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ALTERNATIVE TO LABORATORY ANIMALS FOR ISOLATED TISSUE EXPERIMENTATION USING CHICKEN ILEUM PREPARATION

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ABSTRACT

For the teaching purposes to use isolated strip preparations from various organs, the laboratory animal species has to be sacrificed just for a piece of tissue. Pharmacology as a subject depends largely on experiments conducted in laboratory animals. Experimental animals like rat, guinea pig, rabbit, etc. are used for the biological assay. The present study was aimed to develop *ex vivo* model for pharmacological experimentation, which will mimic the actual laboratory condition without sacrificing the experimental animals. Dose response curve of acetylcholine is carried out in different conditions by changing different parameters and compare the result. The effect of acetylcholine in terms of its type of agonist was predicted based on kymograph and curve had been obtained. The chicken ileum, duodenum and rat ileum were also evaluated for four- and three-point bioassay, respectively. The results suggested that acetylcholine produced a dose-dependent increase in contraction in both chicken and rat ileum preparation. The concentration response curve of acetylcholine in chicken ileum shows good response by changing the parameters other than that used in rat ileum. It is concluded that isolated chicken ileum and duodenum preparation can be employed for routine experiments of pharmacology subject and the use of these isolated preparations is a novel approach for managing pharmacological experiments and importantly, without sacrificing the experimental animals.

KEYWORDS: Acetylcholine, PSS, chicken ileum, four point bioassay, three point bioassay.

1. INTRODUCTION

Many physiological salt solutions have been devised, each of which has been found empirically to maintain different isolated tissue preparations and are frequently named according to the innovator. It is curious that for allegedly the same solution, slight variations in composition are cited in various publications (Table 3.1). The essential features are that they are all isotonic with blood plasma and are designed to maintain a pH close to 7.4. The first synthetic solution designed to maintain an isolated organ was introduced by Sydney Ringer to maintain a frog heart in vitro and sometimes all similar solutions are generically described as Ringer's. Slight modifications in the composition of these salt solutions were found to be beneficial to maintain different organs. These buffers must be bubbled with different gases in order to maintain the correct pH. Thus Krebs', De Jalon's and McEwan's should be gassed with carbogen (95% O_2 / 5% CO_2), whilst Tyrode's is gassed with air. It will be noticed that frog's Ringer has a lower isotonicity than is used for mammalian tissues in order to mimic amphibian body fluids. It is important to note that there will be problems with the precipitation of the calcium chloride if it is not added last and as a solution rather than the solid.

Animal testing which is also known as *in vivo* testing means the use of non-human animals in experiments. About 50 to 100 million vertebrates are used worldwide for the purpose of education and research. The research is conducted inside universities, medical schools, pharmaceutical companies and includes pure research such as genetics, developmental biology, behavioral studies, as well as applied research such as biomedical research, xeno-transplantation, drug testing and toxicology tests, including cosmetics testing. Sources of laboratory animals vary from countries and species; while most animals are purpose-bred, others may be caught in the wild or supplied by dealers. The rodents like mice, rats, rabbit's guinea pigs, hamsters and non human primates are widely used for experimentation. The non judicious use of these animals has affected the eco system and therefore many countries and scientists are looking for the alternatives for animal testing. The two major alternatives to *in vivo* animal testing are *in vitro* cell culture technique and *in silico* computer simulation. But still the alternative to animal testing needs to be developed. The present study was undertaken to exploit the small intestine of chicken for isolated tissue experimentation which is a waste product easily available from slaughter houses. The previous studies on chicken small intestine revealed the presence of tachykinin, histaminic, motile receptors 1. In the present study, ileum portion of chick small intestine was used and various parameters were studied to

optimize the conditions as physiological salt solution, tissue length, magnification value and load/tension. The contractile response of variable doses of Acetylcholine solution (Stalk solution: 1mg/ml) were recorded with the help of frontal writing lever. The experiments were repeated for number of times to check the reproducibility, sensitivity and accuracy. The composition of Physiological salt solution was modified so as to find out most suitable Physiological salt solution that can be used. From the experiments carried out, it was found that chicken ileum may be one of the best, cheapest and easily available non mammalian tissues for experimentation on isolated organs.

