RESEARCH PAPERS

THE ACTIONS OF DIGITALIS LEAF PREPARATIONS AND OF CARDIAC GLYCOSIDES ON THE ISOLATED RIGHT VENTRICLE OF THE GUINEA PIG

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A method is described for recording kymographically the systolic contraction and the resting length of the electrically stimulated, isolated right ventricle of the guinea pig. Two samples of *Digitalis purpurea* leaf and a sample of *Digitalis lanata* have been assayed in terms of the Third International Standard of *D. purpurea* using metameters dependent on changes in the systolic contractions and resting length of the muscle and the time elapsing before the changes occur. In a similar manner digitoxin and ouabain have been assayed in terms of digoxin. The results are compared with those obtained by slow intravenous infusion of the preparations into guinea pigs. The potencies derived from the two methods are in close agreement in the case of the digitalis leaf preparations. Digitoxin, however, is more active than digoxin on the ventricle preparation irrespective of the metameter used and less active by the slow infusion method, while ouabain has the same activity as digoxin on the ventricle and twice the activity by the infusion method. The results for ouabain on the ventricle using the different metameters, are homogenous, those for digitoxin are not. There is little difference between the general actions of the three glycosides on the ventricle but each glycoside is capable of producing different effects on systolic contraction and resting length depending on the dose employed. The possible mechanisms responsible for these effects are discussed.

THE relationship between the therapeutic and toxic actions of cardiac glycosides has long been a problem in animal and clinical pharmacology. While the toxic properties of these substances are easily defined their therapeutic properties, especially in animal experiments, are less easily recognised and open to a variety of interpretations.

Cardiac glycosides may act via the nervous system of the heart for example by inducing a return to normal rate in auricular fibrillation, or they may act directly on the heart muscle itself inducing a more efficient utilisation of energy in congestive heart failure.

Several workers have used isolated portions of the heart to study the direct actions of the cardiac glycosides on muscle. Trevan and Boock[1] first described the use of isolated rabbit auricles for the estimation of the activity of digitalis leaf preparations; the preparations were allowed to act on the auricles for 4 minutes and the increase in amplitude of contraction was recorded. Halpern and others[2] used the rabbit's left auricle only and calculated the increase in work done per second against a given load after the addition of convallotoxin to the organ bath containing the auricle.

Cattell and Gold[3,4] registered photographically the increase in amplitude of the isometric contractions induced by glycosides on the electrically

stimulated, isolated papillary muscle of the right ventricle of the cat. In one series of muscle preparations they determined the threshold concentration of glycoside resulting in an augmentation of amplitude in 50 per cent of the preparations, which they defined as the "therapeutic factor", and in another series they determined for given doses of the glycoside the time taken for the amplitude to decline to 80 per cent of its maximum augmented contraction. This was defined as the "toxic factor".

White and Salter[5], Sciarini, Ackerman and Salter[6], White, Belford and Salter[7], and Ipsen and White[8] using the same preparation in a manner similar to that of Cattell and Gold[3,4] have described statistical procedures which have permitted the evaluation of potency of one glycoside in terms of another. In one series of experiments they determined the minimum dose of glycoside which showed a positive inotropic effect, and in another the dose of one glycoside which could be substituted for another glycoside isodynamically at a given stage where the increase of the amplitude of contraction after substitution continued at the same rate as before. In a final series the dose required to produce toxic effects (decline in amplitude) was determined. The authors define the first two metameters as measurements of therapeutic activity.

Luisada and Diamond[9] studied the action of cardiac glycosides on diastolic and resting length of isolated ventricle strips, papillary muscles and atrial strips of cats and dogs and showed that whereas all glycosides examined induced an isotonic decrease in resting length there was a number which, at various concentrations of glycoside, induced an increase in resting length.

The increase in amplitude of the isotonic contractions of the electrically driven isolated right ventricle of the rat induced by ouabain has been observed by McDowall and Zayat[10,11].

Wedd and Blair[12] found that glycosides were capable of producing a shortening of the QT segment of an electrogram obtained from an electrically stimulated strip of turtle ventricle, and this shortening, which reflected an increased rate of recovery from systolic contraction, was not related to the mechanical contraction of the muscle.

In the present paper a study has been made of the influence of digitalis leaf preparations and of cardiac glycosides on the resting length and amplitude of contraction of the electrically stimulated isolated right ventricle of the guinea pig. Only one dose of the drug is allowed to act on each preparation so as to avoid the assumptions of previous workers regarding rates of action and metabolism of the glycosides.

The results are compared with those obtained for the same preparation administered by slow intravenous infusion to the guinea pig according to the method of biological assay described in the B.P., 1958, p. 943.

