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CHEMICAL STRUCTURE AND PHARMACOLOGICAL ACTIVITY OF SOME DERIVATIVES OF DIGITOXIGENIN AND DIGOXIGENIN

BY

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A series of derivatives of digitoxigenin and digoxigenin were prepared and tested for toxicity in the cat and the guinea-pig and on the isolated heart of the 48-hr chick embryo, and for inotropic activity on the cat isolated papillary muscle and the guinea-pig Langendorff heart. The order of relative potency of the compounds remained the same whether they were tested for toxicity or for positive inotropic activity. There are three molecular centres in the cardiac aglycone that are linked closely with cardiac activity. These are: (*a*) an OH at carbon-3 which can be combined as a glycoside, thus enhancing activity, or esterified or oxidized, producing compounds of lower activity; the maximum intensity of the inotropic response was reduced in the less potent compounds; (*b*) a 14-β-OH associated with a *cis* C-D ring junction, alteration of which abolished activity; (*c*) an unsaturated cyclobutenolide ring which cannot be reduced without a great decrease in activity.

Current knowledge about the relationship between chemical structure and pharmacological activity in the cardiac glycosides is derived principally from the work of Chen and his colleagues (see review by Chen, 1945; Chen & Henderson, 1954), who tested more than 200 naturally occurring cardiac glycosides, aglycones and their derivatives for toxicity in the cat. Toxicity in the frog after injection into the lymph sac (Chen, Chen & Anderson, 1936; Chen & Elderfield, 1940, 1942; Chen, Elderfield, Uhle & Fried, 1943) and the concentration that produced systolic standstill in the isolated frog heart (Chen, Steldt, Fried & Elderfield, 1942) were also tested for some of the compounds. However, such methods do not measure potency in increasing the force of contraction of heart muscle, which is the most important therapeutic action of the cardiac glycosides. The therapeutic ratios of some naturally occurring cardiac glycosides do not differ greatly (Cattell & Gold, 1941; Farah & Maresh, 1948; Walton & Gazes, 1951), and it is frequently assumed that positive inotropic potency is parallel to toxicity. However, some experiments with the synthetic 20:22 dihydroglycosides suggest that it is possible to decrease toxicity without a corresponding decrease in positive inotropic activity (Vick, Kahn & Acheson, 1957); with compounds such as these, assessment of cardiac activity on the sole basis of toxicity to cats would obscure their most interesting properties.

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We selected the aglycones digitoxigenin and digoxigenin as reference compounds, and prepared a series of their derivatives in which three molecular centres were modified: (*a*) the hydroxyl group at carbon-3; (*b*) the β-hydroxyl group at carbon-14, and the *cis* C-D ring configuration; (*c*) the unsaturated lactone ring. Compounds in group (*a*) included the glycosides, acetates and ketones; in group (*b*) the β-anhydro- and 14-deoxygenins; and in group (*c*) the 20:22 dihydrogenins and a 20:22 dihydroglycoside. Toxicity was determined on cats and guinea-pigs, and positive inotropic activity was measured on the cat papillary muscle and the isolated perfused guinea-pig heart. The compounds were also tested on the isolated heart of the 48-hr embryonic chick, which is a method based essentially upon a measurement of toxicity, but which eliminates some of the factors which complicate the estimation of cardiac toxicity in intact animals.

METHODS

Preparation of digitoxigenin and digoxigenin

Digitoxigenin. Digitoxin was hydrolysed by the method of Hasenfratz (1931). The material crystallized from ethanol-water, m.p. 248 to 250° C.

Digoxigenin. Digoxin was hydrolysed in boiling 0.5% hydrochloric acid (Smith, 1930a). After recrystallization the material melted at 217 to 219° C. Paper chromatograms on formamide impregnated paper with a developing solvent of chloroform 78, benzene 12 (Brown, Ranger & Wright, 1955), revealed no unchanged digoxin, although about 0.1% of β-anhydrodigoxigenin was estimated to be present.

Compounds in which the hydroxyl at carbon-3 has been modified

Lanatosides A and C. Obtained from Sandoz A.G., Basel, Switzerland.

Digitoxin and digoxin. Obtained from Burroughs Wellcome & Co. (Aust.), Sydney, Australia.

Digitoxigenin acetate. Digitoxigenin was acetylated with acetic acid in pyridine at room temperature. The crystalline material obtained melted at 218° C (Rangaswami & Reichstein, 1949).

