A Practical Book of

PRACTICAL PHARMACOLOGY - II

As Per PCI Regulations

THIRD YEAR B. PHARM.

Semester V

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Preface

Practical training is an important aspect of Experimental Pharmacology. This book is collection of specific methods used in understanding of basic principles of experimental pharmacology. In this an attempt has been made to highlight the practical areas of experimental pharmacology with intention to help students to learn basics in experimental pharmacology, various screening methods, their models and concept that provide advanced understanding of the subject. It provides a concise account of the preliminary methods and general principles which form the basis of pharmacological experimentation. We tried to achieve maximum coverage in the simplest way.

Practical book of Pharmacology-II is primarily aimed at the course requirements of the T. Y. B. Pharm. students according to PCI Regulations. It would also serve as a valuable resource of information to other healthcare science students. Some important features of the book are given below:

- Exactly as per the new syllabus prescribed by Pharmacy Council of India.
- Easy to follow, stepwise and self explanatory.
- Complete coverage to all topics.
- At the end of each experiment, short questions and answers along with MCQ's are given.

Practical book of Pharmacology-II is the outcome of numerous efforts of authors to assimilate the voluminous knowledge of experimental pharmacology.

Any suggestions for the improvement of this book are always welcome.

Authors

Contents

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Experiment No. 01

Aim: Introduction to *in-vitro* pharmacology and physiological salt solutions.

IN VITRO PHARMACOLOGY

In vitro pharmacology studies are done in the laboratory. *In vitro* pharmacology includes study of therapeutic effects of a drug in an isolated environment, such as cell lines or tissues. This setup conveniently eliminates whole organism's physiological influences allowing for a detailed analysis and a compounds impact.

In vitro pharmacological examinations, (for example, capacity of a medication to treat malignancy) are regularly first performed *in vitro* - either in a test tube or laboratory dish. A model would develop malignant growth cells in a dish outside of the body. This should be possible by utilizing various mediums which permit developing these cells free of the body.

Studies are normally done *in-vitro* first for ethical reasons. *In vitro* investigations enable a substance to be considered securely, as individuals or creatures are not exposed to the conceivable reactions or lethality of another medication. This learns however much as could reasonably be expected about a medication before presenting people to these potential impacts. In the event that a chemotherapeutic medication, for instance, does not chip away at malignancy cells developed in a dish, it is dishonest to have people utilize the medication and hazard the potential poisonous quality.

Advantages: *In vitro* pharmacological investigations are significant, in that they permit increasingly quick advancement of new medicines - numerous medications can be learned at once (and they can be contemplated in countless examples of cells) and just those that seem, by all accounts, to be strong go on to human examinations.

Disadvantage: A non-attendance of pharmacokinetics, in medicinal phrasing, is one of the critical downsides of *in vitro* pharmacological investigations. An absence of pharmacokinetics, just as a few different variables, can make it hard to extrapolate the outcomes to what may be normal when the medication is utilized in vivo.

PHYSIOLOGICAL SALT SOLUTIONS

Physiological salt solution can be defined as artificially prepared solution to keep isolated tissue alive under experimental conditions. They provide isotonicity, nutrition and act as a buffer when drugs are added. As animal experiments have to be done with isolated organs, it is necessary to use a certain number of physiological solutions of different ionic concentrations which almost act as a substitute to the tissue fluid.

It was "Ringer" who first introduced the idea that tissue could be kept alive by providing proper nutrition, oxygen, temperature, pH etc. The content of these solutions carries according to tissues and animals selected for experimentation. These solutions provide food

material i.e. energy, oxygen, electrolytes like proportion as that present in tissue fluid. They exert same osmotic pressure as that of interstitial fluid i.e. isotonic with body fluids.

All PSS are prepared in distilled water. PSS are prepared fresh and utilized within 24 hours. Storage is not suggested because of microbial growth. While preparing the PSS, calcium chloride should be added last in the form of solution in order to prevent the precipitation of bicarbonate and isolated tissue will not live for extended period in cloudy PSS. Cloudy PSS also gives erratic response with drugs.

Any variant from the principle will lead to shrinkage or blotting depending on hypertonicity and loss of physiological function. For these two things should be remembered:

1. Prepare solution carefully with pure material.
2. They can be kept for about 24 hours and as they are good media for the growth of micro-organisms, they must be refrigerated and should be freshly prepared after 24 hours.

