Rabbit

TO observe the effects of different drugs on Rabbit Eye

(Pilocarpine) → Miosis, spasm of accomodation, Reflex that controls diameter ↑ of pupil

Time	Diameter		Colour of ~~Sclera~~ conjunctiva		Conj.' Reflex		Corneal Reflex		Light Reflex	
	Control	Exp.	C	E	C	E	C	E	C	E
0 min	6	6	Grey	White	✓	✓	✓	✓	✓	✓
5 min	6	5	"	"	✓	✓	✓	✓	✓	✓
10 min	6	4	"	"	✓	✓	✓	✓	✓	✓
15 min	6	3	"	"	✓	✓	✓	✓	✓	✓
20 min	6	3	"	"	✓	✓	✓	✓	✓	cant be perceived due to pupil contraction
25 min	6	3	"	Pink	✓	✓	✓	✓	✓	
30 min	6	3	"	Reddish	✓	✓	✓	✓	✓	

(Antimusunic)
Homatropine → Mydriasis → light reflex lost, dilation of pupil

Time	Diameter		Colour of Sclera		Conjunctival Reflex		Corneal Reflex		Light Reflex	
	C	E	C	E	C	E	C	E	C	E
0 min	6	6	White	Pink	✓	✓	✓	✓	✓	✓
5 min	6	9	"	"	✓	✓	✓	✓	✓	✓
10 min	6	10	Pink	"	✓	✓	✓	✓	✓	✓
15 min	6	11	"	"	✓	✓	✓	✓	✓	×
20 min	6	11	"	"	✓	✓	✓	✓	✓	×
25 min	6	12	"	"	✓	✓	✓	✓	✓	×
30 min	6	12	Reddish	"	✓	✓	✓	✓	×	×

Experiment # 14:

To observe effects of different drugs on Rabbit's Eye:

Iris contains two sets of muscles

i) Constrictor pupillae innervated parasympathetic fibres

ii) Dilator pupillae innervated by sympathetic fibres.

Normally a state of balance exists between them.

Cholinergic drugs → **Miosis**

Like pilocarpine, physostigmine causes miosis and spasm of accomodation by stimulating constrictor pupillae by parasym fibres. light reflex is present but sometimes not observed due to constrict

Parasympatholytics or Antimuscarinic drugs

like homatropine, tropicamide produces mydriasis, dilation of pupil due to unopposed action of dilator pupillae. Light reflex is absent.

Adsenergic drugs

like phenylephrine, adsenoline for eye examination, for dilatation of pupil but there is no cycloplegia. Light reflex is present

Local anasthetics

Abolish normal conjunctival & corneal reflexes. Cocaine was used previously but it is a vasoconstrictor & cause pain.

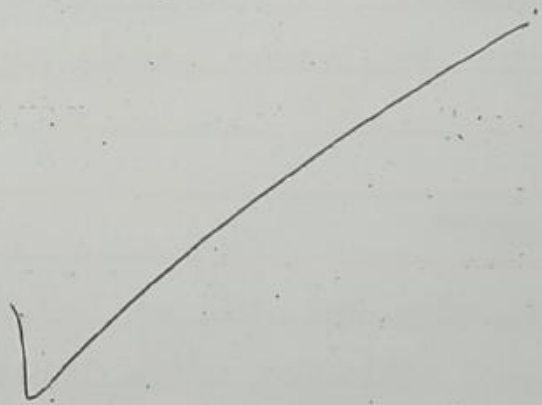
Active

- It is alkaloid, Symmisthetik & local anesthetic
- It causes vasconstriction, colour of conjunctiv becomes pale to white

↑
Xylocaine (local anaesthetic)

Time	Diameter		colour of sclera		Conjunctival Reflex		Corneal Reflex		Light Reflex	
	C	E	C	E	C	E	C	E	C	E
0min	8mm	8mm	Normal	Pink	✓	✓	✓	✓	✓	✓
5min	〃	〃	〃	〃	✓	✓	✓	✓	✓	✓
10min	〃	〃	〃	〃	✓	×	✓	×	✓	✓
15min	〃	〃	〃	〃	✓	×	✓	×	✓	✓
20min	〃	〃	〃	〃	✓	×	✓	×	✓	✓
25min	〃	〃	〃	〃	✓	×	✓	×	✓	✓
30min	〃	〃	〃	Pale	✓	×	✓	×	✓	✓

due to vasoconstriction.



Cholinergic

Pilocarpine :

It produces miosis and spasm of accomodation due to muscarinic action. Colour of conjunctiva becomes pink or red. Light reflex +ve.

Homatropine: Antimuscarinic

Antimuscarinic drug so there is mydriasis It is due to unopposed action of dilator pupillae. Light Reflex Lost.

Cocaine : Local anesthetic

It is an alkaloid, sympathomimetic & local anesthetic. It causes vasoconstriction, colour of conjunctiva becomes pale to white.

Procedure:

A rabbit is (1) provided. Trim (2) the eye lashes of rabbit. Before instillation (3) of drug, diff. parameters are observed - Light Reflex, Conjunctival, Corneal Reflex and colour of Conjunctiva are observed. After (5) observing, instill (4) drug into eye. One drug should be instilled at 1 time in one eye and same parameters are observed. (6) Average 5-30 min. Other eye will be seen as a control. Tabulate the result.