Method

A male albino guinea pig of 400–500 g. in body weight is killed by a sharp blow on the head. The heart is quickly removed and placed on a nylon-foam pad soaked in warm, oxygenated salt solution. The entire outer wall of the right ventricle is cut from the heart. The apex of the

ventricle and the muscle adjacent to it is tied to the glass hook in which is sealed a platinum electrode. A cotton thread is tied around the stump of the pulmonary artery and the ventricle suspended to form a triangular wedge as shown in Figure 1. The organ bath is a modification of that described by Green, Riley and White[13] where the nozzle through which the oxygen enters the fluid and the angle of the two arms connecting the two compartments lead to a greater efficiency of circulation and the prevention of static regions in the more remote parts of the fluid. The bath thus permits the muscle to be oxygenated without agitation, and prevents substances which would form a froth from producing a seal over the top of the fluid in the compartment in which the muscle is housed.

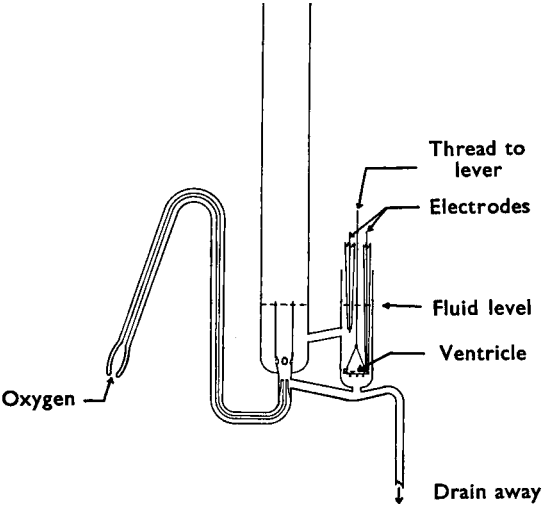

FIG. 1. Isolated organ bath

The platinum electrodes are 0·020 inches in diameter. The free electrode, 2 mm. in length, is placed in the chamber close to the muscle. The organ bath contains 68 ml. (34 ml. in one series of experiments) of salt solution of the following composition: NaCl 9·0, KCl 0·42, $CaCl_2$ 2·015, $MgCl_2$ 0·0025, Glucose 0·5, and $NaHCO_3$ 0·5 g., in one litre of distilled water. The $CaCl_2$ is added last as 1 ml. of a 20·15 per cent (w/v) solution, otherwise precipitation may occur. The final pH is 7·8.

The excess of $CaCl_2$ in this solution produces a very large augmentation of the amplitude of contraction of the muscle thus enabling the contractions to be recorded kymographically, obviating the need for expensive optical cameras and enabling one to observe the progress of the experiment without awaiting a developed photograph before the results can be observed.

The solution is oxygenated with pure oxygen at the rate of 200 ml. per minute. The apparatus is immersed in a water bath maintained at $35 \pm 0{\cdot}2°$. The muscle is stimulated from a square-wave stimulator as follows: duration 5 msec., volts 50, rate 2 per minute. The slow rate of stimulation allows a clear record of the resting length of the muscle to be made, as well as producing large contractions which are recorded isotonically with a spring loaded lever, the long arm of which is made of balsawood weighing 1 g. The fine nickel-silver spring coil is adjusted so that when a 6 g. weight is suspended at the point of attachment of the

cotton, the lever is horizontal. The lever gives a 32-fold magnification of the actual muscular contractions.

The muscle is allowed to settle in the bath for up to 3 hours before any drug is added since during this time the amplitude of contraction often becomes larger.

Preparations Examined

Digitalis leaf preparations. Two samples of *Digitalis purpurea* leaf, one of high and one of low potency as determined by the B.P. 1958 method of assay in guinea pigs, one sample of powdered *Digitalis lanata* leaf and the International Standard of Digitalis Leaf (1949) were extracted with 80 per cent (v/v) ethanol at room temperature according to the B.P. 1958 procedure. The two test extracts of *D. purpurea* leaf were then freeze-dried and taken up again in a similar volume of 80 per cent (v/v) ethanol so that the amount of alcohol administered was constant irrespective of the dose of digitalis. The tincture of the sample of *D. lanata* was diluted with 80 per cent (v/v) ethanol for the same reason. The highest dose of the standard preparation was 0·4 ml. (40 mg. of leaf).

Cardiac glycosides. Digoxin, digitoxin (assaying at 1,000 I.U./g. by the guinea pig infusion method against the International Standard of Digitalis Leaf), and ouabain were each used as a solution of 1 mg./ml. in 70 per cent (v/v) ethanol.

