ORIGINAL RESEARCH PAPER

Pharmacology

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Could Chick Ileum Be Relied For Test Solution Strength Estimation Accurately In MD Pharmacology Practical Examination?

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ABSTRACT

Isolated chick ileum is recently introduced for bioassay during MD Pharmacology examination, without conclusive setup by previous works. Testing the skill of bioassay and getting the accurate strength of unknown in result - are targets of assessment. For this we have designed method using chick solution with modified calcium content. We have observed interpolation method giving more accurate result than three point and four point methods using formulae. Accuracy in 3 out of 10 occasions, whereas phenomenal accuracy by taking mean of percentage errors observed in two methods (interpolation + four point or interpolation+ three point assays) could yield accurate result by minimizing percentage error. Chick ileum tissue doesn't seem to be useful alternative to establish other tissue techniques of bioassay on reliability, reproducibility, sensitivity, accuracy, specificity grounds. However it can fulfill the criteria of bioassay skill testing only, in the examinee.

Introduction:

Long experiment exercise in MD Pharmacology examination carries 150 out of 400 practical marks in MUHS Nasik pattern. Long exercise means minimum 2 hours for experiment proper followed by calculations time of approximately one hour or more- to be consumed by examinee. This is then followed by examination, viva voce etc- time which is consumed by the examiner for examination purpose. This way total 6-8 hours minimum are required to be consumed. In current scenario bioassay is mentioned as long exercise, and very recently board of studies MUHS has also permitted chick ileum- and avian tissue for assessing the skill of the candidate for the purpose of bioassay[1]. Many workers have reported their work on chick ileum wherein either acetylcholine or histamine bioassay have been performed [2,3,4,5,6] We have not come across any study wherein 4 point assay was carried out and was reported along with the graphs.

Chick ileum being newly introduced avian tissue for bioassay experimentation in MD Pharmacology, needs to be set up so that it must give consistent results in the form of contractile responses to acetylcholine for required length of the time i.e. 4 - 6 hours. Easy fatigability has been reported[7] due to which three point and four point assay may not be possible at postgraduate level in MD Pharmacology examination. Four point assay is expected from examinee under heading of long exercise, this can be relaxed to three point assay at the most. In past below this i.e. interpolation, matching methods are the short exercises, which were meant for understanding bioassay at undergraduate level. Some institutions adopt and therefore accept interpolation method during training and MD examination purposes. Accordingly the workers have reported mostly the interpolation method using different physiological salt solutions while performing bioassay of acetylcholine or histamine on chick ileum.((A) Frog Ringer solution (B) Ringer Lock solution (C) De Jalon Solution (D) Tyrode solution(E) Kreb's solution.) For recording graded dose responses[1] we have tried to set up the method with the following objectives

Cost benefit using aerator without use of oxygen Without killing of extra animal - chick ileum is brought from the chicken shop in market Selecting appropriate physiological salt solution with little modifications Ability to conduct experiment in three point and four point methodology Saving the time required for completion of Latin square design mentioned in both three and four point methodology Calculation of the errors, understanding the possibility of minimizing it.

Material and methods: One inch piece of chick ileum was mounted in isolated organ bath assembly in accordance to method of Magnus, using PSS chick solution, aerator, temperature 35 - 37 degree Celsius.

Composition of chick solution used NaCl= 6.92 g/L, KCL = 0.34g/L, CaCl2= 0.30g/L, MgCl2 = 0.11g/L, KH2PO4= 0.16 g/L, NaHCO3 = 2.1 g/L, Glucose =2.0 g/L, Sucrose = 4.5g/L[8]

(To avoid the generation of spontaneous activity, temperature was not allowed to exceed 37 degrees and calcium quantity was restricted to 0.15g/L).Mounted piece of ileum was stretched with one gram weight suspended with long arm of lever, tracings were taken on photo paper using kymograph drum, black sketch pen tip attached to suspending mobile part of frontal writing lever. Frequent washes (3-4) were given. Priming of the tissue with 5-6 doses of acetylcholine (first dose higher than subsequent low graded doses) was done this was followed by another 15 minutes stretch giving washes. Graded dose responses were taken with standard acetylcholine (1000ug/ml, 100ug/ml, 10ug/ml, 1ug/ml) using this solutions. First response of point 0.005 ug or 0.01ug followed by graded doses and their responses were recorded. This was then followed by graded dose responses with unknown strength solution used in fraction of ml - of different dilutions i.e.1:100, 1:10, 1:1 dilutions. After reaching ceiling dose two or more responses of standard in the range of 25- 75% of ceiling were selected showing near double height of contraction and were labeled as S1 and S2 noting applicable log. Similarly two responses of the test graded responses were labeled as T1 and T2, in the range of 25- 75 % of ceiling of test. Now the values of S1, S2, T1, T2 and their applicable log were put in formula, their equations were solved, relative potency was calculated, antilog was taken and finally strength of unknown solution was arrived at.

