Chapter

1

Introduction to Experimental Pharmacology

PHARMACOLOGY

Branch of science, which deals with study of drugs on living systems.

EXPERIMENTAL PHARMACOLOGY

Study of effects of various pharmacological agents on different animal species.

- Originating in the 19th century, the discipline makes drug development possible.
- In the early 19th century, **Francois Magendie** (1809) studied the action of nux vomica (a strychnine containing plant) on dogs, and showed that spinal cord was its site of convulsant action.
- **Claude Bernard** (1842) discovered that the arrow poison curare acts at neuromuscular junction to block the neuromuscular transmission.
- **Rudolph Buchheim** (1847) is remembered for his pioneer work in experimental pharmacology.
- **Oswald Schmiedeberg** is considered as Father of Modern Pharmacology.

AIMS OF EXPERIMENTAL PHARMACOLOGY

1. Find out a therapeutic agent suitable for human use in preclinical studies.
2. Study the toxicity of a drug.
3. Study the mechanism and site of action of drug.

COMMON TERMINOLOGY

- ***Ex vivo:*** Outside the normal living organism (experiment on tissues from an organism in external environment).
- ***In vitro:*** Within glass usually in a cultured system.
- ***In situ:*** In biology and medical science, it means to examine the phenomenon exactly in place where it occurs (without moving it to some special medium).
- ***In vivo:*** Experiment on intact animal.
- ***In silico/in silicio*** (derived from silicon of computer chip): Performed on computer or via computer simulation.
- **The conventional teaching methods in experimental pharmacology involved the use of experimental instruments. Now the animal experiments for teaching purpose have shifted to simulation experiments on CAL (computer assisted learning).**

Experimental Animals

1. Rodents (mouse, rat, guinea pig).
2. Non-rodents (rabbit, monkey, dog, cat).
3. Others (frog, pigeon, zebra fish).

Rodents

1. **Mouse (*Mus musculus*):** Smallest lab animal. (Common strain = Swiss albino mice.) Easy to keep, handle and require small place for housing. There is large similarity in mice and human genome, therefore, they provide good model for study of mammalian biology and also for study on cancer, diabetes, immunological and autoimmune disorders, neurological, endocrine diseases. They are applied widely in acute toxicity studies and testing the drugs for teratogenicity.

- **Nude mice** = Hairless genetic mutant which lacks thymus gland.
- **Biege mice** = lack NK (natural killer) cells, susceptible to cancer.
- **Knockout mice** = Selective suppression of gene.
- **Knockin mice** = Selective introduction of gene.

2. **Rat (*Rattus norvegicus*)**
   - Most commonly used animal in biomedical research.
   - Albino rats
     - **Wistar rats** (wide head, long ear, small tail).
     - **Sprague-Dawley rats** (long and narrow head, longer tail).
   - Nude rats, similar to nude mice, lack a normal thymus and functionally mature T cells, hairless, used in immunological research.
   - Rats do not have tonsil and gall bladder. Tail helps in thermoregulation. Do not vomit (because of lack of vomiting centre and presence of strong sphincter between stomach and oesophagus). Rats are used in—research of behaviour, pharmacology, physiology, neuroscience, immunogenetics, cancer study, cardiovascular diseases, testing of psychopharmacological agents.
   - Study of drugs in acute and chronic BP effects.
   - Evaluation of antiulcer (gastric ulcer) drugs (Shay rat method of pyloric ligation).
   - Study of analgesic drugs on tail flick analgesiometer or Eddy's hot plate.
   - Acute and chronic toxicity studies.
   - Teratogenicity and carcinogenicity.
3. **Guinea pigs (*Cavia porcellus*)**
   - Herbivorous
   - Require vitamin C (ascorbic acid in food because unable to synthesise daily vitamin C requirement).
   - Guinea pigs are sensitive to many infections which make it suitable for the diagnostic tests.

- Ideal model for enteric amoebiasis, bronchial asthma, COPD and for screening of local anaesthetics.
- Susceptible for TB and anaphylactic shock, highly sensitive to histamine and penicillin.