In any test the amount of alcohol administered to a given ventricle preparation was kept constant by using as diluent for lower doses the appropriate concentration of solvent.

Results

Assay of Digitalis Leaf Preparations on the Guinea Pig Ventricle

The three extracts of digitalis leaf were assayed against the International Standard using a randomised block design with three doses of each extract

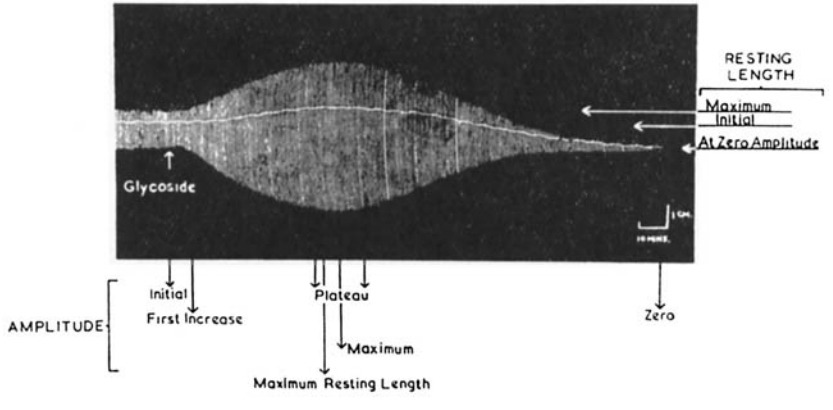

FIG. 2. Kymograph tracing of the electrically stimulated isolated right ventricle of the guinea pig. The arrows indicate the changes in systolic contraction and resting length induced by a cardiac glycoside.

in a dose ratio of 2:1. Each dose was administered twice to each bath and replicated in three different baths. Only one dose was administered to any ventricle. In this way each dose was tested six times. There were 72 preparations in all.

Figure 2 shows a typical kymograph record and the points marked on the tracing indicate the various metameters used to assess activity. The tracing registered above the resting length is due to a recoil of the spring following contraction of the muscle. A study was made of the changes in resting length and systolic contraction of the ventricle induced by the digitalis extracts, by conducting analyses of variance on the following metameters against $\log_2$ dose.

The $\log_{10}$ time to (*a*) the first increase of amplitude, taken as the first point at which the amplitude increases 1 mm. over the initial amplitude and thereafter continues to increase, (*b*) the beginning of plateau of maximum contraction, taken as the time to reach 95 per cent of the maximum contraction, (*c*) the maximum amplitude, (*d*) the duration of plateau, that is, the time embraced by the 95 per cent limits on maximum contraction, (*e*) the maximum length, that is the maximum relaxation of the muscle relative to initial length, and (*f*) the zero amplitude. Other metameters included (*g*) the $\log_{10}$ rate of increase of amplitude obtained by dividing the increase of amplitude from the first observable increase to the maximum amplitude by the time during which this change takes place, (*h*) the maximum resting length of the muscle minus initial resting length, (*i*) the initial resting length minus resting length at zero amplitude,

TABLE I

ASSAY OF DIGITALIS LEAF PREPARATIONS ON THE ISOLATED RIGHT VENTRICLE OF THE GUINEA PIG

POTENCY AS A PERCENTAGE OF THE 3RD INTERNATIONAL STANDARD

Sample	*Digitalis lanata*		*Digitalis purpurea*				
	B		C		D		
Assumed potency as percentage of standard	200		33·3		66·7		
Metameter	Mean potency	Limits of error (P = 0·95)	Mean potency	Limits of error (P = 0·95)	Mean potency	Limits of error (P = 0·95)	$\frac{b^2}{s^2 I^2}$
$\log_{10}$ time to/of							
(*a*) First increase in amplitude	222	174–283	33	31–36	67	52–85	40·9
(*b*) Beginning of plateau	227*	—	42†	—	71	61–82	108·4
(*c*) Maximum amplitude	209*	—	41†	—	69	61–78	164·0
(*d*) Duration of plateau	200†	—	42†	—	74	65–85	137·7
(*f*) Zero amplitude	188†	—	40*	—	71	64–80	202·2
(*g*) $\log_{10}$ rate of increase of amplitude (mm./min.)	177	145–216	34	28–41	65	54–80	61·4
(*h*) Maximum resting length—initial resting length, mm.	280*	—	57*	—	82	65–108	44·4
(*i*) Initial resting length—resting length at zero amplitude, mm.	243*	—	55*	—	72†	—	(130·4)
(*k*) Maximum increase in amplitude corrected for initial amplitude	334	106–2351	70	24–671	111	36–784	2·2

* Significant non-parallelism between test and standard ($P \leqslant 0{\cdot}01$).
† Significant non-parallelism between test and standard ($0{\cdot}01 < P \leqslant 0{\cdot}05$).

