

[EXPERIMENT No. 1]

Date _____

Expt. No. 01

Page No. 1

Ruby

Aim $\Rightarrow$ To study the various Instrument and apparatus used in the Pharmacology Laboratory.

Reference $\Rightarrow$

- ii) Kulkarni S.K. Hand book of experimental Pharmacology. 3rd edition vallabh prakashan page no. 2-7
- iii) Pallai K.K experimental Pharmacology - 1st edition C.B.S publishers and distributors page No. 3-4

Theory $\div$

- ii) Student's organe bath $\div$ The tissue bath used to put the animal tissue for studying the drug action is called "student's" organe bath. This was first designed by Rudolph magnus in 1909. The organe bath essentially consist of $\div$

- (a) An outer jacket (water bath) made up of steel glass perper.
- (b) The inner organe of tissue bath made up of glass. with a capacity varying from 10 ml to some
- (c) Thermostatically controlled heating rod.
- (d) Stirrer to keep the water the water jacket at uniform temperature
- (e) Oxygen an delivery glass tube which also serves as tissue holder.

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(f) Glass coil one of which is connected to the lower lead of the organ bath and the other to the container having the Physiological salt solutions.

The glass coil is usually of double the capacity of inner organ bath to ensure warming up to the solution before it enters the organ bath.

The standard organ bath having two units of inner tissue bath is called double units organ bath.

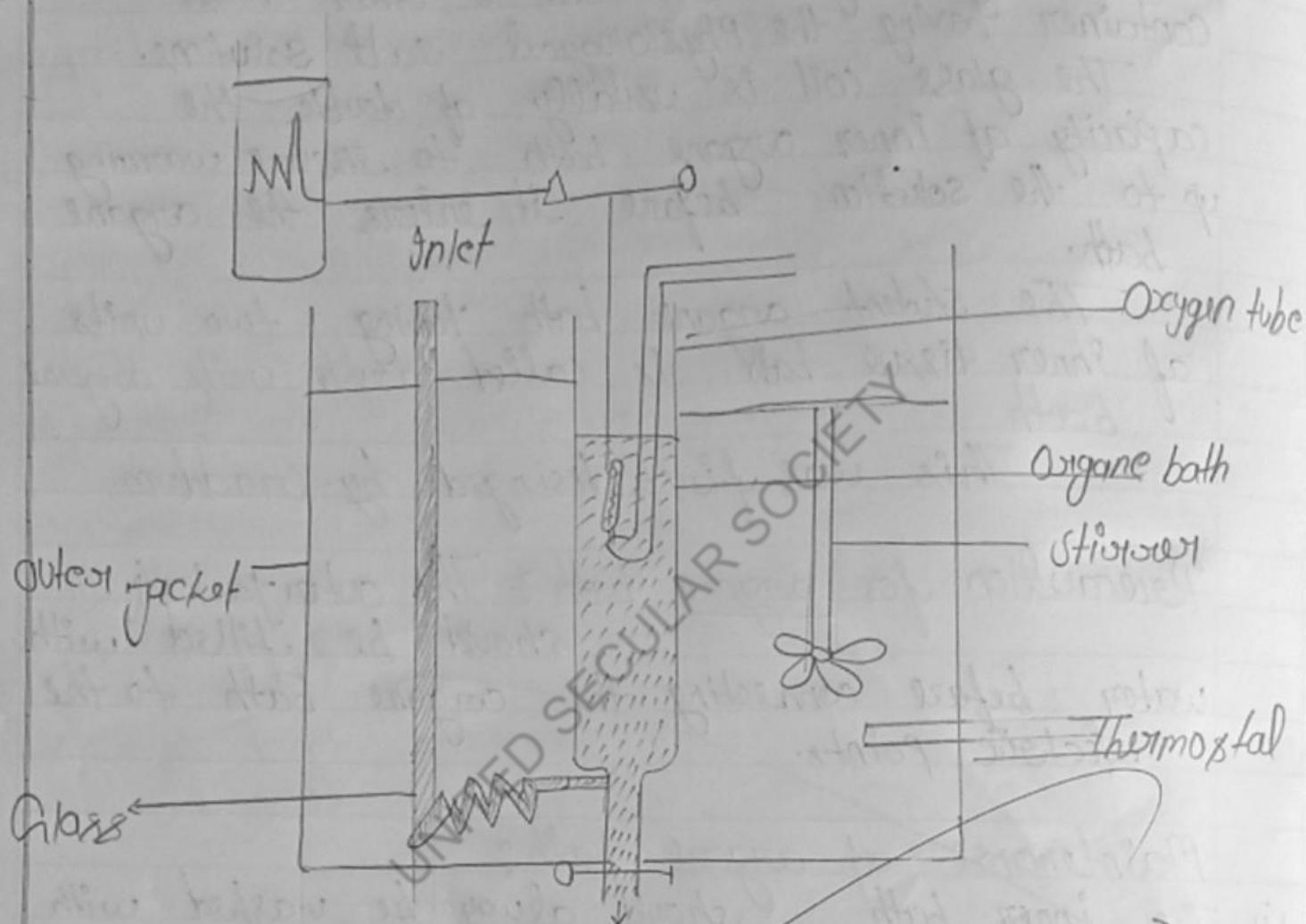
This was first designed by Graddum.

Precaution for organ bath: The outer jacket should be filled with water before connecting the organ bath to the electric points.

Maintenance of organ bath:

- (1) The inner bath should always be washed with distilled water after use to prevent the growth of fungus and other micro-organisms.
- (2) The inner organ bath should be periodically cleansed with HCl or chromic acid.

Recording Levers: They are used to record the contractions or relaxation.



(Structure:- student's organic bath)

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of isolated tissue preparation. The recording is done on smoked papers fixed on circular cylinders and run at different speed of the drum is adjusted depending upon the nature of the experiment.

The writing levers are light in weight rigid and are generally made up of wood light aluminum or stainless steel.

The levers are of two type:

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

(ii) Isotometric type: i.e. isometric recording measures increase in tension of the tissue. When the length of the tissue is kept constant. These are used in special circumstances as recording muscle switches produced by electrical stimulation for recording such observations isometric strain - gauge transducer may be preferred.

The common recording levers used in the laboratory are:

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- (A) Simple lever: It is simplest type of lever made up of wood stainless steel or aluminium. A celluloid writing tip is attached at the end of the longer. The contractions are recorded as curved lines.
- (b) Frontal writing lever: This lever is designed in such a way that the writing point rotates free about its axis. This helps in reducing the tension between the smoked paper and the recording tip. The contraction recorded as straight line.
- (c) Starling's heart lever: It is used to record the contraction of the heart. The difference between this and other isotonic lever is that the fulcrum lies at one end beyond the point of attachment.
- (d) Broadie's universal lever - It is a general utility lever.

