THE DETERMINATION OF THE MINIMAL LETHAL DOSE AND THE AVERAGE RATE OF UPTAKE OF G-STROPHANTHIN AND DIGITOXIN IN THE HEART-LUNG PREPARATION OF THE DOG

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Several attempts to determine the minimal lethal dose of certain cardiac glycosides for the cat heart-lung preparation have been made (1, 2, 3, 4); the average value of the minimal lethal dose (M.L.D.) of g-strophanthin, as determined by these investigators, varied between 1.5 and 2.6 micrograms per gram of cat heart. In all of these investigations the duration of the experiment did not exceed 50 minutes. Rothlin (3) has shown that when the experimental time was 30–40 minutes, the lethal dose (L.D.) of digitoxin was 10.5 micrograms, while an extension of this time to 80–90 minutes reduced the L.D. to 6.9 micrograms of digitoxin per gram of cat heart. In the intact animal a reduction of the rate of inflow of digitalis glycosides produced a prolongation of the experimental time and a reduction in the L.D. (5, 6). It is clear that the L.D. of cardioactive glycosides determined during short experimental periods or with high rates of administration cannot be the true M.L.D. So far as we know, no attempt has been made to establish the exact experimental conditions under which the true M.L.D. of cardiac glycosides may be determined on the heart-lung preparation (H.L.P.)

To determine the M.L.D. of a cardiac glycoside in the intact animal, two methods have been applied. The method used by Fromherz and Welsch (7) frequently needed observation periods exceeding 24 hours and was considered unsuitable for our purposes, since, even under the most favorable conditions, the life of the H.L.P. does not exceed 8–9 hours. Lendle (4), Mehnert (5) and Herre (8) used a method first introduced by Hauptstein (9). The characteristic feature of this method is the slow continuous infusion of the cardiac glycoside by means of a constant infusion pump. It was observed that when rates of administration were rapid, death would occur quickly and the L.D. would be high. When the rates of infusion were very slow, the L.D. would reach a value whereafter any further decrease of the rate of administration would merely lengthen the experimental time without any further lowering of the L.D. This value of the L.D. was considered to be the M.L.D. and the values obtained by this constant infusion method agree fairly well with those determined by the "timeless" method of Fromherz and Welsch (5). We have used the constant infusion method, since the M.L.D. could be determined within the period of viability of the dog H.L.P.; also, with this method, any destruction of cardioactive glycosides by the H.L.P. would be revealed by a gradual rise in the M.L.D. when the rate of administration of the drug was very slow (Hauptstein (9)).

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Weese (1) has shown that in the H.L.P. of the cat, the heart will extract from the blood any quantity of g-strophanthin less than one M.L.D., but no more than one M.L.D. will be extracted regardless of the excess amount of glycoside in the blood. On the other hand, Genuit (10) and Genuit and Eschbach (11) have shown that rat and guinea pig hearts perfused with Ringer solution do not extract strophanthin quantitatively from the perfusion fluid. As an essential preliminary control we performed a number of experiments with the dog H.L.P.[2] and were able to show that the dog heart reacts in a manner similar to that of the cat in the quantitative uptake of glycoside. Since, regardless of the concentration, the dog H.L.P. binds any amount of cardiac glycoside not exceeding the M.L.D. it is possible to determine the *average rate of uptake* per gram heart per minute by dividing the M.L.D. by the experimental time (Herre (8)). By this procedure the specific affinity of cardiac glycosides for the heart can be accurately studied, since all other organs capable of absorbing the drug have been excluded (1).

In this paper we describe the influence of the rate of administration of g-strophanthin and digitoxin on the lethal dose, experimental time, destruction and rate of uptake in the H.L.P.

Materials and methods. The cardiac glycosides employed were crystalline g-strophanthin Thoms (Merck) and crystalline digitoxin (Hoffmann La Roche[3]). According to the manufacturers, g-strophanthin Thoms contains 20 per cent water of crystallization (12). All values given in this paper have been calculated on the basis of anhydrous g-strophanthin. Stock solutions containing 0.5 per cent g-strophanthin or digitoxin in 95 per cent ethyl alcohol were diluted in 0.9 per cent saline. The dilutions in saline were varied from 1:5000 to 1:1,000,000 for g-strophanthin, and from 1:25,000 to 1:200,000, for digitoxin.

All constant infusions were made with a small Marriotte tube into the venous end of the heart-lung circuit and the rate of administration was regulated at the beginning of each experiment by means of a small screw clamp. All though the experiment great care was taken to keep the rate of inflow constant and, to this end, the number of drops of fluid infused per unit of time was frequently checked. The simplicity of this method of infusion does not detract from the accuracy of the results obtained, as a later series of experiments with a more elaborate method of constant infusion did not show any significant differences from the results described below.