(*j*) the maximum increase of amplitude corrected for the initial amplitude in the phase of increase of maximum amplitude with increase in dose, and (*k*) the maximum increase of amplitude corrected for the initial amplitude in the phase of decrease of maximum with increase in dose.

The residual error after removal of sums of squares attributal to baths, doses, and the baths × doses interaction was used to calculate fiducial limits of error for each assay. The analyses also included tests for linearity, parallelism and quadratic components and the interactions of each with drugs and baths.

Table I shows the results obtained for each sample of digitalis leaf assayed against the International Standard using the various metameters. The column on the extreme right of the Table gives the index of precision, b^2/s^2I^2, where b = slope in $\log_2$ of assay, s^2 = variance attributable to error, and I is the correction factor for conversion of $\log_2$ to $\log_{10}$ with respect to the coefficients used in the analysis[14].

In the case of the sample of *D. lanata* only three estimates are valid, the remainder being invalid in virtue of statistical non-parallelism of the dose-reponse curves. Nevertheless the estimates of potency derived have been included, which, although strictly invalid, show the reproducibility of some of the estimates, particularly with those metameters dependent on time. A similar finding holds with the sample of *D. purpurea* leaf of low potency.

The estimates for the sample of *D. purpurea* leaf which has a potency closest to that of the Standard preparation are valid, apart from (*i*), the decrease in resting length. The index of precision is highest when time to zero amplitude is used as metameter, and least when the maximum amplitude corrected for the initial amplitude is used. In general the estimates dependent on time measurements are lower than those based upon the actual effects produced but the non-parallelism or low index of precision of the latter make a firm conclusion impossible.

TABLE II

ASSAYS OF THE DIGITALIS LEAF SAMPLES IN TERMS OF THE 3RD STANDARD FOR DIGITALIS BY SLOW INFUSION INTO GUINEA PIGS

Leaf	Sample	Potency as a percentage of the Standard	Limits of error (P = 0·95) as a percentage of the Standard
D. lanata	B	180·1	153·9–210·6
D. purpurea	C	32·4	23·9– 43·8
D. purpurea	D	67·9	64·7– 71·4

Table II gives the results obtained on the same digitalis leaf preparations assayed by slow intravenous injection in guinea pigs according to the method given in the B.P. 1958. The results are in close agreement to those shown in Table I, the best comparison being given by the $\log_{10}$ rate of increase of amplitude in the case of the sample of *D. lanata*, by the $\log_{10}$ time to first increase in amplitude, and $\log_{10}$ rate of increase in amplitude for the sample of *D. purpurea* of low potency, and by all the metameters involving time measurements in the case of the sample of *D. purpurea* of higher potency.

Investigation of the Actions of Three Cardiac Glycosides on the Right Ventricle Preparation of the Guinea Pig

The effects of digoxin on the isolated ventricle preparation were investigated by injecting into a 34-ml. bath all doses from 2·5 to 160 μg. in a geometric ratio of 2·0. Only one dose was allowed to act on each preparation; there were six preparations at each dose level. Using the same range of doses the effects of digitoxin and ouabain were also studied.

Figure 3 shows the maximum amplitude corrected for initial amplitude plotted against $\log_2$ dose for each glycoside. With each glycoside there are two distinct effects, namely a phase where an increase in dose produces an increase in the maximum amplitude, and a later phase where larger doses produce a decrease in maximum amplitude with an increase in dose.

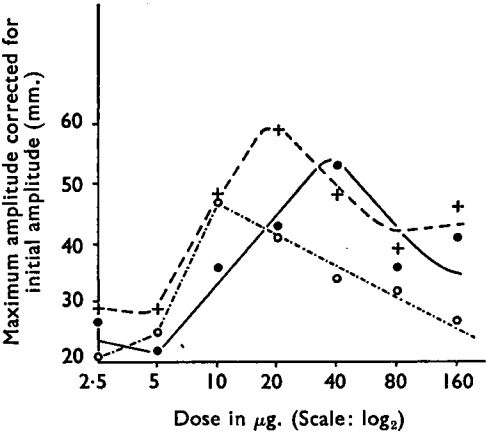

FIG. 3. The influence of the dose of cardiac glycoside on the maximum amplitude of systolic contraction of the electrically stimulated isolated right ventricle of the guinea pig.

●—● Digoxin. ○-·-·-○ Digitoxin.
+ — — + Ouabain.

