EXPERIMENT NO:-02 **DATE**

AIM: - To study the effects of various drugs on the Isolated Heart of Frog.

INTRODUCTION

Frog heart:

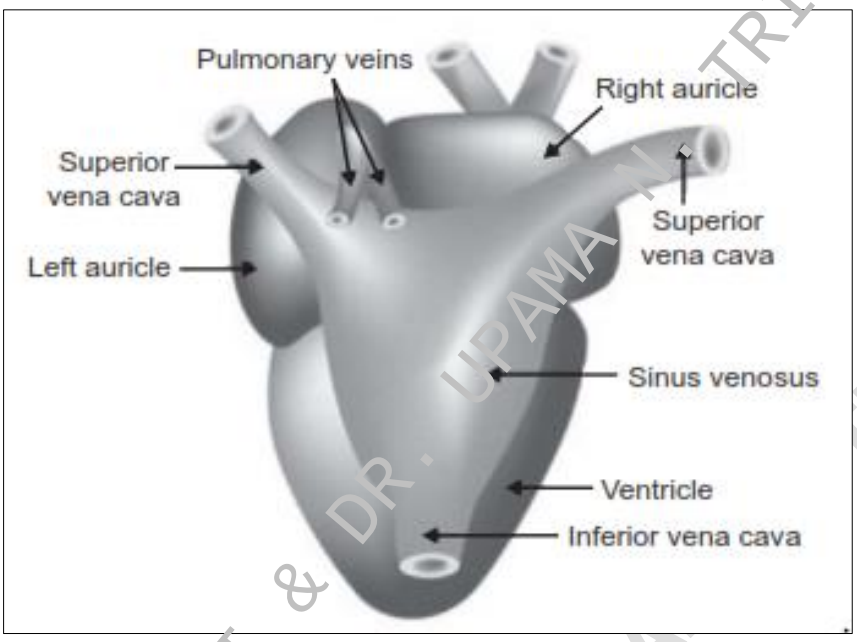
- ✓ Heart of frog is three chambered.
- ✓ It is dark red colored conical muscular organ situated mid-ventrally in the frontal fraction of the body cavity in between two lungs.
- ✓ The heart is enclosed in two membranes an inner epicardium and outer pericardium.
- ✓ The space between these two layers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

External structure of heart:

- ✓ Remotely heart resembles a triangular structure.
- ✓ It is 3 chambered other than sinus venosus and truncus arteriosus.
- ✓ The front more extensive part is called auricles though the back part is called ventricles.
- ✓ Auricles are two-chambered: left and right auricles.
- ✓ Ventricle is single chambered.
- ✓ It is funnel shaped fit as a fiddle with thick solid dividers.

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.



Frog Heart

THEORY:

- ✓ Many drugs act on the heart. Adrenergic and cholinergic drugs produce opposite effects.
- ✓ These drugs act through respective receptors.
- ✓ Some drugs act directly on the heart.
- ✓ This experiment demonstrates the effects of a few drugs (agonists, antagonists, calcium and potassium) on the isolated heart of frog.

PRINCIPLE:

Drugs may influence the rate (chronotropy) and force (inotropy) of contraction of the heart. An increase in the heart rate is called a "positive chronotropic" response, while a "negative chronotropic" response is a decrease in the heart rate. Similarly, an increase in the force of contraction is called a 'positive inotropic' response and a decrease in the force of contraction is called a 'negative inotropic' response. Sympathomimetic amines such as adrenaline and noradrenaline produce positive inotropic and positive chronotropic response. Whereas parasympathomimetics such as acetylcholine produce negative inotropic and negative chronotropic response.

REQUIREMENTS:

Instruments

- ✓ Starling's heart lever with stylet
- ✓ A kymograph with Recording drum
- ✓ Reservoir, Tubing, Screw clips, heart cannula, Clamp, Boss head, Pin Hook, Thread, Syringe and Needle, Stand.

