TECHNOCRATS

Lab Work Book of

Pharmacology-II

(BP - 507 P)

Department of Pharmacy

Lab Manual of

Pharmacology-II

(BP - 507 P)

Price : ₹ ...../-

Edition :

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Printed & Published by :

TECHNOCRATS
PUBLICATIONS

Arera Colony, Bhopal.
e-mail : technocratspublications@gmail.com

TECHNOCRATS
PUBLICATIONS

Lab Work Book
of

Pharmacology-II

(BP - 507 P)

(Strictly According to RGPV Syllabus)

Name : .....

Enrollment No. : .....

Institute : .....

Academic Session : .....

Department of Pharmacy

TECHNOCRATS
PUBLICATIONS

Vision of the Institute

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

Mission of the Institute

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

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

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

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

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

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

PROGRAM OUTCOMES (POs)

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

PEOs

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

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

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

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

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

Course Outcomes (COs):

Student will be able to:

- CO1: Define various terminology used in pharmacology.
- CO2: Identify the pharmacological actions of drugs on the tissues.
- CO3: Identify the unknow concentration of a drugs on the animal model.
- CO4: Compare the potency of standard with the test compound.
- CO5: Demonstrate the animal model or bioassay method by using stimulated softwares.

Index

S NO	EXPERIMENTS	PAGE NO
1	To prepare physiological salt solution for isolated organ experiment	1
2	To study the effect of noradrenaline, acetylcholine and isoprenaline on the coronary blood flow and heart rate using isolated rat heart	4
3	To study the diuretic activity of drugs	7
4	To study matching bioassay of histamine using isolated organ preparation of rat guinea pig ileum	10
5	To study interpolation bioassay of acetylcholine using isolated organ preparation of rat ileum	13
6	To study the analgesic effect of morphine in mice using tail-flick method	16
7	To record the concentration response curve of histamine and its modification by an anti histaminic using guinea pig ileum preparation	19
8	To calculate pA2 value for atropine using acetylcholine as agonist employing guinea pig ileum preparations	22
9	To record a concentration-response curve of acetylcholine using recuts abdominis muscle preparation of frog	25
10	To study the anti-inflammatory activity of indomethacin against carrageenan-induced paw oedema in rats	28
11	Determine the strength of given sample of acetylcholine by three point bioassay method using isolated organ preparation rat ileum/rat duodenum/rat colon	32
12	Determine the strength of given sample of acetylcholine by four point bioassay method using isolated organ preparation rat ileum/rat duodenum/rat colon	35
13	To record the concentration response curve of Oxytocin using rat uterus preparation	39
14	To record the concentration response curve of 5-hydroxy tryptamine (serotonin) using rat fundus strip preparation	42
15	To study the analgesic effect of the morphine in mice using hot plate method	45

Experiment No.1

AIM:

To prepare physiological salt solution for isolated organ experiment.

REFERENCE:

Kulkarni, S.K., "Hand Book of experimental Pharmacology", 3rd Edition, Published by Vallabh Prakashan, Delhi, Page No.10.

REQUIREMENT:

Nacl, KCl, $CaCl_2$, $MgCl_2$, MgSo4, $NaHCo_3$, NaH_2PO_4, KH_2PO4, Glucose.

THEORY:

The Physiological salt solutions are used to keep isolated tissue or organ preparations surviving as long as the experiments are over. It is important to choose the particular type of solution in which tissue is known to survive. These physiological salt solutions are prepared carefully by using analytical grade reagents and distilled water.

PROCEDURE:

Wash the beaker (500 ml).

Weigh all the given salts ingredients accurately in analytical balance.

Dissolve salt one by one in 500ml of distilled water in beaker.

OBSERVATION TABLE:

Compounds	Frog Ringer	Ringer Or Ringer Locke	De Jalon	Tyrode	Krebs-Hensleit (KERBS)

RESULTS AND DISCUSSION:

VIVA QUESTIONS

Q.1. What is the purpose of physiological salt solution?

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Q.2. Discuss importance of physiological salt solution.

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Q.3. Write uses of different salts used in PSS.

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Q.4. What is the importance of buffers used in PSS?

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Experiment no. 2

AIM:

To study the effect of noradrenaline, acetylcholine and isoprenaline on the coronary blood flow and heart rate using isolated rat heart (Langendroff's heart preparation).

REFERENCE:

Kulkarni, S.K., "Hand Book of experimental Pharmacology", 3rd Edition, Published by Vallabh Prakashan, Delhi, Page No.159.

REQUIREMENTS:

Animal- Rat 200-300gm, Anesthetic- Pentobarbitone sodium 45 mg/kg i.p., Drugs- Noradrenaline, isoprenaline and acetylcholine (all 10 mg/kg stock solution, normal saline and heparin 300 IU/ml), Apparatus- Langendroff's apparatus, Physiological salt solution- Kreb's Hanselet solution.

THEORY:

The heart is innervated by both sympathetic and parasympathetic nerves, which alters the basic rhythm of the heart and also that initiated by the pacemaker tissue of the SA node. Blood is supplied to the walls of the heart by the coronary arteries. Postganglionic sympathetic axons pass from the main cardiac sympathetic nerves to the coronary vessels. There is no parasympathetic innervations of the vascular supply to the heart. It is probable that alpha-adrenoceptors mediating vasoconstriction are present in the large coronary arteries and beta2-adrenoceptors mediating vasodialation exist in the smaller vessels. However, coronary blood vessels are very sensitive to vasodiloator metabolities released from the myocardial cells, especially under hypoxic conditions. This effect probably guinea pig hearts can be maintained by perfusing the coronary arteries using the method of Langendroff. The aorta is cannulated and the technique is therefore a retrograde perfusion. The pressure of Ringer solution closes the aortic valve so that the ringer is delivered directly to the mouths of the coronary arteries without passing through the heart. The aortic valves present the left ventricle from filling and the left side of the heart remains empty. The right side received the fluid draining from the coronary sinus and this is expelled through the cut orifices of the inferior vena cava or passes through the right ventricle and is pumped out of the cut pulmonary artery. Although there is no real cardiac output in the perfused heart, the rate at which fluid leaves the heart reflects the coronary flow, and will beat myogenically and the force of ventricular contraction can be measured by attaching a thread from the tip of the ventricle through a pulley system to a transducer and can be recorded on a polygraph or using a kymograph.