Figure 4 shows for digoxin the $\log_{10}$ time for various metameters plotted against $\log_2$ dose. Between 2·5 and 20 μg. the time to zero amplitude remains constant, and between 40 and 160 μg. declines linearly with increase of dose. In the case of $\log_{10}$ time to maximum resting length, between 5 and 20 μg. there is a slight but significant decrease in the time taken to reach this point; thereafter, between 20 and 80 μg. there is a linear decline. With the three metameters $\log_{10}$ time to maximum amplitude, to beginning of plateau, and duration of plateau, the time remains constant up to 20 μg. and thereafter decreases linearly parallel to the curves for the other two metameters. In the case of $\log_{10}$ time to first increase in amplitude there is a linear decrease between 2·5 and 40 μg., thereafter it remains constant. The $\log_{10}$ rate of increase in amplitude does not alter significantly between 2·5 and 20 μg., but between 20 and 160 μg. the rate increases linearly.

With digitoxin and ouabain a similar picture obtains to that shown in Figure 3 for digoxin except that the point of inflexion for digitoxin occurs at a dose of 10 μg., and its $\log_{10}$ rate of increase of amplitude is linear between 2·5 and 160 μg. Also with doses above 20 μg. neither digitoxin nor ouabain produce an increase in resting length of the ventricle. Furthermore whilst for digoxin the $\log_{10}$ time to the first increase in amplitude decreases linearly with increase of dose the $\log_{10}$ time to this effect for digitoxin and ouabain decreases in a non-linear manner.

Figure 5*a* shows the changes in resting length of the ventricle plotted against $\log_2$ dose of glycoside. With digoxin between 5 and 80 μg. the increase in resting length varies inversely with increase in dose; the same holds for digitoxin between 2·5 and 40 μg., but for ouabain between 2·5 and 10 μg. the increase in resting length varies proportionally with increase in dose; above 20 μg. ouabain fails to produce an increase in resting length.

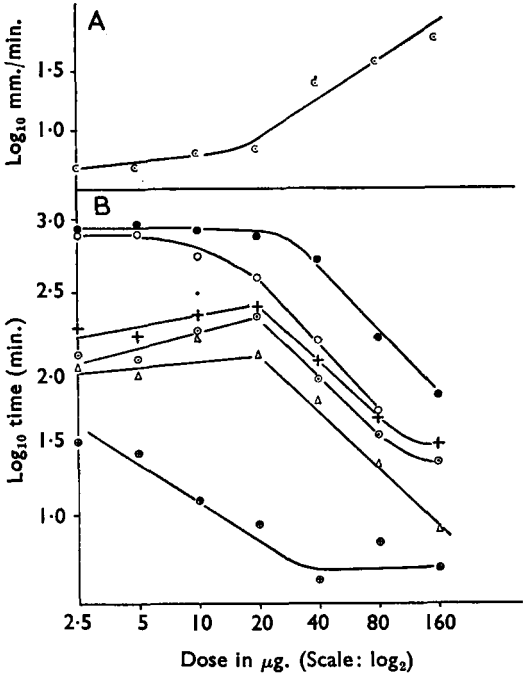

FIG. 4. The influence of the dose of digoxin on the time elapsing before various changes occur in the amplitude of systolic contraction and resting length of the electrically stimulated isolated right ventricle of the guinea pig.
A. Rate of increase of amp. × 100.
B. ●—● Zero amp.
○—○ Maximum resting length.
+—+ Maximum amp.
⊙—⊙ Beginning of plateau.
△—△ Duration of plateau.
⊕—⊕ First increase in amp.

Figure 5*b* shows the decrease in resting length at zero amplitude relative to the resting length initially. With ouabain in doses up to 10 μg., and digoxin and digitoxin up to 20 μg., the action causing an increase in resting length predominates so that, although contracture takes place, the resting length at zero amplitude is greater than that initially. Larger doses induce little or no relaxation of the resting length so that there is an overall shortening of the muscle.

In Table III are shown the potencies of digitoxin and ouabain calculated where possible in terms of digoxin for the various metameters in a manner similar to that described for Table I. Apart from $\log_{10}$ time to maximum resting length all the estimates involving time give greater indexes of precision than those with metameters involving measurement of the effects produced. Once again the index of precision is highest for $\log_{10}$ time to zero amplitude. Using this metameter digitoxin is 2·24 and ouabain 1·11 times as active as digoxin.