The H.L.P. was prepared by the technic of Knowlton and Starling (13), using dogs weighing between 2.15 and 11.6 kg. Anesthesia was induced with ether and followed by 90 mg. per kg. of chloralose given intravenously. In each of the experiments described, all controllable factors were as follows: blood temperature, 38.9–39.2°C.; blood volume, 850–900 cc.; resistance, 75 mm. of mercury; output of the left heart, 400–450 cc. per minute; and the work of the left heart, 0.65–0.72 kg.-m. per minute. The heart rate was not controllable, but varied between 138–192 beats per minute. Right venous pressure varied between 2 and 5 cm. of water. All preparations showing irregular rhythms, early insufficiency, or severe edema of the lungs were discarded. The end point of the experiment was the appearance of ventricular fibrillation, and the infusion of the drug was continued until such fibrillation began.

In experiments of long duration, we occasionally observed that during the period of complete block the ventricles would beat at a rate of 5–10 beats per minute or would stop completely. This stoppage was clearly *not* the end point since mechanical stimulation would

[2] Dogs were employed for our experiments because they were more easily obtained than cats.

[3] Kindly supplied by Hoffmann La Roche, Basel, Switzerland.

keep the ventricles beating until idioventricular rhythm set in, which in turn was followed by the characteristic fibrillation. Since mechanical stimulation was not very satisfactory, we employed 20 microgram doses of adrenaline to overcome this period of stoppage; in most instances only two and rarely three such doses were necessary.

In a series of experiments to be published later it will be shown that such amounts of adrenaline have no effect on either the M.L.D. or the average rate of uptake of g-strophanthin in the H.L.P. of the dog. Furthermore, from the data in table 1, it can be seen that adrenaline did not effect the M.L.D. of g-strophanthin, since in the experiments where adrenaline had to be used (exp. No. 18, 21, 23, 25, 26) the M.L.D. was 1.214 micrograms while in those experiments where adrenaline was not used (exp. No. 19, 20, 22, 24) the M.L.D. was 1.173 micrograms g-strophanthin per gram of heart.

When the experimental period exceeded 150 minutes, many of the hearts showed a marked infiltration of blood into the ventricular muscle, which was most apparent in the left ventricle and the apex of the heart. As a result of this infiltration, the heart weight was disproportionately high, and was corrected by approximating the true weight from the normal ratio of heart weight to body weight. In 38 dogs this ratio averaged 0.84 with a minimum and maximum of 0.58 and 1.12 respectively. Thus multiplying the body weight in kilograms by the factor 8.4 gave the approximate normal heart weight in grams.

Wood and Moe (14) have shown that in the H.L.P. edema of the heart seriously effects the determination of the heart weight. Furthermore they have shown that no accurate predicition of heart weight can be made from any formulae relating heart weight to body weight. According to Wood and Moe the only reliable method for determining heart weight in these preparations is by chloride space determinations, a method which unfortunately could not be used in the present study.

In four control experiments the heart was allowed to beat for 4–6 hours under the conditions described above, but no drug was infused. In none of the four experiments was there any gross infiltration or thickening of the ventricular muscle, although small hemorrhagic spots were observed in the region of the papillary muscles. These lesions were much less severe than those observed in hearts poisoned with cardioactive glycosides, and where the experimental time was of similar duration. In hearts where cardioactive glycosides were infused, and the experimental time was short, no such infiltration could be observed and we conclude that this infiltrative process is caused by the cardioactive glycosides acting over a long period of time. The question whether this infiltrative process has any effect on the M.L.D. cannot be answered definitely, but in the case of g-strophanthin, hearts showing an infiltration had a M.L.D. of 1.184 micrograms while in those experiments where no gross infiltration was apparent the M.L.D. was 1.201 micrograms per gram of heart, a difference which is less than the experimental error.

In some of the longer experiments there was a loss of circulating blood due to the development of edema of the lungs, but by using undiluted defibrinated blood only, and by keeping the inflation of the lungs at the smallest volume still compatible with a proper oxygenation of the blood, this edema was minimized. All experiments where the fluid loss exceeded 20 per cent of the original blood volume were discarded, excepting experiment 24 in table 2 where a fluid loss of about 35 per cent had to be recorded. This leakage would necessarily affect the determination of the M.L.D., but the error is probably not very great, since in most experiments the fluid loss was less then 15 per cent, and usually occurred only during the last 20 or 30 minutes of the experiment.

