FACULTY OF PHARMACY

Pharmacology and Toxicology Department

Fourth Year

Pharmacology II

Laboratory Course

Semester 1

Course Organisers

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First trimester

List of Contents

Lab. No. 1

Handling and restraint of laboratory animals

The pharmacological experiments will be mostly done on laboratory animals (mice, rats, & rabbits), so the animals should be treated well, as improper handling and treatment of animals may lead to failure to observe the effects, and will give rise to bad results. The steps for proper handling of animals we need to notice the followings:-

I- General

1- Health: healthy or sick animal
2- Activity: hyperactive, irritable, aggressive (CNS stimulants e.g. strychnine) / hypoactive (e.g. sedatives) / normally: mouse is hyperactive, rat is aggressive, and rabbit is quite.
3- Gait: ataxic or drunken gait (e.g. alcohol) / normal.
4- Erection of tail: in rats due to stimulation of spinal cord by narcotics (e.g. morphine) called straub phenomenon.
5- Righting reflex: placing the animal on the lateral side, the animal will retain its normal position. Decreased by CNS depressants (e.g. diazepam) and increased by CNS stimulants (e.g. strychnine)
6- Pain reflex: reflex to painful stimulation by twisting the patella in rabbit or pinching the tail by forceps in rats & mice. (Decreased by narcotics & non-narcotics).

II- Vital signs

Each reading should be taken several times by different persons, ignore the odd one and take the mean of remaining readings:

- Respiratory rate (abdominal breathing / nostrils)
- heart rate (flickering)

General Handling Principles:-

1- The use of proper restraint and handling technique reduces stress to animals and also to the researchers.

2- Animals can inflict serious injuries to human and to them selves as a result of improper handling.

3- Acclimation periods of up to one week are recommended for all animals.
4- Most animals, even rodents will respond positively to handling and will learn to recognize individuals.

5-Any person who has bitten or scratched by animal, is at risk of infection and must consult the physician .

6- immunization for tetanus is mandatory for full and part time animal care staff and highly recommended for all other regular animal users..

7- immunization for rabies is recommended only for those animal users likely to come into contact with wild live animals.

8-handle animals gently, do not make loud noises or sudden movements that may startle them.

9- use an assistant whenever is possible .

10- use restraint devices to assist when appropriate.

11- chemical restraint should be considered for any prolonged or potentially painful procedure.

12- Handle animals firmly. The animal will struggle more if it sees a chance to escape.

Handling Methods

The methods described below will assist with performing basic manipulations. Alternate techniques may be needed for special procedures.

MICE

Tail restraint, is adequate for examining animals and transfering them to another cage.as shown in figure(1)

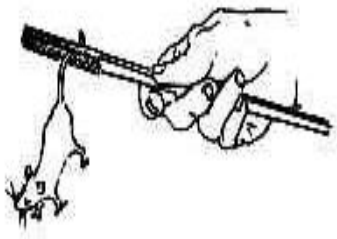


Figure (1) mice tail restraint

The scruff can be grasped between the thumb and forefinger whilst maintaining a grip on the tail. The animal is then secure and can be examined or injected safely as shown in figure 2. These methods may be used to perform minor, non-painful procedures such as injections or ear tagging.

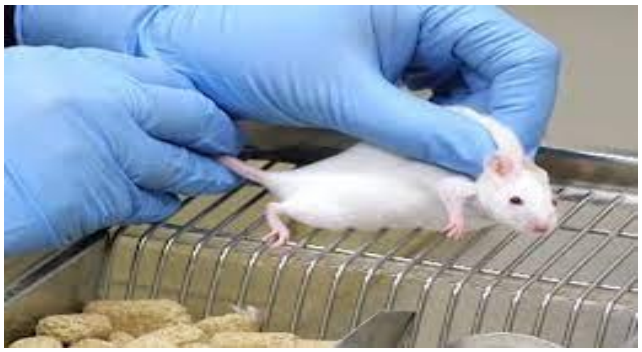


Figure (2) proper mice handling

RATS:-

May be handled by the tail, with precautions similar to those used for mice, with emphasis on only grasping the tail base as shown in figure (3). Holding the tail distal to the base can result in a de-gloving injury to the tail that will require surgical repair or euthanasia.This method should be used to restrain a rat for injections and other minor procedures

Figure (3) rat tail holding

HAMSTERS:-

Because hamsters do not have tails, they must be grasped firmly by the loose skin of its back, or handled in a manner similar to the rabbit as shown in figure (4)

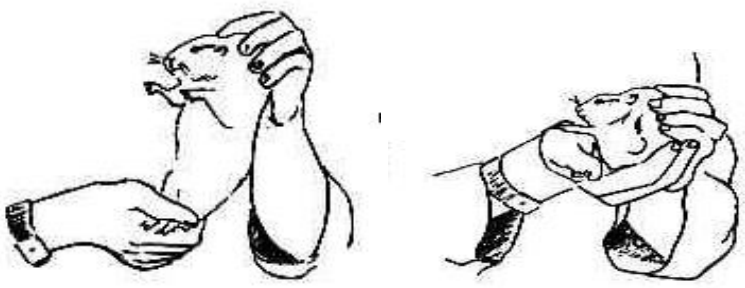


Figure (4) hamster handling

GUINEA PIGS:-

rarely bite, but are very easily frightened and will vocalize and squirm to avoid restraint. The hind limbs must be supported at all times to prevent the animal from injuring its back as shown in figure (5)

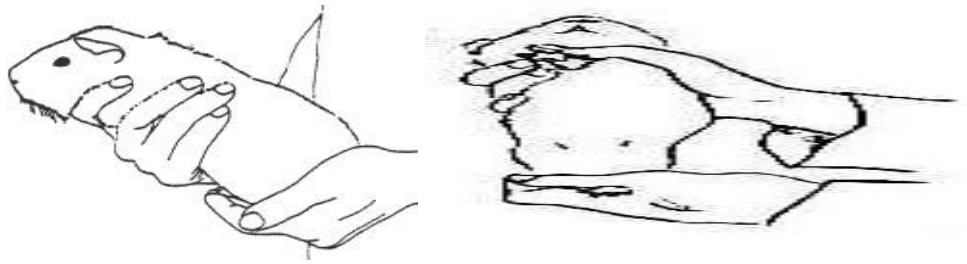


Figure (5) guinea pig proper handling

RABBITS:-

Are very susceptible to lumbar spinal luxation, resulting in paralysis. It is necessary to support the animal's hindquarter at all times as shown in figure (6). Although rabbits seldom bite, they can inflict painful scratches with their hind legs. One way of lifting a rabbit is by grasping the skin over the shoulder with one hand and gently lifting it with the other arm cradling the body, the head nestled in the crook of your arm. Rabbits must never by lifted by the ears.

Figure (6) rabbit proper handling

Restraint Devices:-

Restraint devices such as rabbit or rodent restrainers, are useful for certain non-painful procedures. As shown in figue (7)

However, certain guidelines should be followed when using these devices.

- Animals should be adapted to the restraint devices. This means that for long-term restraint (i.e. more than an hour), it is advisable to "train" the animal to the device by placing it into the device for successively longer intervals until the maximum time of restraint can be achieved without causing distress to the animal.
- Animals in a restraint device be regularly monitored. This means not leaving the area for long intervals unless someone else is available to monitor the animal. Animals have an uncanny ability to attempt escape from devices, if

they don't succeed completely, they may end up with a limb or their head entrapped. This could result in ischemia or hypoxia.

- Animals should have access to food or water at appropriate intervals, even when restrained, unless doing so would interfere with the goals of the experiment. Food or water should be offered twice daily. For rabbits and rodents, water should be offered more frequently.
- Animals should be released from restraint devices at least daily and allowed unrestrained activity to prevent muscle atrophy and skin necrosis.

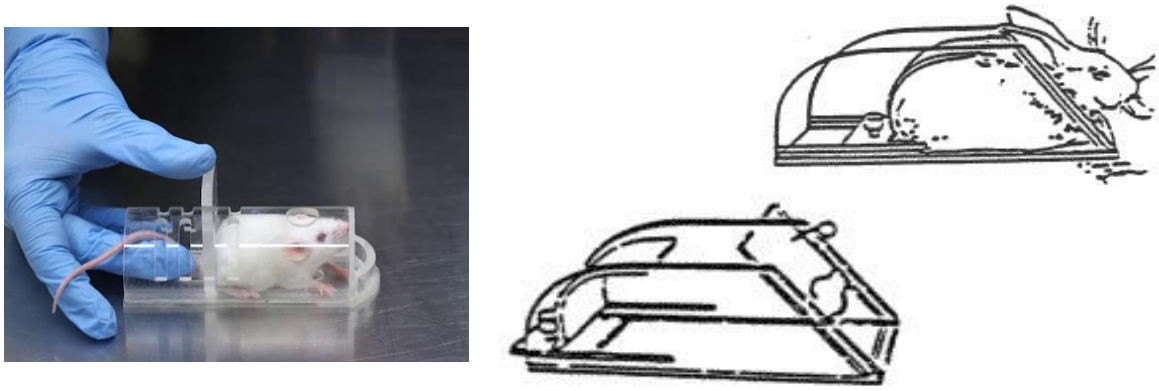
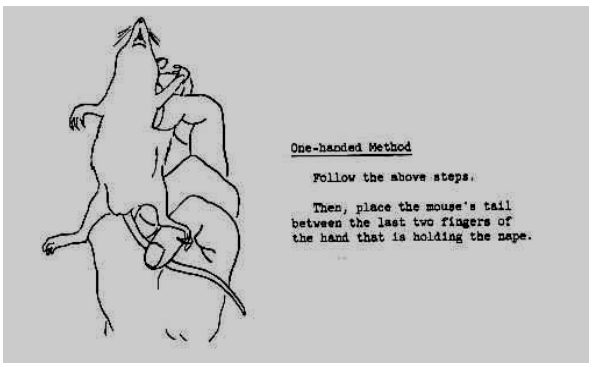


Figure (7) restraint devices

<u>Experiment.</u> training each student on proper handling of animal by two and one hand technique as shown in figure (8) and give them marks on the proper handling .