$$M = (S2-T2) + (S1-T1) / (S2-S1) \times (T2-T1) \times \text{Log}\,(s1/t1)$$ [9]

Concentration of unknown = s1/t1 x Antilog M Similarly three point assay methodology was followed on some occasions using standard technique[10]

Interpolation calculations were carried using S1and S2 which were two responses of consecutive doses in range of 25%- 75% of ceiling. Herein dose in ml of test solution, giving 66% (60% to 70% range) response of the ceiling of standard dose was selected for purpose of interpolation using semi log paper. Antilog of the interpolated point gives us the strength of unknown in specified volume used from here we can arrive at strength of unknown per ml. We have dropped the Latin square design number of the responses (9 or 16) as mentioned in traditional 3 or 4 point assay to save time. Percentage error was calculated.

Conflict of interest: Nil
Institutional animal ethics committee: Approval not required as tissue was brought as waste from chicken market.
Funding: Nil
Statistical analysis test: Not required.

RESULTS AND OBSERVATION

FIGURE 1 showing graded dose responses of standard solution of ach

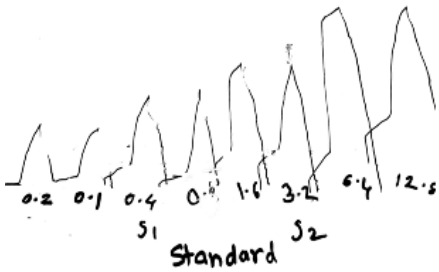


FIGURE 2: Showing graded dose responses of unknown solution of ach

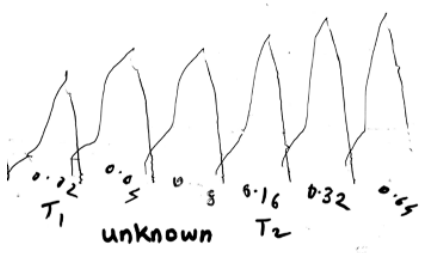


FIGURE 3: showing Latin square design base sequence of doses of S1, S2, T1, and T2 of Ach

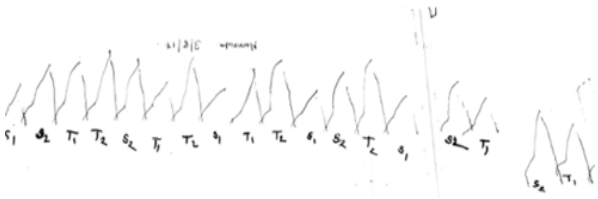
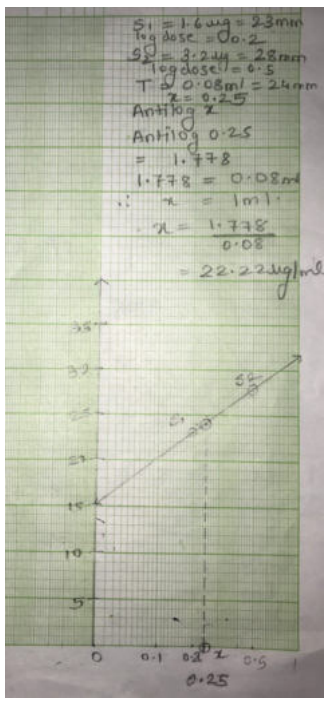


FIGURE 4: figure showing log dose response curve of standard and interpolation of one dose of unknown and its calculations



Sr no	observed strength	actual strength	percentage error	Method
1	407	400	1.75%	Interpolation
2	435	400	8.75%	three point assay without Latin square
3	346	400	13.4%	four point assay without Latin square
4	15.14	20	24.3%	Interpolation
5	25.7	20	28.5%	three point assay with Latin square
6	1.7	2	15%	Interpolation
7	1.6	2	20%	three point assay with Latin square
8	20.25	20	1.25%	interpolation
9	22.22	20	11.01%	interpolation
10	16.59	20	17.05%	four point assay with Latin square

Table showing the estimation of test solution of acetylcholine by different methods (n=10)using isolated chick ileum

Table shows that Four point assay done without Latin square gives 13.4% error whereas with Latin square gives 17.05% error similarly Three point assay done without Latin square shows less percentage error as compared to 3 point assay done with Latin square design (8.75%vs 20% or more) 0.4 mcg is S1 and 3.2 mcg is S2 marked in figure 1 are for purpose of calculations in formula for four point assay 0.02 ml T1 and 0.16ml T2 marked in figure 2 are for purpose of calculations in formula for four point assay 1.6 mcg is S1 and 3.2 mcg is S2, 0.08ml with height 24mm is T for purpose of interpolation.

Figure 3: response 3 (T2) and response 15 (S1) are erratic in this Latin square sequence. Repeat response were taken corresponding to the dose showing erratic response were taken at the end and their height was taken for calculation purpose.
Calculation of four point assay .

