A NOTE ON THE SUITABILITY OF THE INDIAN FROG (*RANA TIGRINA*) FOR THE BIOLOGICAL ASSAY OF TINCTURE OF DIGITALIS

By A. K. Ghosh, R. K. Srivastava and J. N. Tayal

From the Technical Development Establishment Laboratories, Kanpur, India

The relationship between the log of the dose of tincture of digitalis and the effect, in terms of probits of the mortalities (per cent) among the Indian frog, *R. tigrina*, has been found to be linear. Statistical analysis of the data collected from four experiments performed from July to November has not shown any non-linear relationship. The results obtained by applying the B.P. 2 and 2 quantal response assay on the frog method have been found to be in fair agreement with those obtained by using guinea pigs. The fiducial limits of error of potency of the samples at (P = 0·95) have been found to lie within the limits specified in the B.P. 1953. By using *R. tigrina*, potencies of the standard, substandard and abnormal samples of tincture of digitalis have been satisfactorily determined. The advantage of the frog method is the speed and simplicity of the technique: the disadvantage is the restricted availability of the suitable size of the frog during the five months July to November.

Rana temporaria is not available in India, and it therefore seemed desirable to investigate the suitability of an Indian frog *Rana tigrina* for the frog assay of digitalis in the 1953 British Pharmacopoeia. We have also compared the results obtained with *R. tigrina* with those obtained from the B.P. assay method using guinea pigs.

EXPERIMENTAL

Dose Mortality Relation

Specimens of *R. tigrina* weighing from 25 to 50 g. were divided into five groups of 20 such that the average weight of the frogs in the groups were almost equal. Females were equally distributed in the groups; those with distended abdomens were not used. Apart from these restrictions the selection was random.

A tincture was prepared by method (2) in the B.P. using International Standard digitalis leaf powder. The tincture served as the stock solution and was stored in a refrigerator at 5°. For use the solution was diluted with 0·6 per cent saline and injected into the ventral lymph sac in doses of 0·02 ml./g. The number of frogs in each group which died from the specific effect of digitalis on the heart was determined. Four sets of results during the period July to November were obtained.

Comparison of Frog and Guinea Pig Methods

Frog method. Eighty frogs in groups of 20 were injected in a 2 and 2 quantal response assay as described in the B.P. using dilutions of the stock solution and of test preparation. The estimation of potency ratio of test and standard and the calculation of the fiducial limits of error were

made according to the B.P. recommendation under the "2 and 2 dose assay" procedure.

Guinea pig method. Healthy male animals weighing between 300 and 600 g. were selected in groups of 12 for each experiment, such that the individual weights in each group did not differ by more than 100 g. Each group was then divided into two groups of 6 such that the average weights of both were almost equal. The guinea pigs were anaesthetised by subcutaneous injection of 25 per cent urethane in a dose of 1·8 g./kg. body weight, and the standard and test preparation in 0·9 per cent saline infused

TABLE I

DOSE-MORTALITY DATA ON FROGS

Dose (mg. leaf/g. body weight)	Mortality—number dead/number injected			
	I: July	II: September (2nd week)	III: September (4th week)	IV: November
0·250	2/20	0/20	1/20	1/20
0·375	4/20	4/20	4/20	6/20
0·500	14/20	8/20	7/20	13/20
0·625	17/20	15/20	14/20	18/20
0·750	18/20	16/20	19/20	20/20

into the jugular vein at about 1 ml./6 minutes. No artificial respiration was given. Estimation of test and standard, and calculation of the fiducial limits of error, were made according to the Pharmacopoeial method recommended under "Assays depending upon measurement of the effective dose of each animal", Example III.

RESULTS AND DISCUSSION

Dose-mortality Relationship

Probit analysis. The results of the first four experiments to establish the dose-mortality relationship on frogs are given in Table I. The mortalities in each experiment were converted into percentages and plotted against logarithms of the respective doses. The resultant curves assumed typical sinusoidal shape. The data were analysed and the probits of the percentage mortality were plotted against the logarithms of the doses; an examination of the curves thus obtained revealed that in each case a straight line would fit the observations. The regression of the probit of the percentage mortality on the logarithm of the corresponding dose was estimated in each experiment. The relationships observed as a result of this analysis are given in Table II.

