius***
Body Temperature	**37.5 °C**
Respiration Rate	**80-150 Per Minute**
Pulse Rate	**260.450 Per Minute**
Blood Pressure	**130/90**
Blood Volume	**50-65 ml/kg**
Estrous Cycle	**4-5 Days**
Life Span	**2-3 Years**
Humidity Prefer	**44-60%**
Room Temperature	**18.5-27**
Mating Age	**70-84 Days**
Gestation Periods	**21-23 Days**
Body Weight	**120-300 gm**

Feed	**Cracked wheat, Ground nut, Yeast powder, Not Specific**		
Common Disease	**Bronchopneumonia, Parasitic Infection**	Other Strains	Wistar rat, Sprague Dawley rat, Biobreeding rat, Long-Evans rat, Zucker rat, Hairless rats, RCS rats. Shaking rat Kawasaki
USE	1. Evaluation of Psychopharmacological agents 2. Toxicity Studies- Teratogenic and Carcinogenic Activity 3. Anti Ulcer (Gastric Secretion) 4. Liver Physiological Studies 5. Isolation Preparation- uterus, Fundus Strip, Colon, Stomach 6. Antihypertensive Effects 7. Assay of Hormones	Advantage-	I. Small in size 2.Drug required in small quantity 3.Vomiting center is absent so oral administration is easy 4.Gall bladder and tonsils are absent 5.Continuous bile flow in intestine

Experimental Animals Used in Pharmacology (MICE)

Scientific Name	*Mus musculus*		
Body Temperature	37.4°C		
Respiration Rate	90-160 Per Minute		
Pulse Rate	300-750 Per Minute		
Blood Pressure	120/75		
Blood Volume	70-80 ml/kg		
RBC Count	4.9-12.5 MAnm3		
Life Span	1-2 Years		
Humidity Prefer	60-70%		
Room Temperature	20-27		
Mating Age	6-8 Weeks		
Gestation Periods	19-21 Days		
Estrous Cycle	4-5 Days		
Body Weight	25-30 gm		
Feed	Cracked food, Shark liver oil, Yeast powder, and Pallet, Sesame oil, Fish and other General food supplements.	USE	1. Acute toxicity studies. 2.Assay of Insulin 3.Screening of chemotherapeutic and teratogenic agents 4.Cancer and genetic research 5.Isolated preparation- ileum.
Common Disease	Salmonellosis like Mouse typhoid, Small pox infection	Advantages	1. Small 2. Easy in handling and required small dosage

Experimental Animals Used in Pharmacology (HAMSTER)

Scientific Name	*Mesocricetus auratus*		
Body Temperature	36.2-37.5 °C (rectal)		
Respiration Rate	74(33-127)		
Pulse Rate	280-412/min		
Prefer humidity	NA		
Room Temperature	37		
Mating Age	6-8 week		
Gestation Periods	15-18 days		
Estrous Cycle	4 days		
Life Span	2-3 Years		
Body Weight	85-140 gm		
Feed	Soybean meal provides a better protein supplement More complex carbohydrates, such as, cornstarch arc more highly tolerated energy sources. 30-40% corn starch in the ration is ideal		
Common Disease	Enteritis, Pneumonia, Neoplasia, Polycystic disease		
Species	Syrian/Golden and Chinese, Mesocrictus auratus		
USE	1. Immunology and Virology 2. In Diabetes Studies 3.Cytological Investigation 4.Genetics, Tissue culture, and Radiation experiments.	Advantages	1. Availability and Ease in reproduction 2.Relative freedom from spontaneous diseases 3.Anatomical and physiological features with unique potential For study 4.Rapid development with short life cycles 5.Presence of cheek pouch

Experimental Animals Used in Pharmacology (MONKEY)

Scientific Name	*Alacaca mullata*
Body Temperature	37-39 °C
Respiration Rate	76-90
Pulse Rate	Upto 150 Per Minute
Room Temperature	37-40
Mating Age	4-5 Years
Gestation Periods	165 clays
Estrous Cycle	26-28 days
Body Weight	About 5000-6000 gm

Feed	They arc basically vegetarian and they eat banana and other fruits
Common Disease	Herpes 13, Rabies, Cold, Polio, Measles, Tetanus And Tuberculosis
USE	1. Infertility 2. Virology, Parasitology, Immunology, 3. Immunosuppressant, Nutrition etc
Advantages	1. They arc very much similar to humans 2. They have same menstrual cycle as woman 2. They have same emotional behavior as human.