Non-rodents

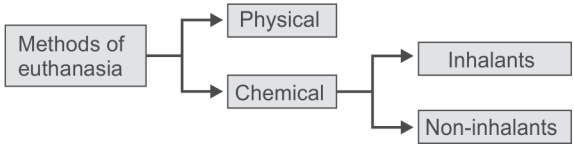
4. **Rabbit (*Oryctolagus cuniculus*)**
   - Most common strain used in lab New Zealand white rabbit.
   - Most suitable model for pyrogen testing of intravenous fluids.
   - Other uses: Screening of diabetes, diphtheria, TB, cancer, heart diseases, genetics, nutrition, physiology, reproduction, and to test toxic effect of cosmetics and pharmaceuticals, good model for production of antibodies and antiserum.
   - Very sensitive to histamine, ideal animal for PK (pharmacokinetic study).
   - Enzyme atropine esterase is present in blood, which degrades atropine.
5. **Monkey (*Macaca mulatta*)**
   - Rhesus monkey (large animal).
   - Used as primate model to study drug metabolism because they show metabolic pattern similar to humans.
   - Ideal model for PK study.
   - Used for action of drugs on CNS (memory, anxiety, antidepressants, etc.), CVS (antianginal, antihypertensives, etc.), GIT and fertility.
   - Require regular check up for rabies, TB and timely immunization.
6. **Dog**
   - Small alimentary tract and easily get trained.
   - Mongrel and beagle variety.
   - Cardiovascular research, drugs acting on BP, vasomotor reversal phenomenon of Dale.
   - Used as model for CVS research, diabetes mellitus, CNS, etc.

7. **Cat**
   - Carnivorous, has nictitating membrane.
   - Nictitating membrane is used in screening of ganglion blocking drugs.
   - Used for behavioural studies, CNS studies, nerve impulse transmission, e.g. reflexes of respiratory system, spinal reflexes, and light perception.
   - Also used in neuropharmacology.
8. **Frog (*Rana tigrina*)**
   - Experiments on frogs are totally banned and they are endangered and protected species.
   - Heart is three chambered (two atria, one ventricle).
   - For CVS experiments or bioassay of acetylcholine on rectus abdominis muscle.
   - Unlike in mammals where noradrenaline is the main neurotransmitter, in frog adrenaline is the main neurotransmitter in sympathetic system. Hence, frog heart is more sensitive to adrenaline.

As per guidelines of UGC (University Grants Commission) and MCI (Medical Council of India), the animal experiments are banned in India by dissection methods in undergraduate medical courses. Animal experiments are now replaced by computer models and simulation experiments (i.e. CAL).

Euthanasia

Humane killing (sacrifice) of an animal which produces rapid unconsciousness and subsequent death without or minimal pain or distress to animal.

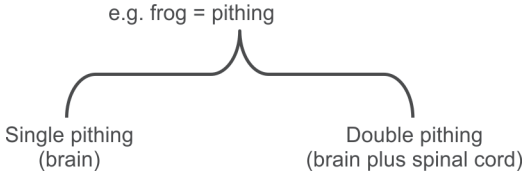


A. Physical methods: Performed by skilled, experienced personnel with appropriate, well-maintained equipment.
1. Cervical dislocation for rodents and small rabbits.
2. Decapitation for rodents and small rabbits.
3. Microwave irradiation.

B. Chemical methods:

1. Inhalant anaesthetics: Halothane, enflurane, sevoflurane, methoxyflurane, isoflurane and desflurane.
   - Ether is not preferred now.
2. Non-inhalational anaesthetics:
   i. Barbiturates (sodium pentobarbital): IP (intraperitoneal) in small animals, IV (intravenous) in non-rodents.
   ii. KCl: Induces immediate cardiac arrest without significant CNS depression, used after the animal is deeply anaesthetised.
   iii. $MgSO_4$: Causes cardiac arrhythmia, neuromuscular blockade and deep anaesthesia.
   iv. Neuromuscular blockers: For example, succinylcholine induces muscular paralysis and death because of respiratory failure, but distress to the animal is more, hence less preferred.

Euthanasia in cold blooded (poikilothermic animal)



ANIMAL USE IN INDIA

- Supervised by **CPCSEA** (committee for the purpose of control and supervision of experiments on animals).
- Objective of CPCSEA: To promote the humane care of animals used in biomedical research and provide the legal aspect for experimentation in the animals.

Institutional Animal Ethics Committee: Members of IAEC

- A biological scientist.
- Two scientists from different biological disciplines.
- A veterinarian involved in care of animals.
- The scientist incharge of animal facility.