Tet

Effect of different concentrations of ACH on Rabbit's ileum

Sr. No.	Dose (ug)	Strength of Ach solution	Volume	Log Dose	Response Amplitude of contraction (mm)
1	1ug	10^{-5}	0.1ml	0	30
2	2	10^{-5}	0.2	0.3	35
3	4	10^{-5}	0.4	0.4	40
4	8	10^{-5}	0.8	0.8	80
5	16	10^{-4}	0.16	0.16	85
6	32	〃	0.32	0.32	90
7	64	10^{-4}	0.64	0.64	95
8	128	10^{-3}	0.128	0.128	100
9	256	10^{-3}	0.256	0.256	100

To see effect of different concentrations of acetylcholine on isolated Rabbit's Ileum

Overnight fasted Rabbit

Rabbit ileum is dissected out

2-3 cm long pieces of ileum are cut.

Put these pieces immediately in a container containing tyrode's solution maintain its temp at 37°C and oxygen is constantly bubbled in it.

Phy. saline: Tyrode's for rabbit ileum strip.

It provide nourishment to tissue

Apparatus

Oxygen cylinder with tube (9)

Thermometer (8)

2-3cm rabbit's ileum strip (1)

Beakers (6)

2 Pipettes 1ml, 10ml (5)

Stop watch (4)

Reservior Bottle (Tyrod's solution) (2)

Isolated organ bath

Kymograph (1)

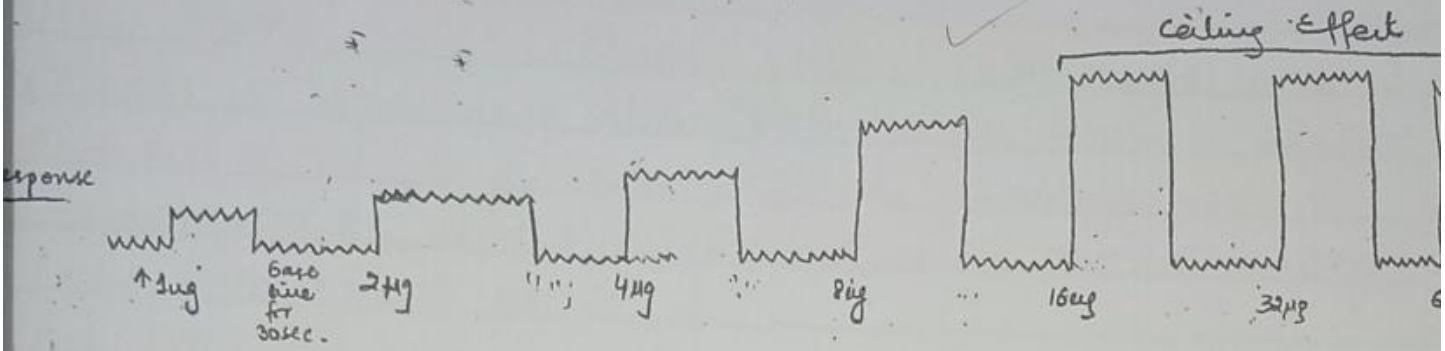
~~Isolated~~ Organ bath (2)

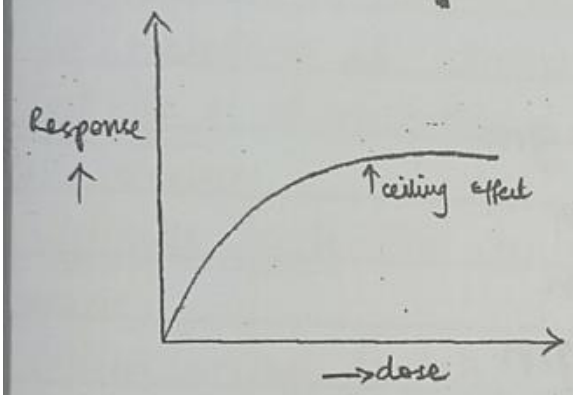
Organ bath: (Tissue bath)

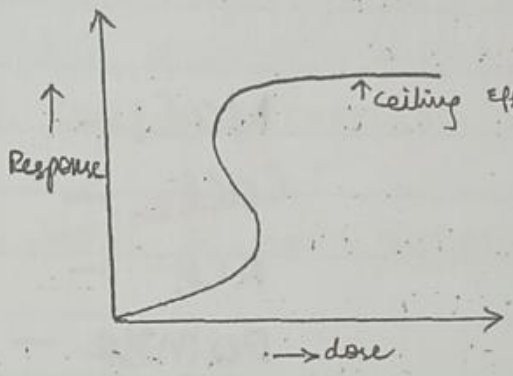
Inner Jacket/Chamber: 50ml, keep level of fluid constant, fill it with tyrode's solution — keep temp 37°C

Outer Jacket/chamber: hot water to keep temp at 38°C check with thermometer

Inlet tube with clamp: Brings tyrodes soln. from reservior bottle (3), dipped in outer chamber and soln. gets warm, then tube coiled around inner chamber then enters into it.


ceiling effect
esponse
1ug
Saro give for 30sec.
2ug
4ug
8ug
16ug
32ug


Response
ceiling effect
dose


Response
ceiling effect
dose

2 mm/sec.

Outlet Tube with clamp: It is attached at base of tissue bath & solution drain out through it.

Tissue Fixing hook: L-Shaped metal tube. longer arm (3 inch) is dipped into tissue bath where hook is attached one end of 3cm long ileum strip is attached with it (thread) and the second is attached with frontal writing lever with an ink pen.