In the case of digitoxin, metameters *b*–*f* inclusive yield results which are heterogenous ($\chi^2 = 14{\cdot}222$, d.f. = 4, $P < 0{\cdot}01$), which is shown to be due to metameter *f*, i.e., $\log_{10}$ time to zero amplitude (χ^2(*f* v. *b* to *d*) = 9·307, d.f. = 1, $P < 0{\cdot}01$). The metameters *g*–*j*, based on actual effects, yield results which are heterogenous ($\chi^2 = 8{\cdot}276$, d.f. = 3,

TABLE III

RELATIVE POTENCY OF DIGITOXIN AND OUABAIN IN TERMS OF DIGOXIN ASSAYED ON THE ISOLATED RIGHT VENTRICLE OF THE GUINEA PIG. DIGOXIN = 1

Metameter	Digitoxin†		Ouabain		
	Mean potency	Limits of error (P = 0·95)	Mean potency	Limits of error (P = 0·95)	$\frac{b^2}{s^2 I^2}$
Log_{10} time to/of					
(*a*) First increase in amplitude	C	—	C	—	—
(*b*) Beginning of plateau	1·68	1·35–2·08	1·44	1·16–1·78	58·6
(*c*) Maximum amplitude	1·67	1·35–2·06	1·42	1·16–1·74	61·5
(*d*) Duration of plateau	1·54	1·18–2·00	1·08	0·84–1·39	37·5
(*e*) Maximum length	3·84	2·02–12·62	0·85	0·40–1·56	7·4
(*f*) Zero amplitude	2·24	1·94–2·59	1·11	0·96–1·28	177·1
(*g*) Log_{10} rate of increase of amplitude (mm./min.)	1·24	0·92–1·66	1·52	1·12–2·06	28·8
(*h*) Maximum resting length—initial resting length, mm.	2·30	1·42–3·92	*	—	11·9
(*i*) Initial resting length—resting length at zero amplitude, mm.	2·18	1·46–3·28	1·55	1·01–2·29	16·2
(*j*) Maximum amplitude corrected for initial amplitude (phase of increase/log dose)	1·58	1·07–2·25	1·39	0·97–2·07	19·4
(*k*) Maximum amplitude corrected for initial amplitude (phase of decrease/log dose)	NS	—	NS	—	—

† = Values should be multiplied by a factor of 1·4 for comparison with results on pure digitoxin.
C = Significant curvature of dose response lines.
* = Non-parallelism ($P < 0·001$).
NS = No slope.

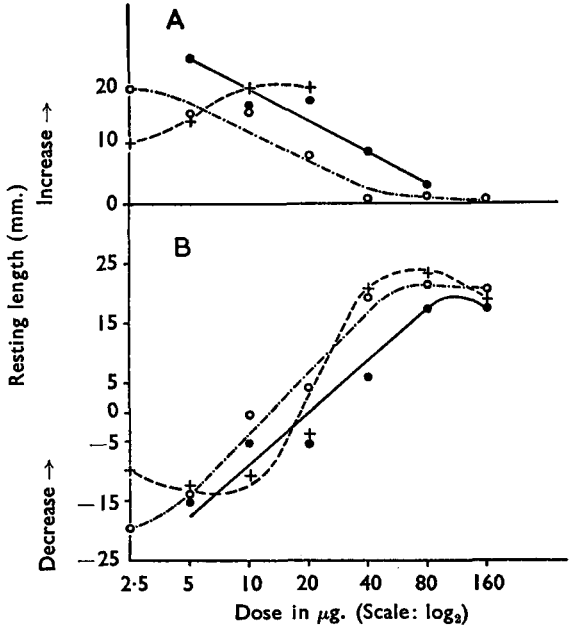

FIG. 5. The influence of the dose of cardiac glycoside on maximum resting length and resting length at zero amplitude of the electrically stimulated isolated right ventricle of the guinea pig. A, increase, B, decrease in resting length.

●—● Digoxin. ○–·–·–○ Digitoxin.
+ – – – + Ouabain.

P <0·05). If, however, the results from metameter *f* are excluded from the rest, the results are homogenous ($\chi^2 = 13{\cdot}233$, d.f. = 7, P >0·05), and the mean potency ratio is 1·66. The results based solely upon time measurements, yield a statistical weight of 2331 for five metameters which is considerably greater than the weight of 673 attributable to the four metameters involving measurements of the effects produced.

In the case of ouabain none of the estimates is significantly different from each other. ($\chi^2 = 12{\cdot}724$ for d.f. = 7, P >0·05). The overall mean potency for ouabain is 1·26 times that of digoxin.

