INTRODUCTION

Bioassay of acetylcholine using rat ileum by four-point bioassay method

The four-point bioassay is a quantitative method used to determine the potency of a drug by comparing its response to that of a standard preparation. In the case of acetylcholine (Ach), this method is commonly used on isolated rat ileum due to its high sensitivity to cholinergic stimulation.

EQUIPMENT REQUIRED

Animal :-	Frog
Drug:-	Acetylcholine stock solution (100 µg/ml)
Instrument:-	Student Organ Bath, kymograph.
Physiological salt solution:-	Tyrode's solution

PRINCIPLE

The **four-point bioassay** is based on the principle that a biological tissue (rat ileum) responds in a reproducible manner to different concentrations of **acetylcholine (ACh)**, a neurotransmitter that stimulates smooth muscle contraction through **muscarinic (M3) receptors**. By comparing the contractile response of the ileum to known standard doses of acetylcholine and an unknown test sample, the concentration of the unknown sample can be determined.

Action of Acetylcholine on Rat Ileum:

- Acetylcholine binds to **M3 muscarinic receptors** on smooth muscle cells in the ileum, leading to an increase in intracellular **calcium ions** and resulting in contraction.
- The magnitude of contraction is directly proportional to the concentration of acetylcholine applied, up to a maximal response.

PROCEDURE:

Preparation of the Rat Ileum:

- Sacrifice the rat using ethical and approved methods (e.g., cervical dislocation under anaesthesia).
- Dissect out a **3-5 cm segment of the ileum** and wash it.
- Mount the ileum in **organ bath** filled with **Tyrode's solution** maintained at 37°C and continuously aerated with 95% O_2 and 5% CO_2.
- Attach the ileum to a lever system connected to a kymograph to record contractions.
- Maintain a resting tension of **0.5–1 g** and allow the tissue to equilibrate for **30 minutes**, washing it every 10 minutes.

Standard Drug (Acetylcholine) Preparation:

- Prepare a stock solution of **acetylcholine (ACh) with** 100 µg/ml concentration.
- From this, prepare a series of known concentrations.

Recording Dose-Response Relationship:

- Administer a small volume (e.g., **0.1 mL**) of the lowest standard concentration of ACh into the organ bath.
- Record the contractile response until it reaches a peak, then wash the tissue with Tyrode's solution.
- Allow a **3–5-minute recovery period** before administering the next higher concentration.
- Repeat this process for at least **two standard concentrations and two test sample concentrations** that produce comparable responses.

Data Analysis (Four-Point Bioassay):

- Measure and record the height of contraction (in mm or mg force) for each dose response (S1, S2, T1, T2).
- Plot a **log-dose response curve** using standard concentrations.
- Determine the unknown concentration of acetylcholine using dilution- factor for test solution.

Conclusion:

The unknown concentration of test sample for acetylcholine is determined by matching its effect with that of the standard solutions.

IDEAL OBSERVATION

Sr. No.	Conc. Of Ach (µg/ml)	Amount Added in Organ bath		Conc. Of Ach in µg/ml (in organ bath contains 20ml Solution)	Response (in mm)	% Response
		In mL	In µg			
1.	100	0.1	1	0.05	10	47.61
2.	100	0.1	1	0.05	10	47.61
3.	100	0.2	2	0.1	14	66.66
4.	100	0.4	4	0.2	17	80.95
5.	100	0.8	8	0.4	21	100.00
6.	100	1.6	16	0.8	21	100.00

Test solution of Ach		
Sr. No.	Amount added to organ bath (ml)	Response (in mm)
1	0.1	7
2	0.2	10
3	0.4	12
4	0.8	16

Sample	Dose (mL)	Conc. (µg)	Height of conc. (mm)	Mean Height
S1	0.1		10	S1= 10.5
S1			11	
S1			11	
S1			10	
T1	0.4		12	T1= 13
T1			13	
T1			13	
T1			14	
S2	0.2		14	S2=14.75
S2			15	
S2			15	
S2			15	
T2	0.8		16	T2= 15.5
T2			15	
T2			15	
T2			16	

Formula:-

$C_t = n_1/t \times \text{Antilog } [(T_2-S_2) + (T_1-S_1)/ (T_2-T_1) + (S_2-S_1) \times \log (n_2/n_1)] \times Cs$

Where :

S_1 = Height of contraction produced by standard dose S_1

S_2 = Height of contraction produced by standard dose S_2

T_1 = Height of contraction produced by standard dose T_1

T_2 = Height of contraction produced by standard dose T_2

n_1 = Dose (mL) of S1

n_2 = Dose (mL) of S2

t = Dose (mL) of T

C_t = Conc. Of test

Cs = Conc. of std.

C_t= 34.82 µg/ml

RESULT:

The four-point bioassay of acetylcholine using rat ileum demonstrated a dose-dependent contractile response. The standard and test doses produced measurable contractions, allowing for interpolation of the unknown concentration. The estimated potency of the unknown acetylcholine solution was found to be within an acceptable range of accuracy.

DISCUSSION:

The four-point bioassay method effectively determined the potency of acetylcholine by comparing the contractile responses of rat ileum to known and unknown concentrations. The dose-dependent increase in contraction confirmed the

activation of muscarinic receptors, which mediate smooth muscle contraction. The method's reliability was evident in the consistent response patterns and acceptable error margins.

However, factors such as tissue sensitivity, desensitization, and potential variability in biological response could influence results. Ensuring proper tissue handling, maintaining optimal physiological conditions, and minimizing external interference are crucial for accuracy. Despite these challenges, the four-point bioassay remains a precise and reproducible method for assessing acetylcholine activity in pharmacological studies.