1.1 Alternatives to Animal Experimentation

Because of the growing concern on the use of animals in biochemical research several of the industrialized countries of Europe and North America have passed legislation to prevent or curtailment of animal experimentation. These include experiments with tissues and body fluids of normal animals and human, use of micro-organisms, primary cell culture and cell lines. Use of models and computer programs are other alternatives though provide valuable information, they are expensive and at times do not represent the exact response or affect that one would get using intact animals2. The laboratory animals have to be sacrificed just for a piece of tissue. However, chick ileum is a tissue that is available easily and animals need not be killed additionally for experimental purpose. The advantages of using chick ileum preparation are that it is economical, very easy to mount in organ bath, gives good response, without sacrificing the experimental animals2.

1.2 Biological Standardization (Bioassays)

Biological standardization or bioassays are procedures by which the potency or the nature of the substance is estimated by studying its effect on living matter. Bioassay procedure are generally employed.

- When a chemical assay for the substance is not available or the substance gets inactivated by interacting with chemicals as the case with hormones.
- When the quantity of the sample is too small. In such situation a matching type of bioassay is conveniently done to compare the biological response with the standard drug.
- To estimate the concentration of active principles present in the tissue extract, the endogenous mediators like acetylcholine, 5HT, prostaglandins.
- To measure the pharmacological activity of new or chemically unidentified substance.

- To measure drug toxicity.
- When the bioassay is more sensitive than the chemical assay

2. MATERIAL AND METHODS

The present study was undertaken using graded type of bioassay.

2.1.1 Procurement of Chick ileum

Chick ileum was procured from the local market.

2.1.2 Chemicals

All other chemicals used are of laboratory grade. Sodium Chloride, Potassium Chloride, Calcium Chloride, Magnesium Chloride, Magnesium Sulphate, Sodium bicarbonate, Sodium hydrogen phosphate, Potassium hydrogen phosphate and distilled water. Acetylcholine stock solution (1mg/ml).

Table 1: Preparation of physiological salt solution (PSS).

Compound	Frog Ringer	Ringer Locke	De Jalon	Tyrode	Krebs
Nacl	6.0	9.0	9.0	8.0	9.6
KCl	0.14	0.42	0.42	0.2	0.35
$CaCl_2$	0.12	0.24	0.06	0.2	0.28
$MgCl_2$	-----	------	------	0.1	------
$MgSo_4$	-----	------	------	-----	1.28
$NaHCO_3$	0.2	0.5	0.5	1.0	2.1
NaH_2PO_4	-----	------	------	0.05	------
$K\ H_2PO_4$	-----	------	------	-----	0.16
Glucose	2.0	1.0	0.5	1 or 2	1 or 2

All values are in g/l. Weighed accurate quantity of the ingredients and dissolved in one liter distilled water. The physiological solution prepared should be clear and if turbid it is advised to prepare fresh solution before the start of the experiment.

2.2 METHOD

a. Fresh entire gastrointestinal tract of healthy chicken was obtained from a slaughter house.
b. The caecum was lifted forwards and the ileocaecal junction was identified.
c. A few centimeters of the ileal portion was cut and removed and immediately placed it in the watch glass containing physiological salt solution. The mesentery and adhering tissues were removed with gentle care. Utmost care was taken to avoid any damage to the gut muscle. The ileum was cut into small segments of 2-3 cm long.

d. To one piece of ileum the thread was tied to top and bottom ends without closing the ileum and mounted the tissue in the organ bath containing PSS maintained at 32-35˚C and bubbled with air. The magnification from 5-7 folds and bath volume of about 25 ml was maintained and the tissue was allowed to equilibrate for 30 min before adding Acetylcholine to the organ bath.
e. The Acetylcholine induces the contraction in the ileal smooth muscles which were recorded on Kymograph by using frontal writing lever. Contact time of 30 sec and 5 min time cycle was kept for proper recording of the responses.
f. The CRC was recorded till ceiling effect to Acetylcholine was obtained.
g. Various parameters were changed and responses were taken as magnification value 3 & 5, load/tension 0.5, 1.0 & 1.5 gm and tissue length 1.5, 2.0 cm.
h. All physiological salt solution was tried and responses were taken.
i. Height of response was measured.