RESULTS. The influence of the rate of administration of g-strophanthin and digitoxin on the L.D. has been studied on the dog H.L.P. A total of 32 experiments were performed with g-strophanthin, of which 26 were considered successful, i.e., less than 20 per cent fluid loss. Twenty four out of 31 experiments were considered satisfactory with digitoxin. The results and data of these experiments are shown in tables 1 and 2.

The relationship between rate of administration and L.D. of g-strophanthin and digitoxin has been graphically represented in figure 1. With the higher rates of administration employed, the L.D. is about ten times the M.L.D. Since, in the heart-lung system, the heart is the only organ which can bind cardioactive

TABLE 1

The effect of the rate of administration of g-strophanthin on the lethal dose, rate of uptake and experimental time in the dog heart-lung preparation

EXP. NO.	WEIGHT OF DOG	TEMPERATURE	HEART WEIGHT IN GRAMS		RATE OF ADMINISTRATION, MICROGRAM PER GRAM HEART, PER MINUTE	LETHAL DOSE, MICROGRAM PER GRAM HEART	EXPERIMENTAL TIME	RATE OF UPTAKE, MICROGRAM PER GRAM HEART, PER MIN.	RATE OF UPTAKE, PER CENT OF MINIMAL LETHAL DOSE	REMARKS
			Uncorrected	Corrected						
	kilograms	°C.					*minutes*			
1	7.45	39	60.5		0.830	14.93	18	0.066	5.55	
2	8.20	39.1	75.5		0.713	9.97	14	0.085	7.14	
3	11.60	39	68		0.619	10.23	16	0.074	6.25	
4	5.6	39	43.5		0.592	10.5	18	0.066	5.55	
5	5.85	39.2	62.5		0.445	6.12	14	0.085	7.14	
6	5.50	39	47.5		0.433	9.1	21	0.056	4.76	
7	9.41	39	66.5		0.337	8.1	24	0.048	4.17	
8	5.51	39.1	41		0.219	6.14	28	0.042	3.57	
9	4.70	39.2	41		0.141	5.62	40	0.030	2.5	
10	5.95	39	56		0.118	4.94	42	0.028	2.37	
11	6.25	39	43.5		0.0972	4.38	45	0.027	2.22	Fluid loss about 50 cc.
12	6.60	38.8	59		0.065	3.57	55	0.022	1.81	
13	7.20	39.2	67.5		0.048	2.89	60	0.0195	1.66	
14	6.15	38.8	53		0.0311	2.23	72	0.017	1.39	Fluid loss about 50 cc.
15	5.00	39	58.5		0.028	1.87	67	0.018	1.49	
16	5.22	39	61	44	0.028	1.99	72	0.017	1.39	
17	5.20	39.2	55		0.0194	1.9	98	0.012	1.02	
18	3.95	39.1	34.5		0.010	1.39	139	0.0085	0.705	Fluid loss about 50 cc. adrenaline 1. × 30 micrograms
19	6.30	39.2	70	53	0.0098	1.23	126	0.0097	0.79	Fluid loss about 50 cc.
20	5.30	39	50		0.0095	1.011	107	0.0111	0.93	
21	5.45	39	46.5		0.0082	1.068	129	0.0091	0.77	Adrenaline 1 × 20 micrograms
22	3.22	39.1	28.5		0.0076	1.334	174	0.0068	0.57	Fluid loss 80–100 cc.
23	7.4	39.2	107	62	0.0069	1.501	213	0.0055	0.47	Adrenaline 2 × 20 micrograms. Fluid loss 100 cc.
24	10.9	39.1	148	92	0.00667	0.901	235	0.0051	0.42	Fluid loss 100 cc.
25	8.3	39	95	70	0.00612	1.134	195	0.0062	0.51	Adrenaline 2 × 20 micrograms. Fluid loss 150 cc.
26	7.01	39.1	91	59	0.0041	1.154	280	0.0042	0.36	Adrenaline 2 × 20 micrograms. Fluid loss ab. 200 cc.

glycosides (1), these high lethal doses are due to the inability of the heart to bind all the drug infused. A proof that the excess glycoside is still in the blood is found in experiment 2 of table 3. Here an overtitration resulted in a determination of a L.D. of 3.44 micrograms per gram heart. The blood from this preparation, when added to a second heart, contained enough glycoside to kill the second preparation within a period of 64 minutes. On the other hand, the blood from a preparation where no overtitration had taken place did not poison the

TABLE 2

The effect of the rate of administration of digitoxin on the lethal dose, rate of uptake and experimental time in the dog heart-lung preparation