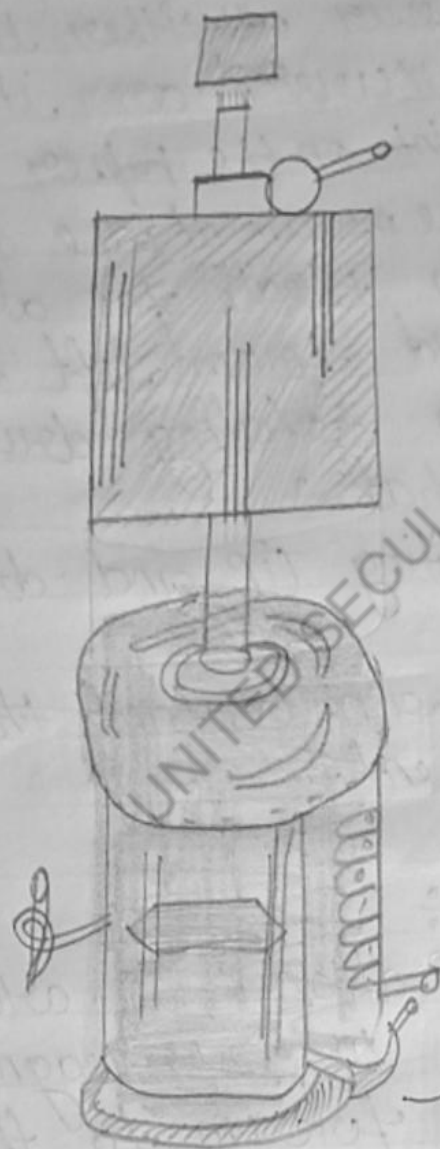
(II) Sherrington rotating drum (Kymograph)
Different models of kymograph are available. Kymograph is an electrical device consisting of gear box with provision for carrying drum. The speed of drum can be controlled by

Clutch and gears while setting up the assembly for any experiment care has been taken to ensure that the Kymograph cylinder is strictly perpendicular to working bench and that the lever moves in the same vertical plane. If the Kymograph is known kept perpendicular to the working bench the lever will either leave the paper at some point with its excursion over it will pass max. heavily at the same point on the paper and increased friction with retards the movement. The drum rotates in clockwise direction therefore for all Kymograph records should be read from left to right. While using Sherrington rotating drum and Kymograph ensure that :-

- (a) Friction between writing tip and drum should be as low as possible.
- (b) The friction must remain constant through out the experiment.

Recording procedure :-

- (A) Adjustment for magnification :- In order to achieve 10 times magnification. the distance from the fulcrum to the point of recording should be 10 times more than the distance from fulcrum to the point of tissue attachment.



(Dig:- Sherrington rotating drum-)

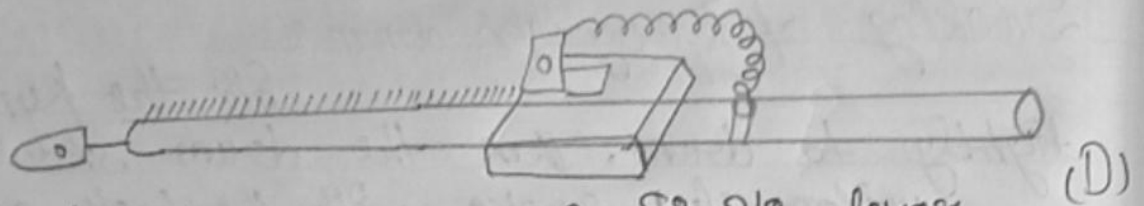
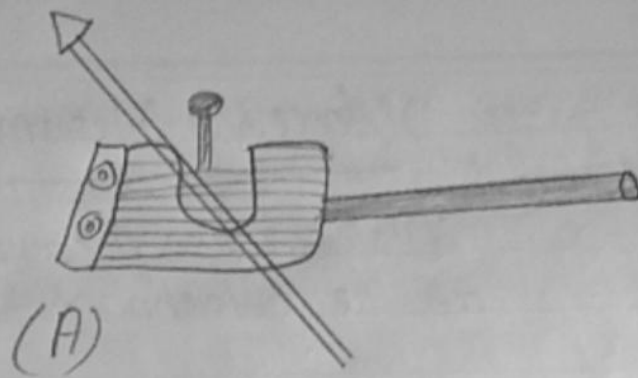
magnification value = $\frac{\text{Distance b/w fulcrum \& writing TPP (A)}}{\text{Distance b/w fulcrum and the point of the attachment to the tissue (B)}}$

Smoking of Kymograph drum:

Fix the Kymograph paper tightly to drum. Then the drum should be uniformly smoke with the back smoke of benzene or Karocine or the mixture of the two. Uniform smoking is essential for proper recording. The recording can be done directly on white paper with the help of ink writing device (pen) attached to the tip of the lever.

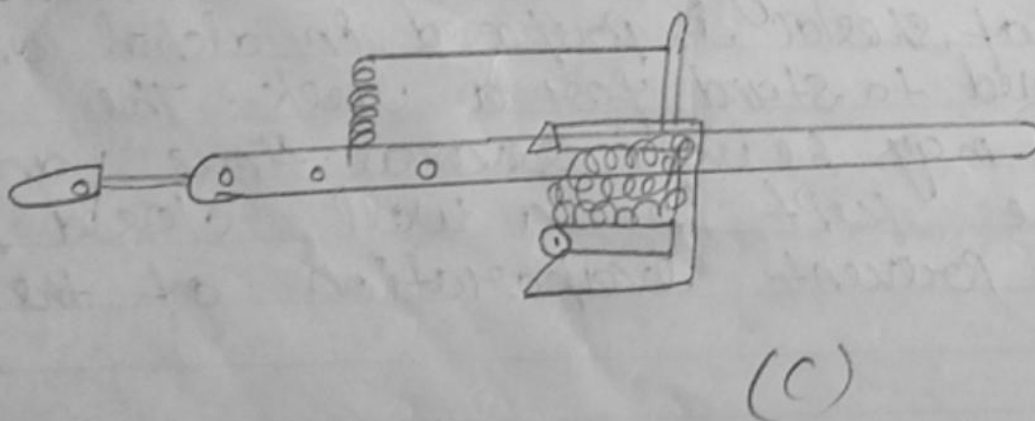
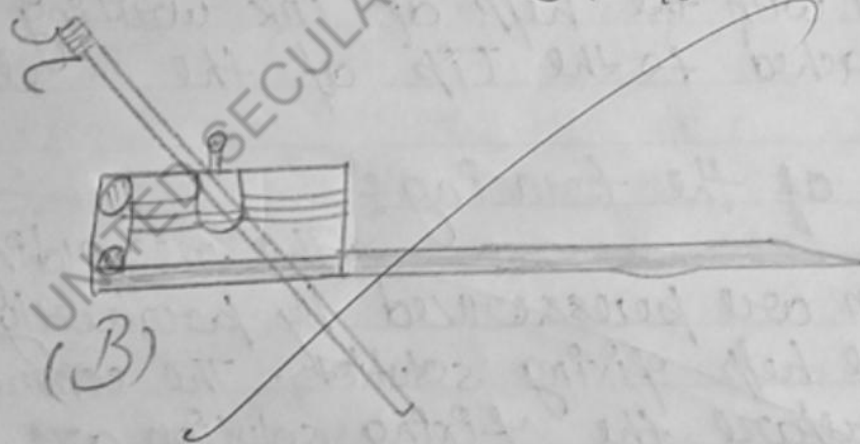
Fixing of the tracing:

The recording on the smoked drum are preserved by properly fixing them with the help of fixing solution. The commonly used resin to prepare the fixing solution are Shellac & storax. Solution of shellac is prepared in alcohol and it is allowed to stand for a week. The solution may be used several times and should be kept in a well closed bottle to prevent evaporation of the solvent.



Recording levers -

- A. Simple lever
- B. Frontal writing lever
- C. Starling's heart lever
- D. Bixby's lever.



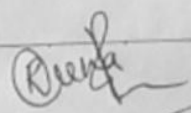
Physiological salt solution (PSS) ÷ The ionic requirement and nutritional supply can be provided by using the suitable solution commonly known as physiological salt solution. Its composition is such that it provides an artificial media resembling the inorganic composition of blood plasma together with a buffer mechanism to maintain the optimum pH 7.0 - 7.2 and glucose facilitates tissue metabolism. Commonly used PSS are frog Tringer tyrod crab's solution etc.

Purpose of each ingredients ÷

1. NaCl ÷ To maintain isomolarity. Isotonic, excitability and contraction of the preparation.
2. KCl ÷ To maintain ionic balance
3. CaCl_2 ÷ To maintain the contractility of preparation
4. NaHCO_3 ÷ To provide energy.
5. $\text{C}_6\text{H}_{12}\text{O}_6$ ÷ To provide alkaline pH
6. Na, K ÷ Act as a buffer
7. Hydrogen phosphate, dihydrogen phosphate, magnesium chloride or sulphate ÷

To stabilise the preparation and deference to reduce the property.