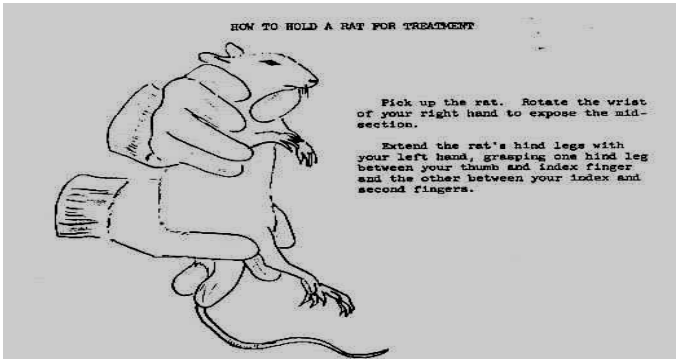


Figure (8) one and two hand handling technique

Lab. No. 2

Routes of drug administration

Definition

Route of drug administration is defined as the mean by which drugs can be delivered into the body.

Factors to be considered when choosing certain route:

1. **Site of drug action**: *e.g., treatment of certain GIT diseases necessitates giving the drug orally.*
2. **Drug nature***: intravenous fluids should be isotonic.*
3. **Onset of action**: *treatment of emergency conditions necessitates the use of intravenous route*.
4. **Duration of action**: *drugs intended for longer duration of action are given by a route when absorption is slow.*
5. **Patient status**: *oral route can not be used when the patient is unconscious, or has difficulty in swallowing, or has repeated vomiting.*
6. **Desire of the patient**.

Routes of administration can be classified as follows: figure (9)

A. Enteral (GIT): oral
B. Parenteral: intramuscular (IM), intravenous (IV), subcutaneous (SC), inhalational.

In the beginning measure the weight of the animals to ensure the precision of dosage of the given drugs by using measuring scale (balance).

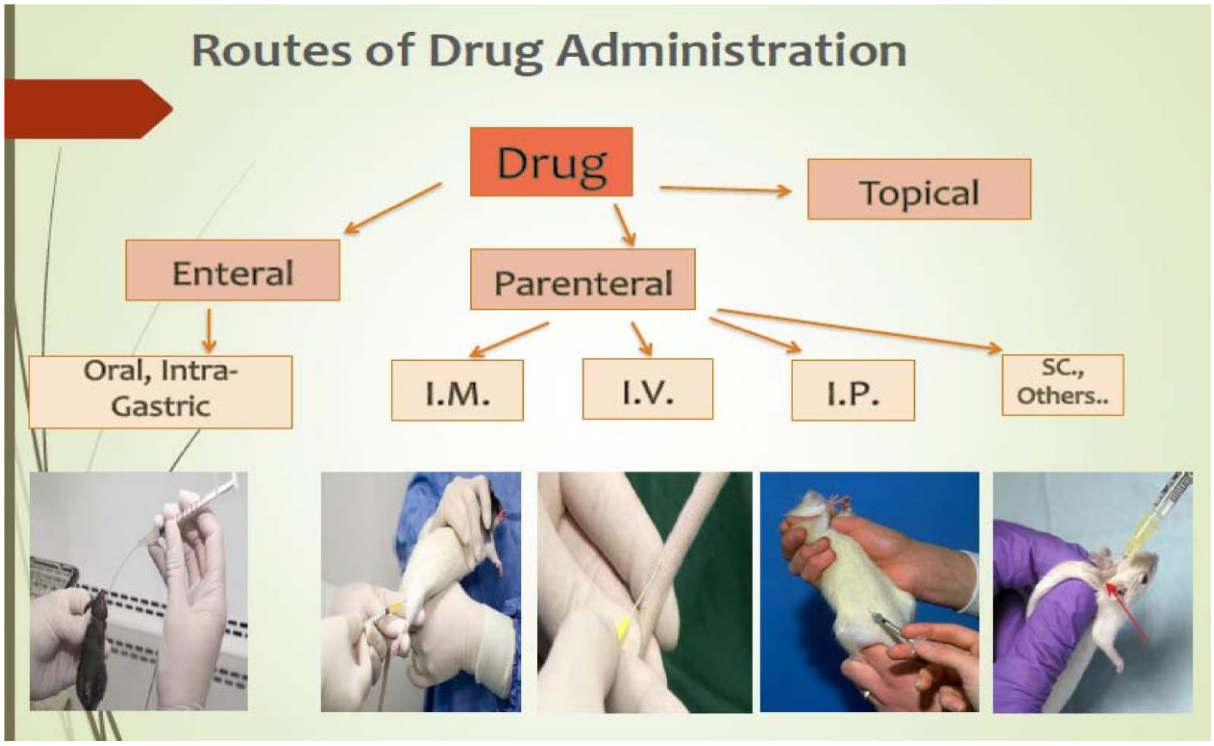
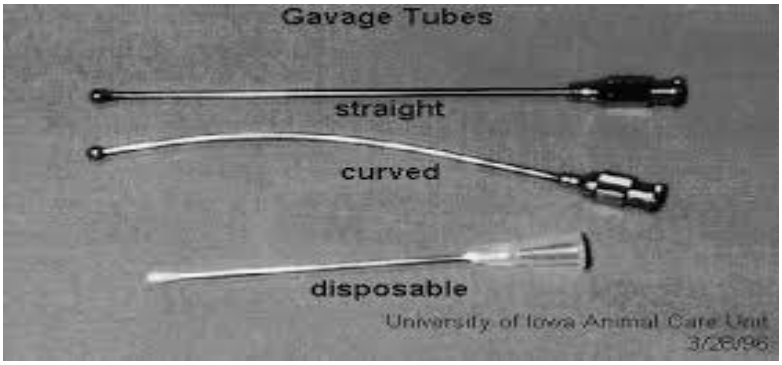


Figure (9) route of drug adminstration

1. Oral route:

By placing mouth gag (needle gavage) into the mouth of rat or rabbit as shown in figure (10). To make sure that the tube is in the esophagus and not in the trachea, dip the end of the tube into a beaker containing water (bubbling indicates wrong position). By using medical syringe, push 2.5 ml of normal saline (N.S) into the stomach through the rubber tube.



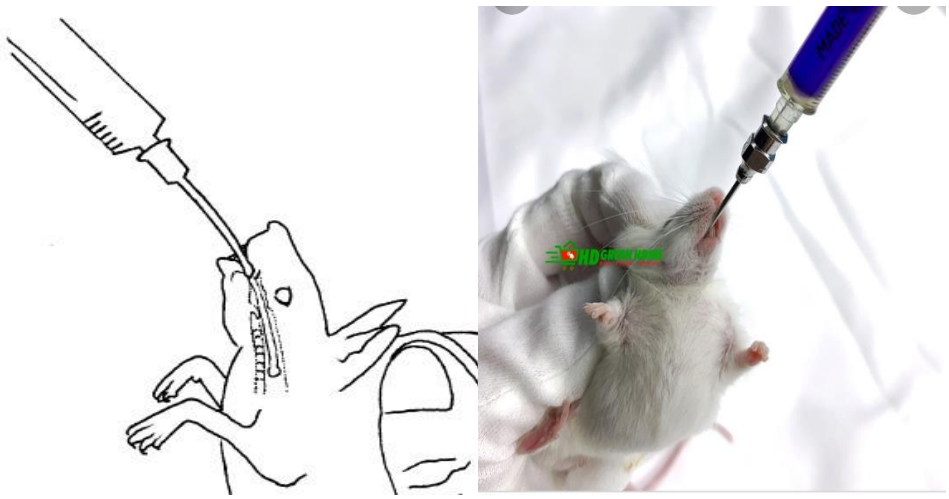


Figure (10) oral gavage

2. Intravenous (IV) route:

- In mice and rat administrations are usually made into **the lateral tail veins** not into the dorsal tail vein , as it is not straight as shown in figure (11). The lateral veins are readily visualized, but have quite small diameters.
- The mouse or rat is either placed in the restrainer or anesthetized and the **tail is then warmed** with a lamp or warm towel, or immersed in warm water (40–45_C^{o}) **in order to dilate the vessels .**
- The tail is swabbed with 70% alcohol on a gauze sponge or swab.
- Insert the needle parallel to the **tail vein** penetrating 2–4 mm into the lumen while keeping the bevel of the needle face upwards .
- The solution is then injected slowly and no resistance should be felt if the solution is properly administered.
- The injection site must be pressed firmly with a swab or fingers to prevent backflow of the administered solution and/or blood . If the same vein must be used several times the first administration should be made as distal as possible
- **The recommended volume is 0.2 ml.**