EXP. NO.	WEIGHT OF DOG	BLOOD TEMPERATURE	HEART WEIGHT IN GRAMS		RATE OF ADMINISTRATION, MICROGRAM PER GRAM HEART, PER MINUTE	LETHAL DOSE, MICROGRAM PER GRAM HEART	EXPERIMENTAL TIME	RATE OF UPTAKE, MICROGRAM PER GRAM HEART, PER MINUTE	RATE OF UPTAKE PER CENT OF MINIMAL LETHAL DOSE	REMARKS
			Uncorrected	Corrected						
	kg.	°C.					*minutes*			
1	6.1	39	55		3.703	100.1	27	0.219	3.71	
2	4.9	39	41		0.721	44.7	62	0.0953	1.61	
3	6.0	39	54		0.551	37.4	68	0.087	1.47	
4	2.45	39	27		0.54	41.06	76	0.0777	1.30	Young animal
5	7.12	39.1	51.2		0.504	33.77	67	0.0882	1.49	
6	5.3	39	49.7		0.472	32.56	69	0.0856	1.45	
7	3.42	38.8	39		0.458	35.64	78	0.0758	1.28	Young animal
8	2.15	39	30		0.443	38.08	86	0.068	1.16	Young animal
9	4.42	39	32.5		0.413	33.16	81	0.0729	1.23	
10	5.7	39	47.2		0.318	26.88	83	0.0712	1.20	Slight fluid loss
11	5.1	39.1	46		0.294	23.85	81	0.0729	1.23	
12	4.52	39.1	48.5		0.276	22.68	82	0.0723	1.22	
13	7.12	39	51.2		0.247	19.12	78	0.0758	1.28	Fluid loss 50 cc.
14	5.50	39.2	42.5		0.166	17.9	108	0.0549	0.92	
15	6.49	39	57.7		0.132	14.38	109	0.0542	0.92	
16	7.60	39	69.0		0.0798	10.37	131	0.0451	0.76	Fluid loss 100 cc.
17	7.12	39	66		0.0761	9.06	119	0.0488	0.84	
18	7.30	39	65.5		0.0521	8.86	170	0.0348	0.59	Fluid loss 50 cc.
19	7.93	39	87	67	0.040	6.53	163	0.0356	0.61	Adrenaline 1 × 20 micrograms
20	5.03	39.1	71		0.0338	6.19	183	0.0332	0.56	Fluid loss 100 cc.
21	5.6	39	75.5	47	0.0265	6.28	237	0.0249	0.42	Fluid loss 150 cc.
22	5.9	39	35		0.0236	5.43	230	0.0257	0.43	Fluid loss 150 cc. Adrenaline 2 × 20 micrograms
23	7.4	39	74	62	0.019	5.44	286	0.0206	0.35	Fluid loss 200 cc. Adrenaline 2 × 20 micrograms
24	5.95	39.1	84	50	0.0152	5.75	375	0.016	0.26	Fluid loss 250–300 cc. Adrenaline 3 × 10 micrograms

heart when the blood was transferred to the second H.L.P. (Experiment 1, table 3).

When the rate of administration is slow, both g-strophanthin and digitoxin showed a flattening of their respective curves; further reduction of the rate of administration did not reduce the L.D. On the basis of these experiments we would designate as *optimal rate of administration* (O.R.A.), that rate below which further reduction fails to lower the L.D. This O.R.A. has a characteristic value for each of the two glycosides studied, and under our experimental conditions is about 0.012 microgram and 0.028 microgram per gram heart per minute for g-strophanthin and digitoxin respectively. The optimal rate of administration should be differentiated from the term *critical rate of administration* (Kritische Einlaufgeschwindigkeit) of Heubner and Nyary (15), since the latter denotes the rate of administration at which elimination and rate of administration are equal.