PROCEDURE:

- Before beginning the experiment, set the Langendroff's apparatus and ensure that the perfusion system is in good condition.
- Inject heparin (300 IU, i.p.) and after 20 minutes, anaesthetize the rat with pentobarbitone sodium.
- Kill the rat by dislocating the neck and exsanguinate (draining of blood) the animal.
- Open thorax immediately, expose and remove the heart along with aorta as rapidly as possible and

plunge the tissue into ice-cold Kreb's Hanselet solution.

- Cannulate the heart through aorta using artery cannula and mount the heart on Langendroff's apparatus by securing firmly in place with button thread.
- Perfused the heart at a constant pressure of 90 cm of water with Kreb's Hanseleit solution, maintained at 37^0C and pH 7.4.
- Maintain a perfusion rate of 5 ml/minute. Saturated the perfusion solution with oxygen bubbles at a constant and slow pace.
- Attach a heart clip complete with a light thread to the tip of the ventricle. Locate the thread around a pulley about 4 mm vertically below the heart. Attach the thread to the transducer or to kymograph for recording the contractions of the heart beats.
- Record the normal coronary output by counting the drops of the fluid leaving the heart rate and record the concentration on kymograph.
- Inject 0.1 ml test drugs and note the coronary out flow (drops per minute) after 10 seconds after the drug injections. Also note the heart rate and record the concentrations of the heart for a minute.
- Fix the kymograph and calculate the readings.

OBSERVATION:

Drug	Coronary flow	Heart rate	Force of contraction
Noradrenaline			
Isoprenaline			
Acetylcholine			

RESULT AND DISCUSSION:

VIVA QUESTIONS

Q.1. What is the composition of Kreb's solution?

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Q.2. Give mechanism of action of isoprenaline.

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Q.3. Define hypodynamic heart?

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Experiment No.3

AIM:

To study the diuretic activity of drugs

REFFERENCE:

Jayasree T, Kiran KK. Evaluation of the diuretic effect of the chloroform extract of the Benincasa hispida rind extract in guinea pigs. J Clini Diagnos Res.2011;5(3):578-82.

REQUIREMENTS:

Animals: Make Albino Wistar rats of 140-200 g of either sex, **Drugs:** 2% CMC in normal saline- 10 ml/kg, Furosemide- 10 mg/kg p.o. in vehicle, **Instruments:** pH meter, volumetric tubes.

THEORY:

Diuretics are the drugs that increase urine output and help in treating mild to moderate hypertension by reducing volume of the plasma and thus venous return to the heart. The aim of the study was to screen the diuretic activity of given test drug.

PROCEDURE:

- Divide selected male Albino Wistar rats into two groups consisting of 3 animals in each.
- Group-1 receives 2% CMC in normal saline and serves as control and the other group receives Furosemide and serves as test group.
- After treatment immediately hydrate the rats with 15 ml/kg of saline and place them in metabolic cages separately and maintain the room temperature to 21 0.5 and avoid access to feed and water.
- Care to be taken to avoid mixing of urine and faeces.
- After 5 hours measure the total volume of urine collected in measuring cylinder and compares the same in two groups.
- Other parameters like urine pH and concentration of electrolytes such as sodium, chloride and potassium are to be estimated by using ion selective electrode method described by the manuals in biochemical kits.

Observation Table:

Group	Parameters				
	Urine volume (in ml)	Urine pH	Concentration of sodium (m.mol/L)	Concentration of potassium (m.mol/L)	Concentration of chloride (m.mol/L)
Group -1 (2 % cmc in normal saline)					
Group-2 (Furosemide-10 mg/kg, p.o.)					

RESULTS AND DISCUSSION:

VIVA QUESTIONS

Q.1. Discuss mechanism of action of Furosamide.

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Q.2. What is the role of Carbonic anhydrase enzyme in urine physiology?

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Q.3. What are osmotic diuretics?

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Experiment no. 4

AIM:

To study matching bioassay of histamine using isolated organ preparation of guinea pig ileum.

REFERENCE:

"Principles of Pharmacology" by H.L. Sharma and K.K. Sharma, First Edition 2007, Published by Paras Medical Publisher, Hyderabad, Page No. 84-89.

"Hand Book of Experimental Pharmacology" by S.K. Kulkarni, Ninth Edition 2007, Published by Vallabh Prakashan, New Delhi, Page No. 102-105.

REQUIREMENTS:

Apparatus- Student's Organ Bath, Sherrington Rotating Drum, Animal- Guinea pig 400-600 g, Drugs- Histamine stock solution 1mg/ml, Physiological salt solution- Tyrode solution.

THEORY:

In graded response assays the responses to varying doses of a drug are graded and measured. Here, the effect of the standard drug and of the unknown are measured repeatedly on the same tissue, e.g. bioassay of histamine on guinea pig ileum and bioassay of acetylcholine on frog rectus abdominus muscle.

Matching Assay is type of graded response assays. It is used when the test sample is too small. In this method, a concentration of the known which matches in its response with the known dose of the standard is found by trial and error. From this the potency ratio of the two can be approximately found and the strength of the unknown test solution can be calculated.

