ASSAY OF DIGITALIS

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The uses of the outer walls of the right ventricles of guinea pigs and of auricles of rabbits for the assay of digitalis have been reinvestigated. The results obtained are compared with those obtained by slow intravenous infusion in guinea pigs (B.P. 1958) and the pigeon method (U.S.P. XV). The results obtained by the use of isolated auricles indicate that this method is reasonably accurate, cheap and less time-consuming than other methods and has the further advantage that the test sample is compared with the standard on the same tissue.

The chemical methods for the assay of the potency of digitalis preparations do not give satisfactory results, as digitalis contains several active principles with different chemical and pharmacodynamic properties. Unless it becomes possible to cultivate leaves with a fixed or nearly fixed content of glycosides, it is necessary to depend on biological methods.

Knaffl-Lenz[1] introduced the guinea pig method which is now official in B.P. 1958[2]. Hanzlik[3] proposed the use of pigeons for the assay of digitalis, as simple, economic and reasonably accurate. Hagg and Woodley[4] described a new intravenous pigeon method for the standardisation of digitalis and showed that results obtained by their method agree reasonably well with those of the cat method of Hatcher and Brody[5]. This method, as modified by Braun and Lusky[6], has been recognised as an official method in U.S.P. XV[7].

Trevan and Boock[8] suggested the use of isolated rabbit auricles for the estimation of digitalis, as they show a reversible increase of amplitude when digitalis is added to the bath and the increase is proportional to the dose added. Stewart[9] has studied the influence of digitalis preparation and of cardiac glycosides on an electrically stimulated isolated outer wall of the right ventricle of a guinea pig. He has shown that a digitalis preparation can be standardised by computing $\log_{10}$ time to zero amplitude for a standard and a test preparation, as the index of precision is highest when time for zero amplitude is taken as the metameter, in preference to first increase in amplitude, beginning of plateau, maximum amplitude, and duration of plateau. In the present study the uses of the outer walls of right ventricles of guinea pigs and of rabbit auricles for the assay of digitalis have been reinvestigated and compared.

The results obtained by these two methods are compared with the results obtained by B.P. 1958[2] method and U.S.P. XV[7] method.

Methods and Materials

Isolated Ventricle Method

Throughout the investigation, male albino guinea pigs weighing between 350 g. and 450 g. were used.

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A suitable wide glass tube closed at one end was used as the isolated organ bath (Fig. 1), which was suspended in a thermostatically controlled water bath to give a temperatue of 35° ± 1°.

Pure oxygen at the rate of 200 ml. per minute was bubbled through the Ringer solution[9]. A spring lever was balanced by a definite load, to keep the tension constant for all the preparations of ventricles. The ventricles were stimulated by a square wave stimulator—duration 5 msec., volts 50, rate one per 20 sec. Platinum electrodes (20 S.W.G.) were used; one was kept free near the ventricle preparation in the organ bath and the other was in contact with the ventricle preparation (Fig. 1). Extracts from two samples of *Digitalis purpurea* leaves were prepared by

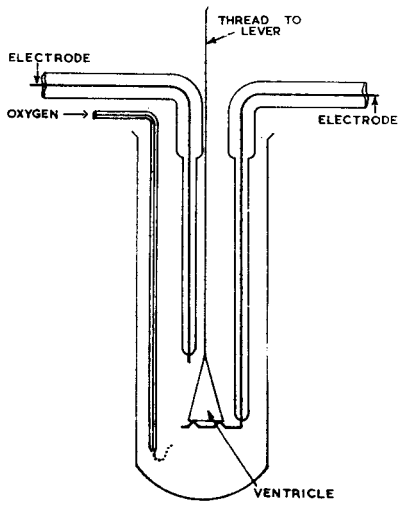

Fig. 1. Isolated organ bath.

the B.P. 1958[2] procedure. To ensure the uniformity of the alcohol content in each preparation, ethanol was completely removed under vacuum from a definite volume of each extract and the residues obtained were taken up in a fresh quantity of a similar volume of ethanol (80 per cent v/v). The samples of *Digitalis purpurea* used had been standardised by B.P. 1958[2] and U.S.P. XV[7] procedures.