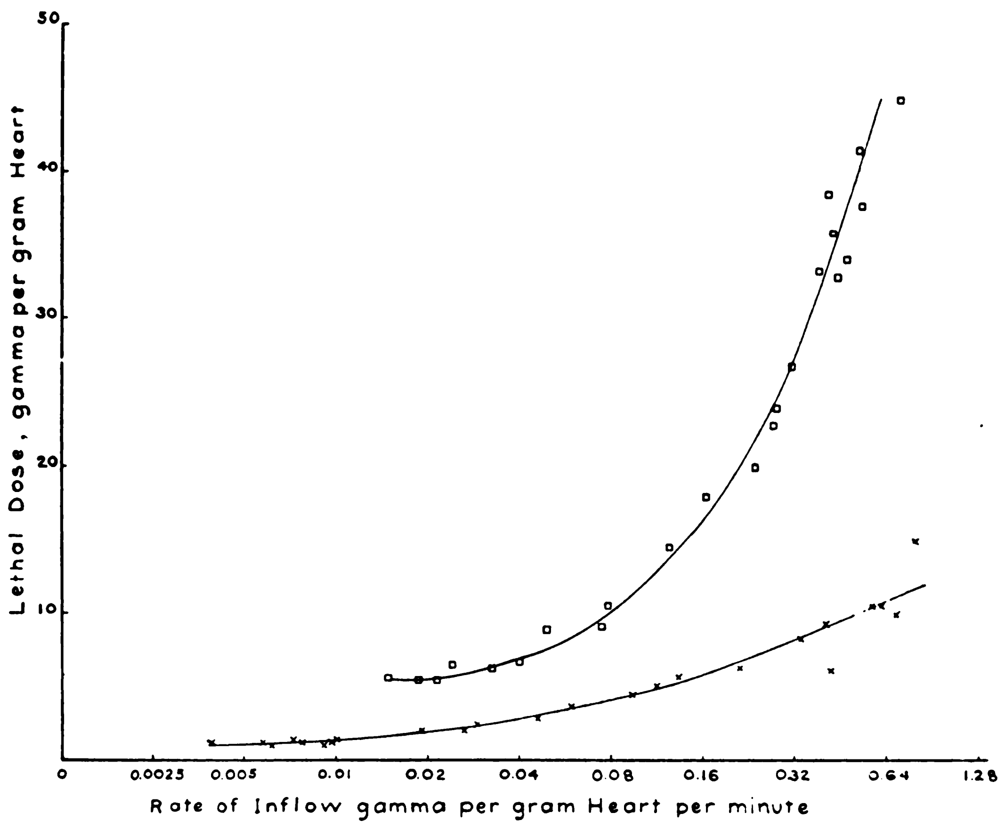

FIG. 1. DOG HEART-LUNG PREPARATION

The relation of rate of inflow of g-strophanthin and digitoxin to the lethal dose. (Gamma = microgram)

□————□ Digitoxin ×————× g-strophanthin

A large number of the experiments in which we tried to determine the M.L.D. of g-strophanthin and digitoxin had to be discarded, because prolonged experimental time produced edema of the lungs which could neither be prevented nor controlled. In 12 experiments with g-strophanthin, 8 were successful, while with digitoxin only 4 out of 9 experiments could be considered satisfactory. From these successful experiments we determined an average M.L.D. of 1.184 micrograms of g-strophanthin and 5.95 micrograms of digitoxin per gram of dog heart. These minimal doses are based on the correction of the heart weight described under methods.

Rothlin (3) has shown that a lengthening of the experimental time lowers the L.D. of digitoxin for the H.L.P. of the cat. Our results confirm and extend this finding for g-strophanthin and digitoxin for the dog H.L.P. (fig. 2). However, the lowering of the L.D. continued until an experimental time was reached, beyond which any further extension did not change the L.D. Under our experi-