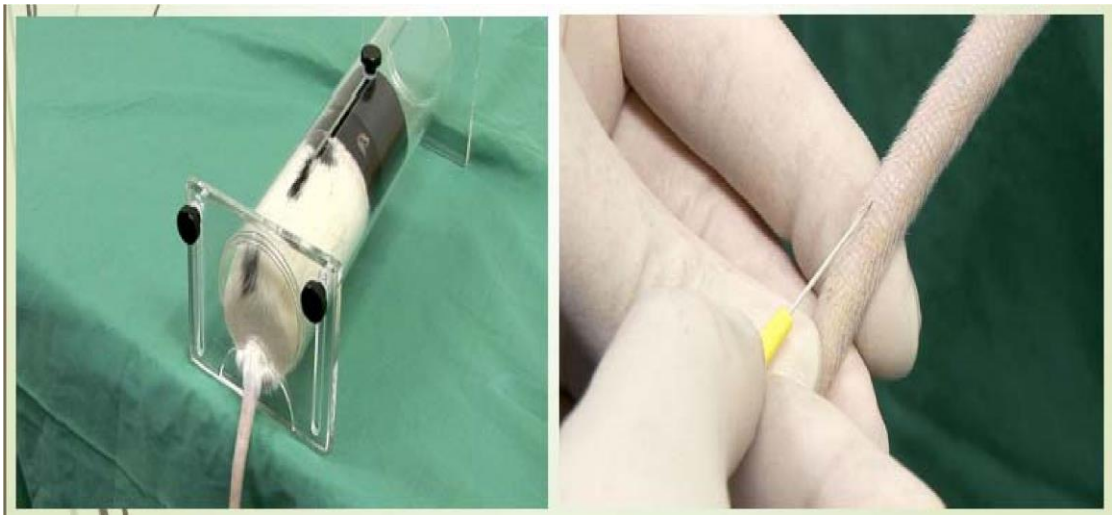


Figure (11) mice or rat I.V route

For rabbit ,shave the hair over the ear of rabbit; rub vigorously the marginal vein with xylol to dilate the vessels, insert butterfly canula beginning from the distal end towards the proximal one. Verify the position of needle and inject (0.02 ml) normal saline by insulin syringe. Drugs should be given slowly. Press cotton onto the wound.

Figure (12) mice or rat I.V route

3. Intramuscular (IM) route:

- Common route, and less affected by peripheral circulatory failure.
- Preparation is about 2 ml, and isotonicity is not essential except for the comfort of the patient.
- Drug can be aqueous or specialized depot preparations.
- Sites of injection: gluteal region in the upper lateral quarter.as shown in figure (12)

Figure (13) mice or rat I.m route

4. Subcutaneous (SC) route:

- Injection (1ml or less).
- Mechanical pumps (insulin).
- The solution to be injected should be aqueous and isotonicity is not essential except for the comfort of the patient.
- Poor absorption in case of peripheral circulatory failure.
- Advantage: reliable and acceptable for self administration.
- Disadvantage: skin reaction.
- Site of injection :loos skin behind ear (the same site of handling) but must suppot on plane surface as shown in figure (13)

Figure (14) mice or rat s.c route

5. Intraperitoneal (I.P) route:

- Relatively large volume of non-irritant drugs.
- Absorption is faster in the peritoneal cavity than IM or SC.

Site of injection

As shown in figure (14)In mice and rats: in the lower half of abdomen.
In rabbits: in the lower left quadrant of abdomen to avoid injuring the liver.
Hold the animal and insert needle at angle of 45 degrees.

Figure (15) mice or rat I.P route

6. Inhalational route:

- The drug is taken through the inspired air (gas, or aerosol).
- Rapid absorption (wide surface area) with rapid effect almost as rapid as IV route.
- Particularly effective for respiratory disorders because the drug is delivered directly at the site of action and systemic S.E. are minimized.

Put a piece of cotton soaked with ether in a closed glass container, place rat or rabbit inside the jar and observe it. Whenever there is a change in behavior, remove the animal.

Experiment:

Animal: mice

Material: insulin syringe, gloves, masks

Train the student about the right route of drug administration by using proper handling technique and applying closed syringe on the proper location and and consider this as daily oral assessment

lab no: -3

Onset and duration of drugs (barbiturates)

Drug dose: Dose is the amount of the active ingredient of a certain dosage form that provides specific effects on an illness or symptoms of an illness.

A maximum dose is the largest dose that can be given to adults without harm.

threshold dose a minimum dose that can provide a real response and the response is called the response threshold.

median effective dose **(ED50):** is the dose of a medication that produces a specific effect in 50% of the population that takes that dose.

Median lethal dose **(LD50):** is the dose that causes death in 50% of individuals

median toxic dose **(TD50):** is toxic dose of 50% of the individuals.

NOTE: Ideal drugs should provide *therapy effects in all patients* without causing toxic effects in a patient.

(PHENOBARBITAL)/LUMINAL

gamma-aminobutyric acid (GABA) : Is the main inhibitory neurotransmitter in develobmentally mature mammalian CNS. Its principal role is reducing neuronal excitability throughout the NS

Phenobarbital (5,5-phenyl-ethyl-barbituric acid) mimics GABA work.

first organic compound used as anticonvulsant.

It acts by restricting the propagation of seizure activity. The main effects of barbiturates are CNS depression.

All levels of depression can be achieved ranging from sedation, hypnosis, various levels of anesthesia, coma, and finally death.

The hypnotic effect of phenobarbital can be reached within 20-60 minutes at a dose of hypnotics

Principle of experiment

Effect of varied doses, route of drug administration, and the individual animal variabilities can be observed following administration of phenobarbital in which level of hypnotic posed is reactive, slow motion, and sleep depends on:

the amount of the dose administered

route of administration

physiologic variations of the experimental animals.

method

Animal:the animals used are mice

Preparation of Na Phenobarbital Solution:

Phenobarbital-Na with concentration of 0.75% is prepared by weighing 0,375 g of Na phenobarbital and dissolved in 50 ml of distilled water.

Experimental Procedure

The experimental animals are weighed and marked

The doses administered are calculated

report

Mouse no.	treatment	Time						note
		10 min.	20	30	40	50		
1	NS per oral route							
2	luminal dose of 80 mg / kg orally							
3	Luminal dose of 80 mg / kg i.p							

Lab.No 4

Absorption and excretion of drugs

This experiment on the excretion of potassium iodide, is an illustration of the considerable variation that exists in the rate of absorption and excretion of drugs when administered orally.

Procedure:-

1- **According to the test below, examine a normal sample of saliva for the presence of iodide. Two students in a group undergo the test.**
2- **One student receive 0.5g of KI in a capsule and another receive 0.5g KI in solution.**
3- **Two samples of saliva are collected every 10 min and 2 samples every 30 min. two samples of urine are collected every 20 min and 2 samples every 30 min. these samples are tested as follow.**

Test :-

Place 4 drops of saliva (or urine) in a test tube. Add 3 drops of 10% sodium nitrate (or 3% hydrogen peroxide), 1 ml of 1% starch solution and 3 drops of 10% sulphuric acid . shake thoroughly and let stand for 30 sec. a purple color indicates the presence of iodide.

Results:- are tabulated as shown below.

Min after	Presence of iodide in	
KI capsule administration	saliva	Urine
10		
20		
30		
40		
50		
60		
70		
80		
90		
100		

Lab. No. 5

General Anesthesia

General anesthesia is essential to surgical practice, because it renders patients analgesic, amnesic, and unconscious, and provides muscle relaxation and suppression of undesirable reflexes. No single drug is capable of achieving these effects both rapidly and safely. Rather, several different categories of drugs are utilized to produce optimal anesthesia. Benefits:- Preanesthetic medication serves to calm the patient, relieve pain, and protect against undesirable effects of the subsequently administered anesthetic or the surgical procedure. Skeletal muscle relaxants facilitate intubation and suppress muscle tone to the degree required for surgery.

Patient Factors in Selection of Anesthesia:-

During the preoperative phase, the anesthesiologist selects drugs that provide a safe and efficient anesthetic regimen based on the nature of the surgical or diagnostic procedure as well as on the patient's physiologic, pathologic, and pharmacologic state.

A. Status of organ systems

Liver and kidney:
Respiratory system:
Cardiovascular system:
Nervous system:
Pregnancy:

Concomitant use of drugs

Multiple adjunct agents: Commonly, surgical patients receive one or more of the following preanesthetic medications: benzodiazepines, such as midazolam or diazepam, to allay anxiety and facilitate amnesia; barbiturates, such as pentobarbital, for sedation; antihistamines, such as diphenhydramine, for prevention of allergic reactions, or ranitidine, to reduce gastric acidity; antiemetics, such as ondansetron, to prevent the possible aspiration of stomach contents; opioids, such as fentanyl, for analgesia; and/or anticholinergics, such as scopolamine, for their amnesic effect and to prevent bradycardia and secretion of fluids into the respiratory tract .These agents facilitate smooth induction of anesthesia, and when administered continuously, they also lower the dose of anesthetic required to maintain the desired level of surgical (Stage III) anesthesia. However, such coadministration can also enhance undesirable

anesthetic effects (for example, hypoventilation), and it may produce negative effects that are not observed when each drug is given individually.