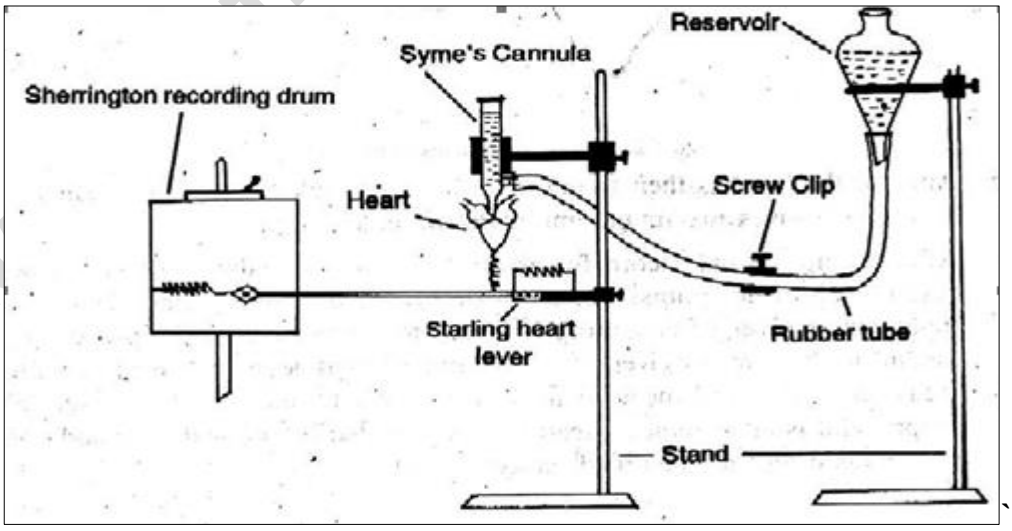
Experimental Condition

- ✓ **PSS**:-Frog Ringer
- ✓ **Temperature:- 37°c**
- ✓ **Aeration:-** Carbogen

Drugs & Solution

S.NO.	NAME OF DRUG	DOSE(MCG)	CONCENTRATION
1.	Potassium Chloride	2000	10 mg/ml
2.	Epinephrine(Adrenaline)	2	10 mcg/ml
3.	Norepinephrine(Noradrenaline)	2	10 mcg/ml
4.	Isoprenaline	2	10 mcg/ml
5.	Propranolol	200	1 mg/ml
6.	Acetylcholine	2	10 mg/ml
8.	Calcium Chloride	2000	10 mg/ml
9.	Atropine sulphate	20	100 mg/ml
10.	Digoxin	2	

***Volume of above solutions to be injected = 0.1, 0.2, 0.3 & 0.8 ml; mcg = micrograms



Isolated Frog heart assembly

Step-1 Pithing of frog:

- Holding the frog in such a way that the thumb of left hand is pressed against its back, right front leg of frog is held between index finger and middle finger of the left hand while rest two fingers are on its back. Left front leg and hind of the frog are free.
- Position for pithing: Pithing is done at the junction between cranium and atlas vertebra (this relates to the foramen magnum). The position of foremen, magnum is decided by sliding the pithing needle along the midline of frog's head. Pithing has to be done at the point where the first slight depression is felt.
- Pithing: Insert a sharp needle in the foremen magnum towards the brain and destroy a part of it. Then remove and reinsert the needle in opened spinal canal and destroy a part of the spinal cord by inserting the needle backwards. This may cause the frog to urinate and throw its hind leg in convulsion.
- Checking the reflux: To see whether the frog has been properly pithed, touch the cornea of eye with the needle and see whether corneal responses have completely subsided. Also 'touch and pain' reflexes can be checked by superficially pricking the hind leg of the frog to see whether jerking movement occurs. A properly pithed frog shows neither corneal nor pain reflexes.

Step-2 Dissection:

- Lay the pithed frog on its back. With a fine scissors, take a small 'V' shaped cut in the abdominal skin at the pelvic girdle. Insert a curved scissors in the 'V' shaped cut and cut the abdominal skin up to pectoral girdle.
- The underlying muscular part shows rectus abdominal muscle. Take a bold cut on one side of the central vein. Through this cut, insert the blunt side of the scissors and take a cut up to pelvic girdle without injuring the visceral organs.
- Cut the pelvic girdle with a bone cutter or larger scissors to expose the heart.

Step 3 Mounting:

- Remove the pericardium with the help of a blunt forceps to avoid any injury to the heart. With the thumb of left hand push upwards the ventricle of heart and locate the sinus venosus.
- Pass a small piece of double thread below the sinus venosus. With a fine scissors take a cut at the central vein in the sinus venosus.

- Start a weak flow of P.S.S through the cannula. Insert the cannula in the central vein of sinus venosus through the cut and tie it in position with the tread. Cut the aorta to let out the perfusate.
- Hold the cannula between the index finger and the middle finger of the left hand and slightly lift it up. Carefully cut off the tissues attaching to the heart with a scissors and isolated heart on the stand as shown. Superficially insert, the pin attached to starling's heart lever, in the wall of ventricle at its tip. Adjust lever to make it horizontal.