PROCEDURE:

- The overnight fasted Guinea pig is sacrificed by cervical dislocation.
- Cut open the abdomen and lift the caecum to trace the ileocaecal junction. Cut & remove a few cm. long of the ileum portion and immediately place it in the watch glass containing Tyrode solution.
- Trim the mesentery and with gentle care clean the contents of the ileum by pushing the tyrode solution into the lumen of the ileum utmost care should be taken to avoid any damage to the gut muscle.
- Cut the ileum into small segments of 3 cm long.
- Take 1 piece of the ileum of 2-3 cm and tie the thread to top and the bottom ends without closing the lumen and mount the tissue in the organ bath containing tyrode solution maintained at 32^{O}c-35^{O}c and bubbled with oxygen air.
- A tension of 0.5g is created or applied and the tissue is allowed to equilibrate for 30 minutes before adding drugs to the organ bath.
- Record the concentration dependent responses due to histamine using frontal writing lever. Contact time of 30 sec and 1 min time cycle are kept for proper recording of the responses.

- Record until peak or sub saturated, concentration dependent responses is obtained.
- Record responses due to 0.1, 0.2, or 0.4 ml of the test substance. See that these responses would fall on the linear portion of the concentration-response curve for the standard solution.
- Label and fix the tracing.
- Plot the concentration-response curve due to standard acetylcholine/histamine solution. Measure the heights of the concentrations (response) due to different doses (A and B) of test solution. Read the corresponding concentration from the standard curve.

OBSERVATIONS:

Dose (ml)	Response	Height in cm	Remark

RESULTS AND DISCUSSION:

VIVA QUESTIONS

Q.1. Define Biological assays?

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Q.2. Write note on acetylcholine as neurotransmitter.

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Q.3. Discuss cholinergic receptors in brief.

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Q.4. Give physiological effects of histamine.

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Experiment No. 5

AIM:

To study interpolation bioassay of acetylcholine using isolated organ preparation of rat ileum.

REFERENCE:

"Principles of Pharmacology" by H.L. Sharma and K.K. Sharma, First Edition 2007, Published by Paras Medical Publisher, Hyderabad, Page No. 84-89.

"Hand Book of Experimental Pharmacology" by S.K. Kulkarni, Ninth Edition 2007, Published by Vallabh Prakashan, New Delhi, Page No. 97-100.

REQUIREMENTS:

Apparatus- Student's Organ Bath, Sherrington Rotating Drum, Animal- Rats 150-200 g/Guinea pig 400-600 g, Drugs- Acetylcholine stock solution 1mg/ml, Physiological salt solution- Tyrode solution.

THEORY:

In graded response assays the responses to varying doses of a drug are graded and measured. Here, the effect of the standard drug and of the unknown are measured repeatedly on the same tissue, e.g. bioassay of histamine on guinea pig ileum and bioassay of acetylcholine on frog rectus abdominus muscle.

It is based on the principles of log dose response (LDR) curves. In this method, the LDR curve of the standard drug is obtained first, later two or three such responses of the unknown, which fall in between the linear portion of the LDR curve, are obtained by trial and error. Then, by interpolation of these responses at the dose axis, and taking the antilog (of x and x1), the concentration of the unknown test solution can be found.

PROCEDURE:

- The rat is sacrificed by cervical dislocation.
- Cut open the abdomen and lift the caecum to trace the ileocaecal junction. Cut and remove a few cm. long of the ileum portion and immediately place it in the watch glass containing Tyrode solution.
- Trim the mesentery and with gentle care clean the contents of the ileum by pushing the tyrode solution into the lumen of the ileum utmost care should be taken to avoid any damage to the gut muscle.
- Cut the ileum into small segments of 3 cm long.
- Take 1 piece of the ileum of 2-3 cm and tie the thread to top and the bottom ends without closing the lumen and mount the tissue in the organ bath containing tyrode solution maintained at 32^{o}c-35^{o}c and bubbled with oxygen air.
- A tension of 0.5g is created or applied and the tissue is allowed to equilibrate for 30 minutes before adding drugs to the organ bath.

- Record the concentration dependent responses due to acetylcholine using frontal writing lever. Contact time of 30 sec and 1 min time cycle are kept for proper recording of the responses.
- Record until peak or sub saturated, concentration dependent responses is obtained.
- Record responses due to 0.1, 0.2, or 0.4 ml of the test substance. See that these responses would fall on the linear portion of the concentration-response curve for the standard solution.
- Label and fix the tracing.

OBSERVATIONS:

Dose (ml)	Responses	Height (in cm)	Remarks

RESULTS AND DISCUSSION:

VIVA QUESTIONS

Q.1. Define Grade Response Biological assays?

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Q.2. Discuss physiological effects of histamine.

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Q.3. Discuss different types of histaminic receptors.

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Q.4. How histamine produce contraction in intestinal smooth muscle.

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Experiment No. 6

AIM:

To study the analgesic effect of morphine in mice using tail-flick method.

REFERENCE:

"Hand Book of Experimental Pharmacology" by S.K. Kulkarni, Ninth Edition 2007, Published by Vallabh Prakashan, New Delhi, Page No. 123-125.

REQUIREMENTS:

Animal- mice 20-25 g, drug- morphine sulphate dose- 5 mg/kg s.c., equipment- Analgesiometer.

THEORY:

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

Painful reaction in experimental animals can be produced by applying noxious (unpleasant) stimuli such as (i) thermal (radiant heat as a source of pain), (ii) chemical (irritants such as acetic acid and bradykinin) and (iii) physical pressure (tail compression). In the laboratory, commonly used procedures are tail-flick (tail-withdrawal from the radiant heat) method using analgesiometer, hot plate (jumping from the hot plate at 55^{o}c) method and acetic acid-induced writhing.

PROCEDURE:

- Weigh and number the mice.
- Take basal reaction time to radiant heat by placing the tip (last 1-2 cm) of the tail on the radiant heat source. The tail-withdrawal from the heat (flicking response) is taken as the end point. Normally a mouse withdraws its tail within 3-5 sec. A cut off period of 10-12 sec is observed to prevent damage to the tail. Any animal failing to withdraw its tail in 3-5 sec is rejected from the study. Take at least 3-5 basal reaction times for each mouse at a gap of 5 min to confirm normal behaviour of the animal.
- Inject morphine and note the reaction time at 5, 15, 30 and 60 min after the drug. As the reaction time reaches 10 sec it is considered maximum analgesia and the tail is removed from the source of heat to avoid tissue damage.
- Calculate percentage increase in reaction time (index of analgesia) at each time interval.