3. RESULTS

From the experiments, it was found that the chick ileum was giving good responses in following optimized conditions:

- Temperature 40 - 42°C
- Physiological salt solution= Ringer lock (Fig.1,Graph 1)
- Load/tension = 1.5gm (Fig.2, Graph 2)
- Tissue length = 2.0cm (Fig. 3, Graph 3)
- Magnification value = 3.0

With magnification value more than 3.0, responses were not proper. Also for the tension 1.0 gm was tried (result not shown), responses were not proper. Tissue length less than 1.5cm was not showing satisfactory results. So the above conditions were optimized for the experiments with the chick ileum. To confirm the optimized conditions, Concentration response curve of Acetylcholine and Histamine was taken with the same conditions (Fig. 4 and Fig. 5).

3.1 FIGURES AND TABLES

Table – 1 and Figure – 1: Concentration response curves of Acetylcholine (1 mg/ml) with Frog Ringer solution.

Dose MCG	Dose (ml)	Ht.,(mm)
100	0.1	24
200	0.2	25
400	0.4	26
800	0.8	27
1600	1.6	27
3200	3.2	27
6400	6.4	27
12800	12.8	27

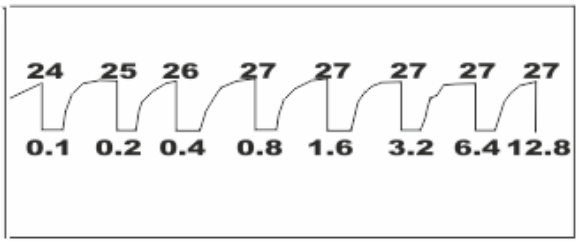


Table – 2 and Figure – 2: Concentration response curves of Acetylcholine (1 mg/ml) with Ringer Lock solution.

Dose MCG	Dose ml	Ht.,mm
100	0.1	28
200	0.2	29
400	0.4	31
800	0.8	37
1600	1.6	42
3200	3.2	45

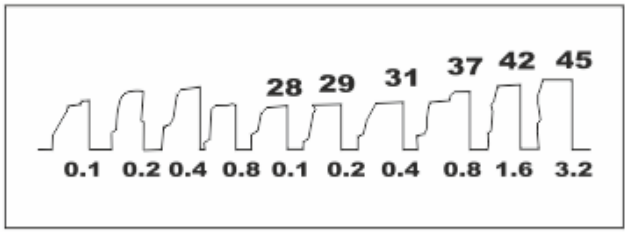


Table – 3 and Figure – 3: Concentration response curves of Acetylcholine (1 mg/ml) with De Jalon Solution.

Dose MCG	Dose ml	Ht.,mm
100	0.1	17
200	0.2	22
400	0.4	24
800	0.8	25
1600	1.6	26
3200	3.2	26
6400	6.4	26

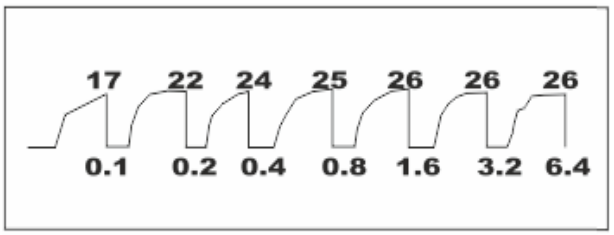
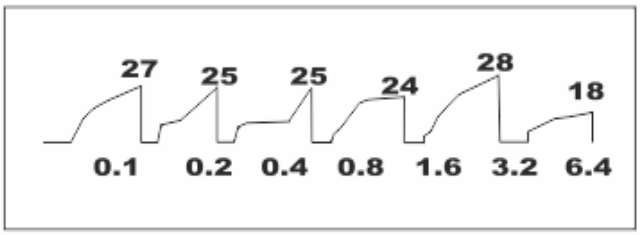


Table – 4 and Figure – 4: Concentration response curves of Acetylcholine (1 mg/ml) with Tyrode solution.

