

## Chicken ileum: a better option for conducting isolated tissue experiments and bioassay

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### Abstract

*In vivo* method of animal testing is the use of non-human animals in experiments. Mice, rats, rabbits, guinea pigs, hamsters and non-human primates are widely used as laboratory animals. *In vitro* cell culture technique and *in silico* computer simulation are alternatives to *in vivo* animal testing. Current limitations on performing experiments with laboratory animals bring about the need for search of alternate tissues for biological testing. Bioassay is an integral part of undergraduate and postgraduate Pharmacology curriculum where various isolated tissues like frog rectus, rat colon, guinea pig ileum etc. were used for which whole animal has to be sacrificed for a small piece of tissue. So, we conducted bioassay of acetylcholine on chicken ileum obtained from chicken sacrificed for food which is easily available from slaughter houses. We found greater height of response and stability with Tyrode solution compared with Ringer Locke solution. We assessed the unknown concentration of acetylcholine by interpolation method of bioassay which we found 82.17% correct of actual concentration of unknown. Isolated chicken ileum is a better option available for conducting isolated tissue experiments considering the restriction with the use of experimental animals under CPCSEA guidelines.

**Keywords:** Chicken ileum, Bioassay, Acetylcholine, Physiological salt solution, Interpolation

### Introduction

*In vivo* method of animal testing is the use of non-human animals in experiments. As an educational and research tool nearly 50 to 100 million vertebrates are used worldwide. For the experimental purpose rodents like mice, rats, rabbits, guinea pigs, hamsters and non-human primates are widely used as laboratory animals. Our eco system has affected to a great extent due to non-judicious use of these animals and therefore many countries and scientists are looking for the alternatives for animal testing.<sup>(1)</sup> Guidelines have been set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) for laboratory animal facility to promote the human care of animals used in biomedical and behavioural research and testing with the basic objective of providing specifications that will enhance animal well being and quality in the interest of advancement of biological knowledge that is pertinent to humans and animals.<sup>(2)</sup> This encourage the search for alternative tissues for biological testing.

*In vitro* cell culture technique and *in silico* computer simulation are the two major alternatives to *in vivo* animal testing. These technique as such do not represent the exact response or effect that one would get using intact animals as well as they are expensive. So, there is a need for some other alternative method.

Bioassay is defined as comparative assessment of relative potency of a test compound to a standard compound on any living animal or biological tissue. Bioassay was started in late 18th century with standardization of diphtheria antitoxin by Paul Ehrlich. Bioassay is an important basic step in drug discovery. Also it is an integral part of undergraduate and postgraduate pharmacology curriculum.<sup>(3)</sup> Bioassay can

be performed on various isolated tissues like frog rectus, rat colon, guineapig ileum etc. But main concern for performing bioassay is that we have to sacrifice whole animal for a small piece of tissue.

Current limitations on performing experiments with laboratory animals bring about the need for search of alternate tissues for biological testing. Tissues from poultry, sheep, goats, cattle, fish, etc., usually consumed for food, were suggested for experiment. The previous studies on chicken small intestine revealed that the small intestine of chicken is long and uniform in diameter. Its circular muscles are 3 times thicker than the longitudinal muscles<sup>(4)</sup> and it contracts in response to acetylcholine, histamine, 5-hydroxytryptamine (5-HT), prostaglandins (E1, E2, F2), bradykinin and bovine slow reacting substance of anaphylaxis (SRS-A). Marked seasonal and individual variations in the responsiveness of gut tissues to these exogenous agonists were noted.<sup>(5)</sup>

**Table 1: Composition of physiological salt solutions<sup>(2)</sup>**

Compound	Ringer Locke	Tyrode
NaCl	9.0	8.0
KCl	0.42	0.2
CaCl <sub>2</sub>	0.24	0.2
MgCl <sub>2</sub>	-	0.10
NaHCO <sub>3</sub>	0.5	1.0
NaH <sub>2</sub> PO <sub>4</sub>	-	0.05
Glucose	1.0	1 or 2

All values are in g/l.

In view of this, we conducted bioassay of acetylcholine on chicken ileum obtained from chicken

sacrificed for food which is a waste product easily available from slaughter houses.

In our department we perform the experiment on isolated chicken ileum using Ringer Locke and Tyrode solution as physiological salt solution (PSS).

