Brit. J. Pharmacol. (1959), **14**, 174.

THE DURATION OF ACTION OF SOME CARDIAC GLYCOSIDES AND AGLYCONES IN THE GUINEA-PIG

BY

M. RAND* AND ANNE STAFFORD†

From the Department of Pharmacology, University of Sydney, Australia

(RECEIVED JANUARY 5, 1959)

A method is described for determining the duration of action of cardiac glycosides and aglycones in the guinea-pig. It is based on their property of potentiating the cardiac response to adenosine. The method is particularly suitable for those drugs with a short duration of action, whereas previous methods are more suitable for those drugs with longer durations of action. The duration of action of one-fifth of the lethal dose has been found for: digoxigenin, lanatoside C, ouabain, digitoxigenin-3-one, digitoxin, 3-acetyldigitoxigenin, digoxin, digitoxigenin, lanatoside A; these drugs are arranged in order of increasing duration of action. The possible relationship between the elimination of these drugs and their duration of action can provide an estimate of their rates of elimination.

The determination of the elimination of cardiac glycosides by direct measurement of the amount of drug lost from the animal, or the amount remaining after certain periods of time, is not yet feasible. The existing methods are based on the persistence of effect of these drugs. In the method of Hatcher (1912, 1913), a known fraction of the lethal dose of the cardiac glycoside under examination is given and, after appropriate periods of time, the lethal dose of another (standard) cardiac glycoside is determined; the difference between this lethal dose and the usual lethal dose of the standard drug gives the equivalent dose as a fraction of the LD remaining effective in the animal after the elapsed period of time. Another method, introduced by Hauptstein (1927) and modified by Hoffman and Lendle (1951) depends on the differences in lethal doses observed with fast and slow rates of infusion of a cardiac glycoside. With these methods, large numbers of animals are required and estimates of precision are difficult to obtain.

Recently we proposed a method for the determination of the duration of action of cardiac glycosides which allows a value to be obtained from each animal used (Rand and Stafford, 1956). In this paper we describe the method in more detail and give results obtained with the following cardiac glycosides, ouabain, lanatosides A and C, digitoxin, and digoxin; with the aglycones digitoxigenin and digoxigenin, and the semi-synthetic derivatives, 3-acetyldigitoxigenin and digitoxigenin-3-one.

*Present address: Department of Pharmacology, Oxford.
†Present address: Nuffield Institute for Medical Research, Oxford.

METHODS

For the determination of the duration of action of cardiac glycosides, we used their property of potentiating the response to adenosine (Rand, Stafford, and Thorp, 1955). The guinea-pig under urethane anaesthesia (1.75 g./kg.) was given artificial respiration, the chest was opened, and a 1 mm. bore polythene cannula tied into the tip of the left atrium. Injections of adenosine were given through this cannula at 2 min. intervals. Adenosine (1 mg./ml.) was dissolved in 0.9% NaCl and 0.01 to 0.05 ml. was injected each time, either with an "Agla" micrometer syringe, or with a Palmer motor-driven syringe which delivered 0.5 ml./min. driven for 1 to 6 sec. With this latter device, pulses of the required duration from a photo-timer could be applied to the motor at 2 min. intervals. In either case, the dose of adenosine (10 to 50 μg.) was adjusted to produce heart block lasting for 3 to 5 sec. This response was measured from the electrocardiogram recorded with a Kelvin-Hughes ink writer. A number of injections of adenosine (6 to 10) were given to determine the mean initial response and its 95% confidence limits. The cardiac glycoside was then injected into a jugular vein and the regular injections of adenosine

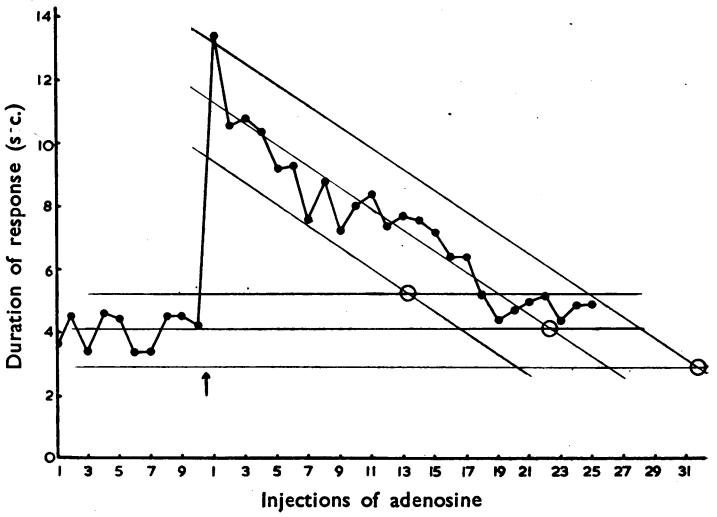

FIG. 1.—The ordinate gives the time in sec. during which heart block occurred in response to injections of adenosine (30 μg.) given at 2 min. intervals in the guinea-pig. There was a temporary potentiation of the response to adenosine after the injection of 1.28 mg./kg. of digitoxigenin-3-one at the arrow. The light horizontal lines are the mean and 95% confidence limits of the initial responses to adenosine. The light sloping lines are the regression and its 95% limits expressing the decrease in the potentiated response to adenosine with time.