OBSERVATION:

S.No.	Basal reaction time (sec)	Reaction time (sec) after Morphine administration	% decrease

RESULTS AND DISCUSSION:

VIVA QUESTIONS

Q.1. Discuss mechanism action of morphine.

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Q.2. Define opioid analgesic and enumerate them.

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Q.3. How morphine produce analgesia defers from nor-narcotic drugs.

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Q.4. What are the adverse effects of morphine?

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Experiment No. 7

AIM:

To record the concentration response curve of histamine and its modification by an anti histaminic using guinea pig ileum preparation.

REFFERENCE:

"Hand Book of Experimental Pharmacology" by S.K. Kulkarni, Ninth Edition 2007, Published by Vallabh Prakashan, New Delhi, Page No. 92-94.

REQUIREMENTS:

Animal: Guinea pig 400-600 g, **Drugs:** Histamine stock solution 1mg/ml, mepyramine stock solution 1mg/ml, **Physiological salt solution**: Tyrode

THEORY:

Histamine is an autocoid having profound physiological effect in the body. Beside the triple response caused by it, histamine has spasmogenic response on intestinal smooth muscle. By acting on H1-histaminic receptors it causes the contraction of intestinal smooth muscle. Guinea pig is highly sensitive to histamine and guinea pig ileum preparation is very commonly used for isolated tissue work. Mepyramine is a selective H1-histamine receptor antagonist.

PROCEDURE:

- The overnight fasted guinea pig is sacrificed by a blow on the head and carotid bleeding.
- Cut open the abdomen and lift the caecum to trace the ileocaecal junction. Cut and remove a few cm. long of the ileac portion and immediately place it in the watch glass containing Tyrode solution. Trim the messentary and with gentle care clean the contents of the ileum by pushing the tyrode solution into the lumen of the ileum utmost care should be taken to avoid any damage to the gut muscle. Cut the ileum into small segments of 3 cm long.
- Take 1 piece of the ileum of 2-3 cm and tie the thread to top and the bottom ends without closing the lumen and mount the tissue in the organ bath containing tyrode solution maintained at 32^{o}c-35^{o}c and bubbled with oxygen air. A tension of 0.5g is created or applied and the tissue is allowed to equilibrate for 30 minutes before adding drugs to the organ bath.
- Record the concentration dependent responses due to histamine using frontal writing lever. Contact time of 30 sec 5 min time cycle are kept for proper recording of the responses.
- Record at least 4 concentration dependent responses due to histamine.
- Add mepyramine to the reservoir containing tyrode solution and irrigate the tissue with mepyramine containing tyrode solution for 30 min.
- Repeat the concentration response curve of histamine in the presence of mepyramine.

- Label and fix the tracing and plot a graph done in the earlier experiment.
- Calculate the EC50 and note the nature of antagonism.

Dose ratio = $\frac{\text{EC50 after mepyramine}}{\text{EC50 before mepyramine}}$

OBSERVATION:

RESULTS AND DISCUSSION:

VIVA QUESTIONS

Q.1. Discuss in brief histaminic receptors.

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Q.2. Write note on guinea pig as experimental animal.

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Q.3. Give adverse effects of H1-anti histaminic.

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Experiment no. 8

AIM:

To calculate pA2 value for atropine using acetylcholine as agonist employing guinea pig ileum preparations.

REFFERENCE:

"Hand Book of Experimental Pharmacology" by S.K. Kulkarni, Ninth Edition 2007, Published by Vallabh Prakashan, New Delhi, Page No. 95-97.

REQUIREMENTS:

Animal: Guinea pig 400-600 g, **Drugs**: Acetylcholine stock solution 1mg/ml, Atropine stock solution 1mg/ml, **Physiological salt solution**: Tyrode

THEORY:

pAx value is calculated to compare the potency of antagonists acting on the same receptor. The pAx value is defined as the negative logarithm of the molar concentration of the antagonist required to reduce the effect of a multiple dose (x) of the agonist to that of a single dose in the absence of antagonist. Higher the pAx value, more potent is antagonist. The determination of pA2 (x=2) and pA10 (x=10) values have wider applications. If the difference between these two values is found to be 0.95 or very near, the antagonism is likely to be of competitive type. An antagonist acting on the same receptor will have same pA2 value in all the tissue or organ preparations.

PROCEDURE:

- The overnight fasted guinea pig is sacrificed by a blow on the head and carotid bleeding.
- Cut open the abdomen and lift the caecum to trace the ileocaecal junction. Cut and remove a few cm. long of the ileac portion and immediately place it in the watch glass containing Tyrode solution. Trim the messentary and with gentle care clean the contents of the ileum by pushing the tyrode solution into the lumen of the ileum utmost care should be taken to avoid any damage to the gut muscle. Cut the ileum into small segments of 3 cm long.
- Take 1 piece of the ileum of 2-3 cm and tie the thread to top and the bottom ends without closing the lumen and mount the tissue in the organ bath containing tyrode solution maintained at 32^{o}c-35^{o}c and bubbled with oxygen air. A tension of 0.5g is created or applied and the tissue is allowed to equilibrate for 30 minutes before adding drugs to the organ bath..
- Record concentration dependent concentrations due to acetylcholine until a peak response is obtained.
- Select two dose bearing 1:2 ratio and elucidating sub maximal responses (A, 2A) for pA2 determination.
- Standardise the tissue with the selected doses of acetylcholine a tissue is said to be standardize when it responds identically to the same dose of an antagonist wehen repeated.