Digoxigenin diacetate. Acetylation of digoxigenin with acetic anhydride in pyridine gave crystalline material, m.p. 218 to 220° C (Smith, 1930b). No unchanged digoxigenin could be detected by paper chromatography, indicating less than 0.1% of free genin.

3-Oxo-14β-hydroxycard-20(22)-enolide (digitoxigenone). Digitoxigenin was oxidized with chromic oxide in acetic acid. The ketone was purified by chromatography on alumina, and after recrystallization melted at 192 to 195° C.

3:12-Dioxo-14β-hydroxycard-20(22)-enolide (digoxigenone). Oxidation of digoxigenin with chromic oxide in acetic acid gave material which, after purification on alumina and subsequent recrystallization, melted at 263° C (Smith, 1935). No unchanged aglycone could be detected (less than 0.1%).

Compounds in which the hydroxyl at carbon-14 has been modified

3β-Acetoxy-card-14:20(22)-dienolide (β-anhydrodigitoxigenin acetate). Digitoxigenin acetate was treated with phosphorus oxychloride by the method described by Hunziker & Reichstein (1945). The material melted at 185° C.

3β,12β-Dihydroxy-card-14:20(22)-dienolide (β-anhydrodigoxigenin). Digoxigenin was treated with sulphuric acid (Smith, 1930b). After chromatography on alumina and recrystallization the 14:15-anhydrogenin melted at 180° C. Chromatography on formamide impregnated paper revealed no unchanged digoxigenin, indicating less than 0.1%.

3β-Acetoxy-14α-card-20(22)-enolide (14-deoxydigitoxigenin acetate) and 3β,12β-dihydroxy-14α-card-20(22)-enolide (14-deoxydigoxigenin). The preparation of these compounds from their respective 14:15-anhydrogenins has been described (Brown & Wright, 1961). The *trans* arrangement of the C-D ring junction which is present in both of these compounds is in contrast to the *cis* junction of the cardiac glycosides and aglycones.

Compounds in which the cyclobutenolide ring has been reduced

20:22-Dihydrodigitoxigenin. Digitoxigenin was reduced with hydrogen, using platinum catalyst. The material melted at 224° C, $[\alpha]_D$ +16.5 (c=1.0 in methanol). Cardwell & Smith (1954) record m.p. 226° C.

22:22-Dihydrodigoxigenin. Digoxigenin was hydrogenated using platinum catalyst. Though the reduced material may be separated into 20α and 20β isomers (Brown & Wright, 1961), a mixture of isomers m.p. 220° C was used for pharmacological testing. M.p. of 20α isomer, 225° C; and m.p. of 20β isomer, 209° C.

20:22-Dihydrodigoxin. Digoxin was hydrogenated using platinum catalyst (Brown & Wright, 1961). The material obtained melted at 162 to 164° C, resolidified and remelted at 268 to 270° C, $[\alpha]_D$ +13 (c=1.4 in methanol).

Determination of cardiac activity

Lethal doses in cats and guinea-pigs. Young cats (0.4 to 1 kg) were anaesthetized with pentobarbitone sodium (40 mg/kg) and guinea-pigs (0.24 to 0.86 kg) with urethane (1.75 g/kg) given intraperitoneally. Artificial respiration was applied through a tracheal cannula. The rectal temperature was maintained at 37° C. The cardiac glycosides, genins and genin derivatives were dissolved in 95% ethanol, and were diluted with at least 19 vol. of 0.9% sodium chloride solution for infusion into a jugular vein. The synthetic compounds that slowly crystallized out from saline solutions were injected by a procedure similar to that described by Chen, Robbins & Worth (1938). A continuous infusion of 0.9% sodium chloride solution was given at 0.4 ml./min into a jugular vein, and small volumes (<0.01 ml.) of the ethanolic solution of the compound were given at 1 min intervals into this flow. In control experiments, the injection of ethanol at this rate had no effect on the heart as judged from the electrocardiogram. The maximum volume of ethanol injected by either method was 0.7 ml./kg. The concentrations and rates of injection of the drugs were adjusted to produce death of the animal within 15 to 30 min. This period of infusion, which is shorter than that generally recommended for measurement of the lethal doses of cardiac glycosides in the cat (B.P., 1953; U.S.P. XII edition), was chosen because of the more rapid rates of detoxication of the aglycones and their derivatives (Rand & Stafford, 1959). Electrocardiograms were taken at first at 30 sec intervals, and then continuously after the onset of ventricular fibrillation. The amount of drug required to cause cessation of electrical activity observed on the electrocardiogram was taken as the measure of the toxicity of the compound. In addition, the electrocardiograph records were analysed for the changes in sinus rate and P-R interval and the onset of A-V dissociation and ventricular fibrillation produced by each compound.