Dose MCG	Dose ml	Ht.,mm
100	0.1	14
200	0.2	14.5
400	0.4	15
800	0.8	13
1600	1.6	13
3200	3.2	13
6400	6.4	13.5

Table – 5 and Figure – 5: Concentration response curves of Acetylcholine (1 mg/ml) with Kreb's solution.

Dose MCG	Dose ml	Ht.,mm
100	0.1	27
200	0.2	25
400	0.4	25
800	0.8	24
1600	1.6	28
3200	3.2	18



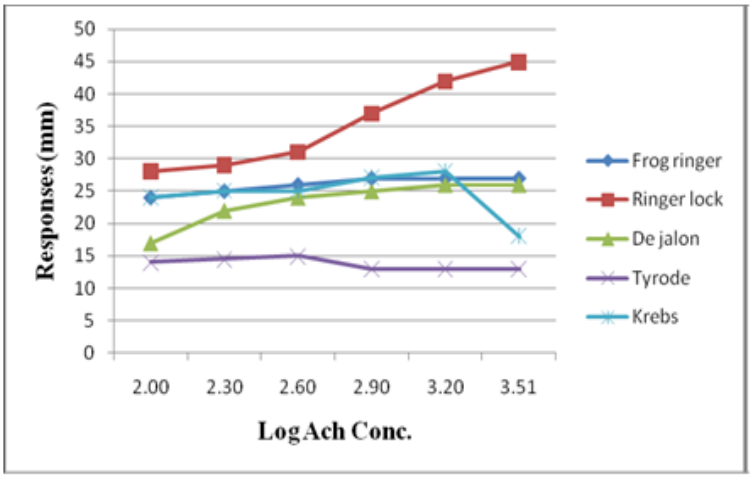


Figure – 6: Comparison for responses of acetylcholine with various physiological salt solutions.

Fig – 3A

Load = 0.5

Fig – 3B

Load = 1.5, Tissue length = 2.0 cm

Conditions: Magnification value = 3, PSS = Ringer lock solution , Acetylcholine (stock)= 1000 ppm

Figure – 7: Concentration response curves of Acetylcholine (1 mg/ml) with different load. (A) Load 0.5gm (B) Load 1.5gm.

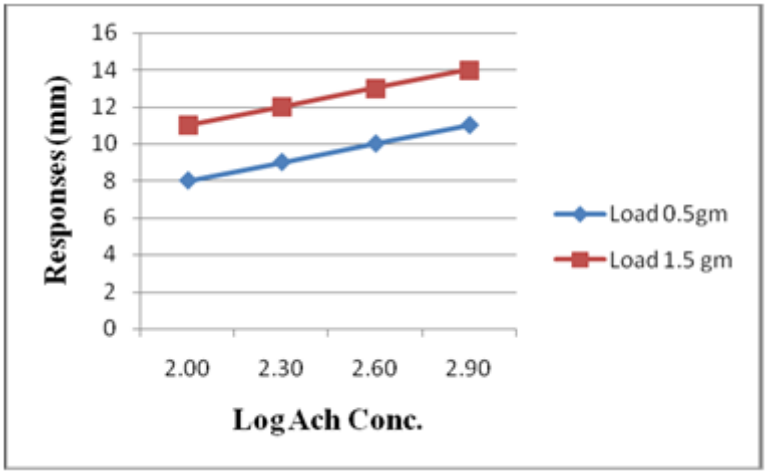


Figure – 8: Comparison for responses of acetylcholine with different load applied.