Experimental Animals Used in Pharmacology (DOG)

Scientific Name	*Canis familiaris*
Body Temperature	37.7 °C
Respiration Rate	14-28 Per Minute
Pulse Rate	77-138 Per Minute
Blood Pressure	140/80
Blood Volume	75-100 ml/kg
Prefer Humidity	60-70
Room Temperature	24-27
Mating Age	90 Days
Gestation Periods	62 Days
Estrous Cycle	180 Days
Life Span	Up to 10 Years
Body Weight	5000-8000 gm

Feed	They are non-vegetarian by habit; they can eat meat, eggs, and other food supplement.
Common Disease	Rabies, Diarrhoea, Arthritis, leprosy, hook worm infection etc
USE	1. Drugs acting on blood pressure 2. Vaccination 3. Diabetes and anti ulcer experiments.
Advantages	1. They can be easily trained for behavioral activity 2. Supportive in nature for the experiments

Experimental Animals Used in Pharmacology (PIG)

Scientific Name	*Sus scrofa domestica*
Body Temperature	37-40 °C
Respiration Rate	15-20 Per Minute
Pulse Rate	56-58 Per Minute
Prefer Humidity	37-40
Room Temperature	22-24
Mating Age	5-6 Months
Gestation Periods	115 Days
Estrous Cycle	21 Days
Body Weight	30-70 Kg

Feed	They can cat vegetable and non vegetable food
Common Disease	Swine Influenza, Cold, Dermatitis, Respiratory Disease and other communicable disease
Species	Sus ahoenobarbus, Sus barbatus, sus celebensis
USE	1. Influenza 2. Respiratory Medicaments 3. GIT Disease
Advantages	1. Similar in Human physiology 2. Immunological reaction can be determined.

Supportive Resources-2

Aim/Object: Study of different physiological salt solution used in pharmacology.

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Theory:

About physiological Salt Solution

- Physiological salt solution is artificially prepared solution used to maintain various tissues under viable state
- It is used to keep isolated tissue alive under experimental conditions
- It helps to maintain tissue outside the body and fulfil their internal environment of ions and nutrients
- It is a solution of salt or salts that is essentially isotonic with tissue fluid or blood
- The salt helps to maintain the isotonicity, isomolarity, contractility and excitability of the tissue.
- We need different physiological salt solution for different tissue or organ

Standard Table

Sr.No.	Composition	Frog-Ringer	Frog-Locke	De Jalon	Kreb	Function
1	Sodium Chloride	6.2	9.0	9.0	6.9	To provide isotonicity, isomolarity, contractility and axcitability
2	Potassium chloride	0.14	0.42	0.42	0.35	To provide ionic balance
3	Calcium chloride	0.12	0.24	0.06	0.28	To provide contractility
4	Magnesium Sulphate	…..	……	….	0.28	To stabilize the preparation
5	Sodium bicarbonate	0.2	0.5	0.5	2.1	To provide alkaline medium
6	Sodium hydrogen phosphate	0.008	….	……	….	As a buffer
7	Potassium dihydrogen phosphate	……	……	…..	0.16	As a buffer
8	Glucose	2.0	2.0	0.5	2.0	To provide energy

Uses of different PSS

- Krebs solution used for nerve muscle preparation
- Tyrode solution rt can be used for experiments involving mammalian smooth muscles
- Ringer solution it is used for amphibian tissue such as frog s heart
- Ringer-Locke solution used for heart muscle preparation
- De-Jalon solution used for mammalian isolated organs

Stepwise procedure

- Weigh all chemicals accurately
- Dissolve alkalies and sodium hydrogen phosphate glucose calcium chloride separately in distilled water in beaker
- Mix all these solutions
- Add the required amount of sodium bicarbonate in sufficient volume of distilled water,
- Add sodium bicarbonate at the time of setting up of the experiment

(Since calcium carbonate is liable to be precipitated if the calcium and bicarbonate are kept long together)

Supportive Resources-3

Aim/Object: Different routes of drug administration in laboratory animals

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Theory: There are number of routes through which drugs can be administered to rodents, some commonly used routes of administration are highlighted below.

1. **Oral**
    A. Through Buccal Cavity
    B. Intra Gastric Gavage
2. **Parental**
    A. Subcutaneous
    B. Intraperitoneal
    C. Intramuscular
    D. Intravenous
    E. Retrobulbar
3. **Miscellaneous:** Many other routes such as inhalation & intra-articular injections are also used to administer drugs in rats and mice.