Toxicity on the isolated embryonic chick heart. Activity on the isolated heart of the 48-hr chick embryo was measured as described by Paff (1940). Fertile eggs were incubated for 48±1 hr at 37 to 38° C. The embryo was removed and the heart was dissected. The required drug concentrations were contained in Tyrode solution in a small heated well (0.05 ml.) on the stage of a dissecting microscope. Three hearts were placed simultaneously into the well and the time taken for the development of auriculo-ventricular block in each heart was measured. Three or four doses of each drug were used, and about 6 hearts were observed at each dose. Concentrations of drugs were chosen so that the times taken for the appearance of auriculo-ventricular block ranged from 3 to 15 min. The regression line of time to block on concentration, and its significance, were calculated and the potency of each compound relative to the corresponding aglycone was determined by the statistical methods described by Emmens (1948).

Positive inotropic activity on cat isolated papillary muscle. Cats were anaesthetized with ether, and papillary muscles of approximately 1 mm diameter were removed and suspended in 15 ml. of modified Krebs-Henseleit solution (Creese, 1950, Solution (a)) in an organ bath at 33° C. The resting tension on the muscle was adjusted to 3 g and supramaximal square wave stimuli (1 or 2 msec duration) were applied to the papillary muscle at the rate of 1/sec. Tension changes produced by contraction of the papillary muscle were detected by a strain gauge transducer which gave a linear record of the developed tension over the range 5 mg to 10 g. The papillary muscles were initially suspended for 1 hr in Krebs-Henseleit bicarbonate solution, and then this solution was replaced by a low-calcium bicarbonate-free solution (Creese, 1950, Solution (d)) of the following composition: 0.154 M sodium chloride 121.5 ml., 0.154 M potassium chloride 5 ml., 0.11 M calcium chloride 1.5 ml., 0.154 M magnesium sulphate 1 ml., disodium hydrogen phosphate duodecahydrate 55 mg, glucose 130 mg, distilled water 1 ml. gassed with oxygen. Under these conditions the developed tension rapidly decreased. Cardiac glycosides and aglycones were then added in ethanolic solutions (final concentrations of ethanol were between 0.03 and 0.3%) and the increase in systolic tension recorded. Systolic tension developed by the muscle in Krebs-Henseleit solution ranged from 1 to 3 g, and, in the low calcium solution, from 0.1 to 0.5 g. Addition of active aglycones and derivatives to the low calcium medium produced a 3- to 10-fold increase in developed tension, which was easily reversible on washing. Comparisons were made on 30 separate papillary muscles, with 3 to 10 responses from each muscle.

Positive inotropic activity on the perfused guinea-pig heart. Guinea-pig hearts were perfused through the coronary circulation, and the amplitude of beat recorded by attaching a thread from the apex of the ventricle to an isotonic lever writing on a smoked drum. The same lever magnification was used in all experiments. The outflow from the heart was diverted through a Gaddum flow recorder before being raised by a small Perspex centrifugal pump to a temperature-controlled reservoir (34° C) 1 m above the heart. The volume of the circulating fluid was 125 ml. and its composition (g/l.) was as follows: sodium chloride 10.8 g, potassium chloride 0.42 g, sodium bicarbonate 0.5 g, calcium chloride 0.24 g, glucose 1.0 g. The solution was gassed with 5% carbon dioxide in oxygen. Heart rate was recorded from light spring contacts connected to an impulse counter. After an equilibration period (approximately 15 min) the drug was added to the perfusion fluid in a small volume (0.05 to 0.7 ml.) of 95% ethanol, and the heart was observed for 1 hr unless it stopped beating before this time. Each heart was used for the measurement of only one response. Observations were made on 90 hearts.

RESULTS

Lethal doses in cats and guinea-pigs. The results are summarized in Tables 1 and 2. Our estimates of the lethal doses of the four glycosides in cats are higher than those reported by others (see Shoppee & Shoppee, 1953). This is because we used higher rates of infusion (Mehnert, 1936) and pentobarbitone instead of ether as an anaesthetic (Holck, Smith & Shuler, 1945). The glycosides, the aglycone acetates and the aglycone ketones produced qualitatively similar electrocardiographic changes both in cats and guinea-pigs.