Aeration Pipe: long arm dips down in the tissue bath & supply oxygen in perfusion fluid. Shorter arm is attached with a oxygen cylinder with a rubber tubbing.

Kymograph

Drum (smoked or it's covered with a kymograph paper. For recording of contractions. 15.2 diameter & 12.5 in height.

Spindle is a metallic rod at top of kymograph that holds the drum.

Cylinder lock: Fix drum with shaft so both rotate at a time.

Main on/off switch

Indicator lamp

Gears and speed regulator (2mm/sec)

Drum should be moved manually only in neutral position indicated by N/none on gear.

Solution Required

Tyrode's solution

Freshly prepared tyrode solution is put in reservoir bottle for tissue perfusion during experiment.

Composition

NaCl 8.00 gm	Dextrose 1.0 gm	KCl 0.2 gm	
$CaCl_2$ 0.2 gm	NaH_2PO_4 0.05 gm	$MgCl_2$ 0.01 gm	
	$NaHCO_3$ 1.00 gm		

To Prepare Graded dose Response curve of Ach on isolated ileum strip

Diff. doses of Ach are taken by using various strengths of Ach solution.

Various strengths of Ach solution

Ach 10^{-3} solution is provided.

It is diluted in 2 steps.

1st step:

1ml of 10^{-3} solution + 9ml will make 10^{-4} solution

1ml of 10^{-4} solution contain 100ug.

2nd step:

1ml of 10^{-4} + 9ml will make 10^{-5} solution.

1ml of 10^{-5} solution contain 10ug.

0.1 ml of solution contain 1ug of drug and 0.2ml of solution contain 2ug of drug and so dose is calculated accordingly.

So,

1ml of 10^{-3} solution contain Ach = 1000ug.

1ml of 10^{-4} " " " = 100ug

1ml of 10^{-5} " " " = 10ug

Procedure II

Uniform cycle of 6 min to record effects of various doses of Ach.

1. At 0 min, start kymograph & record normal contractions for 30 sec.
2. At 30 sec add 1 ug of Ach (0.1 ml of 10^{-5} solution). The drug is added with a pipette on moving drum. Record the contractions for next 30 sec.

Kymograph Cycle of 6 minutes

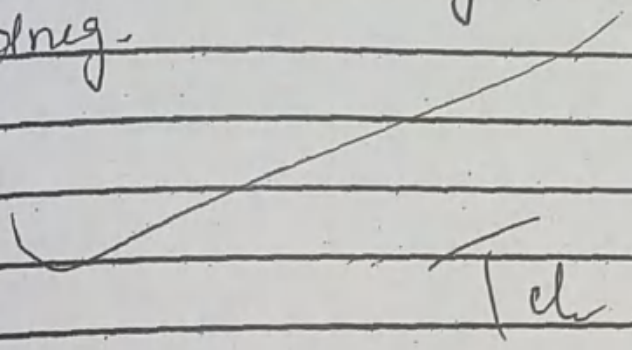
0 min — Start kymograph
(Take normal baseline contractions - 30 sec)

30 sec — Add 1ug of Ach
1 min — stop kymograph
2nd min — 1st washing
3rd min — 2nd washing
4th min — 3rd washing
Wait for 2 min.

3. Stop the drum at completion of 1 min, wash the tissue 3 times in 1st 2nd and 3rd mint. Then give rest to tissue for 2 mint.

4 By end of 6 mint, writing levers will come down to the baseline level (as drug is washed out of drug from tissue).

Similarly, take responses with ↑ing doses of Ach i-e 2, 4, 8, 16, 32 until max effect is achieved, where no further ↑ in response occurs with increasing doses. At this stage, all receptors are occupied by drug.



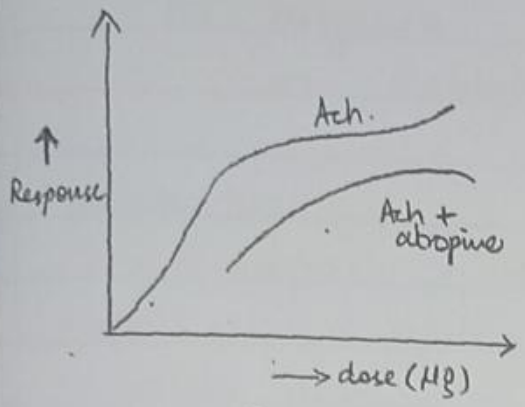
Precautions:

Don't use more than 1ml of Ach because buffer solu. will get diluted

Add drug by dipping pipette in fluid contained in tissue bath & blow.

- Add drug using pipette of 1ml.

Give rest of 15-20 min.



Emax of drug alone = 0.8µg.
Emax of drug + Antagonist = 32µg.