Stages of anesthesia:- as shown in figure (16)

A. Induction:-

During induction, it is essential to avoid the dangerous excitatory phase (Stage II delirium) that was observed with the slow onset of action of some earlier anesthetics . Thus, general anesthesia is normally induced with an intravenous anesthetic like thiopental, which produces unconsciousness within 25 seconds after injection.

B. Maintenance of anesthesia

Maintenance is the period during which the patient is surgically anesthetized.

C. Recovery

Postoperatively, the anesthesiologist withdraws the anesthetic mixture and monitors the return of the patient to consciousness.

D. Depth of anesthesia

The depth of anesthesia has been divided into four sequential stages. Each stage is characterized by increased central nervous system (CNS) depression, which is caused by accumulation of the anesthetic drug in the brain .

Stage I-Analgesia: Loss of pain sensation results from interference with sensory transmission in the spinothalamic tract. The patient is conscious and conversational. Amnesia and a reduced awareness of pain occur as Stage II is approached.

Stage II-Excitement: The patient experiences delirium and possibly violent, combative behavior. There is a rise and irregularity in blood pressure. The respiratory rate may increase. To avoid this stage of anesthesia, a short-acting barbiturate, such as thiopental, is given intravenously before inhalation anesthesia is administered.

Stage III-Surgical anesthesia: Regular respiration and relaxation of the skeletal muscles occur in this stage. Eye reflexes decrease progressively, until the eye movements cease and the pupil is fixed. Surgery may proceed during this stage.

Stage IV-Medullary paralysis: Severe depression of the respiratory and vasomotor centers occur during this stage. Death can rapidly ensue unless measures are taken to maintain circulation and respiration.

Inhalation Anesthetics

Inhaled gases are the mainstay of anesthesia and are used primarily for the maintenance of anesthesia after administration of an intravenous agent. No one anesthetic is superior to another under all circumstances. Inhalation anesthetics have a benefit (1) that is not available with intravenous agents, because the depth of anesthesia can be rapidly altered by changing the concentration of the drug. (2) Inhalation anesthetics are also reversible, because most are rapidly eliminated from the body by exhalation.

A. Common features of inhalation anesthetics:-

Modern inhalation anesthetics are

1- nonflammable,
2- nonexplosive agents that include the gas nitrous oxide as well as a number of volatile, halogenated hydrocarbons.
3- As a group, these agents decrease cerebrovascular resistance, resulting in increased perfusion of the brain.
4- They also cause bronchodilation.
5- The movement of these agents from the lungs to the different body compartments depends upon their solubility in blood and tissues as well as on blood flow. These factors play a role not only in induction but also in recovery.

Experiment

Methods :

1- Put one rat in a glass jar with a piece of cotton soaked in ether.
2- When the animal loses its righting reflex (fall on the side) and respiration becomes regular (3^{rd} stage of anesthesia), remove it from the jar and watch its recovery.
3- Take care during observation of stages as the gap between stage 4 and death is very narrow.

Experimental needs: Jar, chloroform, and cotton

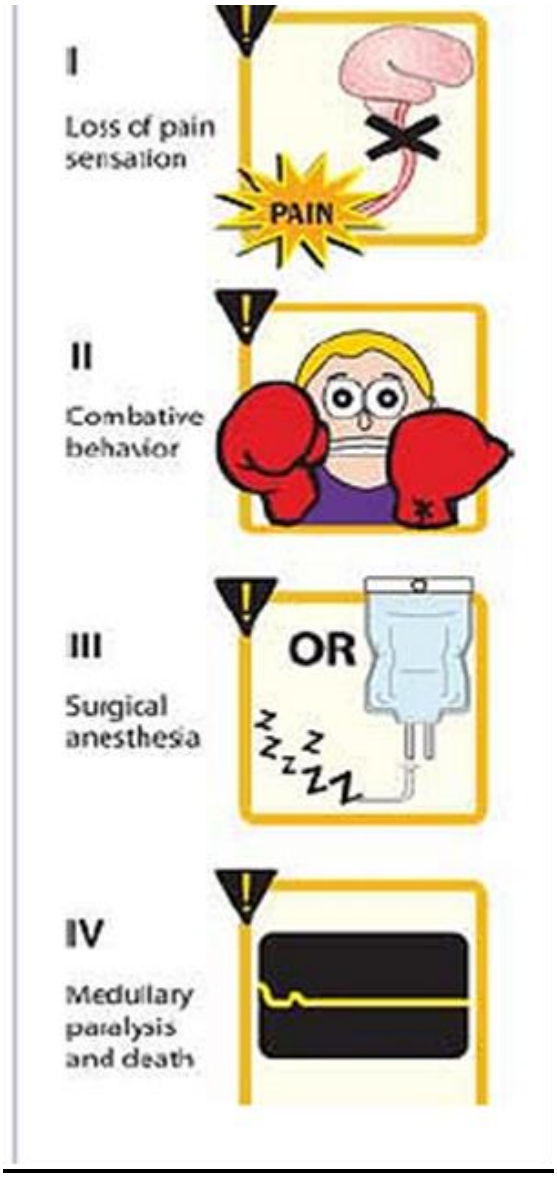
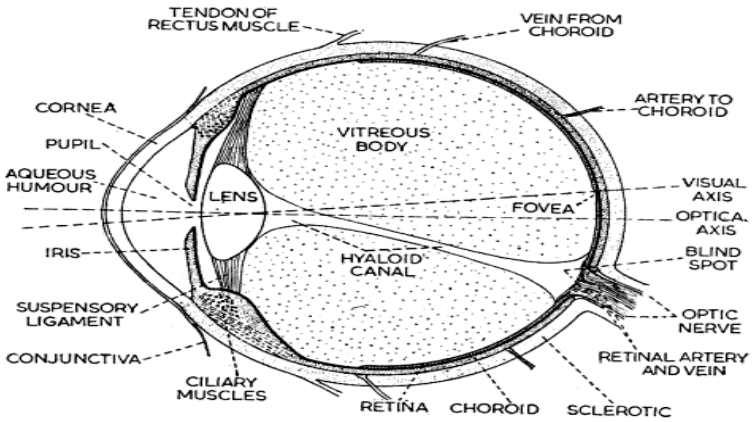


Figure (16) stages of anesthesia

Lab:- No 6

Drugs and human eye

The main compartments of the human eye (as shown in figure 16) are cornea, iris, lens, ciliary body and vitreous humour.



(Figure 17) The composition of the human eye

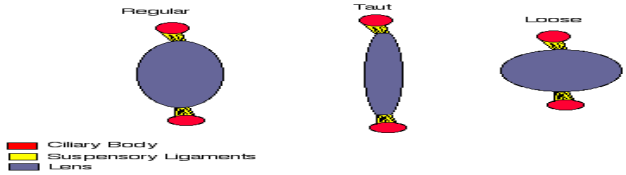
Iris: That involves:
Circular muscle (Muscarinic receptors).
Radial muscle (Alpha-receptors).

Miosis: is due to either contraction of circular muscle or relaxation of radial muscle.
Mydriasis: is due to either contraction of radial muscle or relaxation of circular muscle.

Alpha-agonist → Contraction of radial muscle of Iris (Mydriasis).
Fear (Sympathetic discharge).
Death (Lack of muscular tone due to lack of Ach.)
Except opiod intoxication (M-agonist → Pin point Miosis)

Alpha-Blocker → Relaxation of radial muscles of Iris (Miosis)
Lens: Attached to the ciliary body by ligaments .

Ciliary body: that involves
- Ciliary epithelium (B_2 receptors): responsible for secretion of aqueous humor.
- Ciliary muscle (M receptors): responsible for near or far vision.



(Figure 18) The contraction and relaxation of the lens

Ciliary Muscle (Muscarinic receptors)
M-agonist → Ciliary M. Contraction → Lens contraction → near vision

Anti-Muscarinic → Ciliary M. Relaxation → Lens relaxation → far vision
As shown in figure 17.

Ciliary Epithelium (B2-Receptors)
Responsible for secretion of aqueous humor.

Contraction of ciliary muscle presses trabecular meshwork → enhancing the flow of aqueous humor through canal of Schlemm.

Ciliary muscle contraction → Increases flow → Decreases IOP.

Ciliary muscle Relaxation → Decreases flow → Increases IOP (Glaucoma).

Examination of eye

1. Conjunctival blood vessels: - congested (dilated blood Vessels)
2. pale (constricted blood Vessels)
3. Pupil size: - dilated (mydriasis) or constricted (miosis)
4. 3- Light reflex: by shading the eye for 30 seconds then switch the light on and this will result in miosis.
5. Corneal reflex: attaching cornea by piece of cotton from the lateral side resulting in eye blinking.

Example:
Place few drops of the agents in the following table into the eyes of rabbits or volunteers and check for the parameters mentioned in the same table, and the results are as follows

parameter / Agent	Pupil Size	Light Reflex	Accommodation	Conjunctival Blood vessels	Corneal sensation	IOP
Adrenaline	↔	+ve	↔	Pale	+ve	↔
Phenylphrine	Mydriasis	+ve	↔	Pale	+ve	Inc.
Pilocarpine	Miosis	+ve	Near Vision	Congestion	+ve	Dec.
Atropin	Mydriasis	-ve	Far Vision	Pale (Congested in High Dose)	+ve	Inc.
Xylocaine	↔	+ve	↔	↔	-ve	↔
procaine	↔	+ve	↔	↔	+ve	↔

(+ve) indicates the presence of the reflex
(-ve) indicates the absence of the reflex
(↔) indicates that there is no change

Adrenaline acts on alpha-receptors causing vasoconstriction of the epithelium of conjunctiva, but it does not cause mydriasis as it cannot be absorbed by the iris. This

is also true for procaine (local anesthetic) as the cornea does not absorb it, so it cannot cause loss of corneal reflex.