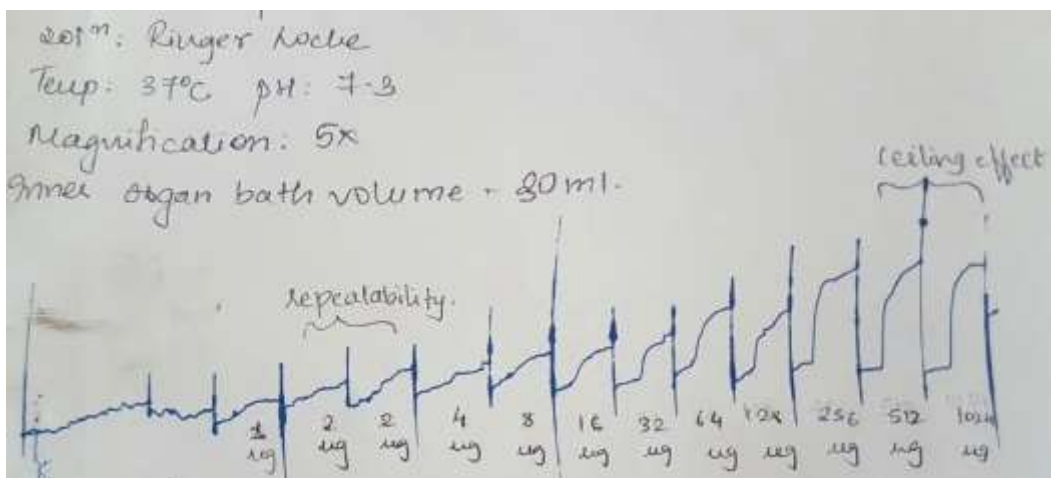
Fresh intestine of Chicken was obtained from slaughter house. The tissue was carried in PSS and immediately transported to the laboratory and kept for aeration. The intestinal content was removed by washing with PSS. The mesentery and adhering tissues were removed with gentle care. The ileum was cut into small segments of 2-3 cm long and mounted in the organ bath containing PSS maintained at 37°C, aeration was given which kept the inner bath bubbled. The tissue was allowed to relax for 30 min using tension/load 1gm and tissue was washed after every 15 min. Strength of stock solution of Acetylcholine was 1mg/ml and solution was diluted serially. The Acetylcholine induced contractions were recorded on kymograph using sideways writing lever. Graded dose response curve was obtained using serial concentration of Acetylcholine like 1, 2, 4, 8, 16µg/ml and so on which means that each selected dose is just the double of its preceding dose. Baseline of 30 sec, contact time of 1min, and 3 min time cycle was kept constant throughout the experiment. Wash was given two times after each response with PSS at an interval of 1 min. We perform the experiment with uniform

laboratory parameters like pH 7.2-7.4, temperature 37°C, inner organ bath volume 30ml and 5-7 times magnification. DRC was recorded till maximum response (ceiling effect) to Acetylcholine was obtained and height of response were measured in terms of millimetre (mm).

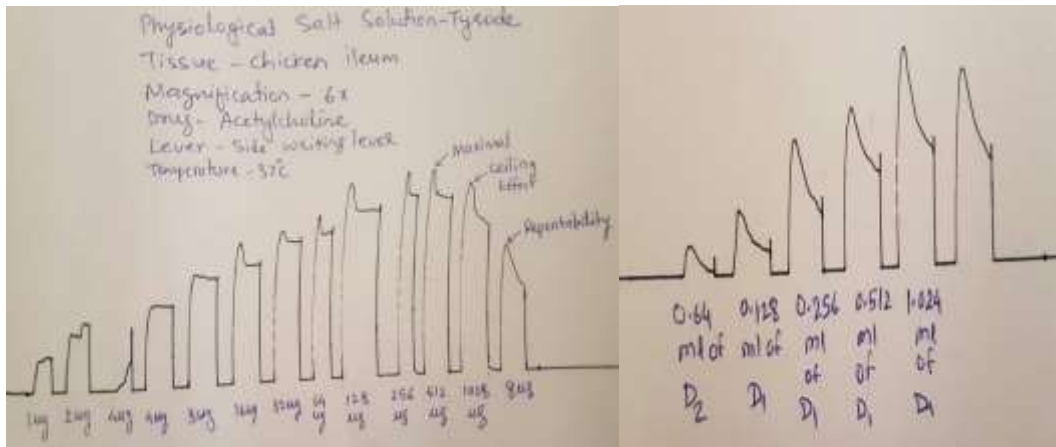
We were not able to perform 3 point and 4 point assay because of easy fatigability of the tissue due to which tissue was not able to sustain for longer period. However many authors have conducted 3 or 4 point assay using chicken ileum.<sup>(6)</sup> Height of response and stability of tissue was greater with Tyrode solution as compared to Ringer Locke solution.(Fig. 3) So interpolation method of bioassay was adopted to measure the concentration of unknown using Tyrode solution.