1. **Oral:** Oral route is most commonly used route of drug administration. It can be of two types.
    A. Through Buccal Cavity
        - This is the most convenient route of drug administration
        - In this swallowing a drug is done through mouth, it can also be done by adding desired drug to the drinking water.
    B. Through Intra-gastric gavage
        - This route of drug administration includes the administration of fluids directly into the lower esophageal or stomach.
        - Gavage is often used in research settings, instead of mixing substances in water or food, to ensure accurate dosing or animals.
        - A small, curved, metal tube, usually with a ball on the end (feeding needle) is often used.

2. Parenteral:

Routes other than enteral are called parenteral routes of administration, Parenteral administration methods typically produce the highest bioavailability because these methods avoid the first pass effect of hepatic metabolism.

A. Subcutaneous
   - The best spot to inject subcutaneously is the loose skin on the back of the neck
   - A mouse may easily be injected by one person, whereas a rat may require restrainment by one person and injection by other
   - Not suitable for large volumes.

B. Intraperitoneal (IP) Injection
   - Commonly used in rats and mice since muscle mass is so small and veins are difficult to find.
   - Rapid absorption (almost as fast as IV) due to large peritoneal surface.
   - IP administration results in a faster absorption into the vasculature than SC administration
   - Comparatively larger volume of in comparison to SC can be given through IP, vehicle ranging between 2 ml/kg to 10 ml/kg can be administered.

C. Intravenous (IV) Injection
   - IV injection is the most efficient means of delivering substances to animals because it provides 100% Bioavailability
   - Technically difficult, and the use of a restraining device with appropriate size for the animal to be injected, is often required.
   - Performed in mice and rats, use the lateral tail vein located on either side of the tail.
   - Suitable for large volumes administration.

D. Intramuscular (IM) Injections
   - IM Injections result in uniform and rapid absorption of substances, because of rich vascular supply as compare to subcutaneous administration.
   - Not recommended in mice and small species due to their small muscle mass.
   - Provides lower bioavailable than Intra Venous route.

E. Retro-orbital injection
   - The Drug is injected in to Retro-orbital Space.

- Before this injection, the mouse should be anesthetized so that it remains still during the procedure.
- The needle is being placed in the retro-bulbar space (the region behind the globe the eye.

2. **Miscellaneous-** Many other routes such as Inhalation & Intra-articular injections are also used to administer drugs in rates and mice.
    - Inhalation: In this method of drug is administered through nasal cavity by inhalation, usually anaesthetics are given through this route.
    - Intra Articular: In this method the drug is administered in Joints section of the animal.

    In addition to above many other routes are also available but the most commonly used routes like oral, parenteral and miscellaneous routes of drugs administration are highlighted.

Supportive Resources-4

Aim/Object: Commonly used blood withdrawal techniques in laboratory animals

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Introduction:

The most commonly used blood withdrawal methods in rodents are:

- Retro-orbital sinus blood withdrawal method.
- Tail snip blood withdrawal method
- Tail blood withdrawal from tail.
- Intracardiac blood withdrawal
- Posterior vena cava blood withdrawal
- Saphenous Sampling (Medial or Lateral Approach)
- Submandibular Sampling
- Jagular Vein Sampling
- Dorsal Metatarsal Vein

Retro-orbital blood withdrawal method

Procedure:

1. The animal is anesthetized with an inhalation anaesthetic, such as isoflurane, in a bell jar.
2. Once the animal is fully anesthetized. The eye is protruded by placing a finger on the top of the head and along the jawline.
3. The capillary tube is placed in the medial part of the eye at a $40\text{-}45^0$ angle.
4. Apply pressure by gently rotating the hematocrit tube. This will cut through the conjunctival membranes.
5. The blood will flow into the hematocrit tube by capillary action.
6. Once blood begins to flow, maintain pressure to keep the eye protruded.
7. After collection completes, to stop bleeding, release the skin and allow the eye to return to the normal position.

Tail snip blood withdrawal procedure

Procedure:

1. The animal is placed into the restraint tube such that tail is accessible.
2. The tail is wiped with slight warm water.
3. For the tail snip, the tail is extended, and the end of the tail i.e. 0.5-1 mm in case of mice and up to 2 mm in case of rats, is cut with the scalpel (10-15 Number)
4. The cut is made over the lateral tail vein.
5. The tail can be stroked from rear end of tip to encourage blood flow.
6. The blood is collected from the tip using hematocrit tubes blood allowed to drip from tail directly into a collection vial.