Result ÷ The various instrument and apparatus used in the Pharmacology Laboratory was studied about successfully.



EXPERIMENT No. 02

Date _____

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Aim: To study about common laboratory animals

Reference:

- (i) Kulkarni S.K., "Hand book of experimental Pharmacology" 11th edition. Vallabh Prakashan. 1999. Page no 12-14
- (ii) Goyal K.K., "Practicals in Pharmacology" 14th edition B.S. Shah Prakashan. 2007 page no- 34-39
- (iii) Pillai K.K., "experimental Pharmacology" 1st edition CBS publishers & distribution. 1999. Page No. 8

Theory:

- (i) The frog (*Rana tigrina*) and the toad *Bufo vulgaris*:

Weighing about 150-200 gm is extensively used in experimental pharmacology in physiology. Frogs have similar response of drug as that human. As frog are small animals, so they are easy to handle inexpensive and easily available.

Frog and toad as experimental animals:

- ① These are extensively used in experimental pharmacology. Isolated frog heart, rectus abdominis muscle preparation study of muscle, nervous preparation and ciliary movement are.

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Parameter	Rat	mouse	Rabbit	Guinea Pig.
Body weight (g)	200-300	25-30	2000-3000	600-800
Life span (yrs)	2-3	1-2	4-5	2
Water consumption (ml/day)	3-5	6	200-300	250-350
Food consumption g/day	20	5	100	30
Urine excretion (ml/day)	10-15	1-3	200-300	100-150
Oestrus cycle (days)	4-5	4-5	Spontaneous ovulator	15-19
Gestation Period (days)	21	19	32	67
Breeding age (days)	60-80	42-56	90-120	365
Breeding life (yrs)	1-1.25	0.75-1.5	3	1
Litter size	10-12	10-12	4-8	2-4
Body Temp (°C)	37.5	37.4	38-3	38-3
Respiratory rate (Breath/min)	80-150	90-180	35-60	60-110
Heart rate (beats/min)	260-450	300-750	205-308	250-300
Blood Press (mm/Hg)	130/90	120/75	-	-
Blood vol (ml/kg)	50-65	20-80	45-70	65-90
Room Temp (°C)	24-27	24-27	24-27	24-27
Relative humidity (%)	50-60	70	70	60-70

Some of the range of frog use. These are also used for the study of nerve block type of local anesthesia, coronarotopic & inotropic studies of cardiovascular system.

- (iii) Frog have been used to determining the retinal toxicity of drug. Light bleaches rhodopsin in frog retina in one hour and the dark certain drugs can be assayed for their toxicity. Since they prevent the resynthesis of rhodopsin in preparation the dose given.

2. The

mouse ÷ (*Mus musculus*) ÷

white mouse are the smallest laboratory animals.

use. mice are also easy to breed and maintain. They are small in size (25-30 gram) and therefore easy to handle. They are sensitive to most of the drug in experimental pharmacology preparation or experiments. Albino mice can be bred uniformly and are cheap and easy to handle and being very small and sensitive to small dosage of all substances. They are also used for the assay of insulin and analgesic, the latter specially against chemically induced pain. General screening in this species. Broad mice are used for the study of problems in genetics and in cancer research.

The Rat :- By white Rat is the commonest laboratory animal used in experimental Pharmacology.

Rats are easy to breed maintain and being small in size (200 gram) resemble man in several organ function and nutrition and sensitive to most of drugs make them very useful experiment animals.

They do not have vomiting center and hence it is not possible to use them study of emetics. The various Rat tissues is extensively employed in radio receptor ligands study. The other strain of rat wistar Sprague Dawley.

Guinea Pig :- Guinea pigs are the commonly used experimental animal. They are docile and easy to handle and maintain. They are highly sensitive to histamine. They are used in experimental of asthma to study bronchodilators. They are also used to study local anesthetic and asmodulus is experimental amoebias and cholera Guinea pig. Isolated heart trachea are sensitive to histamine -

Rabbit :-

Domestic rabbit (2-3 kg) are generally used for pyrogen testing some of the tissue and.

Organs from rabbit used are heart aorta. duodenum and ileum are particular thing about rabbits is that They are resistant to the action of atropin as they are resistant contain atropin esterase are enzyme the presence of which is genetically determined.

Result :- The common laboratory animals was studied about successfully.

Rupin

Aim ÷ To study about handling maintenance and breeding techniques of common laboratory.

Reference: i) Gray R.K "Practicals in Pharmacology."
IVth edition. BS Shah Publications, 2007
page - No. 12, 27, 28.

(ii) Ghosh M.N "Fundamentals of experimental pharmacology"
IIIrd edition - page No 1-3.

Theory ÷ There it is need the biological scientist to conduct animal experiments. For studying pathogenesis of disease, under take drug trials and generate variety of biological to suffering of humans and animals. When such experiment become necessary then increasing concern about the welfare of laboratory animals. The concern of such experimental scientist is to follow certain guidelines for the breeding care and management of such animals involving minimum painment of such and discomfort to such animals.

An Ideal animals facility area should consist of :-

- ① Animal breeding area.
- ② Animal receiving room.
- ③ Storage room.
- ④ Experimental room.

5. Feed storage room.
6. Cleaning area.
7. Local storm toilet facility.
8. Animal disposable are with facility for cold storage.

The animal house should be properly ventilated temperature and humidity controlled and it should have 12 hrs light and dark cycles.

Animal Handling ÷ The handling of the laboratory animal involves to most important responsibilities on the parts of experimenters.

- (1) The animals are handled with most care so that it doesn't suffer only pain.
- (2) Due regard paid towards the health and wellbeing of the animal colony.

The Indian national science academy provide the following guidelines to be follow by all research institution in the country engaged in animal experimentation.

- I Having care breeding and maintenance of experimental animals to keep them in physical comfort and good health and to permit the to grow, reproduce and behave normally.
- II Sources of experimental animal of known.

genetic, health and nutritional status.

Committee for the purpose of control and supervision of experiment on animals: Has also set guidelines for laboratory animal facility.

- I. According to the CPCSEA the guidelines are firstly registered institutions carry out experiment on animals / breeding animals.
2. call for and receive records and other information as required from concern person / institute.
 3. ensure that experiments are carried out by qualified individuals and with the full responsibility of person in charge of the institutions.
 4. monitor and inspect the housing of animals of breeds and ensure that it is as per specified standards.
 5. Give permission to registered institutions for conducting experiments on animals.

Breeding: most of the common laboratory animals such as mice rats, Guinea pigs and rabbits can be breed and breed under can be best ideal conditions to provide are reliable supplied of uniform and health animal for the purpose of experimentation.

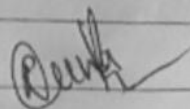
Breeding of animals for research purpose for required the best care & accurate breeding record.

Breeding method :-

- (i) HAND mating :- The male & female are brought together for a breeder period and the separated once the mating is over. Rabbit are mated by this method.
- (2) Pair mating :- In case of mice, one male is mated with one female and left together for the rest of their breeding life. In case of rats, pregnant female should be isolated prior to delivery.
- (3) Trio mating :- one male is mated with two females.
- (4) Harem mating :- up to four female regularly mated with one male. The female is isolated soon after the pregnancy is established, and replaced by fresh female.
Guinea pig is mated by this method.

Result :- The handling maintenance and breeding techniques of common laboratory was studied.

Successfully.



Aim :- To study about different techniques of bleeding drug administration and various anaesthetic agent used in laboratory animals.

Reference :- (i) Ghosh M.N, "Fundamental of experimental Pharmacology." Third edition page No. 15-20
(ii) Pillai K.K, "Experimental Pharmacology." 1st edition Page. No. 16

Theory :-

Route of drug administration :- The onset & duration of action of a drug depend on the route of drug administration. Systemic effect are produced by a drug after it is absorbed in the blood, and then carried by the circulation to the tissue and organs on which it has a selective action. To produce these effects, drugs are administered by orally, i.e., parenteral and inhalation route. To produce local effects drugs are applied topically to the skin or mucous membrane.