Graded Dose Response Curve for Ach :-

Sr. No.	Dose(µg)	Strength of Ach. soln.	Volume(ml)	Log Dose	Response(mm)
1	1	10^{-5}	0.1	0	2
2	2	10^{-5}	0.2	0.3	3
3	4	10^{-5}	0.4	0.6	3.5
4	8	10^{-5}	0.8	0.9	4
5	16	10^{-4}	0.16	1.2	4.2
6	32	10^{-4}	0.32	1.5	4.5
7	64	10^{-4}	0.64	1.8	5
8	128	10^{-3}	0.128	2.1	5.5
9	256	10^{-3}	0.256	2.4	5.5

Graded dose Response Curve for Ach. + Atropine

Sr. No.	Dose (µg)	Strength of Ach. 10^{-x}	Vol (ml)	Log dose	Response (mm)
1	128	10^{-3}	0.128	2.1	4.5
2	256	10^{-3}	0.256	2.4	5.0
3	512	10^{-3}	0.512	2.7	5.0
4	1000	10^{-3}	1	3	5.5

Ceiling effect is achieved at 256µg
Kymograph is started. take 3 ser. baseline cont. add 0.1ml of 1µg of Atropine. Wait 1 min. Add half the dose of Ach i.e 128 of Ach. (Half at which ceiling effect is a

Experiment:

To prepare Drug Antagonism Graph on Rabbit ileum

Apparatus:

- Kymograph
- Isolated organ bath
- Reservoir bottle
- stop watch
- pipettes 1ml, 10ml.
- Beakers 2-3um,
- Rabbit's ileum strip,
- thermometer
- O2. cylinder with tube

Solution: Ach. 10^{-3}, 10^{-4}, 10^{-5} soln.

Atropine 10^{-5} soln.

Procedure:

Ist Responses are recorded with Ach. solution beginning with mini. dose and ending with max dose till ceiling effect is achieved. Note & measure ED_{50}.

1µg of Atropine is added just after the third washing. Atropine is allowed to act for 30sec - 1min. Before tissue is exposed to ACH starting with half of the dose of Ach. at which ceiling effect is achieved. Afterwards all responses of Ach. are recorded in the presence of dose of Atropine. Inc. dose of Atropine till ceiling effect is achieved.

Result:-

In presence of competitive antagonist, same effect of agonist can be achieved, but at a higher dose

Chemical and drugs
1) Frogs ringers solution
2) acetylcholine 100 Mg/ml
3) atropine 10 Mg/ml
adrenaline 100 Mg/ml
propranolone 10 Mg/ml

1) conical

PHARMACOLOGY. PRACTICAL.

TO STUDY THE EFFECTS OF DIFFERENT DRUGS ON FROG'S HEART

Frog Ringer

APPARATUS

FROG'S BOARD, DISSECTION BOX, KYMOGRAPH, DROPPERS, BEAKER, stalling heart lever

OBJECTIVE

THE NERVE SUPPLY OF FROG'S HEART IS THROUGH SYMPATHETIC OR CHOLINERGIC NERVE SUPPLY OF AUTONOMIC NERVOUS SYSTEM THIS EXPERIMENT IS ABOUT ACTION OF SYMPATHOMIMETIC, SYMPATHOLYTIC, PARASYMPATHOMIMETIC AND PARASYMPATHOLYTIC DRUGS ON HEART.

PROCEDURE

(1) PITH THE FROG AND FIX IT ON A WOODEN BOARD.
(2) REMOVE STERNUM AND EXPOSE IT'S HEART AFTER INCISING THE PERICARDIUM. LEAVE A LITTLE OF PERICARDIUM AT IT'S APEX.
(3) PASS THE HOOK THROUGH THIS FOLD OF PERICARDIUM OF HEART'S APEX. AND CONNECT IT TO LEVER WHICH WILL BE MOVING ON THE SMOKED PAPPER OF KYMOGRAPH.
(4) KEEP ON PUTTING RINGER'S SOLUTION ON THE EXPOSED HEART DIRECTLY WITH DROPER TIME TO TIME.
(5) THE FORCE OF CONTRACTION IS MEASURED BY HEIGHT OF CONTRACTION AND RATE / OF NUMBER OF BEATS / MINUTE.

DRUGS	NORMAL CONTRACTIONS		DRUG INDUCED CONTRACTIONS		DIFFERENCE	
	BEATS/30SEC	HEIGHT mm	BEATS/30SECS	HEIGHT mm	BEATS/30SEC	HEIGHT mm
ADRENALINE	30	6	39	8	9	2
PROPRANOLOL	30	6	22	3	8	2
ADRENALINE + PROPRANOLOL	30	6	28	5	2	1/2

DRUGS	NORMAL CONTRACTIONS		DRUG INDUCED CONTRACTIONS		DIFFERENCE	
	BEATS/30SEC	HEIGHT mm	BEATS/30 SEC	HEIGHT mm	BEATS/30SECS	HEIGHT mm
ACETYLCHOLINE	28	4	9	1	19	3
ATROPINE	28	4	32	4	4	0
ACETYLCHOLINE + ATROPINE	28	4	24	2	4	2

6 39 8
22 3
28 5

(1) 30 SECS NORMAL CONTRACTIONS
(2) STOP KYMOGRAPH
(3) ADD ADRENALINE → WAIT FOR 15 SECS
(4) TAKE CONTRACTIONS FOR 30 SECS
(5) STOP KYMOGRAPH (STOP)
(6) WASHING (3) RINGER'S SOLUTION
(7) TAKE NORMAL CONTRACTIONS FOR 30 SEC
(8) STOP KYMOGRAPH, TO ADD PROPRANOLOL

STEPS

(1) SET THE SPEED OF KYMOGRAPH AT 3 mm/sec

(2) ADD RINGER'S SOLUTION DIRECTLY ON HEART WITH THE HELP OF DROPPER AND RECORD NORMAL BASE LINE CONTRACTION FOR 30 SECONDS.