- Record the concentration Due to the double doses of ach (2A) in the presence of varying conc. (B1, B2, B3) of atropine.
- Consider the response due to double dose of Ach i.e before adding atropine as 100% response.
- Plot a graph representing negative log of the molar concentration of an atropine employed along x-axis and % responses along y-axis.
- Read out the pA2 value for atropine from the graph directly. It corresponds to the % responses obtained with half the dose of ach.

OBSERVATION:

pA2 values of Atropine and Mepyramine in different tissue preparations

Tissue	Atropine- acetylcholine	Mepyramine- histamine
Guinea-pig ileum		
Guinea-pig trachea		
Guinea-pig lung (perfused)		

RESULT AND DISCUSSION:

VIVA QUESTIONS

Q.1. What is concentration response curve?

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Q.2. Give physiological effects of acetylcholine.

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Q.3. Write note on choline esterase inhibitors.

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Experiment No. 9

AIM:

To record a concentration-response curve of acetylcholine using recuts abdominis muscle preparation of frog.

REFERENCE:

Kulkarni S.K "Hand book of Experimental Pharmacology", Vallabh Prakashan Ninths edition Page No. 97

REQUIREMENTS:

Animal- Frog, Drugs- Acetylcholine stock solution (1 mg/ml), Frog Ringer solution

THEORY:

Dose (concentration)-response curves demonstrate graded responses to drugs or agonists where an increase in response is recorded with a subsequent increase in the dose or the concentration of the drug. The dose-response curve is sigmoid or S-shaped. The first part (25% of graph) of the curve has poor discrimination between the doses whereas the middle portion of the curve shows greater sensitivity to different concentrations, and the responses to increasing concentrations are linearly differentiated. The last part of the curve (plateau) shows the ceiling effect where no more increase in the response is seen with further increase in the dose.

Sometimes cumulative dose-response curve are employed for the study. The cumulative dose-response curves is obtained by increasing the concentration of the drug in the organ bath step by step without washing the preceding doses. This technique is simple and less time consuming. It is generally employed in those preparations where the tissue is slowly contracting and slowly relaxing. However, this method is not suited for drugs which show fade phenomenon.

When the doses are increased in geometric progression (logarithmic intervals) and the response is plotted against logarithms of doses, the relationship is called log dose-response curve. The logarithmic transformation of doses offer some advantage such as (1) the linear portion of the sigmoid curve becomes more straight; (2) comparison of two dose-response curves is much simpler; (3) large dose ranges can be plotted which is otherwise difficult in dose-response curves; and (4) the error is distributed all through the graph, independent of the dose.

The study of concentration or dose- response curve indicates: (1) relative potency of the drug or agonist, when the curve is more towards the left, it indicates that the drug is more potent. The vice-versa is also true, and (2) slope of the curve indicates error and reliability (precision) of the bioassay. Steeper the slope more precise is the assay and vice versa is also true.

PROCEDURE:

- Pith or stun the frog and lay it on its back on the frog- dissecting board.
- Pin the four limbs and remove the skin on the abdomen and expose the rectus abdominis muscle.

- Cut and prepare two rectus muscle preparations from each frog. Tie a thread to the top and bottom of each muscle preparation before detaching the muscle from the body of the frog.
- Mount the preparation in up-right position in the organ bath containing, frog Ringer solution under a tension of 1 g. There is no need of maintaining the bath temperature since it is an amphibian tissue preparation. Bubble the organ bath with air.
- Relax the tissue for 45 min, during which period wash the tissue with fresh quantum of Ringer for at least four times.
- Record the contractions due to acetylcholine using either simple sideway or frontal writing lever. Ninety second contact time and a total 5 min time cycle may be used for proper recording of the responses.
- Record at least four responses to increasing doses of acetylcholine or till you get the maximum response. The maximum response is achieved if one gets same or slightly less response with a higher concentration. Properly label the graph and fix the tracing with the help of fixing solution.
- Measure the height of the response (mm) and draw a dose (concentration)-response graph. Describe the shape and various parts of the graph.

OBSERVATION:

RESULT AND DISCUSSION:

VIVA QUESTION

Q.1. What is the Dose (concentration)-response curves?

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Q.2. What is the cumulative dose-response curve?

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Q.3. What is the composition of frog ringer solution?

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Experiment No. 10

AIM:

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

REFERENCE:

Kulkarni S.K "Hand book of Experimental Pharmacology" , Vallabh Prakashan Ninths edition Page No. 128

REQUIREMENTS:

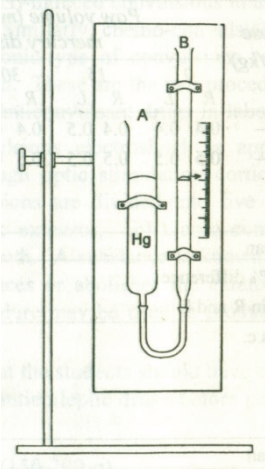
Rats (150-200 g), Carrageenan (Prepare 1% w/v solution and inject 0.1 ml underneath the plantar region), Indomethacin (Dose 20 mg/kg, s.c., ml/100 g of body weight of the animal), Plethysmograph

THEORY:

Inflammation is a tissue-reaction to infection, irritation or foreign substance. It is a part of the host defence mechanism but when it becomes great it is a hopeless condition. Aging is also considered to be an inflammatory response. There are serveral tissue factors or mechanisms that are known to be involved in the inflammatory reactions such as release of histamine, bradykinin and prostaglandins. The development of non-sectoridal anti-inflammatory agents in recent years has contributed a lot in not only overcoming the human suffering such as arthritis but also has helped in understanding the tissue mechanisms of inflammation.

The inflammatory reaction is readily produced in rats in the form of paw oedema with the help of irritants. Substances such as carrageenan, formalin, bradykinin, histamine, 5-hydroxytryptamine, mustard or egg white when injected in the dorsum of the foot of rats they produce acute paw oedema within a few minutes of the injection. Carrageenan-induced paw oedema is the most commonly used method in experimental animals. Carrageenan is a sulphated polysaccharide obtained from sea weed (Rhodophyceae) and by causing the release of histamine, 5-HT, bradykinin and prostaglandins it inflammation and oedema.