Factors governing choice of route of drug administration :-

1. Physical and chemical properties of the drug.
2. Site of desired action localised, and generalised

3. Effect of digestive Juices and first pass metabolism on the drug.
4. condition of subject.
5. Accuracy of dosages required.

Bleeding and Intravenous injection:

1. mouse & rat: Both collection of blood as well as injection can be carried out by pain very puncture in these species. In unanesthetized animal lateral and dorsals veins are dilated by dipping the tail in to the water at 40-50°C or by the rubbing with nylon and then cleaning the part with some disinfectant. The tail is grasped between thumb and the index finger and needle is introduced near the distal position of the tail and the injection made with a uniform speed.

Another way of collecting blood repeatedly is by cutting the tip of the tail with some sharp instrument.

The most efficient method of collecting blood in rats and mice causing least stress to the help of capillary tube.

2. Guinea pig: Small sample of blood may be obtained by simple venisection of the marginal ear veins. Larger quantity of blood may be obtained by card.

Puncture under anaesthesia, in males Intravenous injection can be made through the veins seen along the site of extended penis in anaesthetized animal the lateral margin of the ear is shaved and swabbed with an antiseptic. The ear is grasped between the thumb and the index finger in the well anaesthetized animal using of 20 gauge needle with the behind very puncture is made at a site immediately to the thumb along the marginal vein. Another method is to make out in the ear near its lips. To be injected volume to be administered should be large enough to measure accurately and small enough to avoid under rapid change the blood volume. The usual volume for small animals is between one to ten ml per kg body weight, the maximum for mouse & rabbit being 0.4 & 10 ml respectively.

The volume of intramuscular injection is more limited being about 0.25 ml for mouse 0.5 ml for rat, 1 ml for rabbit and 2 ml for dog. The following formula can be used for the calculation of volume strength or amount of drug to be injected.

$$V = \frac{BW \times A}{S}$$

V = Volume of drug solution
 A = Amount of drug in mg/kg
 S = Strength in mg/ml of drug solution.
 BW = Body weight of animal in kg.

Intra Gastric Administration :

1. Mice : A Polythene tubing 2 to 3 cm long skewed on an 18-20 gauge blunted hypodermic needle may be used for the purpose. The animals are secured by the nape of the neck holding the whole animal with the left hand when it is relatively certain that the tube is desired. The fluid is introduced slowly. A solution or a suspension in cm^3 the Gavage is administered in a volume of 0.5 ml per 20 gram body weight.
 2. Rat and Guinea pig : A 15 to 16 gauge hypodermic needle of 7.5 to 10 cm length is blunted and small ball of solder applied around the tip. And a gentle bent is made about 2 cm proximal to the solder.
- X. LABORATORY Anaesthetics : Animals are given anaesthetic before surgical procedure. The choice of anaesthetic depends on the type of animals and the type of experiment to be performed.

1. Chloralhydrate: It is readily compound of chloral and Glucose. Prepared by heating equal parts of anhydrous glucose and chloral. Chloralose is the active form freely soluble in hot water and slightly soluble in cold water.
2. urethane: (Ethyl carbamate):
It is readily soluble in water giving a neutral solution. Usually 25% solution in water is used.
3. Barbiturates:
Barbiturates interfere with nerve in pulse transmission both in the central nervous system and in the ganglia producing depression of cardiovascular and spinal cord reflexes.
4. Paraldehyde:
It has a wide margin of safety because it depresses only the cerebrum and not the medullary centers. Intravenous injection is likely to produce cardiac dilation and pulmonary congestion and edema.

5. Magnesium Sulphate :

A twenty % magnesium sulphate solution 5 ml per kg intravenously produce anaesthesia fast about an hour. calcium gluconate intravenously will counteract with depressant effect immediately.

Result :- Different technique of bleeding drug administration and various anaesthetic agent was studied successfully.

(Signature)

[EXPERIMENT No. 5]

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Expt. No. 05

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Aim :-

To study the hypnotic activity of pentobarbital in mice.

Reference :- Kulkarni SK "hand book of experimental pharmacology," IIIrd edition Vallabh Prakashan . page- No. 115 - 116

Requirements :-

Animals :- mice (weigh 20 - 25 g)

Drug :- Pentobarbital sodium. (45 mg/kg)

Theory :- Barbiturates induce sleep in man and animal by depressing the central nervous system. The parbiturates induced hypnosis can be easily studied in experimental animal for this purpose generally pentobarbital sodium a short to intermediate acting barbiturate is used. Pentobarbital produce quick onset of sleep as indicated by loss of lighting reflex and the recovery is also easily detected as the animals regain their lighting reflex.

Procedure :-

- (1) weigh and mark the animals
- (2) Inject pentobarbital sodium to each mouse intraperitoneal.

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3. Note the time of injection.
4. Then note the Time of onset of action as the animals loses the fighting reflex & falls sleep. For this place the animal on their back and leaves sufficient space between two animals.
5. Note the Time of recovery from sleep as the animal turn to recover its normal posture.
6. Calculate the onset and duration of action of Pentobarbital sodium.

Result $\Rightarrow$

The hypnotic activity of Pentobarbital in mice was studied successfully.

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* Observation table :- *

Rabbit No.	Drug	Interocular tension		Light reflex		Corneal Reflex		Pupil size	
		Before	After	Before	After	Before	After	Before	After
RE	Saline	Normal	Normal	Present	Present	Present	Present	7 mm	7 mm
LE	Epinephrine	,,	low	,,	,,	,,	,,	,,	9 mm
LE	Atropine	,,	Normal	,,	Absent	,,	,,	,,	,,
LE	Epinephrine	,,	,,	,,	Present	,,	,,	,,	8 mm
LE	Physostigmine	,,	low	,,	,,	,,	,,	7 mm	4 mm
LE	Lignocaine	,,	Normal	,,	,,	,,	Absent	7 mm	7 mm

Aim :- To find out the effect of few drugs on the size of the pupil, light reflex, interocular tension of the rabbit.

Reference :- Rx - Ex - Pharm T. 100 for window 95/98

Requirement :- Rabbit, Rabbit holder, measuring scale, torch, cotton wool & dropper.

Theory :-

The iris is composed of two types of muscle fibers, the circular and 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 produce mydriasis & miosis respectively and their paralytic produce opposite effect.

Procedure :-

- (i) The diameter both the pupils by measured. The interocular tension, light reflex and corneal reflex were noted down.
- (2) The eye was selected.

3. The saline was instilled in control eye and a drug was instilled in the test eye.
4. The measurements were noted such as diameter of pupil intraocular tension & reflexes.
5. The table was prepared.

Result: The rabbit was studied successfully.

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★ Observation table ★

S.N	Drugs	Reading Ist	Reading IInd	Reading IIInd	mean
1-	Ringers	45	44	44	44.3
2-	Acetylcholine	45	28	27	33.3
3-	Physiotigmine	16	17	19	17.3
4-	Atropin	54	60	00:01:03	62.3

[EXPERIMENT No 7]

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Aim : To find out the action of certain drug on the ciliary motility of frog oesophagus.

Reference : Ex Pharma T 4:00 part window

Requirements : Frog. Frog board Poppy seed stop watch.

Theory : Frog oesophagus contain cilia ciliary motility depends on action of acetylcholine (ACh) in mucous membrane acetyl choline causes contraction of cilia leading to increase movement cholinergic drug produced similar affect while acetyl cholinergic paralise cilia & decrease their movement.

Procedure :

i) The distance of seeds movement was determined. The starting point will be : pins fixed winged cephalic end and digital end respectively.

II The frog winged was intilled on the surface of oesophagus a poppy/opium seed was placed at the cephalic end of oesophagus. The seed started across the starting point (cephalic & pins) The stop watch started point and stopped it when the seeds

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reached the digital pins.

III The time taken for the seeds to travel the distance was noted down the step second repeated forgetting & regarding the average of 3 reading was calculated. this would be control.