TABLE IV

THE MINIMAL LETHAL DOSE FOR DIGOXIN, DIGITOXIN AND OUABAIN DETERMINED BY SLOW INTRAVENOUS INFUSION INTO GUINEA PIGS

Glycoside	Minimal lethal dose, mg./kg.		Activity ratio	Activity ratio (digitoxin corrected on basis of pure glycoside, 1·4 I.U./mg.)
	Mean	Limits of error (P = 0·95)		
Digoxin	0·79	0·73–0·86	1·00	1·00
Digitoxin	1·54	1·36–1·73	0·51	0·71
Ouabain	0·39	0·36–0·43	2·03	2·03

Table IV gives the potencies relative to digoxin for digitoxin and ouabain as determined by slow infusion into guinea pigs. The result for digitoxin is in exact agreement to that of Brindle, Rigby and Sharma[15] (1·54 mg./kg.) using guinea pigs. Sellwood[16] has observed that samples of digitoxin may vary in potency from 655 to 1401 I.U./g. The potency of the sample used by Brindle, Rigby and Sharma[15] was 930 I.U./g.; that of the digitoxin used in the present study is 1000 I.U./g. which is close to the value of Brindle's sample. Since the sample examined by Sellwood contained 1·4 I.U./mg. the figures quoted for digitoxin should be multiplied by 1·4 so that the results can be compared with those of other workers using the U.S.P. Standard of digitoxin. The corrected ratio of activity for digitoxin to digoxin is that shown in the extreme right-hand column of Table IV. A similar correction should be applied to the results for digitoxin in Table III.

Table V is a compilation of results obtained from the literature of the activities of digitoxin and ouabain calculated in terms of digoxin. There is considerable variation in the activity ratios depending on the animal species and method used in the assessment of cardiac activity. The ratios shown in Table IV are in excellent agreement with those obtained in cats and pigeons[17–19] using the same assay technique. The lower activity ratio of digitoxin in the dog heart lung preparation[20] is the same as the uncorrected ratio shown in Table IV and may reflect the use of a less pure sample of digitoxin. The two results[7,8] obtained on the cat papillary muscle for ouabain are in good agreement with results from slow infusion assays in cats, pigeons and guinea pigs, whilst only one result is in agreement for digitoxin. Cattell and Gold[4] found ouabain and digitoxin to be equiactive on the papillary muscle preparation of the cat. In frogs[17,19] there is considerable variation in the activity ratios depending upon the method of assay. The ratio of activity for digitoxin using changes in

resting length of isolated ventricle strips and papillary muscles of the cat and dog heart[9] agree closely with the estimates given in Table III for the right ventricle of the guinea pig, though the high ratio of 67·0 for ouabain quoted in Table V for potencies based on the decrease of resting length is vastly different from that quoted in Table III.

TABLE V

THE ASSAY OF DIGITOXIN AND OUABAIN IN TERMS OF DIGOXIN BY VARIOUS INVESTIGATORS

Ref. No.	Method	Activity ratio, digoxin = 1·00		
		Digitoxin	Ouabain	
17	Cat infusion—Lethal dose	0·71	1·99	
18	" " —Minimal lethal dose (optimal infusion rate)	0·75	2·00	
19	Pigeon infusion—Lethal dose	0·77	2·22	
20	Infusion—dog heart-lung preparation, minimal dose for lowering right atrial pressure	0·55	2·24	
20	Infusion—dog heart-lung preparation, minimal dose for producing cardiac irregularities	0·52	2·37	
20	Infusion—dog heart-lung preparation, minimal lethal dose	0·59	2·46	
21	Embryonic chick heart, A-V block	—	3·07	
19	Frog heart-abolition of "staircase" effect, minimal effective dose	0·91	0·42	
17	Frog heart, minimal systolic dose	3·13	5·00	
12	Ventricle (turtle heart)—shortening of Q-T interval	1·00	1·00	
4	Papillary muscle (cat heart)—lowest concentration causing 50 per cent of preparations to respond	—	—	$\frac{\text{Digitoxin}}{\text{Ouabain}} = \frac{1}{1}$
7	Papillary muscle (cat heart)—isodynamic substitution	0·92	2·24	
8	" " "	0·79	2·30	
9	Ventricle strips and papillary muscle (cat and dog), lowest concentration producing a decrease in resting length	2·00	67·00	
9	Ventricle strips and papillary muscle (cat and dog), lowest concentration producing an increase	3·00	*	
22	Man—average digitalising dose (oral and I.V.)	2·21	5·36	
24	" " " " therapeutic	1·88	—	
24	" " toxic dose, daily divided dose	1·38	—	
24	" " " " undivided	1·55	—	
25	" " of range of I.V. dose for moderately complete initial digitalisation	0·7	2·19	
30	" usual initial intravenous dose	2·00	2·00	
31	" mean I.V. dose of range stated for initial digitalisation	1·36	4·05	

* Little or no increase in resting length with ouabain.