A guinea pig is killed by a blow on the head and the chest is opened immediately. The pulmonary artery is tied by means of a cotton thread after exposing the heart. The heart is then removed and put in warm well-oxygenated Ringer solution. The outer wall of the right ventricle is removed as quickly as possible and attached to the platinum electrode and suspended from the pulmonary artery side in oxygenated Ringer solution maintained at a temperature of 35° ± 1°. Before the addition of the drug, the ventricle preparation was allowed to settle for at least 2½ hours, as it was observed that during this period the amplitude of contraction often varies.

Three doses of each preparation were tried in a definite dose ratio. Each dose was tried on six ventricular strips. The preparation of the ventricle could not be used again, as it does not recover its response to stimulation even after repeated washings.

A typical kymographic record of an experiment is shown in Figure 2.

The time in minutes required for the first increase of amplitude (the first point at which the increase in amplitude is 1 mm. over the initial amplitude), beginning of plateau of maximum contraction (taken as the time required to reach 95 per cent of the maximum amplitude), duration

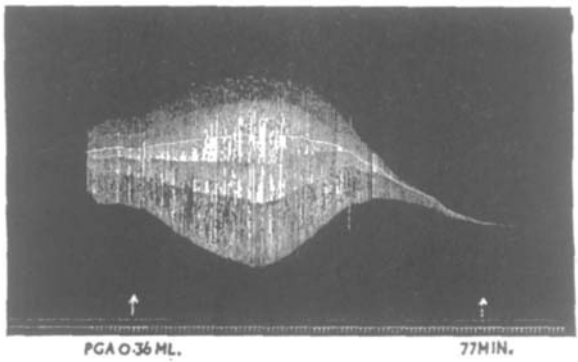

FIG. 2. Effect of digitalis on recorded contractions. Guinea pig, abino male, 370g.

of plateau (time embraced by the 95 per cent limits on maximum contraction), maximum amplitude, half of maximum amplitude and zero amplitude is recorded.

RESULTS

From the results obtained, it was seen that $\log_{10}$ time to maximum amplitude may be taken as a suitable metameter for the assay of digitalis. Analysis of variance[10] worked out for the two samples in comparisons with the standard, with respect to $\log_{10}$ time to maximum amplitude is tabulated in Tables I and II.

From the Tables it is seen that the component "Preparation" shows that there is an overall difference in response between each of the two

TABLE I

VALIDITY TESTS FOR PARALLELISM, CURVATURE AND DIFFERENCE OF CURVATURES WHEN $\log_{10}$ TIME TO MAXIMUM AMPLITUDE FOR STANDARD AND THE TEST A IS COMPUTED

Nature of variance	Degrees of freedom	Sum of squares	Mean square
Preparations	1	1,906·8	1,906·8
Regression	1	1,568·2	1,568·2
Parallelism	1	20·2	20·2
Curvature	1	2·7	2·7
Difference of curvatures ..	1	12·5	12·5
Between doses	5	3,510·4	
Error	30	1,287·0	42·9

preparations and the standard and the component "Regression" indicates that there is an increase in effect with an increase in dose.

Mean square for "Parallelism" is not significantly large for both the samples, indicating that the responses to the two preparations and the standard are similar, and shows that there is no significant deviation from parallelism.

The non-significance of "curvature" indicates that the regression for each of the preparations is satisfactorily linear. This is further enhanced by the non-significance of "difference in curvatures".

The Isolated Auricles Method

An adult rabbit is killed by a blow on the head and the heart removed immediately, following usual procedure, without damaging the auricles. Taking normal precautions, the auricles were separated and suspended

TABLE II

VALIDITY TESTS FOR PARALLELISM, CURVATURE AND DIFFERENCE OF CURVATURES WHEN LOG_{10} TIME TO MAXIMUM AMPLITUDE FOR STANDARD AND THE TEST B IS COMPUTED

Nature of variance	Degrees of freedom	Sum of squares	Mean square
Preparations	1	529·0	529·0
Regression	1	2,185·0	2,185·0
Parallelism	1	7·0	7·0
Curvature	1	48·4	48·4
Difference of curvatures ..	1	36·1	36·1
Between doses	5	2,805·5	
Error	30	2,043	68·10

in an organ bath containing a well oxygenated Ringer solution, as described by Trevan[8], maintained at a temperature of 37° ● 1°. A light lever, consisting of a spindle mounted on centres and having a spring collar for gripping straws, was used. The auricles were allowed to beat until they gave a uniform amplitude.