Tail blood withdrawal from tail

Procedure:

1. The animal is placed into the restraint tube such that the tail is accessible.
2. The tail is wiped with slight warm water.
3. For the blood withdrawal, the tail is extended and the blood is collected by syringe.
4. The tail can be stroked from rear end of tip to encourage blood flow.
5. The blood is collected in the syringe.

Cardiac Blood Collection

Procedure:

1. The animal is restrained by the scruff with the body hanging vertically.
2. The needle is protruded in the notch just to the left of the animal's xiphoid.
3. The needle shall be kept parallel to the spine and placed just under the ribs.
4. Place the needle, protrude up, into the chest, and puncture the heart.
5. Apply slight pressure through the syringe. If the needle is in the heart, blood will flow automatically into the syringe.
6. Wait until the blood gets filled in the syringe before adding more pressure on the syringe.

*For blood collection from a mouse, a 3 cc syringe with a 22-25 gauge x 1" needle is preferred.

*For blood collection from a rat, a 10-12 cc syringe with an 18 gauge x 1.5" needle is preferred.

Posterior vena cava blood withdrawal

Procedure:

1. Animal is placed on surgical platform, dissection tray, injectable anesthesia or inhalation anesthesia is applied.

2. It is ensured that animal is completely anesthetized, as determined by toe pinch or tail pinch.
3. The skin is lifted and a small transverse cut is made through the skin just above the pelvis in females, or just above the prepuce in males.
4. The muscle is lifted, and a small transverse cut is made through the muscle just above the skin cut.
5. Cut transversely along the side of the ribs on both sides.
6. Gently protrude the intestines to the animal's left to expose the posterior vena cava.
7. Insert the needle; protrude upward, into the vena cava midway between the joint of the renal vessels and the iliac bifurcation.
8. Slowly withdraw the blood by applying pressure on the liver.

Miscellaneous

In addition to methods discussed above following methods are also used for blood withdrawal

1. Saphenous Sampling (Medial or Lateral Approach: In this method blood is collected from the Saphenous Vein of the leg of the rodent.
2. Submandibular Sampling: In this method blood is collected from Submedibular Vein of the dental region.
3. Jugular Vein Sampling: Blood is collected from the Jugular Vein of the neck region.
4. Dorsal Metatarsal Vein: Blood is collected from metatarsal region; this method is preferred for Rats over mice.

Supportive Resources-5

Aim/Object: Different methods of anaesthesia and euthanasia in laboratory animals

Reference: "Ex Pharm" Software by "Bureau for Health & Education Status Upliftment", assessed at: https://heb-nic.in/Ex-Pharm/login.php

Introduction:

Commonly used methods of anesthesia & euthanasia

Anesthesia

*Anesthesia is the act of providing sensation free relief from pain or pain producing procedures.

Commonly used anesthetics for laboratory animals - as suggested by CPCESA (CCSEA)

Drugs (mg/kg)	Mouse	Rate	Hamster	Guinea pig	Rabbit	Cat	Dog	Primate
KTEAMINE Hcl	22-24 i/m	22-24 i/m	-	22-24 i/m	22-24 i/m	30 i/m	30 i/m	15-40
PENTOBAR-BITONE SODIUM	35 i/v 50 i/p	25 i/v 50 i/p	35 i/v -	30 i/v 40 i/p	30 i/v 40 i/p	25 i/v -	20-30 i/v -	35 i/v -
THIOPENTONE SODIUM	25 i/v 50 i/p	20 i/v 40 i/p	20 i/v 40 i/p	20 i/v 55 i/p	20 i/v	25 i/v	25 i/v	25 i/v 60 i/p
URETHANE	-	0.75 i/p	-	1. 5 i/p	1.0 i/p, i/v	1.25 i/v 1.50 i/v	1.00 i/v	1.0 i/v

ATROPINE: Dose 0.02-0.05 mg/kg for all species by s/c or i/m or i/v routes used to reduce salivary and bronchial secretions and protect heart from vagal inhibition, given prior to anaesthesia.

i/m = intramuscular, i/v = intravenous, i/p=intraperitoneal, s/c=subcutaneous

Sourse : www.cpcsea.nic.in (www.ccsea.gov.in.)

Euthanasia

Euthanasia is the act of inducing death in an animal by gentle procedure causing minimum physical and mental suffering; it is also called as painless killing.