There is considerable variation for the activity ratios of digitoxin and ouabain in terms of digoxin quoted for man. Lown and Levine[23], and Herrmann[27] state that digoxin is rapidly and fairly completely absorbed from the alimentary tract, while Goodman and Gilman[26] state that 50 per cent or more is absorbed. Even allowing for incomplete absorption of digoxin when given orally digitoxin appears to be more potent in man and not less so as the results from slow intravenous infusion in animals might suggest. The activity ratio of digitoxin in terms of digoxin on the guinea pig right ventricle is therefore in good agreement with that obtained in man; the ratio for ouabain in terms of digoxin is higher in man.

DISCUSSION

The results of the assays of digitalis leaf preparations on the right ventricle of the guinea pig, particularly when obtained from metameters dependent on time, are in close agreement with those obtained by slow intravenous infusion into the same animal. From this it would appear

that the lethal dose determined by infusion is an assessment of the direct action on the heart muscle of the glycosides in the tinctures, or if it is not due solely to this, then any vagal or extracardiac actions must bear the same relationship to each other as the relative activities obtained from a direct action on the muscle.

The analyses of the assays on the ventricle of the crude leaf preparations revealed that the maximum amplitude decreased with increase in dose, and suggests that the doses induced predominantly toxic actions which may have minimised the therapeutic actions. The most precise estimates from the ventricle method were obtained by using $\log_{10}$ time to zero amplitude as metameter.

In contrast the relative potencies of digitoxin and ouabain to that of digoxin were different for the two methods of assay. Primary glycosides are extracted by the method used to prepare the digitalis tinctures, and Brindle, Rigby and Sharma[15] have found purpurea glycoside A to be three times as active as digitoxin, and purpurea glycoside B as active as digitoxin, by slow intravenous infusion into guinea pigs. It may be, therefore, that the other potent glycosides in the tinctures possess the same activity relative to each other by both methods of assay. Digitoxin is absorbed by plasma proteins more readily than the shorter acting glycosides such as digoxin and ouabain, and it may be because of this and its relative absorption by other tissues that it appears in acute tests less active than digoxin or ouabain in the intact animal.

Cattell and Gold[3] expressed the maximum systolic contraction as a percentage of the initial value and failed to find any relationship between the dose and the values so obtained. It is only when the maximum amplitude is corrected by covariance analysis for the initial amplitude and the effects over a wide range of doses are studied, that the biphasic relationship between dose and increase in systolic contraction becomes evident. This biphasic action occurs because with the lowest doses the appearance of the first increase of amplitude appears earlier the larger the dose, while the time to reach the maximum amplitude is constant, whereas with the higher doses the supervention of the toxic action results in an earlier negative inotropic response so that the time to reach maximum amplitude becomes shorter the greater the dose.

When the amplitude of contraction is declining there is a decrease in the resting length of the ventricle which is proportional to the dose. This decrease in resting length is not the cause of the reduction in systolic contraction since in a series of experiments in which the resting length was kept constant by mechanical adjustment the doses of glycoside produced a reduction to zero amplitude at the same rate as in ventricles in which the resting length was allowed to decrease.

It is presumably because of the variability of all these factors that the phase of decline of maximum amplitude is a poor metameter on which to base estimates of potency.

Thus each glycoside is capable, depending on the dose used, of producing different effects which are not directly correlated, though the three glycosides studied appear to have similar actions to each other.

With lower concentrations, the drug may be actively taken up by the muscle and utilised at a rate which reaches the optimum with the dose which produces the greatest increase in amplitude of contraction. With doses greater than the optimum there may be an additional passive diffusion which produces too high a concentration at the site of action which then results in a negative inotropic action. In addition the process controlling resting length must utilise the glycoside differently from that (or those) controlling the systolic contraction since the shape of their dose reponse curves are different.

The possibility that there are two mechanisms controlling systolic contraction should not be ruled out, one controlling increase of contraction dependent on low concentrations of glycoside, the other producing the negative inotropic effect dependent on toxic concentrations of glycosides. The rate of decline of amplitude is always greater than the rate of increase.

The glycosides were able to increase the amplitude of systolic contraction on the unfatigued guinea pig ventricle. A similar finding has been observed with ouabain on isolated strips of ventricle from the same species by Sanyal and Saunders[28], and by Cotten and Stopp[29] on the non-failing heart of the intact dog. Thus it is possible that glycosides act on the same mechanisms in the normal as well as the failing heart.

There is much work in the literature on the effects of glycosides on respiration, organic phosphates containing high energy bonds, carbohydrate metabolism and inorganic ion transport of heart tissue, and the actions of cardiac glycosides must eventually be explained at this level. The different mechanical effects produced on the guinea pig right ventricle show some of the stages at which the biochemical picture should be studied.

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