A measured volume of test extract and standard extract prepared as per B.P. 1958[2] procedure was taken and evaporated to dryness on a water bath, taking care not to over heat. The residues obtained were taken up in similar volumes of normal saline and filtered through cotton wool plugs.

Different doses of the extracts prepared as above were then added to the organ bath containing the auricles to find out a suitable dose which would give a measurable increase in amplitude. Two doses of test extract and two doses of the standard extract in the dose ratio of 1:2 were added to the organ bath and the increases in amplitude recorded. The speed of the drum is adjusted in such a way that each beat is recorded separately and clearly. Each dose was duplicated, triplicated or added four times, depending upon the working of the auricles. Normally the doses were duplicated, as it was observed that at a later stage the auricles lose sensitivity and also that the first few doses are considered as trial doses.

The kymograph record (Fig. 3) is taken, for each dose, first before the addition of the drug, then 1 minute after the addition of the drug and, finally, 5 minutes after the addition. The actual increase in the amplitude during the last 4 minutes is measured for comparison purposes.

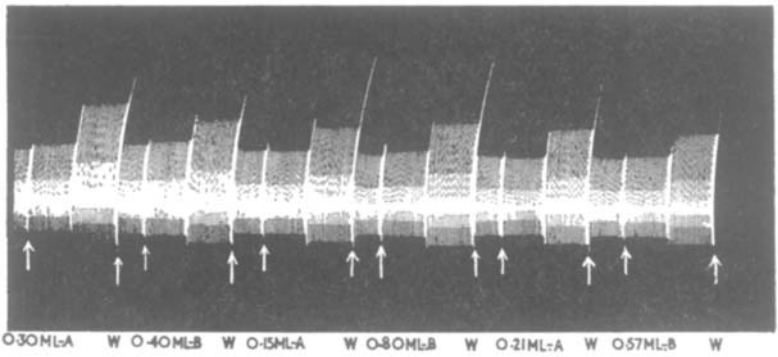

FIG. 3. Tracing showing the increase in auricular beat of isolated rabbit auricles after addition of digitalis in saline. A = Extract from leaf sample A. B = Extract from sample B. W = wash.

The auricles are washed in four changes of Ringer and allowed to beat for 20 minutes before another dose of the drug is added. Used in this way, the same auricles can respond to give satisfactory records for up to sixteen doses of the drug.

TABLE III

RESULTS OF ASSAY OBTAINED BY B.P. 1958, U.S.P. XV, ISOLATED OUTER WALL OF RIGHT VENTRICLE AND ISOLATED AURICLES METHODS

Digitalis purpurea	B.P. I.U./g.	U.S.P. XV I.U./g.	Isolated right ventricle I.U./g.	Isolated rabbit auricles I.U./g.
A	10·78	9·91	9·34	9·10
B	4·60	4·68	5·50	3·20
*C	—	11·12	—	9·77

* A third sample of *Digitalis purpurea* leaf

DISCUSSION

One important advantage of the auricle method over the right ventricle method is that only one animal is required for the assay and that the same auricles can be used for several doses of test and the standard; whereas in the case of ventricles, only one dose of one drug could be tried on one preparation.

In the case of the isolated ventricles, the study of the analysis of variance for digitalis leaf A shows that there is a linear relationship between dose and $\log_{10}$ time to the maximum contraction of ventricle and $\log_{10}$ time to beginning of plateau. When digitalis leaf B was studied, it was found that a linear relationship existed between dose and $\log_{10}$ time to maximum amplitude and $\log_{10}$ time to zero amplitude.

Log_{10} time to first increase for both the samples hardly alters with the increase in dose, therefore this could not be considered as a possible metameter.

Difference in time required for different metameters with different doses of the drug shows that there is a direct relationship of concentration of digitalis and the time required for its action.

In the case of isolated auricles, the initial amplitude increases with the addition of the drug, showing probably that some drug is fixed on or in the tissue, even after repeated washings. However, this does not interfere with the rise in amplitude, which remains proportional to the quantity of the drug added.

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