EXPERIMENT: 6 **DATE:**

AIM: TO STUDY THE EFFECT OF K+, CA++, ACETYLCHOLINE AND ADRENALINE ON FROG'S HEART

REQUIREMENTS:

Reservoir, Starling's Heart lever, Sherrington's Revolving drum machine, Kymograph, Syme's Cannula, Beaker, Thread, Tray, Dissection box.

THEORY:

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 on 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 figure and the speed of drum is adjusted to minimum.

2.The frog is sacrificed as per CPCSEA recommended guidelines and the abdominal and thoracic cavities are opened to the heart.

3.The frog is placed on the frog's board and it is tied to it. Pericardium over the heart is removed and the inferior venacava is exposed and a thread is passed under it.

4.A small 'V' shaped cut is given in inferior venacava and the venous cannula is passed in to it and is tied firmly. Immediately both aortae are cut just before they form branches.

5.Apex of the heart is taken attached to the lever and the tension is adjusted such that it gives maximum amplitude of concentration.

6.After taking normal record for about 2-3 cms, calcium chloride, potassium chloride, acetylcholine, adrenaline are given sequently in graded dose and observe the response.

OBSERVATION TABLE:

Drug and dose		Heart rate (Beats/min)	Amplitude	Tone
PSS		65	Normal	Normal
CaCl2 (1%)	0.1ml	72	Increased	Increased
	0.2ml	70	Increased	Increased
	0.3 ml	60	Increased	Decreased
	0.8 ml	Stops in systole	--------	---------
KCL (1%)	0.1ml	72	Normal	Normal
	0.2ml	70	Decreased	Decreased
	0.3 ml	60	Decreased	Decreased
	0.8 ml	Stops in Diastole	--------	---------
ACH (10ug/ml)	0.1ml	72	Increased	Increased
	0.2ml	70	Increased	Increased
	0.3 ml	60	Increased	Decreased
	0.8 ml	Stops in systole	--------	---------
Adr ((10ug/ml)	0.1ml	72	Increased	Normal
	0.2ml	70	Decreased	Decreased
	0.3 ml	60	Decreased	Decreased
	0.8 ml	Stops in Diastole	--------	---------

RESULTS:

1. CaCl2 in low dose (< 1%) increase heart rate (Positive Chronotropic) and force (Positive ionotropic) of contraction but in high dose it inhibits the heart in systole characterized by straight line recording on upper margin.
2. KCl in low dose (< 1%) Decreased heart rate (Negative Chronotropic) and force (Negative ionotropic) of contraction but in high dose it inhibits the heart in Diastole characterized by straight line recording on lower margin.
3. Acetylcholine (Ach) in low dose reduces heart rate (Negative Chronotropic) and force (Negative ionotropic) of contraction but in high dose it inhibits the heart in Diastole characterized by straight line recording on lower margin.
4. Adrenaline in low dose increase heart rate (Positive Chronotropic) and force (Positive ionotropic) of contraction but in high dose it inhibits the heart in systole characterized by straight line recording on upper margin.

SUMMARY OF SOME OTHER DRUGS EFFECTS:

	Mechanism	Effect on heart	Effect on contractility	Clinical applications
Digoxin (Digitalis)	Inhibits Na^+/K^+ ATPase pump which leads to increase CA^{++} in SR and increased release of CA^{++} in action potential	Decrease heart rate	Increase	Used to regulate arrhythmias in atrial fibrillation or flutter
Atropine	Binds to acetylcholine receptors and inhibit parasympathetic response	Increase	Increase	Dilate pupils, used to treat bradycardia, 2nd & 3rd degree heart block, cardiac arrest
Caffeine	Adenosine receptor antagonist sympathomimetics	Increase	Increase	Treat apnea, bronchopulmonary dysplasia & fecal incontinence
$Cacl_2$	Increase available Ca^{++}	No effect	Increase	Treatment of hypocalcaemia, neonatal tenancy, insect bites, spider bites
Acetylcholine	Hormone or NT binds to cholinergic receptors	Decrease	Decrease	Parasympathetic response
Epinephrine	Hormone binds to adrenergic receptors	Increase	Increase	Sympathetic response
Nicotine	Activate sympathetic nervous system	Increase	Increase (In high conc.)	Antimigraine drug, Alzheimer's, Parkinson's
KCl		Stop heart		Used for lethal injections

OBSERVED RESULT:

Drug and dose		Heart rate (Beats/min)	Amplitude	Tone
PSS				
CaCl2 (1%)	0.1ml			
	0.2ml			
	0.3 ml			
	0.8 ml			
KCL (1%)	0.1ml			
	0.2ml			
	0.3 ml			
	0.8 ml			
ACH (10ug/ml)	0.1ml			
	0.2ml			
	0.3 ml			
	0.8 ml			
Adr ((10ug/ml)	0.1ml			
	0.2ml			
	0.3 ml			
	0.8 ml			

TEACHER'S SIGNATURE