Plethysmograph: It is a simple apparatus containing mercury. The mercury displacement due to dipping of the paw can be directly read from scale attached to the mercury column or adjusting the mercury level in the arm B to the original level by moving arm B up/down and noting the volume required to bring the level in both the arms equal.



Pletsysmograph

PROCEDURE:

- Weigh the animals and number them.
- Make a mark on both the hind paws (right and left) just beyond tibio-tarsal junction, so that every time the paw is dipped in the mercury column up to the fixed mark to ensure constant paw volume.
- Note the initial paw volume (both right and left) of each rat mercury displacement method.
- Divide the animals into two groups each comprising of at least 4 rats. To one group inject saline and to the second group inject indomethacin subcutaneously.
- After 30 min inject 0.1 ml of 1% (w/v) carrageenan in the plantar region of the left paw of control as well as indomethacin treated group. The right paw will serve as reference with inflammed paw for comparison.
- Note the paw volume of both legs of control and indomethacin treated rats at 15, 30, 60 and 120 min after carrageenan challenge.
- Calculate the percent difference in the right and left paw volumes of each animal of control and indomethacin-treated group. Compare the mean percent change in paw volume in control and drug-treated animals and express as percent oedema inhibition by the drug.

Observations:

Anti-inflammatory Action of Indomethacin in Carrageenan-induced Rat Paw Oedema.

S.No.	Body wt. (g)	Treatment	Dose (mg/kg)	Paw volume (ml) as measured by mercury displacement at									
				0		15		30		60		120	
				R	L	R	L	R	L	R	L	R	L
1.	150	Control	--										
2.	155	Control	--										
3.		Control											
4.		Control											
5.			Mean										
			% difference in R and L										
	Indomethacin 20, s.c.												
1.													
2.													
3.													
4.													
			Mean										
			% difference in R and L										

R= Right paw.*L= inject 0.1 ml of 1% (w/v) carrageenan 30 min after indomethacin to the left paw by sub-plantar route.

Calculate % odema inhibition at different time intervals.

RESULT AND DISCUSSION:

VIVA QUESTIONS

Q.1. What is the Plethysmograph?

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Q.2. Explain autacoids.

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Q.3. What is the mechanism of indomethacin?

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Q.4. Write the name of irritant drug which produces paw oedema.

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Q.5. What was the onset of action and peak effect seen?

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Experiment No. 11

AIM:

Determine the strength of given sample of acetylcholine by three point bioassay method using isolated organ preparation rat ileum/rat duodenum/rat colon.

REFERENCE:

"Principles of Pharmacology" by H.L. Sharma and K.K. Sharma, First Edition 2007, Published by Paras Medical Publisher, Hyderabad, Page No. 84-89.

"Hand Book of Experimental Pharmacology" by S.K. Kulkarni, Ninth Edition 2007, Published by Vallabh Prakashan, New Delhi, Page No. 106-107.

REQUIREMENTS:

Apparatus- Student's Organ Bath, Sherrington Rotating Drum, Animal- Rats 150-200g, Drugs- Acetylcholine stock solution 1mg/ml, Physiological salt solution- Tyrode solution

THEORY:

Multiple point bioassays are type of graded response bioassays. Which are based on the dose-response relationship, includes- three points, four point and six point bioassay methods? In multiple point bioassays, the responses are repeated several times and the mean of each is taken. Thus the chances of error are minimized in these methods.

In three point bioassay two dose of standard and one dose of test are used for dose-response curve. The sequence of responses is followed as per the Latin Square method of randomization in order to avoid any bias. The mean responses are calculated and plotted against log-dose and amount of standard producing the same response as produced by the test is determined graphically as well as mathematically.

Acetylcholine is major neurotransmitter in our body and having various physiological effects. In smooth muscles of G.I.T like ileum, duodenum, colon, acetylcholine produces contractions in dose dependant manner. This is because presence of cholinergic muscuranic receptors in smooth muscle of G.I.T.

PROCEDURE:

- The rat is sacrificed by cervical dislocation.
- Cut open the abdomen and lift the caecum to trace the ileocaecal junction. Cut and remove a few cm. long of the ileum portion and immediately place it in the watch glass containing Tyrode solution.
- Trim the mesentery and with gentle care clean the contents of the ileum by pushing the tyrode solution into the lumen of the ileum utmost care should be taken to avoid any damage to the gut muscle.
- Cut the ileum into small segments of 3 cm long.
- Take 1 piece of the ileum of 2-3 cm and tie the thread to top and the bottom ends without closing the lumen and mount the tissue in the organ bath containing tyrode solution maintained at 32^{o}c-35^{o}c and

bubbled with oxygen air.

- A tension of 0.5g is created or applied and the tissue is allowed to equilibrate for 30 minutes before adding drugs to the organ bath.
- Record the concentration dependent responses due to acetylcholine using frontal writing lever. Contact time of 30 sec 5 min time cycle are kept for proper recording of the responses.
- Record until peak or sub saturated, concentration dependent responses is obtained.
- A log-dose response (LDR) curve is plotted with varying concentrations of the standard acetylcholine solution. The doses are plotted in log scale (on X-axis).
- Two such doses s1 and s2 are then selected whose responses S1 and S2 lie in between linear part of this LDR curve. Care should be observed in selecting these doses so that their concentration is in simple ratio (e.g. two as in 1:2 or 2:4). Since its log value has to be used in the calculations.
- Subsequently, a dose t of the test sample, which produces a response T in between S1 and S2 is found.
- Bioassay is then performed in four sets using three doses s1,s2 and t, in a randomized fashion, in each set e.g. using- (s1,s2,t) (t,s1,s2) (s2,s1,t) (t,s2,s1).
- The result have been arbitrarily tabulated and mathematically computed to find out the strength of the test solution.