IV The acetyl choline was instilled and 3 reading was taken.

(V) The step 2nd & 3rd was repeated.

VI The step Physistigmine was instilled & 3 reading was taken.

VII Acetylcholine (without using for winger after step) was instilled observed its effect.

Result: The action of certain drugs on the ciliary motility of frog oesophagus was find out.

Inference: Acetylcholine increase the ciliary movement while atropin decrease the ciliary movement and Physistigmine increase the ciliary movement. that is peristalsis movement of GIT.

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★ Observation table. ★

S.No	Drugs	HRT Beats Per minute	Amplitude	Tone
1.	Control	75	Normal	Normal
2.	Epinephrine	95	Increased	Increased
3.	Nor epinephrine	84	"	"
4.	Isoproterenol	114	"	"
5.	Calcium chloride.	(systole)	"	"
6.	Propionylcholine	73	decrease	decrease
7.	Acetylcholine	63	"	"
8.	Potassium chloride	(diastole)	"	"
9.	Atropin Sulphate.	91	Increase.	Increase.

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Aim :- To find out the action of certain drugs on the isolated heart preparation of frog.

Reference :- Ex Pharm T 1.00 year window 95/98.

Theory :- many drugs act on the heart, adrenergic & cholinergic drugs produced opposite effect on it. These drug act through respective receptors. Some drugs act directly on the heart.

eg :- Epinephrine, nor-epinephrine - increase heart rate.

Proprietary acetyl choline - decrease heart rate.

Requirement :-

A Big size frog starting heart liver. stand A kymograph with drum and smoked paper with chart recorder.

Procedure :-

The drug were injected one by one - The following observation were observed before & after the Administration.

- (A) Force of contraction - Amplitude (Normal - increase - decrease.)
- (B) Tone - (Normal Increase - decrease)
- (C) Heart rate :- (Beats Per minute)

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Parameter A & B were observed by observing the recording. The amplitude of contraction reflect the force left in mid point of systolic and diastolic contraction indicate the change in tones.

Result ÷ The action of certain drug on the isolated heart preparation of frog was studied successfully.

Rush

[EXPERIMENT No. 9]

Date _____

Expt. No. 9

Page No. 30

Aim :- To determine the bioassay of histamine by using matching method.

Reference :- Kulkarni SK "Hand book of experimental pharmacology" Third edition Vallabh prakashan 1999 page no 15-17.

App Requirement :-

EX - Pharma T 4.00 for window 95/98.

Theory :-

Bioassay :- Bioassays are procedure by which the potency or the nature of the substance or constituents is estimated by studying its effect on living tissue or animals.

Importance of bioassay :- Bioassay are elaborated, more laborious and more expensive as comparative other method. However, bioassay is only method of assay if :-

- Active principle of drug is unknown or can be not isolated or to measure the pharmacology activity of new or chemically unidentified substances.
- Chemical method is either not available or if available it is too complex and

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are required high doses as the substance get activated by interacting with chemical for example $\rightarrow$ Insuline.

- $\rightarrow$ chemical component of drug differs but have same pharmacological action viceversa.
- $\rightarrow$ To measure drug efficacy and drug toxicity.

Methods of bioassay $\div$

Direct method $\div$

- $\rightarrow$ End point method
- $\rightarrow$ matching method.
- $\rightarrow$ Bracketing method.

Indirect method $\div$

- $\rightarrow$ Graphical method
- $\rightarrow$ multiple point method:
 - (i) Three point method
 - (ii) four point method
 - (iii) Six point method.

Matching method bioassay $\div$

- $\rightarrow$ In this method the responses obtain from standard drug & test drug are matched by titrating process until they produced equal effect.

Standard response = Test response $\div$

Advantage ÷

It does not depend upon dose relationship.

Disadvantage ÷

It is purely subjective.

Experimental error can not be determined.

Procedure ÷

- (i) Choose a drug dose of histamine & inject obtain a dose response curve (DRC) By increasing the dose in geometric progression.
- (ii) Once the maximum response is reached start the matching assay.
- (iii) When you start the matching assay you will be give a solution of known conc. of histamine which produced the standard curve. This standard curve is chosen on the bases that the response produced by the is about 50% of maximal response.
4. Then inject the standard & unknown alternatively then starting dose can be 0.1 ml adjust the dose of unknown until it match with the standard. matching is achieved by trial error.
5. matching can be confirmed by giving half or double the dose of unknown that matches the standard. After matching

The confirm calculate the conce of histamine in the unknown solution.

Result: bioassay of histamine by using of using matching method was determinated successfully.

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Aim:- To record the dose response curve of Acetylcholine (ACh) employing cock ile

Tissue:- Cock Ileum

Ass:- Tyrode

Drug:- Acetylcholine

Temp:- $37 \pm 1^\circ$

Aeration:- 18-20 Bubbles/min

Magnification:- 10

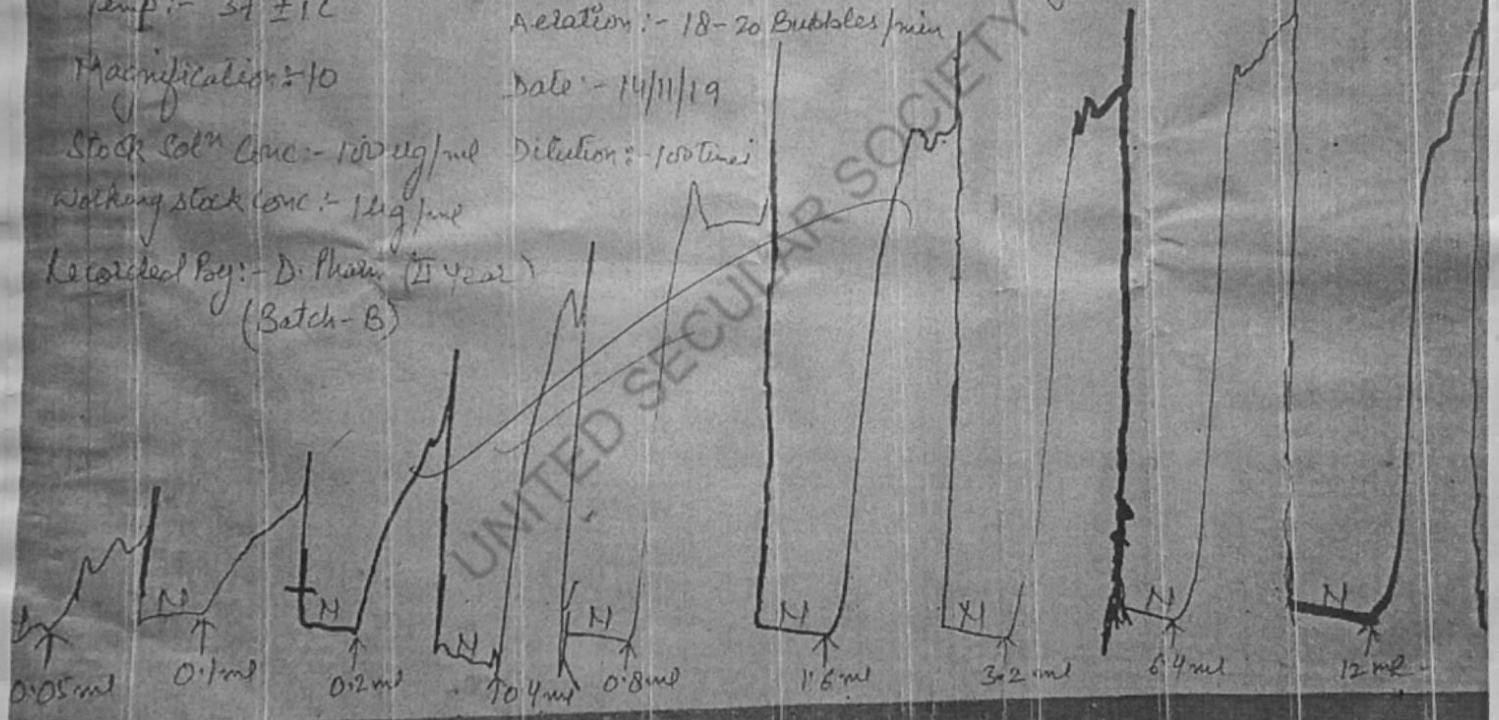
Date:- 14/11/19

Stock solⁿ conc:- 100 μ g/ml

Dilution:- 100 times

Working stock conc:- 1 μ g/ml

Recorded By:- D. Phani (II Year)
(Batch-B)



[EXPERIMENT No. 10]

Date _____

Expt. No. 10

Page No. 34

Aim :- To record the DRC of acetylcholine on cock ileum preparation.