Examples of drugs

Drug name	Action	Pupil size	Light reflex	Corneal reflex	Conjunctival blood vessels	Accommodation
Pilocarpine	M-agonist	myosis	normal	+ve	normal	Near vision
Tropic-amide	Antimuscarinic	mydriasis	absent	+ve	normal	Far vision
phenylephrine	α-agonist	mydriasis	normal	+ve	Pale	No effect
proparacaine	Local anesthetic	normal	normal	-ve	normal	No effect

Exp. Needs:

Animal :rabbit

Drugs:Atropine and Tropic-amide drops

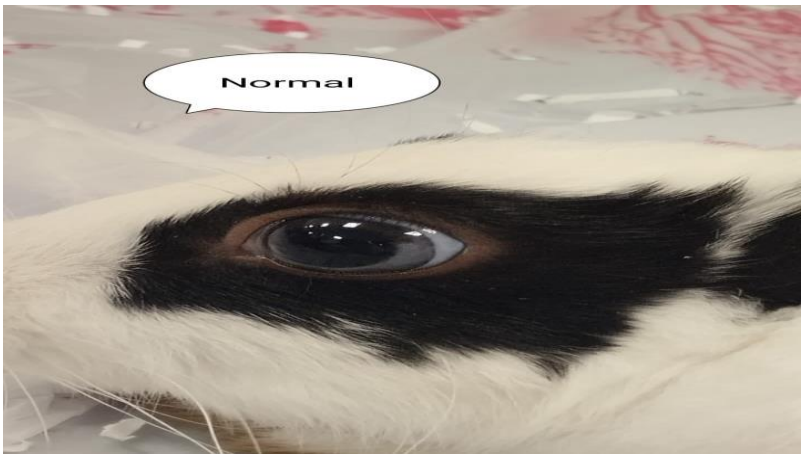
Material : gloves and cotton.

Method: use two rabbit and two different actions drugs

At the beginning training students on proper rabbit handling as shown in figure (18)

Figure (19) proper rabbit handling

and then do the experement as follow
one eye consider control while second eye put drops of drug and wait result and do report in the lab and record result and discussion (miosis or mydriasis)



Atropine effect **tropic amide effect**

Figure (20) effect of drugs on rabbit eye

Lab. No. 7

Evaluation of analgesic drug activity

Pain is an uncomfortable sensory and emotional feeling, related to tissue damage.
Pain in general is a symptom that serves as a hint of the danger of disruption in tissues such as inflammation, microorganism disease or muscle spasms.
Pain caused by mechanical, chemical or physical stimulation (heat, electricity) can cause tissue damage where the stimulation causes the release of chemicals (eg: Bradykinin, Prostaglandins, ATP, protons) that stimulate receptors in patients.

Analgesics are substances that reduce or eliminate pain without losing consciousness.analgesics are divided into 2 major groups peripheral analgesic or non narcotics narcotic analgesic (work centrally , sever pain)

Based on the process of occurrence, the pain can be overcome in several ways, namely:

a.Peripheral analgesics
b. Local analgesics
c. Central analgesics
d. Tricyclic antidepressants
e. Antiepileptic

Pain perception is a difficult situation to define or measure. It is a subjective phenomenon, so it can not be known how the image of experimental animals is experiencing pain.
Most techniques involve the use of nociceptive tests in which painful stimuli, mechanically or electrically are used to produce pain.

Hot plate method

In this method the animal is placed slowly onto a hot plate with a fixed temperature of 55°C.
Response time (usually 4-10 seconds for normal state is calculated as the initial distance of time the animal puts its feet on the plate and time is recorded when the animal starts licking feet or jumps to escape from the heat). Animals that did not show a response within 30 seconds were not used in the experiment.

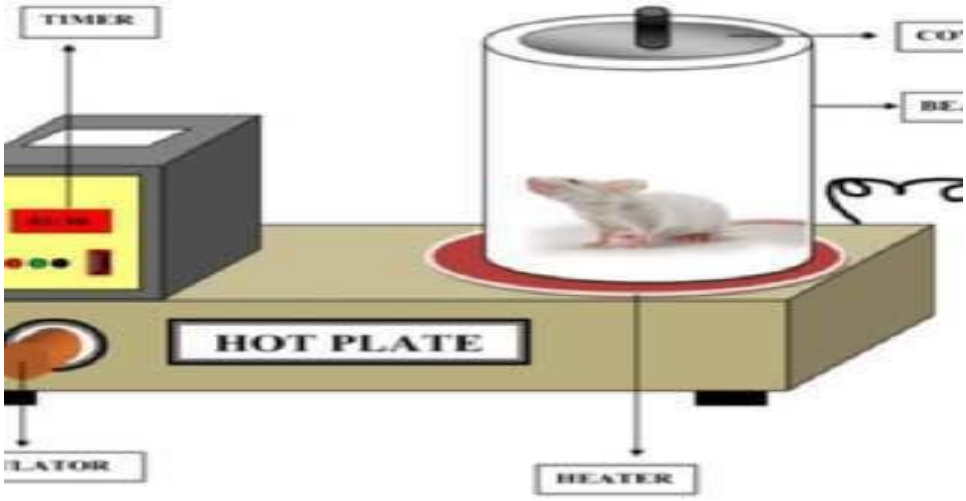


Figure (21) hot plate method

Writhing test

Another method is to use a chemical compound such as **3% acetic acid**which is injected i.p.

•**Acetic acid acts as a stimulus for pain**. The pain of giving acetic acid can be seen from the existing **stretching from** observation to mice (animals).

•This stretch is calculated starting if the mice stretch her legs back and pressing her stomach down. This stretch is calculated 1, and so on. So that the end of the specified time will get the total number of stretch animals in a particular time.

Figure (22) stretching of animal receiving acetic acid

Paw pressure test

The paw pressure test consists in applying a uniformly increasing mechanical pressure on the animal paw.This pressure induces pain leading to an escape reaction.

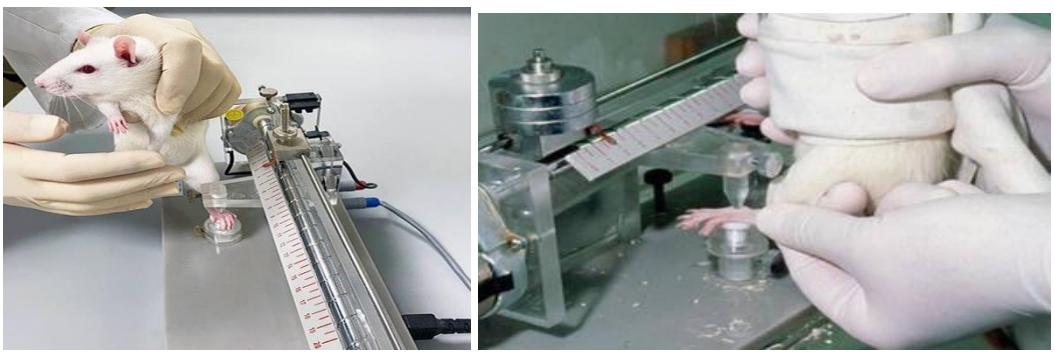


Figure (23) paw pressure method

Tail flick test

The tail flick test is a thermal hyperalgesia test in which the tail of the animal is subjected to a heat source.

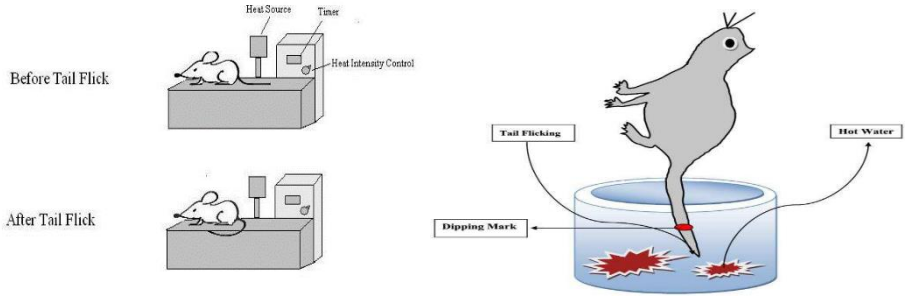


Figure (24) tail flick test

Experiment:

Hot Plate Method:

1.Animals are weighed and marked

2. Dose administration are calculated:

Mice 1: Control NS at the dose of 1% of body weight (i.p)

Mice 2: nefopam, 15 mg/kg BW (i.p)

Mice 3: sodium diclofenac 10 mg/kg BW (i.p)

3. The animals are placed onto a hot plate at 55 C.

4. The time when the animals start to lick its feet from the device (10 minute to 90 minute) is observed and calculated.

5. Then plot a graph based on period of response vs time

6. Data is analyzed statistically.

Acetic Acid Method

1. Animals are weighed and marked

2. Dose administration are calculated:

Mice 1: Control NS at the dose of 1% of body weight (i.p)

Mice 2: Morphine SO4 0.1%, 10 mg/kg BW (i.p)

Mice 3: Morphine SO4 0.1%,15 mg/kg BW (i.p)

3. After 30 minutes, 3% acetic acid at a dose of 1% body weight is injected (i.p).

4. The amount of stretching interval from 10 minutes to 90 minutes is observed and calculate

5. Plot a Graph based on the amount of stretching vs time

6. Data is then analyzed statistically.

Report

no	treatment	Pain induction	Time		no
1	NS	Mice placed on hot plate 55 ° C			
2	Nefopam				
3	Na Diclofinac				

Lab. No. 8

Effects of anticonvulsant drugs
(Antiepileptics)

Epilepsy is the tendency to have recurrent seizures unprovoked by systemic or acute neurologic insults. Antiepileptic drugs (AEDs) are those which decrease the frequency and/or severity of seizures in people with epilepsy. The older term, anticonvulsant drug, is still sometimes used as a synonym for AED, but is less accurate because many seizures do not involve convulsive movements. There is no convincing evidence that AEDs "cure" or alter the natural history of epilepsy. However, many patients whose seizures have been completely controlled for two or more years can be successfully withdrawn from AEDs. The therapeutic goal is maximizing seizure control while minimizing adverse drug effects, thus improving the patient's quality of life.