Commonly used methods of euthanasia in laboratory animals - As suggested by CPCSEA (CCSEA)

IP intraperitoneal; IV Intravenous; IM Intramuscular

Species	Mouse	Rat	Hamster	Guinea pig	Rabbit	Cat	Dog	Primate
a) PHYSICAL METHODS								
Electrocution	NR	NR	NR	NR	NR	NR	NR	NR
Exsanguination Decapitation (for	NR	A	A	A	A	A	NR	NR
analysis of stress)	A	A	A	NR	NR	NR	NR	NR
Cervical dislocation	A	A	A	NR	NR	NR	NR	NR
b) INHALATION OF GASES								
Carbon Monoxide	A	A	A	A	A	A	A	A
Carbon Dioxide	A	A	A	A	A	A	NR	NR
Carbon Dioxide plus	A	A	A	A	A	A	NR	NR
Chloroform/Halothane	A	A	A	A	A	A	A	A
c) DRUG ADMINISTRATION								
Barbiturate Overdose (route)	A(IP)	A(IP)	A(IP)	A(IP)	A(IV,IP)	A(IV,IP)	A(IV,IP)	A(IV,IP)
Chloral hydrate Overdose (route)	NR	NR	NR	NR	A(IV)	A(IV)	A(IV)	A(IV)
Ketamine Overdose (route)	A(IMP)	A(IMP)	A(IMP)	A(IMP)	A(IM/IV)	A(IM/IV)	A(IM/IV)	A(IM/IV)
Sodium Pentothol [Overdose (route)]	IP	IP	IP	IP	IV	IV	IV	IV

Methods Not Acceptable for any species of animals

a) Physical Methods: (1) Decompression (ii) Stunning

b) Inhalation of Gases:(i) Nitrogen Flushing (ii) Argon Flushing

c) Drug Administration:(i) Curariform drugs (ii) Nicotine Sulphate (iii) Magnesium Sulphate (iv) Potassium Chloride (v) Strychnine (vi) Paraquat (vii) Dichlorvos (vii) Air Embolism

Source: www.cpesea.nic.in (www.ccsea.gov.in)

Practice-

Anaesthesia Methods:

1. **Inhalation:**
   Theory:
   Inhalation Method: In this method apropriate agents (as mentioned in theory) are inhaled by animals as per appropriate protocol (as mentioned in theory).
2. **Injection: Injection (Anaethesia)**
   Theory:
   Injectable methods: In these methods appropriate agents (as mentioned in theory section) are injected through appropriate routes. Techniques for S.C,I.M., I.V. & I.P. are already detailed in the "Routes of Administration" Section.

Euthanasia Methods

1. **Chemical Methods:**
   a. **Inhalation:**
      Theory:
      Inhalation Method: In this method appropriate agents (as mentioned above) are inhaled by animals as per appropriate protocol (as mentioned above).

b. **Injection:**

Theory:

Injectable methods: In these methods appropriate agents (as mentioned in theory section) are injected through appropriate routes. Techniques for S.C,I.M., I.V. & I.P. are already detailed in the "Routes of Administration" Section.

2. **Physical Methods:**

a. **Cervical dislocation:**

Theory:

Cervical dislocation involves dislocation of head part above the neck by stretching.

b. **Decapitation:**

Theory:

It involve the action of cutting off head of animal.

c. **Exsanguinations**

Theory:

In this method first the animals are anaesthetized by suitable method after that large volume of blood is extracted/drainage is done to kill the animal.

Not commonly Used / Not recommended /Not Acceptable Methods

1. **Electrocution:**

Theory:

Electrocution, using electric shock, has been used as a method to induce death. In this method electric shock is given to animal to induce death Electrocution induces death by cardiac fibrillation, which causes carebral hypoxia.

2. **Penetrating captive bolt/pithing**

Theory:

Pithing is designed to cause death by increasing the destruction of brain, brainstem and the upper spinal cord tissue. It is performed by inserting a pithing rod through the entry site produced in the skull by the projectile (i.e., bullet or bolt).

3. **Microwave irradiation**

Theory:

Microwave instruments have been specifically designed for use in euthanasia of laboratory mice and rats.

3. **Euthanasia by a blow to the head/stunning**

Theory:

In this method a heavy blow to head of animal is applied.

2. **Thoracic (Cardiopulmonary, cardiac) compression**

Theory:

Thoracic compression involves compressing the thoracic part of animal. Thoracic (cardiopulmonary, cardiac) compression is used to kill animals when alternate techniques are not practical.

Technical Support & Queries :

For further queries & technical support mail us at :

support@heb-nic.in

HEALTH EDUCATION BUREAU

www.heb-nic.in