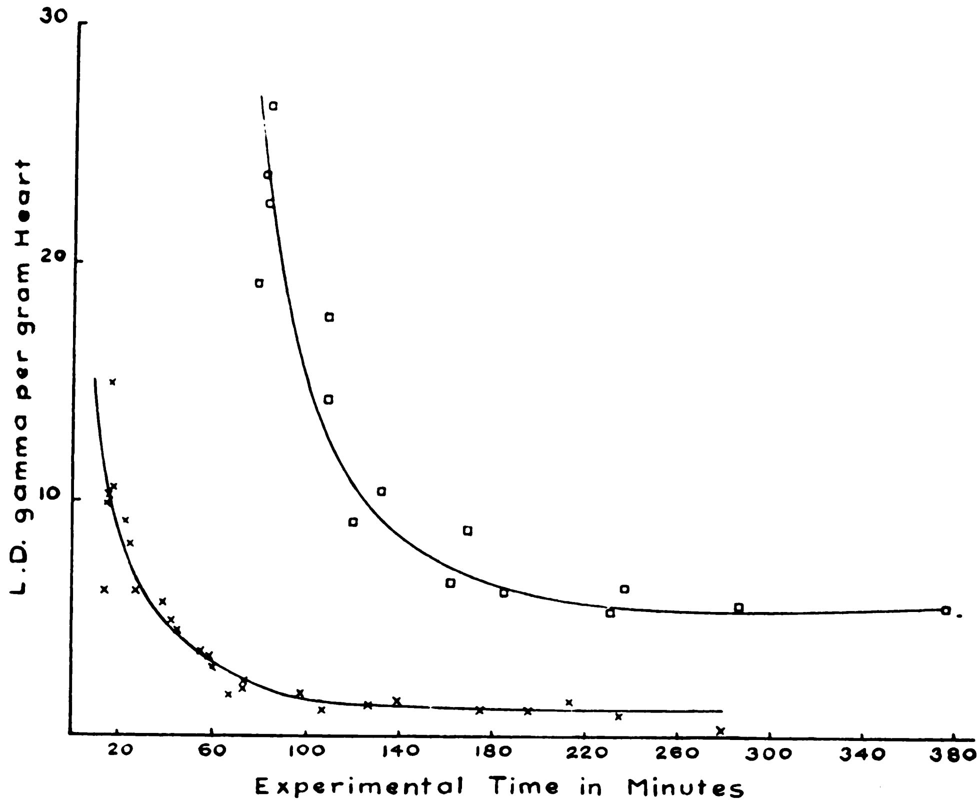

FIG. 2. DOG HEART-LUNG PREPARATION

The relation of experimental time to the lethal dose of g-strophanthin and digitoxin. (Gamma = microgram)

□————□ Digitoxin ×————× g-strophanthin

mental conditions, the minimal experimental time needed for determining the M.L.D. was about 120 minutes for g-strophanthin, and 230 minutes for digitoxin. Weese and Rothlin did not use an experimental time greater than 90 minutes, and thus presumably did not determine the true M.L.D.

Weese (1), while studying the action of scillaren on the H.L.P. of the cat, noticed that this substance was inactivated fairly rapidly. Any destruction of either g-strophanthin or digitoxin in the dog H.L.P. would manifest itself by a gradual rise in the M.L.D. when rates of inflow are less than the optimal. As can be seen from our results there is no rise in the M.L.D. under such conditions, and it is likely that neither g-strophanthin nor digitoxin have been destroyed within the experimental periods used by us.

A number of experiments designed to see whether the dog H.L.P. binds only an amount of g-strophanthin equal to the M.L.D., regardless of the concentration, have been tabulated in table 3. The technic employed by us is similar to that used by Weese for studying the same point in the H.L.P. of the cat (1). From the first experiment it can be concluded that the glycoside infused was sufficient only to poison the first preparation, since the blood transferred to the second heart showed no digitalis action. From experiment 2 it can be concluded that when a rate of administration greater than the optimal is used, a g-strophanthin

TABLE 3

Passage experiments with g-strophanthin in the dog heart-lung preparation

	EXPERIMENT I
	Heart No. 1 = 34.5 grams. Blood volume = 900 cc.
9:06	Continuous infusion of 1:500,000 g-strophanthin at a rate of 0.0101 microgram per gram heart per minute.
11:15	Cardiac arrest in 139 minutes. L.D. = 1.390 micrograms g-strophanthin per gram of heart.
	Heart No. 2 = 49 grams. (corrected)
12:06	850 cc. of blood from Heart No. 1 was added. No change in cardiac action.
1:44	Started infusion of g-strophanthin 1:500,000 at a rate of 0.0091 microgram per gram heart per minute.
3:08	Irregularities set in.
3:51	Ventricular fibrillation. L.D. = 1.16 micrograms of g-strophanthin per gram heart.
	EXPERIMENT II
	Heart No. 1 = 43.0 gram. Blood volume = 850–900 cc.
9:21	Infusion g-strophanthin, 1:100,000, at a rate of 0.06 microgram per gram heart per minute.
9:52	Irregularities set in.
10:19	Ventricular fibrillation. L.D. = 3.49 micrograms per gram heart.
	Heart No. 2 = 53 grams. Blood volume = 200 cc.
11:20	Added 870 cc. of blood from heart No. 1.
11:55	Irregularities.
12:24	Ventricular fibrillation.
	EXPERIMENT III
	Heart No. 1 = 53 grams. Blood volume = 720 cc.
1:51	Added 96 micrograms g-strophanthin.
2:54	Ventricular fibrillation.
	Heart No. 2 = 69 grams. Blood volume = 300 cc.
3:41	Added 520 cc. of blood from Heart No. 1.
4:41	Started infusion of 1:500,000 g-strophanthin at a rate of 0.00477 microgram per gram heart per minute.
6:50	20 micrograms adrenaline injected.
7:25	Ventricular fibrillation. L.D. 0.783 microgram per gram heart.