Epilepsy is a disorder that is best viewed as a symptom of disturbed electrical activity in the brain, which may be caused by a wide variety of etiologies. Approximately 1% of the world's population has epilepsy, the second most common neurologic disorder after stroke. Seizures occur because a group of cortical neurons discharge abnormally in synchrony. Anything that disrupts the normal homeostasis of neurons and their stability can trigger hyperexcitability and seizures. There are thousands of medical conditions that can cause epilepsy, from genetic mutations to traumatic brain injury. The mechanisms of action of anti-seizure drugs fall into three major categories.

I. The first mechanism is to limit the sustained, repetitive firing of neurons, an effect mediated by promoting the inactivated state of voltage-activated Na+ channels.

II. A second mechanism appears to involve enhanced γ-aminobutyric acid (GABA)–mediated synaptic inhibition; an effect mediated either by a presynaptic or postsynaptic action. Drugs effective against the most common forms of epileptic seizures, partial and secondarily generalized tonic-clonic seizures, appear to work by one of these two mechanisms.

III. Drugs effective against absence seizure, a less common form of epileptic seizure, work by a third mechanism, inhibition of voltage-activated Ca2+ channels responsible for T-type Ca2+ currents.

Clinical Pharmacology of anti-epileptic drugs:-

-Tonic-clonic seizures / Partial seizures: Carbamazepine , Lamotrigine , Phenytoin and Valproic acid.

-Myoclonic seizures: Clonazepam , Lamotrigine and Valproic acid.

-Absence seizures: Clonazepam , Ethosuximide and Valproic acid.

-Back-up & adjunctive drugs: Felbamate, Gabapentin, Phenobarbital, Levetiracetam, Topiramate, Zonisamide, Tiagabine and Vigabatrin.

Principle:

Lidocaine is a local anesthetic that is well known to cause tonic – clonic seizure.

Aim:

To determine the action of diazepam as antiepileptic in the chemical model of generalized convulsive **Status Epilepticus** in rats.

Method:

1- Check the weight of two rats / mice.
2- Inject one animal with normal saline I.P and mark it as control, and another animal with diazepam I.P in a dose of 2 mg / kg.
3- 15 minutes later, inject both animals with Lidocaine I.P in a dose of 150 mg / kg.
4- Observe and record the following parameters:

   a- Threshold of seizure.
   b- Duration of seizure.
   c- The cardio – respiratory status.
   d- Death if occur.

Exp. Needs: Lidocaine, Diazepam, N.S., Needles, and Balance.

Lab. No. 9

Effect of drug and its antagonists
(Histamine and its antagonists)

Histamine affects the human skin in the term of Triple response of Lewis .This action can be antagonized by

a - Antihistamines (e.g. diphenhydramine) by blocking histamine receptors in the skin, so this is called competitive antagonism.

b – Adrenaline by producing action that reverses the action of histamine, so this is called physiological antagonism.

Histamine is a chemical messenger that mediates a wide range of cellular responses, including allergic and inflammatory reactions, gastric acid secretion and neurotransmission in parts of the brain.

Location:

- All tissues.
- High amounts in lung, skin and GIT (where the inside meets the outside)
- High concentration in mast cells and basophiles
- It is a component of venoms and found in secretions from insect stings.

Release:

Stimuli:

1. Destruction of cells as a result of cold, toxins, bee sting venoms, or trauma.
2. Allergic and anaphylactic reactions.

Mechanism of action:

Histamine acts by binding to one or more of its receptors:

1. H1 receptors: mediate edema and vascular effects of histamine. Antagonists: H1 antagonists (e.g., diphenhydramine)
2. H2 receptors: mediates the effects on gastric secretion. Antagonists (e.g. cimetidine)
3. H3 receptors.
4. H4 receptors.

Effects of histamine:

Clinically important effects of histamine are those on smooth muscles, blood vessels, skin, and secretary glands.

- IV injection (even small amount e.g., 0.1 mg) will produce the followings; systemic vasodilation with rapid fall in blood pressure, increased heart rate, flushing of face, headache, stimulate gastric acid secretion ., theses changes last for few minutes.
- Intradermal injection (even of a relatively high amount e.g., 10 mg) will produce : *Triple response of Lewis*: which consist of the followings:

1. *Flush*: localized erythema due to dilation of capillaries.
2. *Wheal*: localized edema due to increased permeability of capillaries and post capillary venules.
3. *Flare*: erythema caused by arterial dilation, (it is due to axon reflex).

Histamine antagonists:

The effects of histamine can be opposed in 3 ways:

1. By using a drug with opposite effects e.g., histamine constricts bronchi, causes vasodilation and increase capillary permeability. Adrenaline opposes these effects by a mechanism unrelated to histamine (α and β receptors). This is a physiological antagonism.
2. H1 , H2 receptor antagonists (competitive) (preventing histamine from reaching its sites of action)
3. by preventing the release of histamine from cells in which it is stored: adrenal corticosteroids and sodium cromoglycate (membrane stabilizing action)

Role of histamine in allergy and anaphylaxis:

The symptoms resulting from IV injection of histamine are similar to those associated with anaphylactic shock and allergic reactions.
Anaphylactic shock occurs with penecillins and other drugs.
Signs and symptoms of anaphylactic shock: severe hypotension, bronchospasm, edema, (including laryngeal edema), and sometimes death due to loss of fluid from the intravascular compartment.
Drugs used in treatment of anaphylactic shock:

1. adrenaline
2. antihistamines: (e.g., diphenhydramine)
3. adrenocorticosteroids
4. IV fluid infusion.

Procedure of the experiment

Aim: To show the effect of Histamine and its antagonists on the human skin.

1. make scratched on volunteer skin. Cleanse the forearm with an alcohol-soaked cotton wool and draw 3 circle(control,histamine agonist and histamine antagonist) wait the result and record discussion in the lab.

(To obtain better result, it is preferable to do the above procedure on persons with fair skin.This experiment is not to be done on atopic individuals.)

Exp. Needs: volunteer ,N.S, anti-histamine, histamine agonist , Drops, lancet, cotton, alcohol.

Figure (25) effect of intradermal injection of histamine and its antagonist

Lab. No. 10

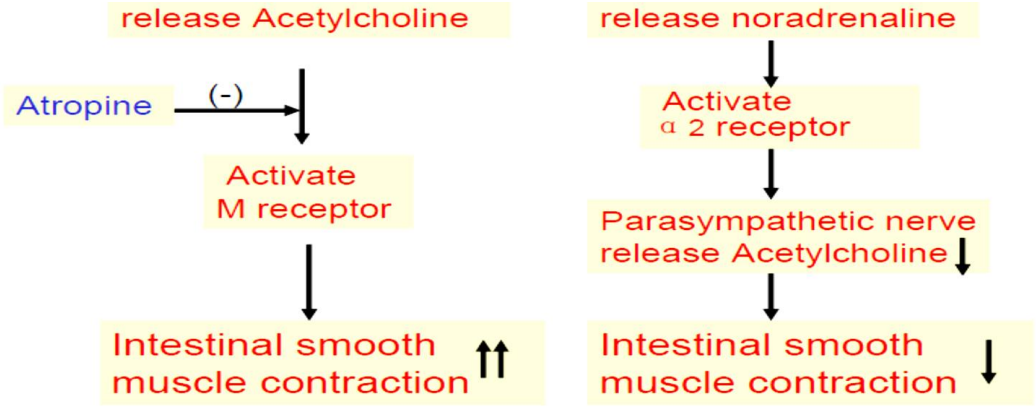
Isolated organ (Guinea pig intestine)