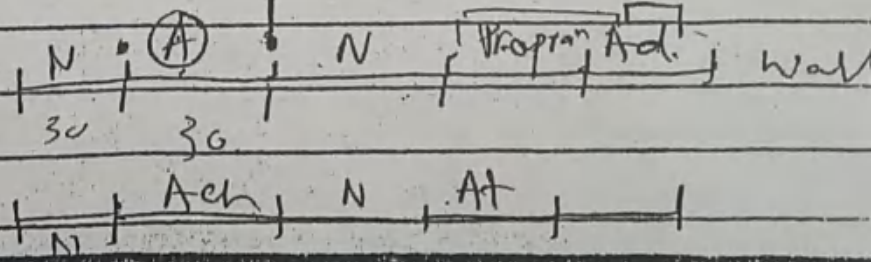
(3) STOP KYMOGRAPH TO ADD ADRENALINE, WAIT FOR 15 SEC, THEN RECORD CONTRACTIONS FOR 30 SECS. THERE WILL BE ↑ IN HEART RATE AND AMPLITUDE OF CONTRACTION.

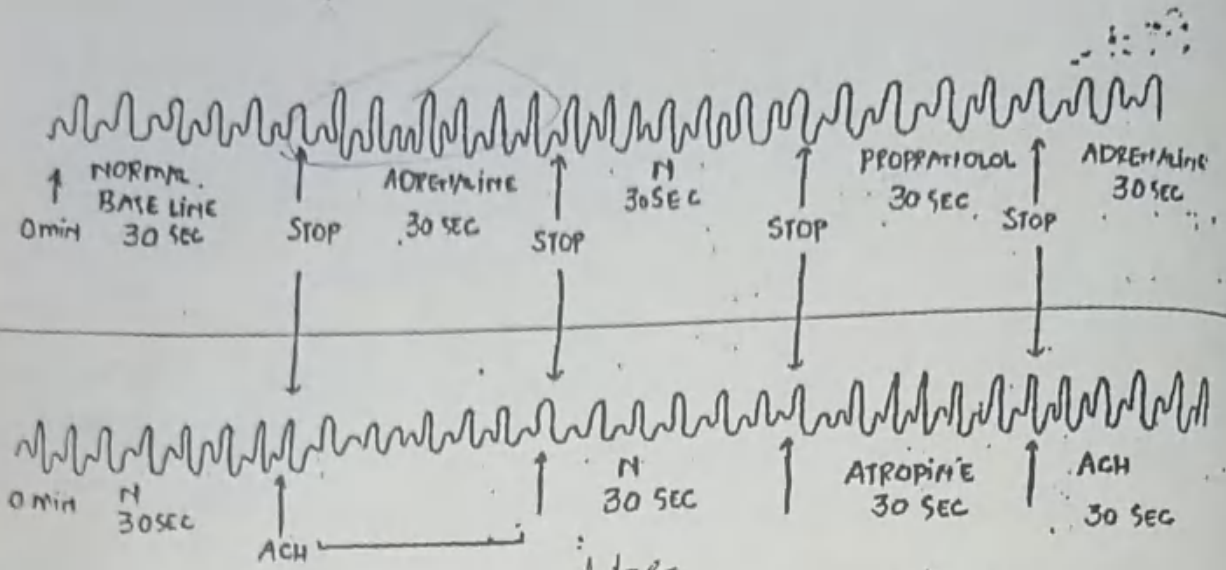
(4) STOP KYMOGRAPH. WASH WITH RINGER'S 2-3 TIMES (1/2 MIN) AND THEN TAKE NORMAL CONTRACTIONS WITH BASE LINE CONTRACTIONS. IF RATE AND AMPLITUDE OF PEAKS IS SIMILAR IN BOTH, THEN PROCEED. IF NOT, AGAIN WASH WITH RINGER'S SOLUTION 2-3 TIMES THEN TAKE CONTRACTIONS FOR 30 SECS.

(5) STOP KYMOGRAPH TO ADD PROPRANOLOL, WAIT 15 SECS, THEN RECORD CONTRACTIONS FOR 30 SECS. THERE WILL BE ↓ IN HEART RATE AS WELL AS IN AMPLITUDE OF CONTRACTION.

(6) STOP KYMOGRAPH, DO NOT WASH AND ADD ADRENALINE, WAIT 15 SEC, RECORD CONTRACTION FOR 30 SEC. NOTE THE DIFFERENCE IN HEART RATE AND AMPLITUDE OF CONTRACTIONS B/W ADRENALINE ALONE AND ADRENALINE + PROPRANOLOL. THERE WILL BE NO ↑ IN HEART RATE AND AMPLITUDE WHEN COMPARED TO NORMAL ADRENALINE CONTRACTION. WASHING

(7) STOP KYMOGRAPH TO ADD ACETYLCHOLINE (NORMAL CONTRACTIONS) SOLUTION, WAIT FOR DECREASE IN RATE AND AMPLITUDE OF CONTRACTIONS.