can be detected in the blood if this is transferred to a second preparation. Experiment 3 is similar to experiment 2, but the results are quantitative. To 720 cc. of blood in the first preparation, 95 micrograms of g-strophanthin were added. This heart weighed 53 grams; therefore, had the M.L.D. been 1.18 micrograms, it should have bound 62 micrograms of g-strophanthin, thus leaving approximately 33 micrograms unbound in the 720 cc. of blood. To a second preparation we added 520 cc. of the blood from the first preparation but in order to stop the second heart it was necessary to add an additional 54 micrograms g-strophanthin. The second heart weighed 69 grams, therefore its M.L.D. could be expected to be approximately 81.5 micrograms of g-strophanthin. Since 54 micrograms of the drug were added to the blood of the second heart, it may be concluded that 26.5 micrograms of g-strophanthin were present in the 520 cc. of blood from the first heart. Hence, the 720 cc. of blood from H.L.P. No. 1 must have contained 36.5 micrograms, a value which agrees well with the calculated amount of 33 micrograms. It may be concluded, therefore, that the dog H.L.P. is similar to that of the cat, in that it binds no more than one M.L.D. of g-strophanthin, regardless of excess concentration.

Since, regardless of the concentration, the heart only binds an amount of cardioactive glycosides equal to the M.L.D., we have calculated the average rate of uptake by dividing the M.L.D. by the experimental time. This rate of uptake is the amount of glycoside bound by one gram of heart in one minute. It should be remembered, however, that the rate of uptake may not be the same during all the stages of the poisoning process. Gehlen (16), from purely mathematical considerations, believes that the uptake of digitalis glycosides by the heart is more rapid during the earlier than during the later stages of this poisoning process. The binding of cardiac glycosides by the heart is a complex mechanism which has been divided by some investigators into an adsorptive and a chemical phase (17). Our term *average rate of uptake* is the resultant of the processes of transfer of the glycoside from the blood to the cardiac cells and the various reactions which they undergo in the cell to produce the characteristic pharmacological manifestations.

In fig. 3 we have plotted the average rate of uptake against the rate of administration. It can be seen that with a reduction in the rate of administration, the rate of uptake decreases. At the *optimal rate of administration* the average rate of uptake equals the rate of administration. The average rate of uptake of digitoxin, expressed in micrograms, is higher than that of g-strophanthin. If, however, the rate of uptake is expressed in per cent of the M.L.D., g-strophanthin has a higher rate of uptake. This is to be expected, since the speed of reaction of g-strophanthin is greater (1), and the M.L.D. less, than that of digitoxin.

Discussion. The results of this investigation on the H.L.P. of the dog clearly show that, within certain limits, the L.D. of a cardioactive glycoside is dependent on its rate of administration. If the M.L.D. is being determined by continuous infusion, it is necessary to extend the experimental periods to 120 minutes for g-strophanthin and 240 minutes for digitoxin. The high values of the M.L.D. of some cardioactive glycosides determined previously (1, 2, 3, 4) are probably due to the fact that these investigators were not extending their experimental periods long enough. Moe (18) has determined the minimal irregularity dose (M.I.D.) for some cardiac glycosides in the dog H.L.P. His value for the M.I.D. for g-strophanthin was 0.37 micrograms per gram heart. According to Krueger and Unna (19) the M.I.D. is about 60 per cent of the M.L.D. in the intact anesthetized cat. If the same relationships should hold for