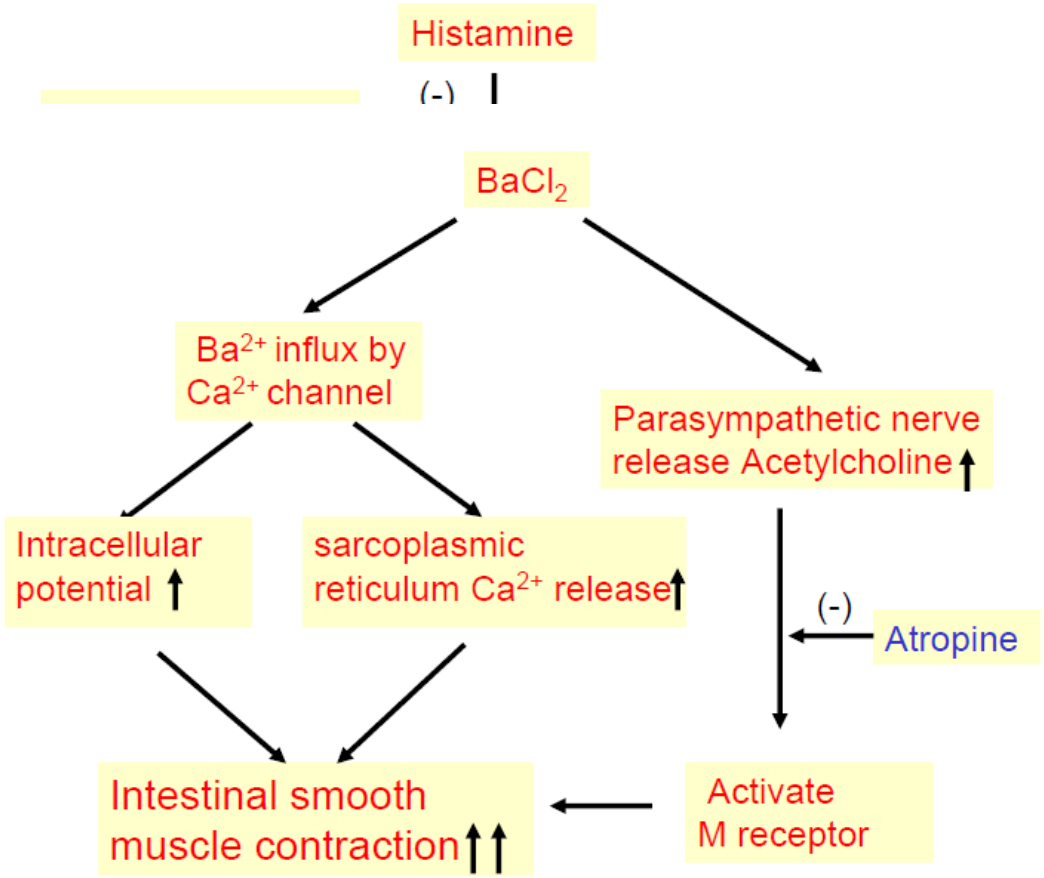
Experimental purpose

To master the experiment condition of isolated intestinal smooth muscle and effects of different drugs on them.

To master the fundamental method of designing drug interaction and to improve the designement ability by observing the relationship between Barium Chloride and receptors

- **Motility in the small intestine, as in all parts of the digestive tube, is controlled predominantly by excitatory and inhibitory signals from the enteric nervous system.**
- These motility are however modulated by inputs from the central nervous system (sympathetic nerve, parasympathetic nerve),
- A number of gastrointestinal hormones (Gastrin, Cholecystokinin) or other mediators (Histamine) appear to affect intestinal motility to some degree.




Histamine
(-)
$BaCl_2$
Ba^{2+} influx by Ca^{2+} channel
Parasympathetic nerve release Acetylcholine
Intracellular potential
sarcoplasmic reticulum Ca^{2+} release
(-)
Atropine
Intestinal smooth muscle contraction
Activate M receptor

[Principle]

- The isolated intestinal tract from many species of animal can maintain active rhythmic movements for a long time under suitable conditions.
- By using different drugs that bind to the receptors on intestinal smooth muscular cells, we can observe the actions of drugs and analyze their action mechanism.
- In this experiment, the effects of acetylcholine and histamine on the motility of isolated ileum in guinea pig are investigated. Atropine and chlorpheniramine are used as tool drugs to analyze the underlying mechanisms.

Animal: guinea pigs, 300-400 g

Drugs:

1:100000 acetylcholine chloride (ACh), ➢

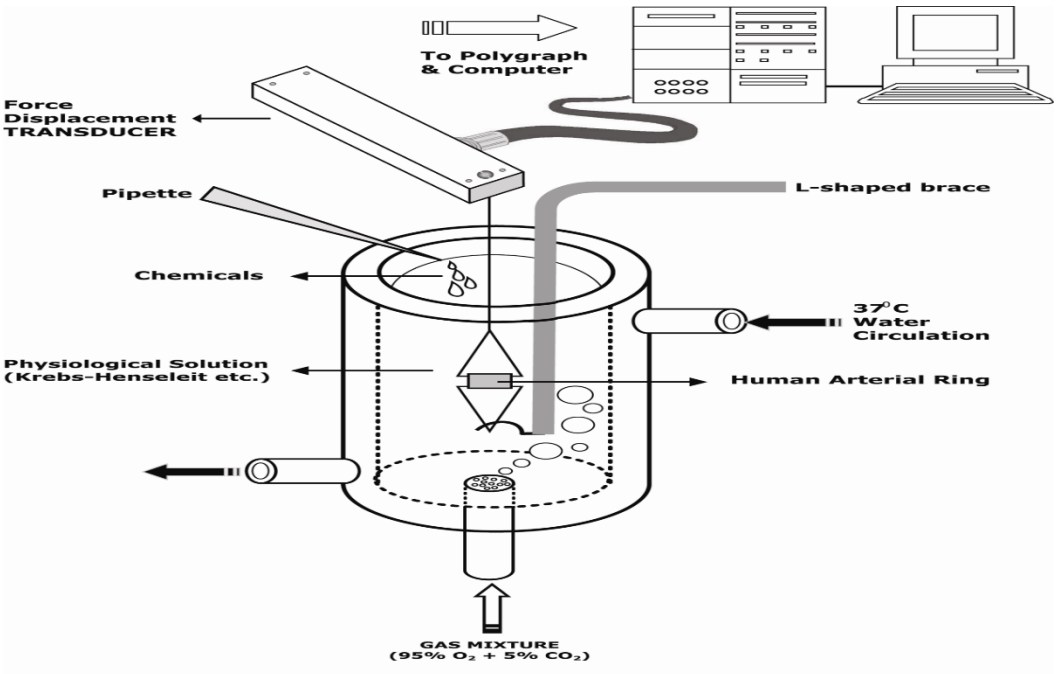
0.1% atropine sulfate, ➢

1:100000 histamine phosphate, ➢

1:1000000 chlorpheniramine , ➢

1%barium chloride (BaCl2), ➢

Tyrode's solution. ➢



Figure(26) principle of isolated organ instrument

Preparation of isolated intestine

- **Take a guinea pig, stun it to cause death, immediately open the abdominal cavity with surgical scissors and clip off 10 cm of ileum, starting at or near the ileocecal junction.**

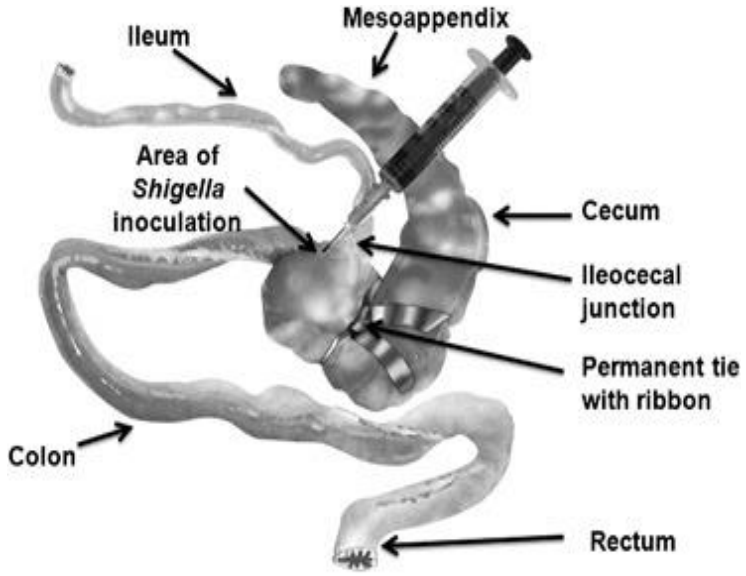


Figure (27) isolated intestine preperation •

- **Wash out the contents inside the intestine with Tyrode's solution aerated with 95% oxygen and 5%carbon dioxide.**

- **Cut the intestine into several sections, 1-1.5 cm per section, and put them in Tyrode's solution aerated continuously with 95% oxygen and 5% carbon dioxide.**