Write on lined page about before after procedure

DRUGS REQUIRED

ACH : 100ug/mL

ADRENALIRIE 100ug/mL

ATROPIIE 10ug/mL

PROPRANIOLOL 10ug/mL

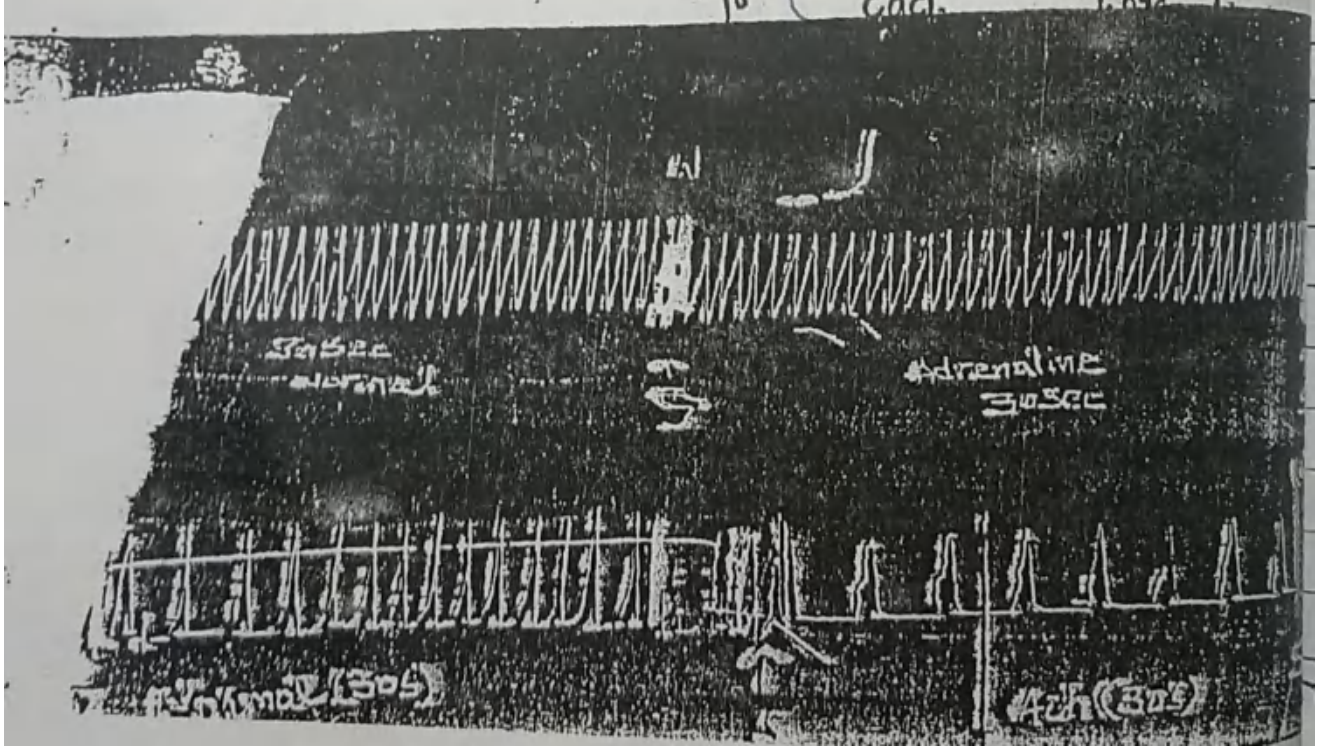
Frog's Ringer's lactate solution

DISTILLED H_2O

18.8.16

COMPOSITION OF FROG'S RINGER'S SOLUTION

NaCl	6.5gm	8
KCl	4.14gm	0.2
NaH_2PO_4	0.05gm	0.05
GLUCOSE	2.0gm	1
$NaHCO_3$	0.4gm	1
$CaCl_2$	1.09gm	1



(9) STOP KYMOGRAPH, WASH WITH RINGER'S SOLUTION AND TAKE NORMAL CONTRACTIONS FOR SEC. (30).

(10) STOP KYMOGRAPH TO ADD ATROPINE TO HEART, WAIT 15 SEC, RECORD CONTRACTIONS FOR 30 SEC. THERE WILL BE AN INCREASE IN RATE OF CONTRACTION.

(11) STOP KYMOGRAPH, DONOT WASH AND ADD ACETYLCHOLINE SOLUTION, WAIT 15 SEC, RECORD CONTRACTIONS FOR 30 SEC. THERE WILL BE NO CHANGE IN HEART RATE AND AMPLITUDE OF CONTRACTION.

PRECAUTIONS

(1) THE HEART SHOULD BE PERFUSED AT CONSTANT RATE OF 20-30 DROPS/MINUTE.

(2) LEVER SHOULD BE HORIZONTAL AND THREAD VERTICAL.

(3) BEFORE RECORDING CONTRACTIONS WITH EACH DRUG, RECORD THE NORMAL CONTRACTION.

(4) TIME TRACING IS TAKEN TO CALCULATE HEART RATE.

CONCLUSIONS

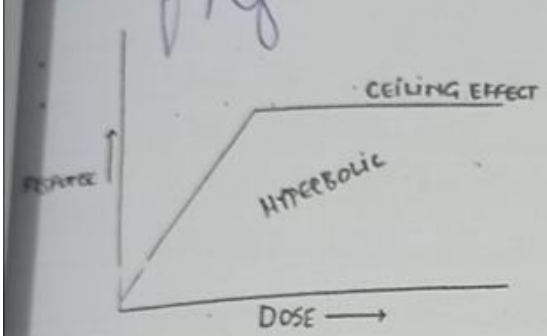
(1) ADRENALINE ACTS ON β-RECEPTORS (β_1, β_2) ON HEART. IT HAS +VE IONOTROPIC, CHRONOTROPIC AND DROMOTROPIC ACTION. WHEREAS, PROPRANOLOL IS A COMPETITIVE ANTAGONIST (NON-SELECTIVE β BLOCKER) OF ADRENALINE. RESULTS OF EXPERIMENT SHOW THAT ADRENALINE ↑ FORCE OF CONTRACTION AND HEART RATE. THIS EFFECT IS COMPLETELY BLOCKED OR REDUCED AFTER PROPRANO