Reference :- Kulkarni SK "Hand book of experimental Pharmacology." Third edition Vallabh Parakash. an 1999 page No 85-88.

Pillai KK "Experimental Pharmacology". edition Ist CBS Publishers & distributors. Page No. 51

Requirement :-

Physiological salt solution - Tyrode solution
Chemical :- Acetylcholine.

Apparatus :- Organ bath, Sherrington rotating drum, frontal lever.

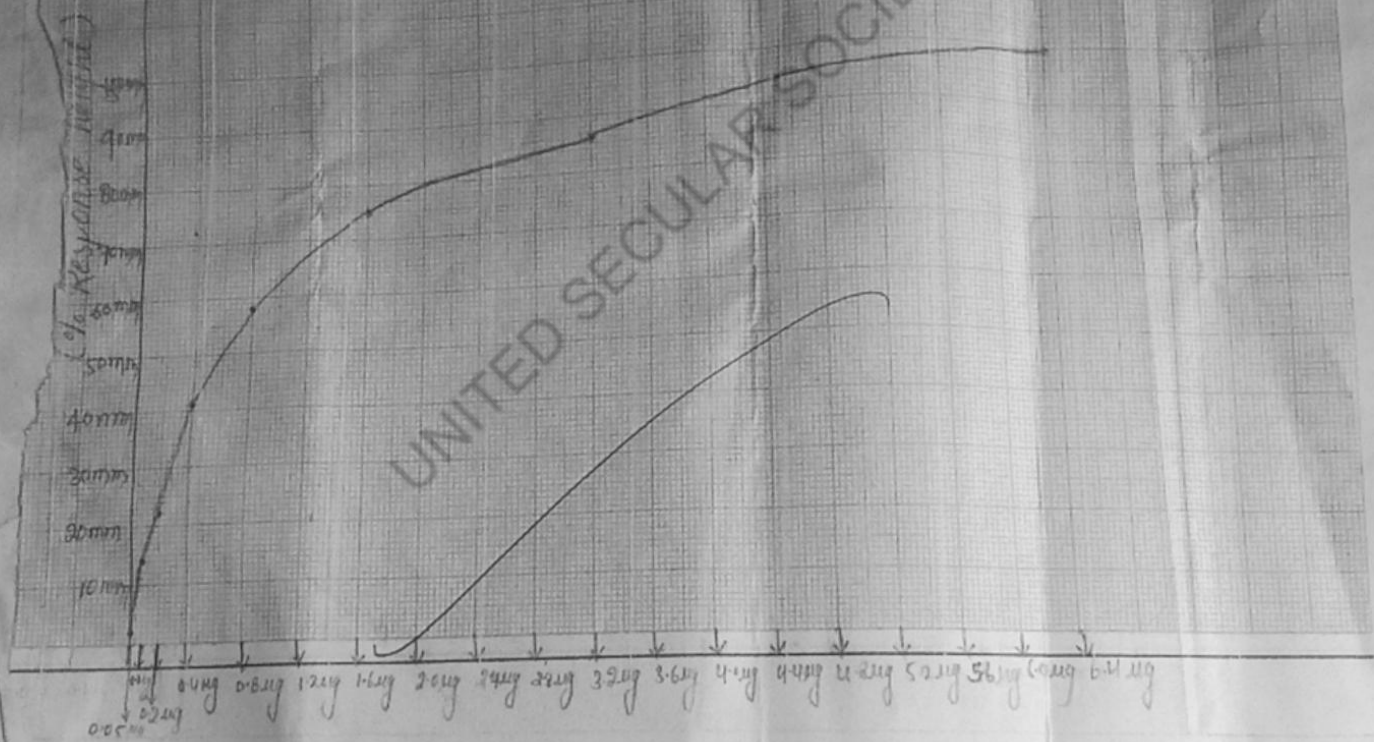
Principle :- Dose response curve demonstrate graded response to drug. An agonist where an increase in response is recorded with a increase in the dose or the conc. of drug. The dose response curve is 'S' shaped. The first part of the curve has poor discrimination between the dosage where as the middle portion of curve shows greater sensitivity to different concentration are linearly differentiated. The last part of the curve shows the

Teacher's Signature : _____

5

1 unite of γ Response height = 10mm

1 unite of γ Concentration = 4mg



of the sealling effect where no more increasing in the response is seen with further increase in dose. The study of conc of dose response curve indicated :-

- ① → Relative Potency of the drug agonist, when the curve is more towards the left side it indicates that the drug is more potent, the vice-versa is also.
- ② → The slope of the curve indicates error and precision of bioassay. Steeper the slope more precision the assay & vice-versa is also true.

Procedure :-

- (i) The thread was tied to both the ends of ileum which was washed with physiological salt solution.
- (2) The preparation was mounted in a bright position in the organ bath containing tyrode solution under a tension of 4 gram. The organ bath was bubbled with air.
- 3 → The base line was recorded.
4. The first dose of Ach was injected into inner organ bath. (0.05 ml)
5. The response was noted down.
6. The drum was stopped & the washed out
- 7 → After complete washing the second dose

0.1 ml was injected the procedure was repeated for recording equipotent response. sub maximal response & ceiling effect with different successive dosage of Ach.

Result :- The DRC of Ach on cock ileum preparation was recorded the ceiling effect was found at the dose of (6.4 ml).

[Signature]

Aim: To record the DRC of BaCl_2 using cock ileum preparation.

Tissue: - Cock Ileum

PSS: - Tyrode

Drug: - BaCl_2

Conc.: - 1% (W/V)

Dilution: - NIL

Temp: - $37^\circ \pm 1^\circ \text{C}$

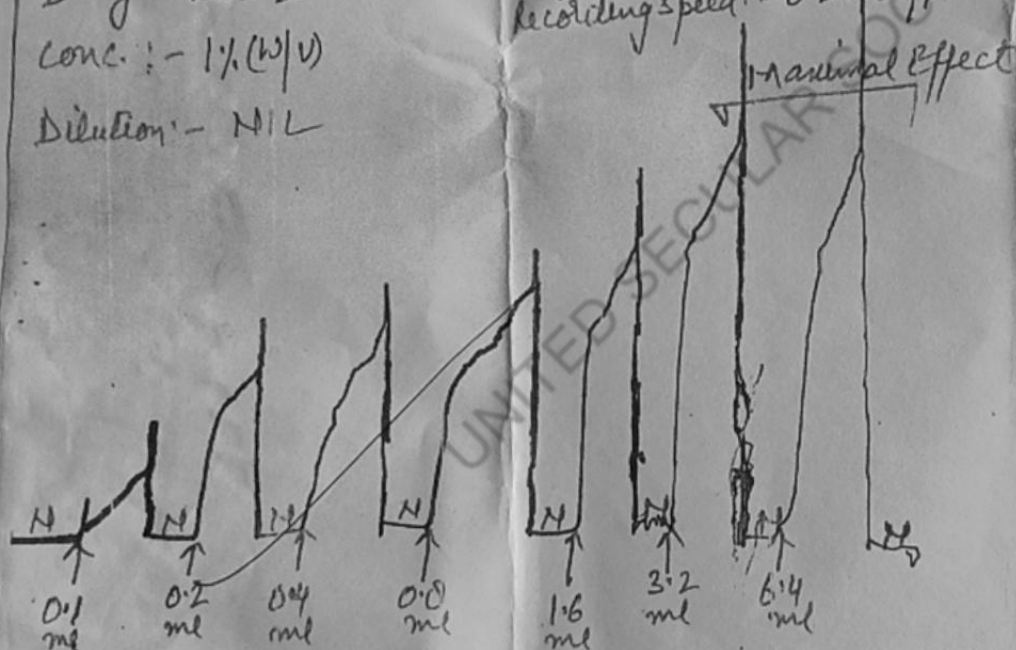
Aeration: - 18-20 Bubbles/min

Magnification: - 10 Times

Recording Speed: - 0.25 mm/sec.