- A scientist from outside the institute.
- A socially aware non-scientific member.
- A representative or nominee of the CPCSEA.

KYMOGRAPH (SHERRINGTON–STARLING KYMOGRAPH) (Fig. 1.1)

It records contraction/relaxation movements of a tissue on moving surface.

- Used to obtain a graphical, amplified, measurable response of a muscle or tissue (contraction and relaxation), against a given concentration of drug or stimuli.
- It consists of the following parts:

1. **Motor box**: The important parts are:
   - **On/off switch** for power supply.
   - **Speed setting lever/variable speed lever**, to control the speed of clockwise rotating drum. The speed of drum depends on the type of tissue used.
   - **Clutch lever**, to disengage or engage the gear.
2. **Drum**: For most of the experiments, the speed of drum is kept at one revolution in 96 minutes.
3. **Spindle and screw**: Rod-like structure which holds the drum in the vertical position.

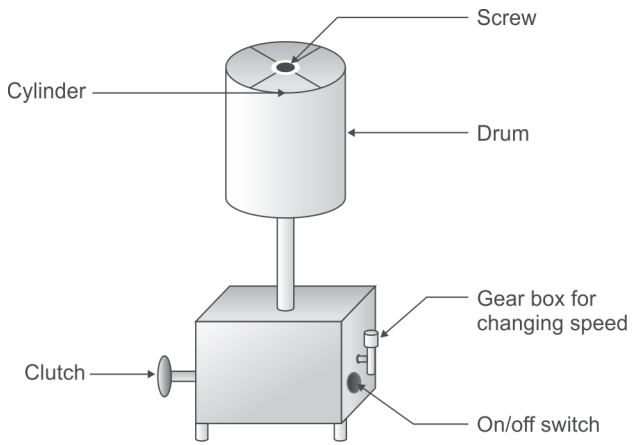


Fig. 1.1: Sherrington-Starling kymograph

- Height of the drum can be adjusted with lift screw attached at the top of the spindle.
- The kymograph paper (glossy surface out) is fixed tightly on the drum.
- Although smoking of kymograph paper is no more practiced due to health hazard, but can be performed where ink-writing device (pen) is unavailable at the tip of the lever.
- The drum is uniformly smoked with black soot (smoke) of benzene or kerosene or the mixture of the two.
- Uniform smoking is essential for proper recording.
- The recordings (tracings) on the smoked paper are preserved by properly fixing them with the help of fixing solution made of saturated solution of shellac and alcohol.
- No fixing is required for the ink-written tracings on the unsmoked paper.

Organ bath: **Rudolph Magnus** (1904) was the first to design the arrangement of bath for excised organs (Fig. 1.2).

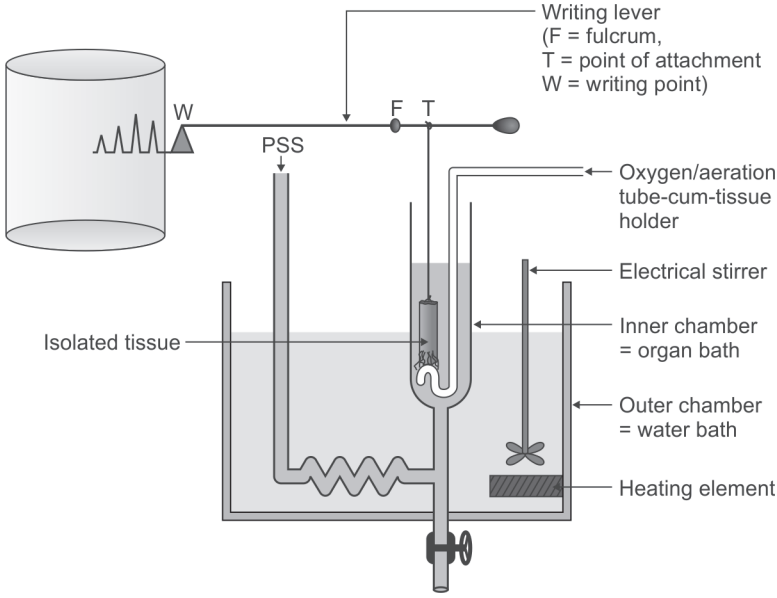


Fig. 1.2: Assembly of organ bath/student's organ bath for recording of contractions of isolated tissue (PSS = physiological salt solution)