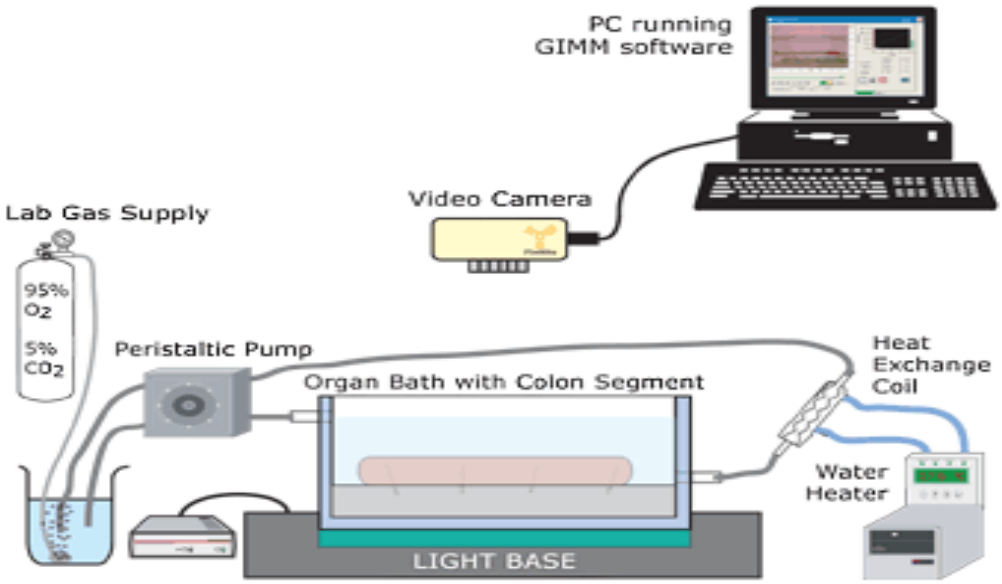


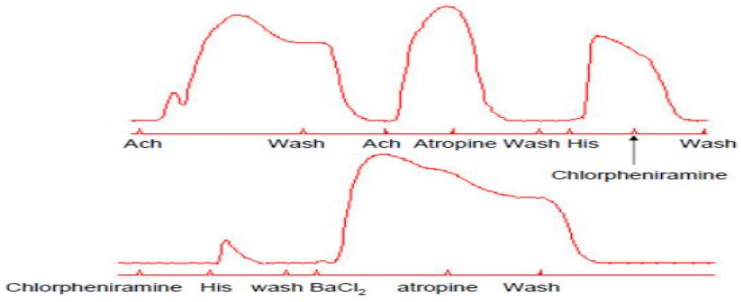
Figure (28) isolated organ instrument

- **Fix the specimen (1-1.5 cm) in the isolated organ bath. The specimens are mounted vertically, one end is connected to the lower hook of the bath and the other end is connected to a force transducer.**

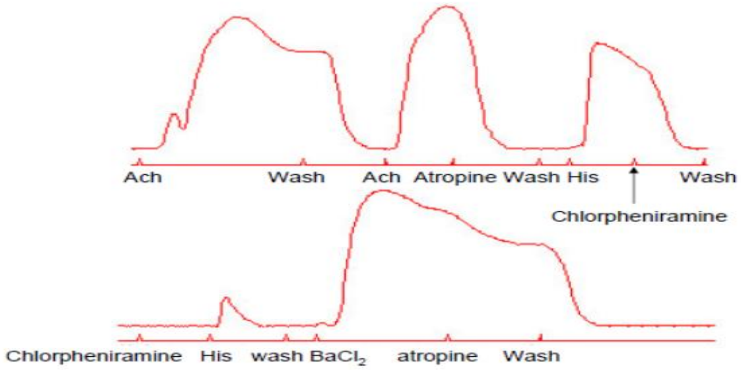
- Tyrode's solution, 10 ml (37±0.5℃), is added into the bath. The specimens are allowed to equilibrate at 2-3 g tension for 30 min.

- Observe the rhythmic contraction and tension level of the intestinal muscle, trace out the normal contraction curve until the specimen becomes stable.

- **Add a drop of Ach into the bath solution and observe for 2-3 min, record the peak of the change of the contractive tension, and then wash off.**

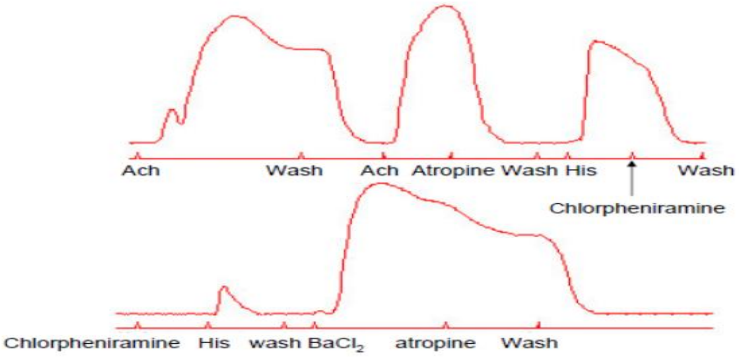


- **Add a drop of Ach into the bath solution.**
- **When the contraction of intestinal smooth muscle reaches the peak, add a drop of Atropine.**
- **After the contraction of intestinal smooth muscle becomes stable, record the tension and then wash off.**



- **Add a drop of Histamine into the bath solution, observe and record the peak of the change of the contractive tension, and then wash off.**

- **Add a drop of Chlorpheniramine into the bath solution and then add a drop of Histamine.**
- **Record the tension of the contraction peak and then wash off.**



- **Add a drop of $BaCl_2$ into the bath solution.**
- **When the contraction of intestinal smooth muscle reaches the peak, record the tension and then add a drop of Atropine.**

After the contraction of intestinal smooth muscle becomes stable, record the tension and then wash off

[Discussion]

- Try to compare the actions on isolated intestinal smooth muscle of acetylcholine, histamine and barium chloride with or without adding Chlorpheniramine (H_1 receptor antagonist) or Atropine (M receptor antagonist), and analyze the mechanisms of action of acetylcholine, histamine, barium chloride on intestinal smooth muscle.
- What is clinical significance of the effect of atropine on intestinal smooth muscle motility?

Appendix

I.Laboratory safety instructions

1. Personal Protective Equipment (PPE)
-Wear Lab Coats: Always wear a lab coat to protect your clothing and skin.
- Safety Goggles: Use safety goggles to protect your eyes from chemical splashes or flying debris.
- Gloves: Wear appropriate gloves for handling chemicals or biological materials.
- Closed-Toe Shoes: Wear closed-toe shoes to protect your feet from spills or dropped objects.

2. Know the Emergency Procedures
- Emergency Exits: Familiarize yourself with the location of all emergency exits.
- Emergency Equipment: Know the locations of fire extinguishers, safety showers, eyewash stations, and first aid kits.
- Fire Evacuation Plan: Understand the fire evacuation plan and practice it regularly.

3. Chemical Safety
- Labeling: Ensure that all chemicals are properly labeled with hazard information.
- Material Safety Data Sheets (MSDS): Review MSDS for chemicals you will be using to understand hazards and first aid measures.
- Proper Storage: Store chemicals according to their compatibility and hazard class.
- No Eating or Drinking: Never eat or drink in the laboratory to avoid contamination.

4. Safe Handling of Equipment
- Proper Use: Use equipment only for its intended purpose and follow operational guidelines.
- Inspect Equipment: Check equipment for damage before use. Don’t use damaged equipment.
- Report Issues: Immediately report any malfunctions or accidents to a supervisor.

5. Waste Disposal
- Segregate Waste: Dispose of hazardous waste in designated containers.
- Follow Protocols: Adhere to your institution’s waste disposal protocols.

6. General Laboratory Conduct.
- Keep Work Areas Clean: Clean up spills immediately and keep work

areas organized.
- Limit Personal Items:** Keep personal items (bags, phones) out of the work area.

7. Training and Awareness
- Mandatory Training: Attend all required safety training sessions.
- Stay Informed: Keep updated on safety protocols and procedures.

8. Handling Biological Materials
- Biosafety- Biosafety Guidelines: Follow biosafety levels appropriate for the materials you are working with and adhere to all related protocols.
- Decontamination Procedures: Always decontaminate surfaces and equipment after working with biological materials.
- Sharps Disposal: Use designated sharps containers for needles and other sharp objects.

9. Electrical Safety
- Avoid Overloading Circuits: Do not overload electrical outlets or use damaged cords.
- Unplug Equipment: Disconnect equipment when not in use and before performing maintenance.
- Use Ground Circuit Interrupters (GFCIs): Ensure that electrical equipment in wet areas is equipped with GFCIs.

II. How to write a laboratory report

Each student should write his report by himself using his own words to improve his language.

DO NOT COPY. The report should be brief, precise and including the following items:

1- The name of the student and the experiment.

2- The principle of the experiment.

3- The aim of the experiment.

4- Materials and methods used.

5- Results, including the measurements and observations during the experiment that sometimes should be arranged in a form of table.

6- Discussion and conclusion, which is the **most important** item in the report as it shows the ability of the student to discuss his findings and compare them with those mentioned in the textbook.

Pharmacology 4th Stage
PRACTICAL LAB REPORT

Student name: **Date and group:**

Name of the experiment:

Aim:

Equipment:

Materials:

Results:

Discussion

III.Oral assessment

Practical Pharmacological Evaluation

Student name ---------- Student Group-------

task	One	Zero
1. The student wearing the lab coat		
2. The student wearing gloves		
4. The student doesn't hesitate before picking the animal		
5. The student doesn't request changing the animal		
6. The student grab the animal from the first attempt		
7. The student hold the animal in a correct manner		
8. The student isn't violent towards the animal		
9. The student knows the injection technique(2)		
10. The student doesn't take a long time to complete the evaluation		
Total grade		

References

1-Lipincott pharmacology 3rd edition ,2006
2-laboratory manual of pharmacology by Assistant Professor Mazin Hamid Ouda

3-https://mlrip.ac.in/wp-content/uploads/2022/03/PHARMACOLOGY-I-LAB-MANUAL.pdf

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5https://www.researchgate.net/publication/378286842_Laboratory_Manual_of_Pharmacology_I

6-https://mlrip.ac.in/wp-content/uploads/2022/03/PHARMACOLOGY-III-LAB-MANUAL.pdf