- **Following things should be carefully noted at the time of preparation of solution:**
  1. **Balance of ions:** Absolute quantity of each ion and preparation among each other especially with calcium and potassium must be maintained.
  2. **pH of solution/reaction of solution:** pH of various PSS varies from 7.3-7.8 depending upon organ. At lower pH value, tone of preparations tends to decrease and impact of medicament is also altered. pH affects tissue directly and by ionization. At elevated pH ionization is less and leads to alkalinity and thus improves cardiac and smooth muscle activity. During experiment there can be accumulation of metabolite which may change the pH. Buffering agents like HCO_3 and PO_4 are added in saline solution and solutions are changed frequently.
  3. **Glucose:** Introduced by "Locke" and serves as source of energy, increases contractility of tissue. Glucose is not essential constituent for amphibian's tissue, but essential for mammalian tissues.
  4. **Distilled Water:** Distilled water serves as a vehicle to dissolve various ingredients.
  5. **Control of temperature:** For consistent effect, it is important to maintain the temperature of PSS, particularly for mammalian tissue. For instance, when temperature of solution is below 37°C, tone of intestine is decreased, increased contracts become smaller and contraction and relaxation time increases; whereas amphibian tissues survive for longer time at normal environment.
  6. **Aeration:** Air, oxygen or oxygen + 5% carbon dioxide are required for the correct working of the tissues. Other than giving oxygen to the tissues, the flood of gas bubbles likewise blends the arrangements in the shower in this manner encouraging dissemination of the medications. The arrangement in the shower ought to be changed oftentimes in light of the fact that delayed air circulation will in general modify pH.

COMMON IONS USED IN PSS AND THEIR USES

1. **Sodium:** Responsible for maintenance of excitability, contractibility, rhythmicity of muscles and nerves.
2. **Potassium:** Responsible for increased relaxation of heart, increased neuromuscular transmission and excitability of nerves.
3. **Calcium:** Responsible for contraction of smooth muscle.
4. **Magnesium chloride and magnesium sulphate:** Responsible for relaxation of smooth muscles.
5. **Glucose:** Provides energy to the cell.
6. **Sodium bicarbonate:** Maintains the alkaline pH.
7. **Potassium dihydrogen phosphate or sodium dihydrogen phosphate:** Acts as a buffer.

DIFFERENT PSS AND THEIR USES

1. **Ringer Locke solution:** For isolated rabbit heart perfusion.
2. **Frog Ringer solution:** Used in rectus abdominis muscle, heart and other preparations of frog.
3. **Tyrode solution:** For experimentation in rabbit intestine and guinea pig ileum, rat ileum, etc.
4. **De-Jalon solution:** Used in rat uterus etc.
5. **Kreb's Henseleit solution:** For tracheal chain of guinea pig, vas deference, fundus strip of rat and aortic strip preparation of rabbit.

Table 1.1: Composition of Physiological Salt Solutions (PSS) (g/*l*)

Salts (g/*l*)	Ringer/ Ringer Locke	Frog Ringer	Tyrode	De Jalon	Kreb's-Henseleit	Mc Ewen	Hukovic
NaCl	9.00	6.5	8.0	9.0	6.9	6.6	6.6
KCl	0.42	0.14	0.2	0.42	0.35	0.42	0.34
$CaCl_2$	0.24	0.12	0.2	0.06	0.28	0.24	0.28
$NaHCO_3$	0.5	0.2	1.0	0.5	2.1	2.1	2.1
$MgCl_2$	-	-	0.1	-	-	-	-
$MgSO_4 \cdot 7H_2O$	-	-	-	-	0.28	-	0.26
NaH_2PO_4	-	0.01	0.05	-	-	0.16	-
KH_2PO_4	-	-	-	-	0.16	-	0.15
Glucose	1.0	2	1.0	0.5	2.0	2.0	2.0
Sucrose	-	-	-	-	-	4.5	-
Aeration	O_2	Air	O_2/Air	O_2 + 5% CO_2	O_2 + 5% CO_2	O_2	Air

VIVA VOCE QUESTIONS

1. Define PSS.

Ans. Physiological Salt Solution can be defined as artificially prepared solution to keep isolated tissue alive under experimental conditions.

2. Enlist the various ingredients present in PSS.

Ans. NaCl, KCl, $CaCl_2$, $NaHCO_3$, $MgCl_2$, $MgSO_4 \cdot 7H_2O$, NaH_2PO_4, KH_2PO_4, Glucose and Sucrose.

3. Give the uses of various PSS.

Ans. **(i) Ringer Locke solution:** It is used in isolated rabbit heart perfusion.

(ii) Frog Ringer solution: Used in rectus abdominis muscle, heart and other preparations of frog.

(iii) Tyrode solution: For test of rabbit intestine and ileum of guinea pig, rat ileum etc.

(iv) De-Jalon solution: Used in rat uterus etc.