The regression equations in the case of experiments II and IV were obtained by iteration as mortalities of 0·0 per cent and 100 per cent respectively were observed in these cases.

Linearity of regression in the experiments. The assumption of linearity of regression in each experiment was tested by calculating the value of χ^2 according to the following formula.

$$\chi^2 = \Sigma\ nw\ (y-\bar{y})^2 - \frac{[\Sigma\ nw\ (x-\bar{x})\ (y-\bar{y})]^2}{\Sigma\ nw\ (x-\bar{x})^2}$$

The degrees of freedom associated with χ^2 were three in respect of experiments I and III and two for experiments II and IV. The values of χ^2 in the experiments are given below Table II. As the values of χ^2 corresponding to 5 per cent level of probability and 2 and 3 degrees of

TABLE II

ANALYSIS OF THE DATA IN TABLE I

	Dose of digitalis leaf mg./g. body weight	Log dose of digitalis leaf mg./g. body weight	Percentage mortality	Probit	Regression coefficient
I	0·250 0·375 0·500 0·625 0·750	−0·60 −0·43 −0·30 −0·20 −0·12	10 20 70 85 90	3·7 4·15 5·66 6·05 6·25	6·05
II	0·250 0·375 0·500 0·625 0·750	−0·60 −0·43 −0·30 −0·20 −0·12	0 20 40 75 80	— 4·15 4·80 5·65 5·85	6·47
III	0·250 0·375 0·500 0·625 0·750	−0·60 −0·43 −0·30 −0·20 −0·12	5 20 35 70 95	3·35 4·15 4·62 5·50 6·62	6·26
IV	0·250 0·375 0·500 0·625 0·750	−0·60 −0·43 −0·30 −0·20 −0·12	5 30 65 90 100	3·35 4·48 5·40 6·25 —	7·87

Values of χ^2 in the four experiments:—I = 2·19; II = 1·37; III = 2·57; IV = 0·56.

freedom were 5·991 and 7·815 respectively, none of the values of χ^2 in the above experiments were significant, showing that there is no evidence of non-linearity of regression in any of the four experiments. Therefore the 2 and 2 quantal response assay method described in the Pharmacopoeia was considered applicable in this case.

*TABLE III

COMPARISON OF THE RESULTS OBTAINED BY ASSAYING TINCTURE OF DIGITALIS ON FROGS (R. tigrina) AND GUINEA PIGS

	Frog method		Guinea pig method		B.P. limits	
Sample No.	Potency ratio (T/S) per cent	Fiducial limits of error (P = 0·95) per cent	Potency ratio (T/S) per cent	Fiducial limits of error (P = 0·95) per cent	Potency ratio (T/S) per cent	Fiducial limits of error (P = 0·95) per cent
1*	149·3	72·5 to 139·8	134·2	90·6 to 110·4		
2	98·6	89·6 to 111·4	96·2	93·0 to 107·4	90 to 110	70 and 140
3	100·7	86·8 to 113·5	99·6	99·7 to 100·3		
4	77·3	77·5 to 117·9	83·8	78·7 to 127·1		

Sample 1 was obtained locally and was freshly manufactured. Samples 2 and 3 were standard tinctures prepared from International digitalis powder by the B.P. method (2). Sample 4 was a known dilution (80 per cent potency) of the standard.

Comparison of Frog and Guinea Pig Methods

Four samples of tincture of digitalis were assayed against the standard preparations by using the technique described. The results are tabulated in Table III.

It will be seen that the results of the frog method, using *R. tigrina*, in these four experiments compare very favourably with the method using guinea pigs; the limits of error of the estimated potency (P = 0·95) lie within the tolerances prescribed in the Pharmacopoeia. The frog method also appears to be suitable for assessing the potency of the samples which is beyond the acceptable range recommended in the Pharmacopoeia.

The frog method has the advantage that the technique employed is simpler and less time-consuming than the other B.P. methods, while a disadvantage is that the frogs of required body weight are available only during July to November in the year.