EXPERIMENT NO.: 8 **DATE:**

AIM: TO STUDY THE DOSE RESPONSE CURVE OF ACH USING RAT ILEUM

REQUIREMENTS:

Animal: Rat (of either sex weighing between 200-250g.)

Drugs: Acetylcholine (1 µg/mL, 10 µg/mL, 100 µg/mL)

Apparatus: Reservoir, Tubing, isolated organ bath, organ tube, heating coil, Thermostat, Isotonic frontal writhing lever, Recording drum, Aeration tube cum tissue holder, Haemostatic forceps, sketch pen tip, ink etc.

EXPERIMENTAL CONDITION:

Physiological Salt Solution	: Tyrode
Temperature	: 37 ± 1° C
Basal Tension on Lever	: 500 mg
Contact time	: 30 sec.
Aeration	: Carbogen (95% O_2 and 5% CO_2)
Magnification of the response	: 10 times
Drug	: Acetylcholine Chloride (1, 10 or 100 µg/mL)
Molecular weight of drug	: 181.78

PRINCIPLE:

Acetylcholine produces a dose dependent concentration of rat ileum smooth muscle. First taken the two equipotent response of same dose and then taken the graded response.

THEORY:

Graded Dose Response Relationship Curve of Acetylcholine on Frog Rectus Muscle:

In graded dose response curve,

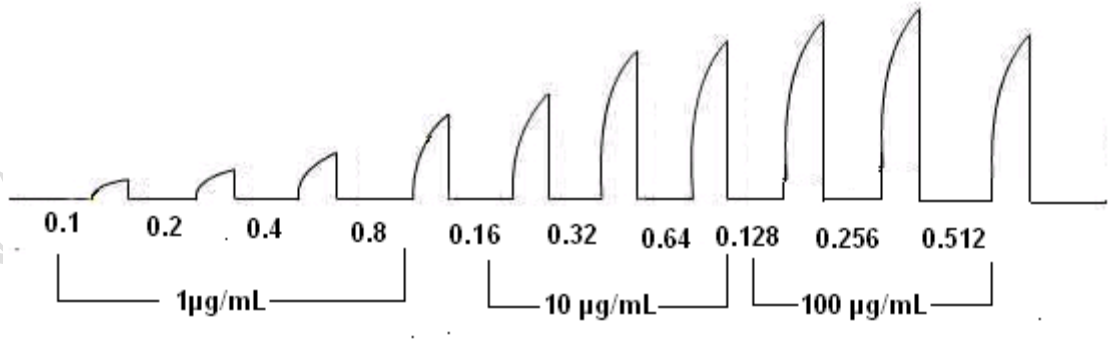
- Single biological unit, either a single animal or an isolated tissue is used.
- It depends upon an observation that graded increase (in geometric proportion) in the dose of drug gives proportional rise in the magnitude of biological response.
- Actually, beyond a specific dose level, biological response increases in proportion to the increase in dose. This dose level is known as ‘Threshold dose’.
- Such proportional rise in biological response occurs only up to a dose level known as ‘Ceiling dose’, beyond which a steady biological response is achieved even after increasing the doses.
- Shape of Graded DRC, when plotted as ‘dose Vs Response’ is a ‘Parabola’

- Shape of 'Log Dose Vs Response' curve is a 'Sigmoid' line or is having 'S' like shape.

PROCEDURE:

- The assembly is set up and the arrangements are made for the above mentioned condition.
- A rat fasted over night was anaesthetized by chloroform and sacrificed by as per CPCSEA recommended guidelines.
- The abdominal cavity was quickly opened through a midline incision, Ileum is separated and mounted in the organ bath.
- One end of the ileum is tied to the aeration tube and the other is connected to the isolated frontal writing lever.
- The ileum is allowed to stabilize for half an hour. During this period the PSS is changed after every ten min. Once the tissue is stabilize, graded doses of Ach are added to at defined time period of interval for obtain contractile responses.
    - 00 sec: Start the drum and record a base line for 30 sec.
    - 30 sec: Add the first dose of drug in organ bath and take the response for another 30sec.
    - 60: Stop the drum and give wash until the tip of lever rich to baseline.
    - Continue above procedure for next doses.
- Measure the height of concentration at different doses of Ach.
- Tabulate the observations into three columns as Dose of Ach, Height of concentration (in mm) and % response.

GRAPH:



OBSERVATION TABLE:

Standard example:

Sr. No	Drug Name	Conc. of drug	Dose of drug in mL	Response in mm	% Response
1.	Ach	1 µg/mL	0.1	2	20
2.			0.2	5	25
3.			0.4	7	35
4.			0.8	9	45
5.		10 µg/mL	0.16	12	60
6.			0.32	14	70
7.			0.64	16	80
8.		100 µg/mL	0.128	17	85
9.			0.256	20	100
10.			0.512	12	60

Observed result:

Sr. No	Drug Name	Conc. of drug	Dose of drug in mL	Response in mm	% Response
1.	Ach	µg/mL			
2.					
3.					
4.					
5.		µg/mL			
6.					
7.					
8.		µg/mL			
9.					
10.					

TEACHER'S SIGNATURE