

READER'S NOTE

TO SEE THE EFFECT OF DIFFERENT CONCENTRATION OF ACETYLCHOLINE ON RABBIT'S INTESTINE

- Apparatus used
- Preparation of Animal
- Preparation of Dilutions
- Functioning of apparatus i.e. kymograph organ bath

APPARATUS:

- Animal i.e. rabbit in this experiment
- Kymograph
- Organ bath with tubing & clamps
- Beakers of various strength eg 5, 10 mls etc
- Pipettes of various dilutions i.e. 1, 2 & 10 mls etc
- Chemical used i.e. Atropine & ACH
- Thread, scissors, stop watch
- Tyrode's solution contained in Bell Jar
- Oxygen.

PROCEDURE:

Rabbit's intestine dissected, 2cm piece taken. Thread is passed through both ends. One end tied to oxygen tube and other end to the lever. Record normal reading for 30 sec - Add 0.5ml of acetylcholine 1:2000 (62.54g) mix drug thoroughly. & record effect for 30 sec. Repeat procedure with different conc. of acetylcholine measure heights of conc. of each response with different conc. make table & plot graph on linear scale & log scale

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PREPARATION OF DILUTIONS:

We have stock solution with known conc. i.e. 10^{-3} which means that 1ml of this solution contains 1000 μ gs

Suppose we have solution whose conc is

1g/litre or 1000 mg/1000mls

or 1 mg/ml or 1000 μ gs/ml

or 1/1000 or 10^{-3} which means that 1ml of this

solution contains **1000 μ gs**. This is stock sol (known)

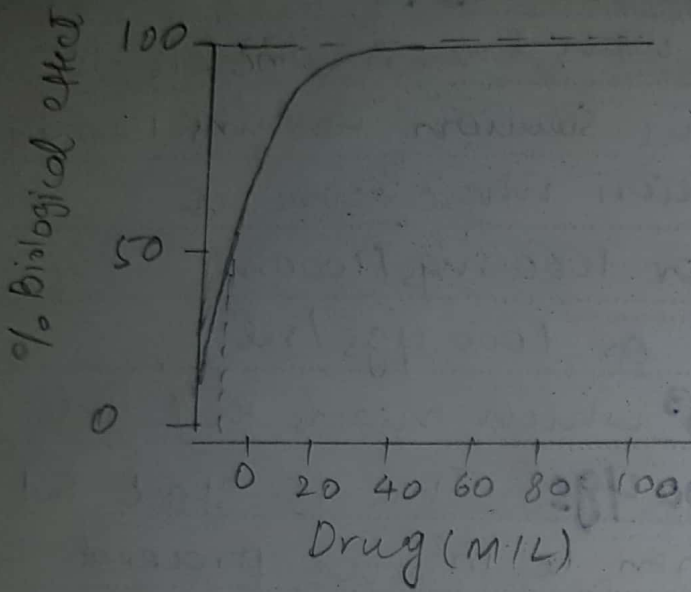
- From this stock solution i.e. 10^{-3} , we proceed to prepare 10^{-4} & 10^{-5} solutions
- Take 1ml of 10^{-3} sol & add 9ml of water. i.e. 1ml of 10^{-3} + 9ml of H_2O = 10mls of 10^{-4} means that 1ml of this sol contains **100 μ gs**
- Similarly take 1ml of 10^{-4} sol & add 9ml of water i.e. 1ml of 10^{-4} + 9mls of H_2O = 10mls of 10^{-5} which means that 1ml of this sol contain **10 μ gs**.

PRECAUTIONS:

- Oxygen must be flowing continuously in inner jacket
- Maintenance of Temp. at $38^{\circ}C$ in outer jacket
- Lever must be vertical
- Tyrode's solution & all dilutions must freshly prepared.
- Tissue (piece of ileum) must be properly submerged in Tyrode's solution. • Speed of kymograph kept at 2 which means 0.25 mm rotations/min - This means 1ml covered in 4 mins