OBSERVATION TABLE:

Responses	Height of conc. (mm)	Mean Height (mm)	Dose (ml)	Dose ratio (d)
S1				
S2				
T				

CALCULATION:

From these data, log potency ratio M is calculated from the following formula-

M = T-S1/S2-S1 X log d where d is dose ratio.

From M, the strength of unknown can be calculated as follows-

Strength of test solution = s1/t X antilog of M

RESULT & DISCUSSION:

VIVA QUESTIONS

Q.1. Define Biological assays?

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Q.2. Enumerate different biological assays.

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Q.3. Write note on acetylcholine as neurotransmitter.

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Q.4. Discuss cholinergic receptors in brief.

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Q.5. Give purpose of physiological salt solution used in bioassays.

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Experiment No. 12

AIM:

Determine the strength of given sample of acetylcholine by four point bioassay method using isolated organ preparation rat ileum/rat duodenum/rat colon.

REFERENCE:

"Principles of Pharmacology" by H.L. Sharma and K.K. Sharma, First Edition 2007, Published by Paras Medical Publisher, Hyderabad, Page No. 84-89.

"Hand Book of Experimental Pharmacology" by S.K. Kulkarni, Ninth Edition 2007, Published by Vallabh Prakashan, New Delhi, Page No. 106-107.

REQUIREMENTS:

Apparatus- Student's Organ Bath, Sherrington Rotating Drum, Animal- Rats 150-200 g, overnight fasted, Drugs- Acetylcholine stock solution 1mg/ml, Physiological salt solution- Tyrode solution

THEORY:

Multiple point bioassays are type of graded response bioassays. Which are based on the dose-response relationship, includes- three points, four point and six point bioassay methods? In multiple point bioassays, the responses are repeated several times and the mean of each is taken. Thus the chances of error are minimized in these methods.

In four point bioassay two dose of standard and two doses of test are used for dose-response curve. The sequence of responses is followed as per the Latin Square method of randomization in order to avoid any bias. The mean responses are calculated and plotted against log-dose and amount of standard producing the same response as produced by the test is determined graphically as well as mathematically.

Acetylcholine is major neurotransmitter in our body and having various physiological effects. In smooth muscles of G.I.T. like ileum, duodenum, colon, acetylcholine produces contractions in dose dependant manner. This is because presence of cholinergic muscuranic receptors in smooth muscle of G.I.T.

PROCEDURE:

- The rat is sacrificed by cervical dislocation.
- Cut open the abdomen and lift the caecum to trace the ileocaecal junction. Cut and remove a few cm. long of the ileum portion and immediately place it in the watch glass containing Tyrode solution.
- Trim the mesentery and with gentle care clean the contents of the ileum by pushing the tyrode solution into the lumen of the ileum utmost care should be taken to avoid any damage to the gut muscle.
- Cut the ileum into small segments of 3 cm long.
- Take 1 piece of the ileum of 2-3 cm and tie the thread to top and the bottom ends without closing the

lumen and mount the tissue in the organ bath containing tyrode solution maintained at 32°c-35°c and bubbled with oxygen air.

- A tension of 0.5g is created or applied and the tissue is allowed to equilibrate for 30 minutes before adding drugs to the organ bath.
- Record the concentration dependent responses due to acetylcholine using frontal writing lever. Contact time of 30 sec 5 min time cycle are kept for proper recording of the responses.
- Record until peak or sub saturated, concentration dependent responses is obtained.
- Constructed log-dose response (LDR) curve for both, the standard and the test solution. The doses are plotted in log scale (on X-axis).
- Two such doses s1 and s2 and two such doses of the test solution t1 and t2 are then selected whose respected responses S1,S2 and T1T2 lie in between linear part of their LDR curve, respectively.
- In selecting these doses care should be taken so that the responses due to the lower doses of the standard S1 and the test solution T1 and due to the higher doses of the standard S2 and the test T2 should be similar amongst themselves.
- Furthermore, the ratio between the higher and the lower doses of the standard i.e. s2/s1 and the test t2/t1 should not only be the same but simpler too (i.e. s2/s1= t2/t1= 2, as in 1:2 or 2:4 ratio) because their log values are to be used in calculation.
- Bioassay is then performed in four sets using four doses s1,s2 and t1,t2 in a randomized fashion, in each set i.e. using- (s1,s2,t1,t2) (s2,t1,t2,s1) (t1,t2,s1,s2) (t2,s1,s2,t1).
- The result have been arbitrarily tabulated and mathematically computed to find out the strength of the test solution.

OBSERVATION TABLE:

Responses	Height of conc. (mm)	Mean Height (mm)	Dose (ml)	Dose ratio (d)
S1				
S2				
T1				
T2				

CALCULATION:

Note that the log dose ratio d of t1 and t2 and s1 and s2 are same. Lower doses, s1 and t1 are providing similar response. The same is true for higher dose s2 and t2.

From these data, log potency ratio M is calculated from the following formula-

M = T1-S1+T2-S2/S2-S1+T2-T1 X log d where d is dose ratio.

From M, the strength of unknown can be calculated as follows-

Strength of test solution = s1/t1 X antilog of M

RESULT & DISCUSSION:

VIVA QUESTIONS

Q.1. Define multiple point biological assays?

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Q.2. Give composition of Tyrode solution.

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Q.3. Discuss effect of acetylcholine on smooth muscle.

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Q.4. Write note on rat as experimental animal.

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Q.5. Give principles of bioassays.

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Experiment No. 13

AIM:

To record the concentration response curve of Oxytocin using rat uterus preparation.

REFERENCE:

"Hand Book of Experimental Pharmacology" by S.K. Kulkarni, Ninth Edition 2007, Published by Vallabh Prakashan, New Delhi, Page No. 107-108.