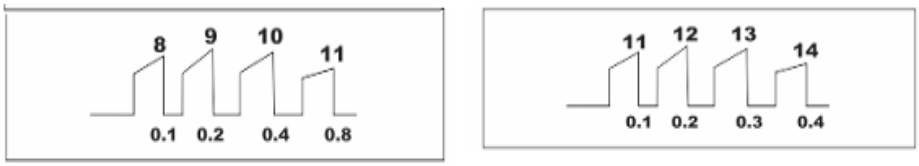


Fig – 5A

Load = 1.5, Tissue length = 1.5 cm

Fig – 5B

Load = 1.5, Tissue length = 2.0 cm

Conditions: Magnification value = 3, PSS = Ringer lock solution , Acetylcholine (stock)= 1000 ppm

Figure – 9: Concentration response curves of Acetylcholine (1 mg/ml) with different tissue length. (A) Tissue length 1.5cm (B) Tissue length 2.0cm.

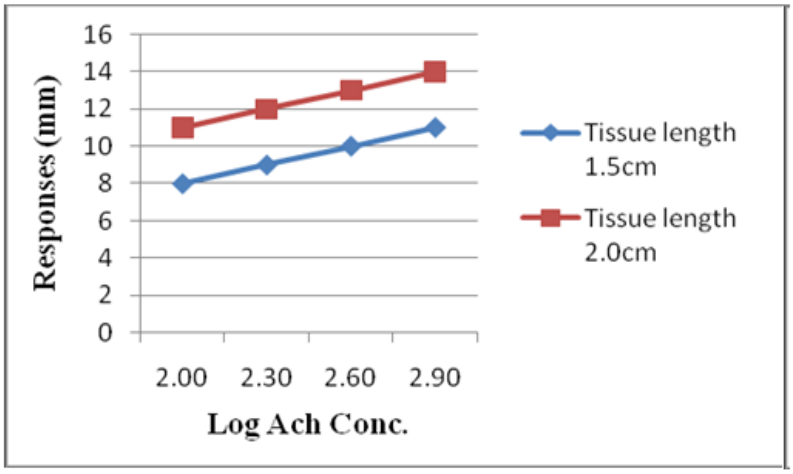
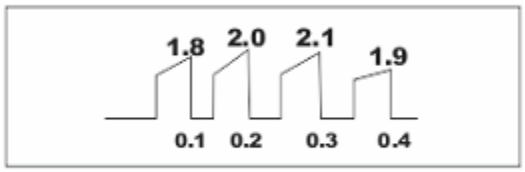


Figure – 10: Comparison for responses of acetylcholine with different tissue length used.

Conditions:
Magnification value = 3
Load = 1.5
Tissue length = 2.0 cm
PSS = Ringer lock solution
Acetylcholine (stock)= 1000 ppm

Figure – 11: Concentration response curve for acetylcholine with optimized conditions using isolated chick ileum tissue.



Conditions:
Magnification value = 3
Load = 1.5
Tissue length = 2.0 cm
PSS = Ringer lock solution
Acetylcholine (stock)= 1000 ppm

Figure – 12: Concentration response curve for Histamine with optimized conditions using isolated chick ileum tissue.

4. DISCUSSION

In preclinical studies rat/guinea pig ileum is widely used isolated tissue for various laboratory experimentations due to presence of various receptors as muscarinic, serotonin, histaminic, GABAnergic and adrenoreceptors 3. As cock ileum contains tachykinin, histaminic, motile receptors, isolated ileum is suitable for carrying out various experiments like determination of pA2 value, three point/four point bioassay, demonstration of potentiating effect of acetylcholine by neostigmine, comparison of activities of different agonists like acetylcholine

and barium chloride4. For its use in educational purpose and new drug discovery process, optimization of experimental conditions would help. In the present investigation, conditions such as tissue length, load/tension to be applied, magnification value, PSS to be used were optimized. It was found that with the optimized conditions as tissue length= 2.0cm, load/tension =1.5gm, magnification value= 3.0 and Ringer lock as physiological salt solution CRC of Acetylcholine and Histamine recorded correlates with the CRC of Acetylcholine and Histamine recorded using isolated rat ileum.

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