Date: - 21st NOV-2019

Recorded by: - D. Phelan.
(II Year)



Signature
21/11/19

Signature
21/11/19

EXPERIMENT No. 11

Date _____

Expt. No. 11

Page No. 37

Aim : To record the DRC of Barium chloride on cock ileum preparation.

Reference : Kulkarni S.K. "Hand book of experimental pharmacology," third edition. Vallabh Prakashan 1999, page No - 105.

⇒ Prillal K.K. "Experimental pharmacology," edition 1st CBS Publishers & distributors page - No. 40

Requirements :

PSS : Tyrode solution

Chemicals : Barium chloride

Apparatus : Organe bath, Sherwington statary drum
Flortal lever.

Theory :

Barium chloride : Shows its action by more receptor mechanism as it does not bind to the receptors.

Procedure :

- 1 → The organe bath was setup and of ileum organe bath was filled with PSS (Tyrode solution)
- 2 → It was made at temperature $37 \pm 1^\circ$ and aeration was also maintain.
- 3 → The thread was tied to the bath and of ileum which was washed with PSS.

Teacher's Signature : _____

SN	Dose in ml	Dose in mg	Response height	% Response
1.	0.02 ml	0.02 mg	50 mm	71.42%
2.	0.08 ml	0.05 mg	82 mm	74.28%
3.	0.25 ml	0.25 mg	55 mm	78.57%
4.	0.5 ml	0.5 mg	60 mm	85 - 71%
5.	1.1 ml	1.1 mg	65 mm	92 - 85%

Ceiling effect :- 70 mm

- 4) The Preparation was mounted in right position. In the organ bath containing tyrode solutions. under tension of 1 gram - The organ bath was bubbled with air.
- 5) The base line was recorded.
- 6) The first dose of Barium chloride was injected into inner organ bath.
- 7) The response was noted down.
- 8) The drum was stopped the tissue was washed out.
- 9) After complete washing the second dose in i.e 0.2 ml was injected and the same procedure was repeated for recording sufficient response sub maximal response and clearing effect with different successive dosage of Barium chloride.

Result :- The DRC of on cock ileum Preparation was recorded clearing effect was found to be 2.2 ml.

Qulfa

NP 12

AIM:- To record the DRC of Acetylcholine in absence & presence of Neostigmine using Cock ileum preparation

Tissue:- Cock Ileum

PSS:- Tyrode

Drug:- (A) ACh (B) Neostigmine

Conc:- (A) ACh - (100 µg/ml)

(B) Neostigmine (100 µg/ml)

Dilution:- 10 times

Temp:- $37 \pm 1^\circ\text{C}$

Aeration:- 18-20 Bubbles/min.

Magnification:- 10 Times

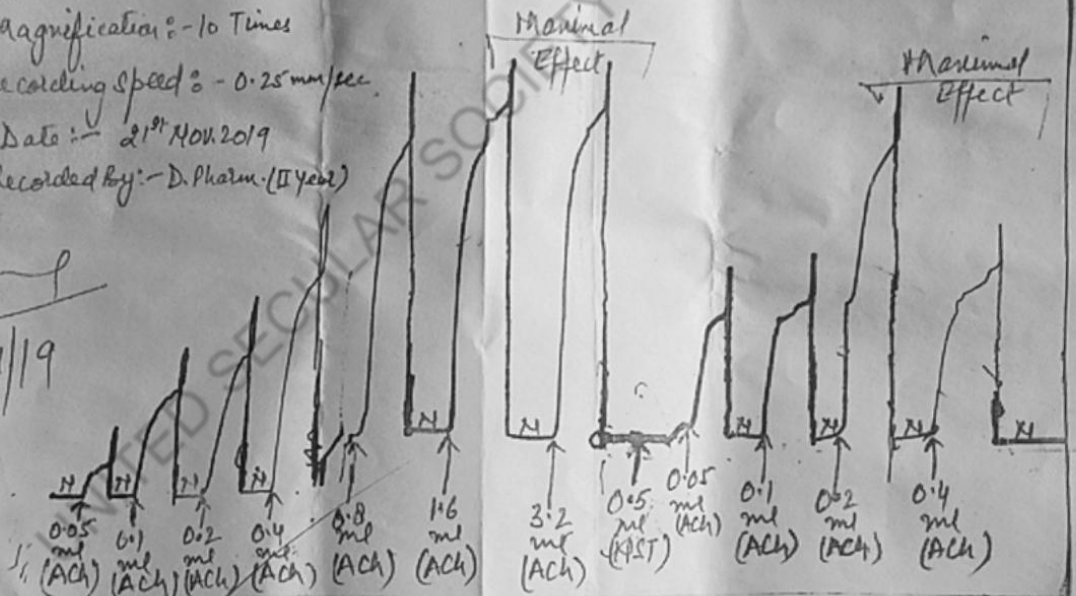
Recording Speed:- 0.25 mm/sec.

Date:- 21st Nov 2019

Recorded by:- D. Pharm. (II Year)

R.O. P
21/11/19

Lacmi
21/11/19



[EXPERIMENT No 12]

Date _____

Expt. No. 12

Page No. 39

Aim: To record DRC of acetylcholine in the presence of neostigmine on cock drum preparation.

Reference: Kulkarni SK. "Hand book of Pharmacology" Edition Third Vallabh Prakashan Page No. 87.

Requirements:

Apparatus: Organe bath, Sherrington rotating drum, Frontal lucer.

Chemicals: Acetylcholine & Neostigmine.

Theory:

Principle: Neostigmine is an anticholinesterase substance and it inhibit the metabolic breakdown of ACh. as a result the action of ACh is potentiated. The conc. response curve of ACh be shifted to the left in the presence of neostigmine.

Procedure:

- (1) The organe bath was setup.
- (2) The thread was tied to both the ends of ileum which washed with PSS.
- (3) The preparation was mounted in a beight. position in the organe bath containing tyrode solution under a tension to 1 gram.

Teacher's Signature : _____

- The organ bath was bubbled with air.
4. The base line was recorded
 5. The first dose of Ach was injected in to inner organ bath (0.05ml)
 6. The response was noted down.
 7. The drum was stopped & the tissue washed out.
 8. After complete washing the second dose 0.1ml was injected & the procedure was repeated for recording equipotent response, sub maxime response & ceiling effect with different successive dose of Ach.
 9. After that 0.5 ml of neostigmine was injected into inner organ bath and waited for 5 to 10 minute.
 10. 0.05 ml of ach was added & repeated for successive doses of Ach untill the ceiling effect reached.

Inference :- From the following result, the graph shifted to the left side which shows the neostigmine potentiated the effect of Ach.

Result $\Rightarrow$ The DRC of Ach & neostigmine was recorded dose ratio was found.