continued. The response to adenosine was potentiated by the cardiac glycoside. The time taken until the potentiated responses to adenosine returned to their initial value has been taken as the duration of action of the dose of cardiac glycoside which had been given.

RESULTS

Constancy of the Response to Adenosine.—This method of determining the duration of action of cardiac glycosides depended upon the assumption that the guinea-pig would respond to successive injections of adenosine with constant periods of heart block for the duration of the experiment, that is, for 1 to 2 hr. The responses to injections of adenosine, given at 2 min. intervals, remained constant for 3 hr. in 5 experiments and for 6 hr. in one experiment. The constancy of the response was judged by the fact that the expected number of the responses were contained within the 95% confidence limits of the mean of the initial 10 responses.

The Determination of the Duration of Action.—The effect of a single dose of digitoxigenin-3-one on the responses to adenosine is shown in Fig. 1. In this experiment, the mean initial response was determined from 10 successive injections of adenosine. The mean time during which heart block occurred was 4 sec. with a standard deviation of 0.52 sec. The mean and the 95% confidence limits enclosing the results ($\pm$1.18 sec.) are shown as 3 horizontal lines.

Immediately following the injection of 1.28 mg./kg. of digitoxigenin-3-one, the response to adenosine was increased from a mean duration of 4 sec. up to 13.4 sec. The responses to successive doses of adenosine then decreased until (after 44 min.) they had the same duration as initially. To determine the point at which the potentiating action of digitoxigenin-3-one had worn off, we used the following procedure. The decay of the responses to adenosine was calculated as the regression of the response (duration) on time (in 2 min. intervals equal in number to the number of injections). This calculation was made beginning at the first response to adenosine showing the maximum potentiation up to the first response to adenosine which fell inside the 95% confidence limits of the mean initial values. In Fig. 1 the regression line for the decay of the responses to adenosine ($b = -0.332$) is drawn on the graph together with the lines representing the 95% confidence limits of the scatter about the regression line. The point chosen as the best estimate of the return of the potentiated responses to adenosine to their initial value is taken as the point of intersection of the extension of the mean initial responses with the extension of the regression line of decay. The greatest limits of this estimate are the extreme points of intersection of the lines of the 95% confidence limits. In Fig. 1, these points are represented by the open circles. In this experiment the effect of 1.28 mg./kg. of digitoxigenin-3-one persisted for 44 min. with a possible variation of 26 to 62 min. based on the extreme intersections of the lines enclosing the 95% confidence limits. The procedure described above has been carried out to provide an objective basis for judging the point of return

TABLE I

LETHAL DOSE OF SOME CARDIAC GLYCOSIDES AND AGLYCONES IN THE GUINEA-PIG

Drug	No. of Animals	Rate of Infusion (μg./kg./min.)	Lethal Dose (mg./kg.)	Standard Error
Lanatoside C ..	6	23– 41	0·679	0·038
Digoxin	7	21– 50	0·588	0·059
Digoxigenin ..	6	200– 300	6·80	0·83
Lanatoside A ..	5	62– 88	1·04	0·04
Digitoxin	6	50– 60	1·22	0·18
Digitoxigenin ..	7	150– 390	2·65	0·21
3-Acetyldigitoxigenin	7	300– 750	6·12	0·61
Digitoxigenin-3-one	4	580–1,100	6·41	0·70
Ouabain	6	11– 25	0·232	0·011

of the potentiated responses to their initial value and also to give an estimate of the precision of this point.

Lethal Doses of the Compounds Studied.—We have shown previously that the persistence of action of digoxigenin in the guinea-pig is proportional to the dose injected (Rand and Stafford, 1959). Therefore it is necessary to compare the durations of action of different cardiac glycosides and aglycones on some equitable basis. We have chosen to use one-fifth of the lethal dose of each compound. The lethal doses were determined in the guinea-pig under urethane anaesthesia with artificial respiration. A continuous infusion of the drug was given into a jugular vein until cardiac arrest, which was estimated from the electrocardiogram. The results are given in Table I.