Height of various responses was measured in mm and the graph was plotted using log paper with log doses of standard on X-axis and height of response in mm on Y-axis. The response of unknown was interpolated on linear portion on the DRC

Concentration of unknown was calculated by considering the point on X-axis which is plotted from point on DRC where height of unknown intersect with DRC. By interpolation method 0.8ml of D2 (1:100 dilution of unknown) coincided with  $(0.6021+0.1505)=0.7526$  log dose of standard.(Fig. 4)



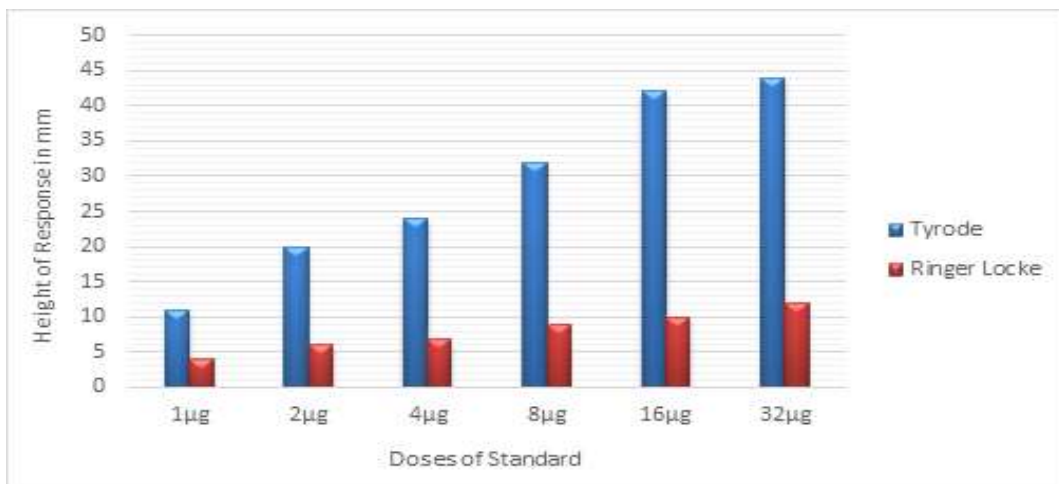
**Fig. 1: Graded dose responses of Acetylcholine on isolated chicken ileum using Ringer Locke as Physiological salt solution**



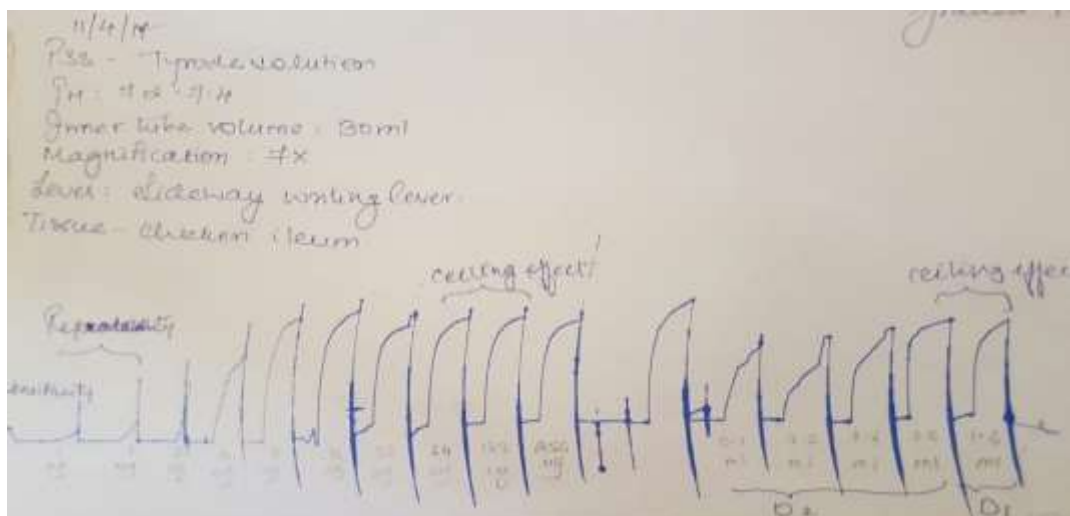
**Fig. 2: Graded dose response of Acetylcholine with standard dose and unknown on chicken ileum Using Tyrodesalt solution**