(v) Kreb's Henseleit solution: For tracheal chain preparations of guinea pig, vas deferens, fundus strips of rat and aortic strip preparation of rabbit.

4. Write significance of PSS.

Ans. As experiments in animals are performed with isolated organs, it's essential to utilize a certain number of physiological solutions of different ionic concentration which almost operate like a substitute to the tissue fluid. They provide isotonicity, nutrition as well as work like a buffer when drugs are added.

5. Define *In vitro* pharmacology with advantage and disadvantage.

Ans. ***In vitro* pharmacology** is the study finished in the laboratory and to study therapeutics outcome of a drug in an isolated environment, such as cell lines or tissues.

Advantages: *In vitro* pharmacological investigations are significant in that they permit increasingly quick advancement of new medicines - numerous medications can be learned at once (and they can be contemplated in countless examples of cells) and just those that seem, by all accounts, to be strong go on to human examinations.

Disadvantage: A non-attendance of pharmacokinetics, in medicinal phrasing, is one of the critical downsides of *in vitro* pharmacological investigations. An absence of pharmacokinetics, just as a few different variables, can make it hard to extrapolate the outcomes to what may be normal when the medication is utilized in vivo.

MULTIPLE CHOICE QUESTIONS (MCQ'S)

1. PSS means :

(a) Physiological Salt Solution (b) Physiological Sugar Solution

(c) Both (a) and (b) (d) None of these

2. Role of Glucose in PSS :

(a) Maintains the alkaline pH (b) Acts as a buffer

(c) Provides energy to the cell (d) All these

3. While preparing the PSS, calcium chloride should be added last as a solution for prevention of precipitation of bicarbonate.

(a) True (b) False

4. Tyrode solution contains …… g/*l* of NaCl.

(a) 6.0 g/*l* (b) 6.6 g/*l*

(c) 8.0 g/*l* (d) 9.0 g/*l*

5. All PSS are prepared in distilled water.

(a) True (b) False

Answers :

1. (a)	2. (c)	3. (a)	4. (c)	5. (a)

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Experiment No. 2

Aim: To study effect of drugs on isolated frog heart.

INTRODUCTION:

Frog:

Frogs are creatures of land and water, animals that occupy both land and water situations similarly effectively. There are believed to be around 5,000 unique types of frogs far and wide. Frogs are outstanding for their looped, sticky tongue which they anticipate out of their mouths to get creepy crawlies. Frogs are likewise notable for having the option to inhale through their skin just as their lungs.

Most types of frogs have projecting eyes, no tail, and solid and have webbed hands and feet, which help the frog in swimming, bouncing and notwithstanding climbing. Frogs tend to lay their eggs (known as frog bring forth) in lakes, however a few frogs have been known to likewise lay their eggs in enormous puddles. Infant frogs are called tadpoles and are totally water-based until the tadpoles create arms and legs and can move out of the water.

Most frogs eat creepy crawlies, other little arthropods, or worms, however various they additionally eat different frogs, rodents, and reptiles.

Frog heart:

Heart of frog is three chambered. It is dark red colored conical muscular organ situated mid-ventrally in the frontal fraction of the body cavity in between two lungs. The heart is enclosed in two membranes an inner epicardium and outer pericardium. The space between these two layers is called pericardial cavity in which pericardial fluid is present.

External structure of heart:

Remotely heart resembles a triangular structure. It is ruddy shading. It is 3 chambered other than sinus venosus and truncus arteriosus. Its foremost end is wide and back end is to some degree pointed. The front more extensive part is called auricles though the back part is called ventricles.

Auricles are two-chambered: left and right auricles. These auricles are separated remotely by extremely black out longitudinal interauricular groove. So it remotely seems one.

Ventricle is single chambered. It is funnel shaped fit as a fiddle with thick solid dividers.

Heart of frog consists of two additional chambers:

1. Sinus venosus
2. Truncus arteriosus

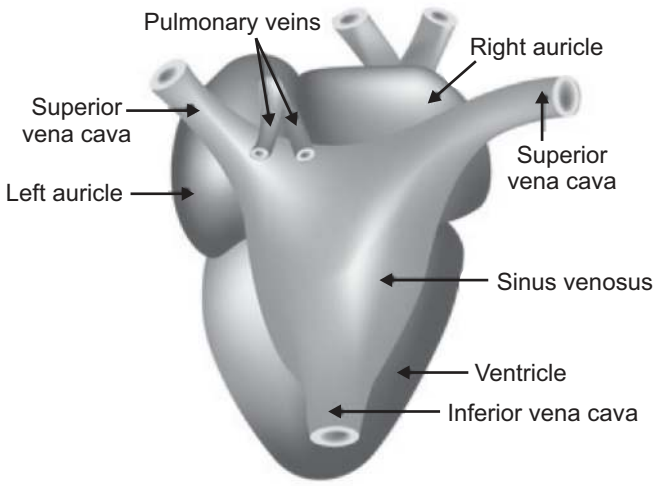

Fig. 2.1: Frog heart

Internal structure of heart of frog:

The ventral perspective on inward structure of heart appears two auricles, one ventricle, truncus arteriosus and the valves, to keep the blood streaming one way. The mass of heart comprises of three layers external epicardium, center mesocardium and inward endocardium.