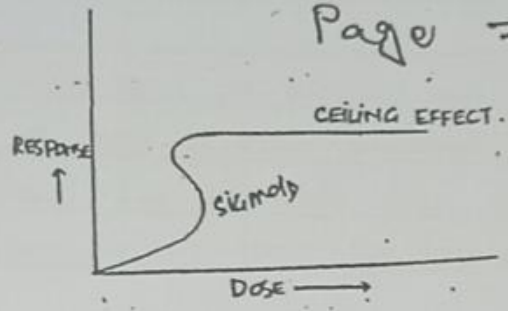
(2) ACETYLCHOLINE ACTS ON MUSCARINIC RECEPTORS (M_2) ON HEART. IT HAS -VE IONOTROPIC, -VE CHRONOTROPIC AND DROMOTROPIC. ATROPINE IS ANTIMUSCARINIC DRUG. IT BLOCKS MUSCARINIC RECEPTORS AND PREVENTS ACETYLCHOLINE FROM IT'S ACTION.

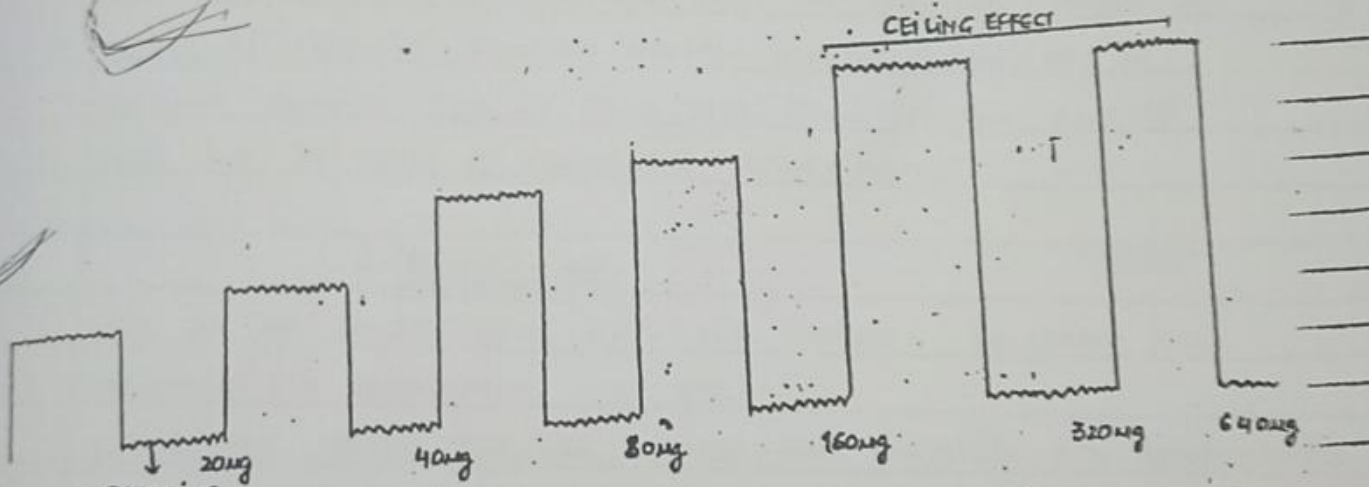
PHM-D-III Experimental Study On Frog's Ileum.

By (Dr. Sir Javeed Nazeer)

Fig





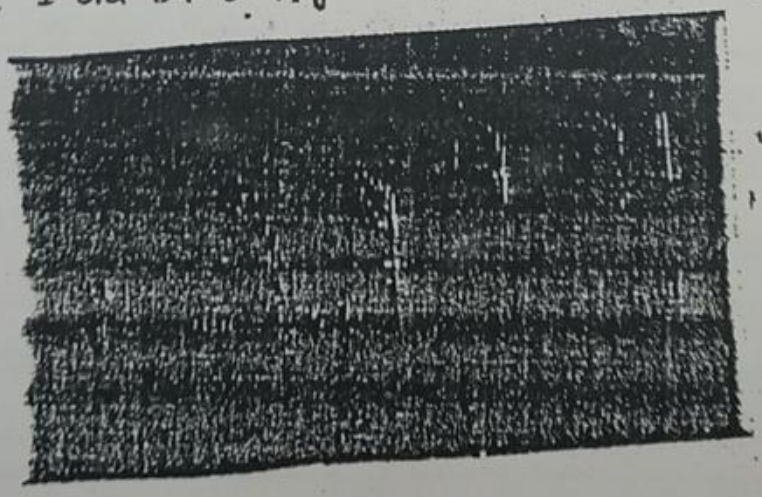


→ ALONG X Axis (DOSE)

1 Big Box = 10 mm , 1 Small Box = 1 mm

→ ALONG Y Axis) AMPLITUDE (mm)

1 Big Box = 1 µg , 1 Small Box = 0.1 µg



DIFFERENT DOSES OF ACETYLCHOLINE ARE PREPARED BY FOLLOWING STEPS, ACETYLCHOLINE 10^{-3} IS PROVIDED TO YOU- IT IS DILUTED IN 2 STEPS.