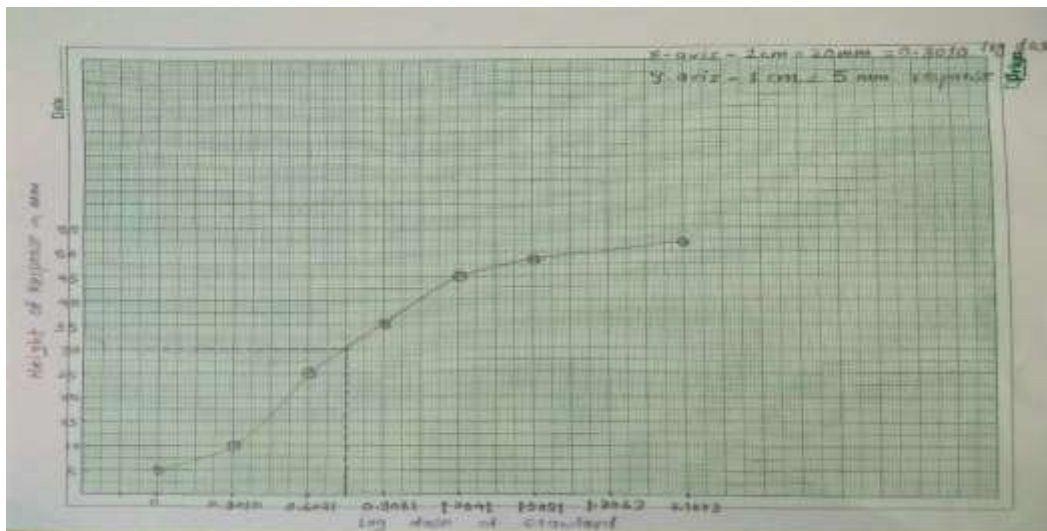
D2- 1:100 Dilution of unknown

D1- 1:10 Dilution of unknown



**Fig. 3: Comparison of height of response obtained with standard doses of Acetylcholine using different salt solutions**





**Fig. 4:** Calculation of unknown concentration of Acetylcholine by interpolation method of bioassay

Antilog of 0.7526=5.657

0.8 ml of 1:100 unknown solution contains 5.657  $\mu\text{g}$

1ml of unknown solution contains  $(5.657 \div 0.8) \times 100 = 701.1 \mu\text{g/ml}$

The actual concentration of unknown was 600  $\mu\text{g/ml}$  and calculated concentration was 701.1  $\mu\text{g/ml}$  which is 82.17 % of actual concentration of unknown.

Initially we tried with Ringer Locke solution as PSS for this tissue but the height of responses was not satisfactory, also the responses were not uniform and tissue showed spontaneous activity. Later when Tyrode solution was used as PSS instead of Ringer Locke height of responses was greater and tissue did not show spontaneous activity. Better performance of the tissue with Tyrode as PSS might be because of the presence of  $\text{MgCl}_2$  in Tyrode solution which gives the stability to the tissue. **Daude et al**<sup>(6)</sup> also used the Tyrode solution for Histamine bioassay and found significant stability and results with isolated ileal preparation.

One of the important advantage of using isolated chicken ileum for experiment is its easy availability from the slaughter houses. We can elicit the exact response by using tissue preparation which could not be done with *in vitro* and *in silico* technique. There is no need to sacrifice the animal for small piece of tissue needed for experimental and demonstration purpose. Other advantages of using chicken ileum preparation is that it is economical, very easy to mount in organ bath, gives good response. Use of chicken ileum might reduce, refine and replace the number of animals for laboratory experiment just for teaching purpose.<sup>(6)</sup>

Although there are certain limitations as we could perform the interpolation bioassay, however the accuracy is less as compared to 3 point and 4 point assay.

In our department we routinely conduct the bioassay on isolated chicken ileum using different drug solutions to explore the nature of receptors present in chick gut preparation. Recently Maharashtra university of Health Sciences (MUHS) Nashik has incorporated

chicken ileum bioassay in MD Pharmacology curriculum.

To conclude, isolated chicken ileum is a better option available for conducting isolated tissue experiments considering the restriction with the use of experimental animals under CPCSEA guidelines.

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