Frog heart is 3-chambered with two auricles and one ventricle. The two auricles are isolated from one another by interauricular septum. Right auricle is bigger than left.

PRINCIPLE:

Heart is provided by ANS. Adrenaline goes about as an agonist. It follows up on beta receptors and builds pulse and plentifulness. Acetylcholine follows up on muscarinic receptors as an agonist and diminishes the pulse and abundance. Abundance convergence of potassium chloride stops the heart beat during diastolic stage. Overabundance centralization of calcium particle stops heart beat during systolic stage. Potassium and calcium particle follow up on cardiovascular muscle through non-receptor component of activity.

REQUIREMENTS:

Apparatus: Mariotte bottle, Syme's cannula clamp, recording drum, Starling heart lever, pin hook, thread, syringe and needle etc.

Drugs: Adrenaline (10 µg/ml and 100 µg/ml)
Acetylcholine (10 µg/ml and 100 µg/ml)
Potassium chloride (KCl – 10 mg/ml)
Calcium chloride ($CaCl_2$ – 10 mg/ml)
Distilled water

PSS: Frog ringer solution.

PROCEDURE:

1. Set up the assembly.
2. Sacrify the frog by pithing or by stunning.

3. Place the frog in a tray with ventral side facing up.
4. Make an incision to skin longitudinally and then expose the rectus muscle.
5. Make incision around the rectus muscle without damaging the frontal abdominal vein.
6. Expose the heart after cutting the sternum, then pericardial membrane remove and tie one part of aorta.
7. Put a knot around the inferior vena cava, then make a small cut for cannulation.
8. After cannulation with Syme's cannula, cut the other part of aorta and isolate the heart and perfuse with frog ringer solution. Supply frog ringer solution through the horizontal arm of the syme's cannula.
9. Place the heart clip on the heart apex, later connect it to a starling heart lever.
10. Record the normal heart beat on a smoked drum.
11. Inject 0.05 – 0.1 ml of adrenaline solution into syme's cannula. Immediately switch on the kymograph and record result of adrenaline for 2 minutes period. After 2 minutes turn off the kymograph till the heart beat and amplitude comes to normal.
12. Inject 0.05 – 0.1 ml of acetylcholine solution into syme's cannula. Immediately switch on the kymograph and record the outcome of acetylcholine (ACh) for 2 minutes period. After 2 minutes turn off the kymograph till the heart beat and amplitude comes to normal.
13. Administer 0.1 ml of KCl solution into syme's cannula. Immediately switch on the kymograph and record the result of KCl for 2 minutes period. After 2 minutes turn off the kymograph till the heart beat and amplitude comes to normal.
14. Inject 0.1 – 0.4 ml of calcium chloride solution into syme's cannula. Immediately switch on the kymograph and record the result of calcium chloride for 3 minutes period. After 3 minutes turn off the kymograph till the heart beat and amplitude comes to normal.
15. Observe the onset and duration of action of all, i.e. Adrenaline, acetylcholine, potassium chloride and calcium chloride.

OBSERVATIONS AND CONCLUSION:

1. Adrenaline: It is responsible for increasing heart rate and amplitude. Heart contains beta receptors. Adrenaline stimulates beta receptors, thereby enhance the heart rate and amplitude. Drugs which block beta receptors (Propranolol, Atenolol etc.) are clinically used in hypertension and tachycardia.

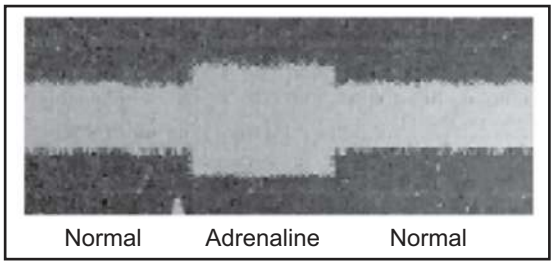

Fig. 2.2: Effect of Adrenaline on isolated frog heart