Duration of Action.—The durations of action of one-fifth of the lethal doses of the cardiac glycosides and aglycones are shown in Table II. In each experiment, the procedure described above for digitoxigenin-3-one was carried out. In some experiments, the potentiated responses to adenosine did not decrease with time, or did so only slowly. In these, the regression line for decay was not significant and the result has been entered in Table II as greater than 2 hr. In all other experiments, the regression line for decay was highly significant.

DISCUSSION

Many previous workers have considered that the persistence of effect or duration of action of a cardiac glycoside-like drug is related to its rate of elimination from the animal (Lendle, 1936; Östling, 1947; Giertz, Hahn, and Schunk, 1954). If this is correct it is possible to present the results obtained on duration of action as rates of elimination of the various compounds studied as shown in Table III. This method of expressing

TABLE II

DURATION OF ACTION OF ONE-FIFTH OF THE LETHAL DOSE OF SOME CARDIAC GLYCOSIDES AND AGLYCONES

Drug	Dose (μg./kg.)	Duration of Action (min.)	95% Confidence Limits (min.)
Ouabain	46	42	39– 52
		40	32– 47
		37	15– 59
		37	20– 54
		27	17– 36
Lanatoside C	136	37	26– 48
		34	28– 40
		27	12– 42
		24	13– 34
		22	15– 28
Digoxin	118	>120	
		>120	
		>120	
		119	77–160
Digoxigenin	1,360	40	16– 47
		37	17– 63
		31	19– 45
		26	16– 36
		22	13– 28
		13	11– 16
Lanatoside A	208	>120	
		>120	
		>120	
		>120	
		>120	
		>120	
Digitoxin	244	>120	
		>120	
		113	50–126
Digitoxigenin	530	>120	
		>120	
		>120	
		>120	
		>120	
		>120	
		79	50–107
3-Acetyldigitoxigenin ..	1,224	>120	
		>120	
		140	57–214
		285	148–430
Digitoxigenin-3-one ..	1,282	84	34–135
		60	24– 94
		44	26– 62
		33	19– 46
		24	15– 34

TABLE III

MEAN RATES OF ELIMINATION OF SOME CARDIAC GLYCOSIDES AND AGLYCONES

Drug	No. of Expt.	Rate of Elimination		
		μg./kg./hr.	% LD/hr.	95% Limits
Ouabain	5	78·4	33·8	25·0–56·9
Lanatoside C ..	5	295	43·6	32·3–71·7
Digoxin	4	17·3	2·5	1·9– 3·9
Digoxigenin ..	6	3,387	43·9	36·7–79·0
Lanatoside A ..	6	0	0	
Digitoxin	3	55·7	4·5	3·2– 8·1
Digitoxigenin ..	7	57·7	2·2	1·6– 3·4
3-Acetyldigitoxigenin	4	197	3·2	2·1– 7·3
Digitoxigenin-3-one	5	1,909	29·7	20·4–54·5

the results allows comparisons to be made with other results recorded in the literature on the rates of elimination of these drugs in which the actual measurement made was on persistence of the effect.

The average rate of elimination of ouabain was found to be 34% of the lethal dose/hr., or 78.4 μg./kg./hr. The rates of elimination of ouabain given by Hoffman and Lendle (1951) were 68 to 180 μg./kg./hr., and their mean value was 35% LD/hr. Calculations made from the results of Herre (1937) gave a rate of elimination of ouabain of 92 μg./kg./hr., which was 30% of the lethal dose/hr. as measured by the same author.

Herre (1937) showed that digitoxin was not eliminated by the guinea-pig, though more recently Hoffman and Lendle (1951) found that digitoxin was eliminated by the guinea-pig at a rate of 20 to 29% of the lethal dose/hr. We have found that digitoxin was only eliminated in 1 out of 3 experiments, at a rate of 13.6% of the lethal dose/hr.

Hoffman and Lendle (1951), who used the method of Hauptstein (1927), estimated that the rate of elimination of digitoxigenin in the guinea-pig is 1.0 to 1.5 mg./kg./hr. However, we were unable to demonstrate any appreciable elimination of digitoxigenin by the guinea-pig, the response to adenosine remaining potentiated for at least 2 hr. following the injection of digitoxigenin in 6 out of 7 animals tested. When we determined the toxicity of digitoxigenin in the guinea-pig, we found that, unless it was infused rapidly (>150 μg./kg./min.) so that cardiac arrest was produced within approximately 15 min., it was impossible to attain a lethal dose. With slower rates of infusion an ectopic ventricular rhythm was produced which persisted until enormous doses of digitoxigenin had been injected (up to 20 mg./kg.) and the experiments were frequently terminated without death of the animal. These observations suggest that digitoxigenin is eliminated very rapidly in the guinea-pig. In order to explain the discrepancy between our results using the effect of digitoxigenin on the response to adenosine and the results of Hoffman and Lendle (1951) which are in accord with our observations made during the determination of the lethal dose of digitoxigenin, we offer the following explanation. The slope of the line relating dose to rate of elimination may be considerably steeper for digitoxigenin than was shown for digoxigenin (Rand and Stafford, 1959). This would mean that although no appreciable elimination of digitoxigenin could be shown with the doses which we used (20% of LD), with the higher concentrations of digitoxigenin which would be present if rate of elimination were measured by the method of Hauptstein (1927), a more rapid rate of elimination would be observed. We have not yet, however, tested this possibility experimentally. Another possibility is that the direct toxic effect of digitoxigenin itself on the heart which Hoffman and Lendle (1951) measured is transient, whereas the indirectly observed action of potentiating the response to adenosine is longer lasting.