Quifa

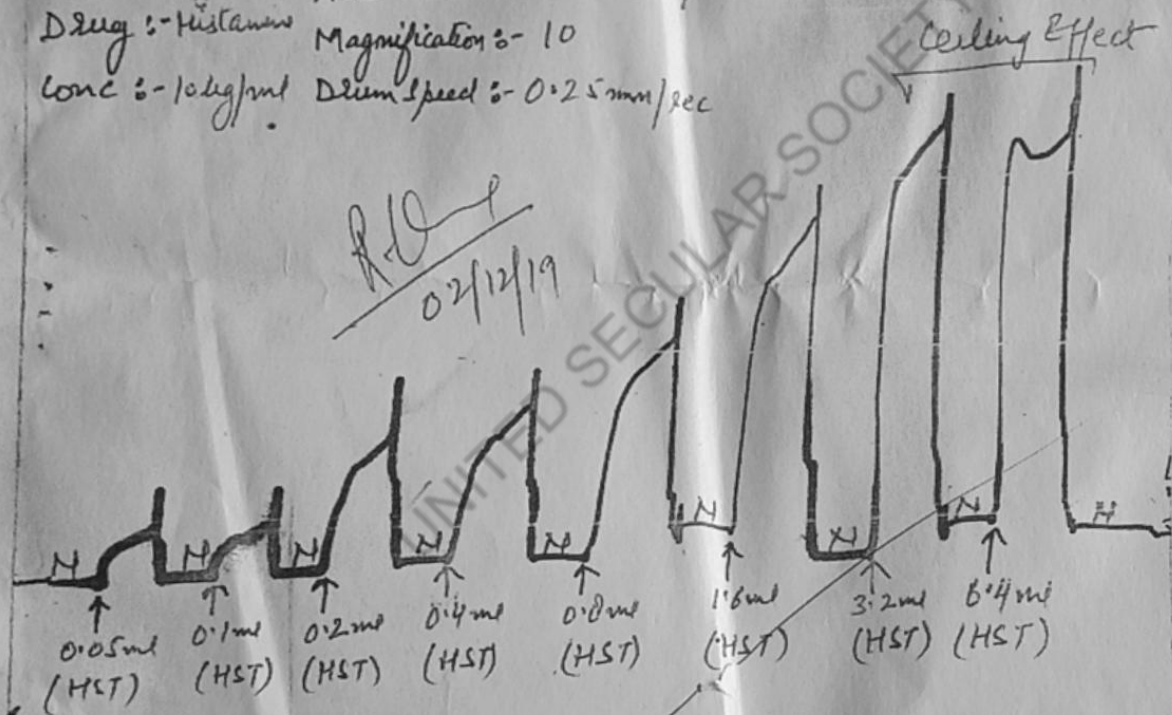
AIM:- To record the DRC of Histamine employing Cock ileum preparation.

Tissue: Cock Ileum Temp.: - $37^{\circ} \pm 1^{\circ}C$

PSS :- Tyrode Aeration :- 18-20 Bubbles/min

Drug :- Histamine Magnification :- 10

Conc :- 10 $\mu g/ml$ Drum Speed :- 0.25 mm/sec



[EXPERIMENT NO. 13]

Date _____

Expt. No. 13

Page No. 41

Aim :- To record the DRC of histamine on cock PLUM preparation.

Reference :- Kulkarni S.K. "Hand book of experimental pharmacology" third edition, Vallabh

Prakashan. 1999 page No. 105

⇒ Pillai K.K "EXPERIMENTAL pharmacology" edition 181 CBS Publisher & distributors page. No 70.

Requirements :-

Apparatus :- Sherrington rotating drum organ bath.

Chemicals :- Histamine.

PSS :- Tyrode solution.

Theory :-

Histamine :- Histamine tissue amine is almost present in animal tissue and in certain plants. It is β amidazolethylamine.

Histamine is present mostly with in storage granules of most cells. Tissue rich in histamine are skin, gastric & intestinal mucosa, lungs, liver & placenta.

Histamine receptors :- It is G-protein coupled receptor. It is of 3 sub types.

H_1 , H_2 & H_3 -

Teacher's Signature : _____

- Procedure :- $\rightarrow$ The Organe bath was setup and the inner Organe bath was filled with PSS.
- 2) It was made a temperature $37 \pm 1^\circ\text{C}$ and aeration was also maintained.
 3. The thread was tied to the both end of ileum which was washed with PSS.
 4. The preparation was mounted in a right position in the Organe bath containing tyrode solution under tension of 1 gram. The Organe bath was bubbled with air.
 5. The base line was recorded.
 6. The first dose of histamine was injected in to inner Organe bath.
 7. The response was noted down.
 8. The drum was stopped & the tissue was washed out.
 9. After complete the washing the second dose i.e 0.2ml was repeated for recording equipotent response, sub maximal response & ceiling effect with different successive dose of histamine.

Result $\Rightarrow$ The DRC of histamine on cock ileum preparation was recorded & ceiling effect was found to be.

Duff

Aim: To study the technique to identify the presence of pyrogen that is telethermometer.

Reference $\rightarrow$ Pillai K.K "experimental pharmacology" edition 1st page No.

Requirements:- Rabbit cage, telethermometer, needle & syringe.

Principle $\Rightarrow$ Rabbits are very sensitive to pyrogen. there fore when pyrogen containing solution is given intravenously it produce a rise in body temperature within three hours.

If the parenteral solution is sterile. it will not produce any rise in body temperature.

If the sample produce a rise in temperature (2.4°) from mean value then the sample is considered pyrogenic. The end is discarded.

Procedure:-

- 1) select healthy rabbit & keep them in the animal room maintain at a temperature of $22 \pm 2.8^{\circ}$ for 3 week period.
- 2) Feed the rabbit with pellet diet & filtered drinking water.
- 3) perform test in an air condition room (ie inject pyrogen free solutions 10 ml / kg & record the

- Record the rectal temperature by using telethermo-meter for there has at 30 mint interval cric there days just for rabbit.
- 5) Record the initial temperature of each rabbit at 30 minute interval for a period of 90 min.
 - 6) Do not select rabbit. initial temperature about at 39.5° below 38°
 - 7) Administered the preparation to be tested for Pyrogen by iv route (0.1 ml/kg) through the marginal ear veins
 - 8) Record the Temperature for each rabbit at 30 min. interval for a period of 3 hrs.

Significance :- Rabbit Pyrogen test is conducted for products such as blood product or derivatives. Immune serum & anti/oxing.

Result -> The technique to identify the presence of Pyrogen that is telethermometer was studied successfully.

Dev

Aim → To perform the bioassay of Acetylcholine by using graphical method.

Reference:- Kulkarni sk. "Hand book of experimental pharmacology" 11th Edition Vallabh prakashan. 1999 Page no. 15-19.

Requirements:-

Apparatus:- Organe bath shearwater rotating drum.

Chemicals:- Acetylcholine.

Theory →

Bioassay are procedure by which the potency or the nature of substance all constituents is estimated by studying its effect on living process.

Importance of bioassay:-

- 1) Bioassay are less elaborate more laborious more fatalism and more expensive as compare to other method.
2. However bioassay is only method of assay if.
 - (a) Active principle of drug is unknown or can not be isolated or to measure the pharmacologically activity of few or unidentified substance.

- 6) chemical method is either not available or if available it is too complex insensitive or required higher dose or the substance get inactivated by interacting with chemicals eg. insulin.
- 3) chemical component of drug different but have some pharmacological action & vice-versa
- 4) To measure drug efficacy & toxicity.

method of bioassay.

- (i) endpoint method or quantal response.
- (ii) Gradiat response $\rightarrow$ matching method.
 $\Rightarrow$ Indirect method.

Indirect method-

- $\Rightarrow$ Graphical method.
- $\Rightarrow$ Three point method.
- $\Rightarrow$ Four point method.

Graphical method $\Rightarrow$

In this method 1st the DRC of standard drug is recorded. The test dose in should lie between the maximum and maximum height of the standard. This method gives us information about the concentration of test dose/drug by plotting graph between log.

Concentration & response height of standard.

Procedure $\Rightarrow$

- 1) The DRC of standard drug was recorded till the ceiling effect.
- 2) 0.1 ml of test drug was added & response height was taken.
- 3) It lied between the minimum & maximum height.

Result $\Rightarrow$ The bioassay of Neetylcholine. by using graphical method was performed successfully.

Paula