1ST STEP

TAKE 1mL OF 10^{-3} SOLUTION, ADD 9mL OF DISTILLED H_2O, IT MAKES 10^{-4} SOLUTION AND 1mL OF THIS SOLUTION CONTAINS 10µg/mL. 0.1mL OF 10^{-5} SOLUTION CONTAINS 1µg OF DRUG AND 0.2mL CONTAIN 2µg OF DRUG AND SO THE DRUG I.e 100µg/mL

2ND STEP

TAKE 1mL OF 10^{-4} SOLUTION, ADD 9mL OF DISTILLED WATER, IT MAKES 10^{-5} AND THIS SOLUTION CONTAIN 10µg/mL. 0.1mL OF 10^{-5} SOLUTION CONTAINS 1µg OF DRUG AND 0.2mL CONTAIN 2µg OF DRUG AND SO DRUG IS CALCULATED ACCORDINGLY.

PROCEDURE

1×10^{-5}

(•) FILL IN THE INNER TISSUE BATH WITH TYRODE'S SOLUTION AND MAINTAIN IT'S TEMPERATURE AT 37°C.

(•) FILL THE OUTER CHAMBER WATER AND MAINTAIN IT'S TEMPERATU AT 38°C WITH HELP OF THERMOMETER.

(•) A 3cm LONG ISOLATED EMPTY ILEUM STRIP IS TAKEN AND THREAD IS PASSED THROUGH BOTH ENDS. ONE END IS ATTACHED TO WRITING LEVER AND OTHER END IS ATTACHED TO TISSUE FIXING HOOK OF OXYGEN TUBE IN TISSUE BATH.

(•) OXYGEN IS CONTINOUSLY BUBBLED THROUGH TISSUE BATH. THIS KEEPS CONCENTRATION OF DRUG UNIFORM IN TYRODE'S SOLUTION AND SUPPLY OXYGEN TO TISSUES.

(•) AFTER MOUNTING THE TISSUE, GIVE IT REST FOR AT LEAST 10-20 MINUTES.

(c) THE LONGITUDINAL CONTRACTIONS ARE RECORDED ON SMOKED DRUM.

(2) NORMAL CONTRACTIONS AS WELL AS INDUCED BY DIFFERENT DOSES OF ACH ARE RECORDED.

UNIFORM CYCLE OF 6 MINUTES FOR RECORDING CONTRACTIONS OF RABBIT'S ILEUM

(1) AT 0 MINUTE, START KYMOGRAPH AND RECORD NORMAL BASELINE CONTROL. I-e (FOR 30 SECONDS).

(2) AT 30 SECONDS ADD 1µg (0.1mL) OF ACETYLCHOLINE ON MOVING DRUM. DRUG IS ADDED WITH 1mL PIPETTE WHICH IS DIPPED IN TISSUE BATH AND DRUG IS BUBBLED IN IT. RECORD THE CONTRACTIONS FOR 30 SECONDS. AVOID TO TOUCH THE THREAD WHILE ADDING DRUG.

(3) STOP THE DRUM AT 1 MINUTE, WASH THE TISSUE WITH TYRODE'S SOLUTION FOR 3 TIMES (1 MIN IS REQUIRED FOR 1 WASH) AND WAIT FOR 2 MINUTES.

BY THE END OF 6 MINUTES, WRITING LEVER WILL COME DOWN TO BASELINE LEVEL. SHOWING THAT DRUG HAS MOVED FROM RECEPTORS.

(4) SIMILARLY TAKE THE RESPONSES WITH INCREASING DOSES OF ACH I-e 2µg, 4µg, 8µg, 16µg, 32µg, 64µg, 128µg AND 256µg OF DRUG UNTIL MAXIMAL EFFECT IS ACHIEVED WHERE NO FURTHER INCREASE IN RESPONSE IS RECORDED WITH INCREASING DOSES. IT IS ALSO CALLED THE "CEILING EFFECT" SHOWING THAT ALL THE RECEPTORS ARE OCCUPIED BY DRUG.

(5) THE RESPONSES RECORDED BY VARIOUS CONCENTRATIONS ARE MEASURED IN MILLIMETER (mm) AND A DOSE RESPONSE CURVE IS PREPARED BY PLOTTING THE DOSE ON HORIZONTAL X-AXIS AGAINST RESPONSES ON VERTICAL Y-AXIS. A HYPERBOLIC CURVE WILL BE OBTAINED ON ARITHMETIC SCALE. THEN PREPARE ANOTHER DOSE RESPONSE CURVE BY TAKING LOG OF SAME DOSES, IT WILL MAKE A SIGMOID CURVE.

PRECAUTIONS; WHILE ADDING DRUG DOSES

(1) DONOT USE MORE THAN 1ml OF ACH SOLUTION TO ADD DRUG IN TISSUE BATH OTHER WISE,

(2) BUFFERING CAPACITY AND NUTRITION PROVIDED BY TYRODE'S SOLUTION WILL GET DILUTED.

(3) ADD DRUG BY USING PIPETTE OF 1mL.

(4) ADD DRUG BY DIPPING PIPETTE IN FLUID CONTAINED IN TISSUE BATH.

(5) BLOW / BUBBLE OUT DRUG INTO INNER CHAMBER WITHOUT TOUCHING THE THREAD ATTACHED TO WRITING LEVER.

(6) KEEP THE LEVEL OF FLUID CONSTANT IN TISSUE BATH AFTER EACH WASH.

PRECAUTIONS

(1) DONOT MISHANDLE TISSUE.

(2) MAINTAIN TEMPERATURE IN TISSUE BATH AT 37°C.

(3) MAINTAIN CONTINOUS OXYGEN SUPPLY.

(4) AFTER MOUNTING TISSUE GIVE REST TO IT FOR 15-20 MIN.