PROCEDURE

1. The assembly is being set up as shown in the figure.
2. The frog is scarify by pithing as per CPCSEA recommended guidelines and then placed in a tray with ventral side facing upwards.
3. The skin and then the abdominal wall are incised and after cutting sternum heart is exposed. Pericardium of heart is removed. The inferior vena cava and aortae are cleaned.
4. A thread is passed under inferior versa cava.
5. A small 'V' shaped cut is given in inferior vena cava and the tip of Syme's cannula is passed into it. It is tied firmly with inferior vena cava. Immediately the aortae are cut and carefully heart along with cannula are isolated from the animal.
6. Horizontal arm of the Syme's cannula is connected to the reservoir while the vertical firm is fixed with the clamp. The flow of the PSS is adjusted such that the level of fluid in the vertical arm remains constant.
7. Place the heart clip on the heart apex, later connect it to a starling heart lever.
8. The tension is adjusted with the spring such that gives maximum contraction.
9. After taking the normal records for about 2-3 cm, various drugs are added and the effects are recorded.
10. The next drug is given only after recovery from the effect of the preceding drug. Heart rate is recorded before and after addition of various drugs.

OBSERVATION TABLE: Write result in terms of increase/decrease/normal

Sr. No	Drug and dose		Heart rate (Beats/min)	Amplitude	Tone
1.	PSS		65	Normal	Normal
2.	KCL (1%)	0.1ml			
		0.2ml			
		0.3 ml			
		0.8 ml			
3.	CaCl2 (1%)	0.1ml			
		0.2ml			
		0.3 ml			
		0.8 ml			
4.	Epinephrine	0.1ml			
		0.2ml			
		0.3 ml			
		0.8 ml			
5.	Norepinephrine	0.1ml			
		0.2ml			
		0.3 ml			
		0.8 ml			
6.	Isoprenaline	0.1ml			
		0.2ml			
		0.3 ml			
		0.8 ml			
7.	Propranolol	0.1ml			
		0.2ml			
		0.3 ml			
		0.8 ml			
8.	ACH (10ug/ml)	0.1ml			
		0.2ml			
		0.3 ml			
		0.8 ml			
9.	Atropine ((1ug/ml)	0.1ml			
		0.2ml			
		0.3 ml			
		0.8 ml			
10.	Digoxin	0.1ml			
		0.2ml			
		0.3 ml			
		0.8 ml			

GRAPH/RESULT

Discussion:

1. **Adrenaline & Noradrenaline** produces increase in heart rate (positive chronotropic action) and force of contraction (positive inotropic action). The recordings are characterized by an increase in amplitude and the beats come closer.
2. **Isoprenaline** produces increase in heart rate (positive chronotropic action) and force of contraction (positive inotropic action).It having more potent effect on heart as compare to Adr and NA. It strong agonist of b receptor.
3. **Acetylcholine** (Ach) (in low dose) reduces heart rate (negative chronotropic action) and force of contraction (negative inotropic action). In high doses heart stop giving straight line on lower border (i.e., in the diastolic condition). On recovery initially there is increase in force of contraction. (as per Starling's law)
4. **Potassium chloride** (KCl) produces the same effect as that produced by Acetylcholine.
5. **Calcium chloride** ($CaCl_2$) in lower doses (less than 1%) increases heart rate and force of contraction but in high doses (1% or more) it inhibits the heart in systole characterized by straight line recording on upper margin. Another characteristic of $CaCl_2$ commonly observed is that 2-3 beats show increased force of contraction before the heart stops in systole transiently.
6. **Atropine** as such does not produce any effect except a slight increase in heart rate and force of contraction. This action may not be seen many a times. However, in presence of atropine, ACh fails to produce any inhibition. The responses to other agents are not affected.
7. **Propranolol** also as such does not produce any effect except that sometimes there may be slight increase in heart rate and force of contraction. If highest doses are used slight inhibition (not stopage) may also be observed. The characteristic is that in the presence of propranolol adrenaline fails to produce any increase in heart rate or force of contraction. The effects of other agents are not affected.
8. **Digoxin** (Digitalis act on hypodermic heart) Inhibits Na+/K+ ATPase pump which leads to increase CA++ in SR and increased release of CA++ in action potential Effect on contractility- Decrease heart rate. Effect on contractility Increase. Clinical applications Used to regulate arrhythmias in atrial fibrillation or flutter

QUESTIONS

1. Name the cannula and site for inserting the cannula for isolated frog heart experiments.
2. Define:
    a. Inotropic
    b. Chronotropic
    c. Dromotropic
    d. Bathmotropic
    e. Lusitropic
3. Which receptor are present in Heart?
4. Discuss effects of Ach, Adr, Propanolol on heart.