	DOSE	HEIGHT
S1	0.4mcg	16mm
S2	3.2mcg	28mm
T1	0.02ml	21mm
T2	0.16ml	29mm

M= {(S2-T2) + (S1-T1) / (S2-S1) x (T2-T1)} x Log (s1/ t1)
M= {(28-29) + (16-21) / (28-16) x (29-21)} x Log (0.4/0.02)
M= {(-1) + (-5) / 12 x 8} x Log 20
M= {(-6) / 96} x 1.301
M= (-0.0625) x 1.301
M= - 0.081

Concentration of unknown = (s1/t1) x Antilog M = (0.4/0.02) x Antilog (-0.081) = 20 x 0.8298
Concentration of unknown =16.59 mcg/ml

From sr no 9 and 10 of table of observation the percentage error observed can be minimized in following manner
Percentage error = (Actual - Observed) / Actual

E1: percentage error of four point assay = {(20-16.59) / 20} x 100
= 17.05%
E2: percentage error of interpolation = {(20-22.22)/20} x 100
= 11.01%
Mean= (22.22+16.59) / 2 =19.40
Mean Error = [(20-19.40) / 20] x 100 = 3%

Similarly sr no 4 and 5 in the table which shows percentage error by two methods can also be minimized in the following manner

E1: percentage error of three point assay = {(20-25.7) / 20} x 100
=28.5%
E2: percentage error of interpolation = {(20-15.14)/20} x 100
= 24.3%
Mean= (25.7+15.14) / 2 =20.42
Mean Error = [(20-20.42) / 20] x 100 = 2.1%

The above mentioned technique of minimizing the percentage error needs to be confirmed by more number of the experiments and respective calculations.

If such type of phenomenon is repeatedly observed it can establish the manner to reduce the percentage error to less than 10% i.e. acceptable by the examiners.

Discussion:

Observed phenomenon is :(Sr. no 9 and 10 in table). Similarly sr no 4 and 5 in table indicate the reduction in the percentage error when we take the mean of the calculation done by interpolation with three point and four point respectively. On the other hand percentage error with interpolation have been observed (sr no1 and 8)less than 2% which is more accurate than the average calculation technique. This way time can be saved as there is no need now to carry out experiment by taking 16 responses sequentially in Latin square design, the chance of fatigability of tissue, erratic response have been overcome. Figure number 3 we have taken 16 responses out of which 2 responses were erratically misleading (S2 and T1 i.e. 3 and 15 Latin square response) and therefore were required to be repeated. This repetition was not mentioned in standard Latin square design methodology, therefore is likely to interfere with the accuracy of the result as the average height of response will be altered.

Conventionally it is believed that interpolation is less accurate method than three point and four point methods of bioassay, all these observations raise the question of reliability of isolated chick ileum as far as accuracy of result is concerned. This is another drawback of this tissue.

MUHS has accepted and permitted this avian tissue for purpose of bioassay in MD Pharmacology examinations. If the examiners do not consider the accuracy of result (about strength of test solution) then this method may be helpful in testing the skill only. However if both the factors i.e. skill and accuracy are taken into account by examiners this method may not prove out to be a useful substitute/alternative of established methods of bioassay using frog rectus abdominis, dorsal muscle of leech for acetylcholine and isolated guinea pig ileum for histamine .

Drawbacks of chick ileum:

1) It is not a mammalian tissue however the response to Ach is contractile.
2) Difficult to maintain temperature and oxygenation/aeration in transit time i.e. bringing from chicken shop to laboratory.
3) Possibility of rough handling of the tissue and no surety of overnight fasting of animal.
4) Long time is required for the stretching and priming
5) Spontaneous contraction is likely to develop if temperature is more than 37 degree Celsius or calcium quantity is higher in PSS, or when kerb solution is used
6) Baseline goes on shifting gradually down and down and as per law of initial values the responses accordingly will be deceiving.
7) 24% desensitization observed from high dose responses comparison one before and another after the experiment. (Indicating maximum contractility) this makes the tissue unreliable for purpose of bioassay. Nevertheless the experiment is sufficient to test the skill of bioassay, during training and examination of MD Pharmacology.

From the table of observation it is clear that using different methods the percentage error range is 1-25%. It has been mentioned that percentage error should not exceed 10% [6] there are 2 such observations in the table i.e. 1, 2 and 8 (1 and 8 observed with interpolation and 2 with three point assay without Latin square).

This amounts to 30% of the experiments showing acceptable percentage error whereas 70% time this tissue has shown more than 10% error.

When animal handling and dissection is not to be tested (as in this method) we conclude that as this tissue is not reliable on all above mentioned criteria it will be better to adopt the animal experiment software technique to test the skill of bioassay in examinee, which is more time saving.

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