Linear Scale

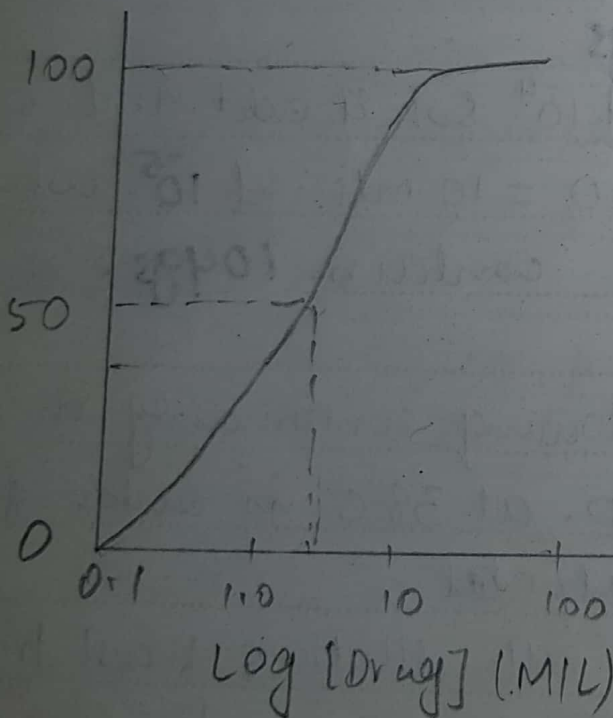
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Composition of Tyrode's solution

NaCl	8gms
KCl	0.2gms
CaCl ₂	0.2gms
MgCl ₂	0.01gms
NaH ₂ PO ₄	0.05gms
NaHCO ₃	1gm
Dextrose	1gm
H ₂ O upto	1L

Semilog Scale



Draw a dose response curve with acetylcholine alone and in presence of atropine.

APPARATUS:

- Tissue Bath with optimal Temp.
- Kymograph
- Oxygen cylinder
- Directing instruments
- Tyrode's solution
- ACh solution (different conc) 10^5 10^4 10^3
- Atropine solution
- Rabbit's ileum

PROCEDURE:

- Rabbit's ileum is dissected out about 2cm piece is taken out.
- Thread is passed through both ends of the strip.
- Mount one end of the oxygen tube and remains fixed in tissue bath whereas the other end tied to the lever which moves on the kymograph.
- Record the normal reading for 30 sec.
- Add 0.1 ml of aCh and record contractions for 30 sec.

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For Antagonism:

- Record normal contractions 30 sec
- Then add 0.1 ml of Atropine and 0.1 ml of Ach. Rest the tissue for 15 sec then record contractions.
- Wash tissue with Tyrode's solution and take normal contractions.
- Again add 0.1 ml of Atropine with successively increasing doses of Ach until maximum response is obtained.
- In the presence of atropine continuous increasing dose of Ach shows antagonism.
- Graph would be shifted to right.

PRECAUTIONS:

- Temperature of tissue bath should be maintained throughout the experiment at 37°C
- There should be continuous oxygen supply
- Level should be horizontal
- Make the tissue bath for constant level of solution.
- Always record normal contractions before adding next Ach concentration.

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TO STUDY THE EFFECTS OF DRUGS ON FROG'S HEART

APPARATUS:

- ⇒ Kymograph
- wooden board
- Dropper
- Dissecting box
- ⇒ Ringer's solution
- Adrenaline
- Propranolol
- Acetylcholine
- Atropine
- ⇒ Frog

PROCEDURE:

- A frog is pitted and placed on wooden board.
- Heart is exposed by a midline incision and pericardium is removed.
- A Thread is passed through the tip of ventricle the other end is attached to the horizontal lever to record contractions on moving drum.
- Now take normal contractions.
- Add 0.5 cc of Aev and wait for 2 mins - Now Record contractions.

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- Wash the specimen with Ringer's solution.
wait for 2 minutes.
- Add 0.5cc of Atropine
- wait for 2 mins and record contractions.
- Again wash the specimen with the Ringer's solution.

PRECAUTIONS:

- The Heart should be perfused at the constant rate ~~greater than~~ of 20-30 drops/minutes.
- Lever should be horizontal & thread vertical.
- Before recording contractions with each drug, record the normal contractions.
- Time Tracking is taken to calculate the Heart Rate.