REQUIREMENTS:

Apparatus- Student's Organ Bath, Sherrington Rotating Drum, Animal- Female rat 120-150 g, Drugs- Oxytocin, stilboestrol, Physiological salt solution- De Jalon solution

THEORY:

Oxytocin is a hormone secreted by the posterior pituitary. The rat uterine preparation is commonly used for the bioassay of Oxytocin. The sensitivity of the uterus is depends upon the oestrus cycles. The various stages of oestrus cycle can be identified by preparing the vaginal smears and observing under microscope.

An adult female rat has an oestrus cycle of 5 days. The oestrus cycle can be divided into different stages:

i) Dioestrus, ii) Oestrus, iii) Frank oestrus and iv) Meta or late oestrus.

Frank oestrus uterus is highly sensitive to Oxytocin and hence preferred for the bioassay.

PROCEDURE:

- Examine the vaginal smear under microscope to know about the proper stage of oestrus cycle. If the rat is not in frank oestrus, injected 0.1 mg/kg of stiloestrol and wait for 24 hrs.
- Vaginal smear is prepared by taking a drop of the vaginal wash and putting on the slide glass to confirm oestrus cycle.
- Sacrifice the animal by a cervical dislocation and cut open the pelvic region and expose both the horns of uterus.
- Separate them gently from the surrounding fatty material and transfer them to a dish containing De Jalon's solution.
- When the rat is in oestrus generally the uterus is fleshy and pink in color. The two separate pieces 2-3 cm long of uterine preparations can be made for experimental use.
- Mount the uterine preparation in the organ bath containing De Jalon at 30-32^{o}c.

- Apply a tension of 0.5 g and allow the tissue to equilibrate for 30 min.
- Record contractions due to different concentrations of Oxytocin using frontal writing lever.
- Contract time of 30 seconds and 3 min time cycle is used for proper recording of the responses.
- Record at least four responses due to different concentrations of Oxytocin.
- Label and fix the tracing and draw the concentration-response curve.

RESULT & DISCUSSION:

VIVA QUESTIONS

Q.1. Define oestrus cycle? Enumerate different stages of oestrus cycle.

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Q.2. Discuss how Oxytocin causes contraction in uterine muscle.

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Q.3. What are ecbolics or oxytocics?

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Q.4. Discuss physiological effects of Oxytocin in brief.

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Q.5. Define uterine relaxants with examples?

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Experiment No.14

AIM:

To record the concentration response curve of 5-hydroxy tryptamine (serotonin) using rat fundus strip preparation.

REFERENCE:

"Hand Book of Experimental Pharmacology" by S.K. Kulkarni, Ninth Edition 2007, Published by Vallabh Prakashan, New Delhi, Page No. 101-102.

REQUIREMENTS:

Animal- Rats 150-200 g overnight fasted, Drugs- 5- hydroxytryptamine stock solution 10 micro g/ml, Physiological salt solution- Krebs solution

THEORY:

Rat fundus is a very sensitive tissue for the study of the action of several naturally occurring substances like 5-hydroxytryptamine, histamine, acetylcholine and bradykinin. Unlike the intestinal smooth muscle (ileum) this preparation is slow contracting and slow relaxing type. Rat fundus is generally employed for the bioassay of serotonin. The fundus is grey in colour and pyloric part pink in colour. A zig-zag preparation of the fundus strip is prepared so as to expose maximum portion of the tissue to drug.

PROCEDURE:

- Sacrifice the animal (rat) by a blow on the head and carotid bleeding.
- Cut and open the abdomen and expose the stomach.
- Identify the fundus of the stomach incise it from the junction of pyloric part b& put it in the dish containing krebs solution.
- Incise the fundus from the lesser curvature and open it longitudinally. Give alternate zig zag cuts to make a fundal strip preparation. Tie on both the ends with the thread and mount in the organ bath containing Krebs solution at 37^{o}c then aerate the tissues.
- Apply 1g load and allow the preparation to equilibrate for 30 minutes. Using frontal writing lever with 10-12 magnifications record the contractions due to increasing concentrations of serotonin. Since the muscle contracts slowly and relaxes slowly a contact time of 90 sec, and 5 min time cycle are followed for the proper recording of concentration response curve.
- Label & fix the tracings.

OBSERVATION:

RESULT AND DISCUSSION:

VIVA QUESTIONS

Q.1. Discuss effects of serotonin.

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Q.2. Write in brief about 5-HT receptors.

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Q.3. How serotonin cause contraction in smooth muscle.

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Experiment No. 15

AIM:

To study the analgesic effect of the morphine in mice using hot plate method.

REFERENCE:

"Hand Book of Experimental Pharmacology" by S.K. Kulkarni, Ninth Edition 2007, Published by Vallabh Prakashan, New Delhi, Page No. 125-126.

REQUIREMENTS:

Animals- Mice 20-25 g**,** Drugs- Morphine sulphate, dose- 5 mg/kg s.c. and prepare a stock solution 0.5 mg/ml**,** Equipment- Eddy's hot plate

THEORY:

In this method heat is used as a source of pain. Animals are individually placed on a hot plate maintained at constant temperature (55^0C) and the reaction of animals, such as paw licking or jump response is taken as the end point. Analgesics increase the reaction-time. The method was first described by Eddy and Leimbach (1953).

PROCEDURE:

- Weigh and no. the mice.
- Take the basal reaction time by thus observing hind paw licking or jump responses in animals when placed on the hot plate method maintained at the constant temperature (55^oc). Normally animals show responses in 6-8 seconds.
- A cut off period of 15 sec. is observed to avoid damage to the paws.
- Inject morphine to animals and then note the reaction time of the animals on the hot plate at 15, 30, 60 and 120 min. after the drug administration, as the reaction time increases with the morphine, 15 sec is taken as maximum analgesia and the animals removed from the hot plate to avoid injury to the paws.
- Calculate % increase in the reaction time at each interval of time.

OBSERVATION:

RESULT AND DISCUSSION:

VIVA QUESTIONS

Q.1. How morphine cause analgesia.

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Q.2. Discuss CNS effects of morphine.

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Q.3. Discuss adverse effects of morphine.

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