Our results give no information about the location of the cardiac glycosides or aglycones or their cardio-active metabolites which are potentiating the responses to adenosine. It is unlikely that potentiation of the response to adenosine would be proportional to the concentration of cardiac glycoside in the blood, as there are observations in many species which indicate that cardiac glycosides are removed from the blood within a few minutes after injection (Hatcher and Eggleston, 1919; Hanzlik and Wood, 1929; and others mentioned by Okita, Talso, Curry, Smith, and Geiling, 1955). It is thought that this is due to their being taken up by various body tissues, particularly by the liver. The potientiated response of the heart to adenosine may reflect the concentration of drug in the heart muscle.

There has been much work concerning the "binding" of cardiac glycosides to the heart muscle of various species. Recent experiments with radioactive drugs indicate that the heart takes up cardiac glycosides, although to a lesser extent/g. of tissue than many other organs (Okita *et al.*, 1955). It may be that only the fraction of the drug actually fixed in the heart muscle is responsible for the potentiation of the response of the heart to adenosine, in which case the concept of elimination of the drug as an explanation for the decrease in effect with time after injection has the more limited sense of elimination from the heart.

The unexpected resistance of digitoxigenin to elimination has already been considered. Acetylation of the 3-OH group of digitoxigenin reduces its toxicity by 60%, but acetyldigitoxigenin is only eliminated slightly more rapidly than digitoxigenin. However, digitoxigenin-3-one, with approximately the same cardiac activity as 3-acetyldigitoxigenin, acts much more briefly. This would suggest that the 3-OH group is a point of attachment to receptor sites for pharmacological activity, and that either acetylation or oxidation of the 3-OH group weakens this

attachment, which is reflected in the lower toxicity of these compounds, and places them in a position where they are more likely to be lost from the heart. However, acetylation of the 3-OH only slightly increases the rate of elimination, whereas oxidation greatly facilitates it. This may be because oxidation of the 3-OH is one of the stages in the detoxication of the aglycones.

This work was done by M. R. as the Drug Houses of Australia Research Fellow in Experimental Pharmacology and by A. S. as Burroughs Wellcome & Co. (Aust.) Ltd. Research Fellow. We wish to thank Professor Wright and his colleagues of the Department of Pharmacy, University of Sydney, for giving us chromatographically pure samples of 3-acetyldigitoxigenin and digitoxigenin-3-one, and Professor R. H. Thorp for his encouragement and interest in this work.

References

Giertz, H., Hahn, F., and Schunk, R. (1954). *Arch. exp. Path. Pharmak.*, **221**, 34.
Hanzlik, P. J., and Wood, D. A. (1929). *J. Pharmacol. exp. Ther.*, **37**, 67.
Hatcher, R. A. (1912). *Arch. intern. Med.*, **10**, 268.
—— (1913). *J. Amer. med. Ass.*, **61**, 386.
—— and Eggleston, C. (1919). *J. Pharmacol. exp. Ther.*, **12**, 405.
Hauptstein, P. (1927). *Arch. exp. Path. Pharmak.*, **126**, 121.
Herre, E. (1937). Ibid., **184**, 716.
Hoffman, G., and Lendle, L. (1951). Ibid., **212**, 376.
Lendle, L. (1936). Ibid., **180**, 518.
Okita, G. T., Talso, P. J., Curry, J. H., Smith, F. D., and Geiling, E. M. K. (1955). *J. Pharmacol. exp. Ther.*, **115**, 371.
Östling, G. (1947). *Acta pharmacol. (Kbh.)*, **3**, 275.
Rand, M., and Stafford, A. (1956). *Nature (Lond.)*, **177**, 278.
—— —— (1959). Ibid., **183**, 688.
—— —— and Thorp, R. H. (1955). *J. Pharmacol. exp. Ther.*, **114**, 119.