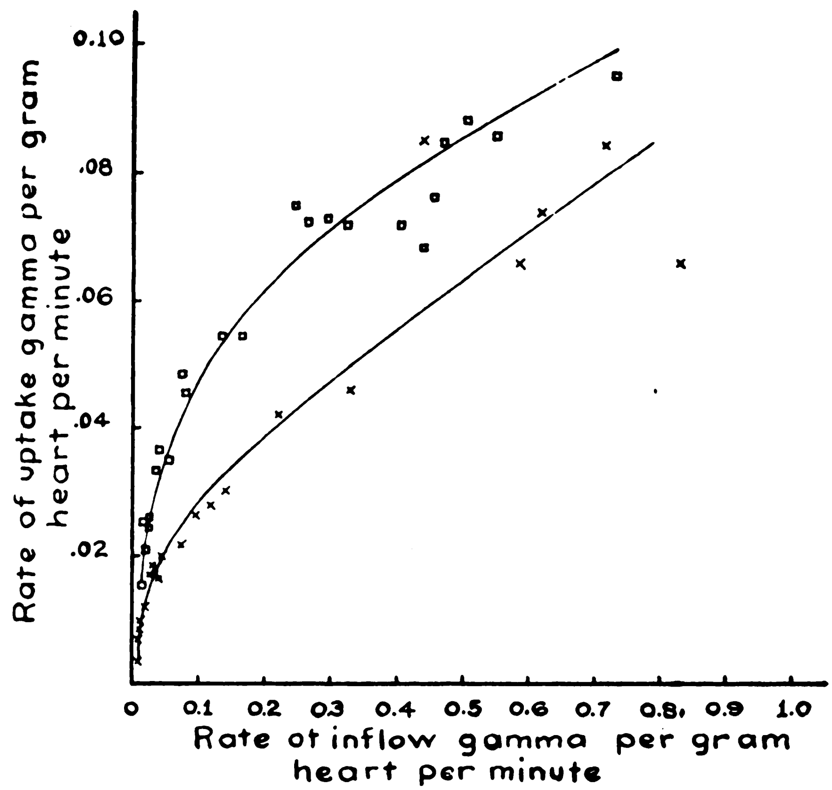

FIG. 3. DOG HEART-LUNG PREPARATION

The relation of the rate of inflow of g-strophanthin and digitoxin to the rate of uptake. (Gamma = microgram)

□————□ Digitoxin ×————× g-strophanthin

the dog H.L.P., the M.L.D. of g-strophanthin would be 0.617 micrograms per gram of heart. This value is about 50 per cent lower than the one determined by us. It is not possible to discuss any further this discrepency since the methods of Moe are somewhat different from those described in this paper.

From our data the partition between the heart and other organs in the intact dog can now be calculated. For comparative purposes the most reasonable values to be used are the M.L.D. of both the intact animal and the H.L.P. In the intact dog the reported lethal doses vary between 0.084 and 0.19 for g-strophanthin, and between 0.37 and 0.5 mg. per kg. of digitoxin (20, 21). The lowest of these values are probably the closest to the true M.L.D., and have been used in our calculations. Since in the dog, the heart is on the average equal to 0.84 per cent of the body weight, the amount of glycoside bound by 8.4 grams of heart should be compared with the M.L.D. per kg. of the intact dog. For g-strophanthin we found that 11.8 per cent, and for digitoxin 13.5 per cent of the M.L.D. of the intact animal is bound by the heart. These values are somewhat higher than those calculated by Weese (1) and Rothlin (3) for the cat, but their values are based on the Hatcher-Magnus dose, and thus the values of the percentage utilization by the cat heart are too low. Since in the dog, the ratio of heart weight to body weight is about twice that in the cat, some species differences in this partition between the heart and other organs would be most interesting, but a final decision on this point must await the accurate determination of the M.L.D. of these glycosides in the cat H.L.P. and the intact dog.

While studying the influence of caffeine on the L.D. of strophanthin in the cat H.L.P. Kohn (22) expressed the opinion that the reduction of the L.D. by caffeine was due to an increase in the permeability of the cardiac cell to cardiac glycosides. Weese and Wiegand (23) have shown that a large dose of caffeine (5 mg. per gram heart) markedly lowered the L.D. of g-strophanthin in the cat H.L.P. Both Weese and Kohn were probably working with short experimental times, so that values higher than the M.L.D. were being determined. It is conceivable that at rates higher than the *optimal rate of administration*, a modification of the L.D. may be due either to a change in the rate of uptake or a change in the true M.L.D. Thus caffeine may have either increased the rate of uptake or decreased the M.L.D. To decide which of these two factors is operating, one would have to determine the M.L.D. and also the L.D. and experimental time at various rates of administration, with and without caffeine. From this data it would be possible to calculate the rate of uptake and thus determine whether the change in the L.D. is due to a reduction in the M.L.D. or an increase in the uptake. A similar reasoning could be applied to any substance which has the ability to change the L.D. of a cardiac glycoside in the H.L.P., when rates greater than the optimal rate of administration are being used.

SUMMARY

The effect of rate of administration of g-strophanthin and digitoxin on the L.D., experimental time, destruction and rate of uptake has been studied in the H.L.P. of the dog.

With high rates of administration the L.D. is high. This is due to an over-titration resulting from the inability of the heart to bind all the glycoside offered per unit of time. To determine the M.L.D., rates equal to or lower than the *optimal rates of administration* have to be employed. The M.L.D. as determined under our experimental conditions is 1.18 micrograms g-strophanthin (0.002 micromols) and 5.95 micrograms of digitoxin (0.008 micromols).

In our experiments it was not possible to detect any destruction of either g-strophanthin or digitoxin by the H.L.P. of the dog.

The dog heart in the H.L.P. binds only an amount of cardiac glycoside equal to the M.L.D. regardless of the concentration in the blood. It was thus possible to calculate the *average rate of uptake* of g-strophanthin and digitoxin by dividing the M.L.D. by the experimental time. The *average rate of uptake* thus determined increased with an increase in the rate of administration.

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