The relative insolubility of the 14-deoxy-, β-anhydro- and 20:22 dihydrogenins limited the determination of their lethal doses. With these compounds, injections were stopped after the animals had received 0.01 ml. of the ethanol solution/min for 30 min, by which time there were no changes in the electrocardiogram. Then, in some of the experiments with cats, an infusion of ouabain was given immediately after the apparently inactive derivatives to see whether the lethal dose of ouabain had been altered by previous administration of these drugs. The results are shown in Table 3. These values for the lethal dose of ouabain (0.248 to 0.292 mg/kg)

TABLE 1

LETHAL DOSES OF DERIVATIVES OF DIGITOXIGENIN AND DIGOXIGENIN IN THE CAT

	Number of animals	Lethal dose	
		mg/kg±s.e.	μM/kg±s.e.
Lanatoside A	5	1·16±0·046	1·20±0·047
Digitoxin	6	1·71±0·066	2·23±0·086
Digitoxigenin	12	0·60±0·031	1·61±0·083
Digitoxigenin acetate	5	1·41±0·11	3·38±0·26
Digitoxigenone	5	1·86±0·16	4·99±0·44
20 : 22-Dihydrodigitoxigenin	1	>13	>34
Lanatoside C	5	1·25±0·12	1·26±0·12
Digoxin	6	0·73±0·019	0·93±0·024
Digoxigenin	11	0·70±0·044	1·79±0·11
Digoxigenin diacetate	7	4·79±0·50	10·1 ±1·1
Digoxigenone	3	9·65±1·40	24·9 ±3·6
β-Anhydrodigoxigenin	1	>19	> 51
14-Deoxydigoxigenin	1	>43	>116
20 : 22-Dihydrodigoxigenin	1	> 8·4	> 33
20 : 22-Dihydrodigoxin	1	> 5·6	> 7·1

TABLE 2

LETHAL DOSES OF DERIVATIVES OF DIGITOXIGENIN AND DIGOXIGENIN IN THE GUINEA-PIG

	Number of animals	Lethal dose	
		mg/kg±s.e.	μM/kg±s.e.
Lanatoside A	5	1·04±0·04	1·07±0·04
Digitoxin	6	1·22±0·18	1·60±0·24
Digitoxigenin	7	2·65±0·21	7·08±0·56
Digitoxigenin acetate	7	6·12±0·61	14·7 ±1·5
Digitoxigenone	4	6·41±0·70	17·2 ±1·9
20 : 22-Dihydrodigitoxigenin	1	>25	> 66
Lanatoside C	6	0·68±0·038	0·69±0·039
Digoxin	7	0·59±0·059	0·75±0·076
Digoxigenin	6	6·80±0·83	17·4 ±2·1
Digoxigenin diacetate	4	20·2 ±4·0	42·6 ±8·5
Digoxigenone	1	>14	> 36
β-Anhydrodigoxigenin	1	>30	> 80
14-Deoxydigoxigenin	1	>47	>126
20 : 22-Dihydrodigoxigenin	1	>60	>153
20 : 22-Dihydrodigoxin	1	>21	> 31

TABLE 3

LETHAL DOSES OF OUABAIN IN THE CAT AFTER ADMINISTRATION OF INACTIVE AGLYCONE DERIVATIVES

			Ouabain	
	Rate of infusion (mg/kg/min)	Amount infused mg/kg	Rate of infusion (μg/kg/min)	Lethal dose mg/kg
Dihydrodigitoxigenin	0·475	13·3	11·4	0·292
β-Anhydrodigoxigenin	1·0	19·0	16·3	0·289
14-Deoxydigoxigenin	0·97	43·5	12·1	0·248

fall within the range of control lethal doses in cats (0.266 ± 0.019 mg/kg, 8 cats). Therefore it can be concluded that, in the dose levels used, 14-deoxydigoxigenin, β-anhydrodigoxigenin and 20:22 dihydrodigitoxigenin neither potentiated nor protected against the lethal dose of ouabain.

The 48-hr embryo chick heart. Each compound produced auriculo-ventricular block in the isolated chick embryo heart except β-anhydrodigitoxigenin acetate, which was inactive at a concentration of 20 μg/ml. in Tyrode solution and insoluble at higher concentrations. β-Anhydrodigoxigenin was toxic at 100 μg/ml., but was only tested at this concentration because of insolubility. Fig. 1 illustrates the results

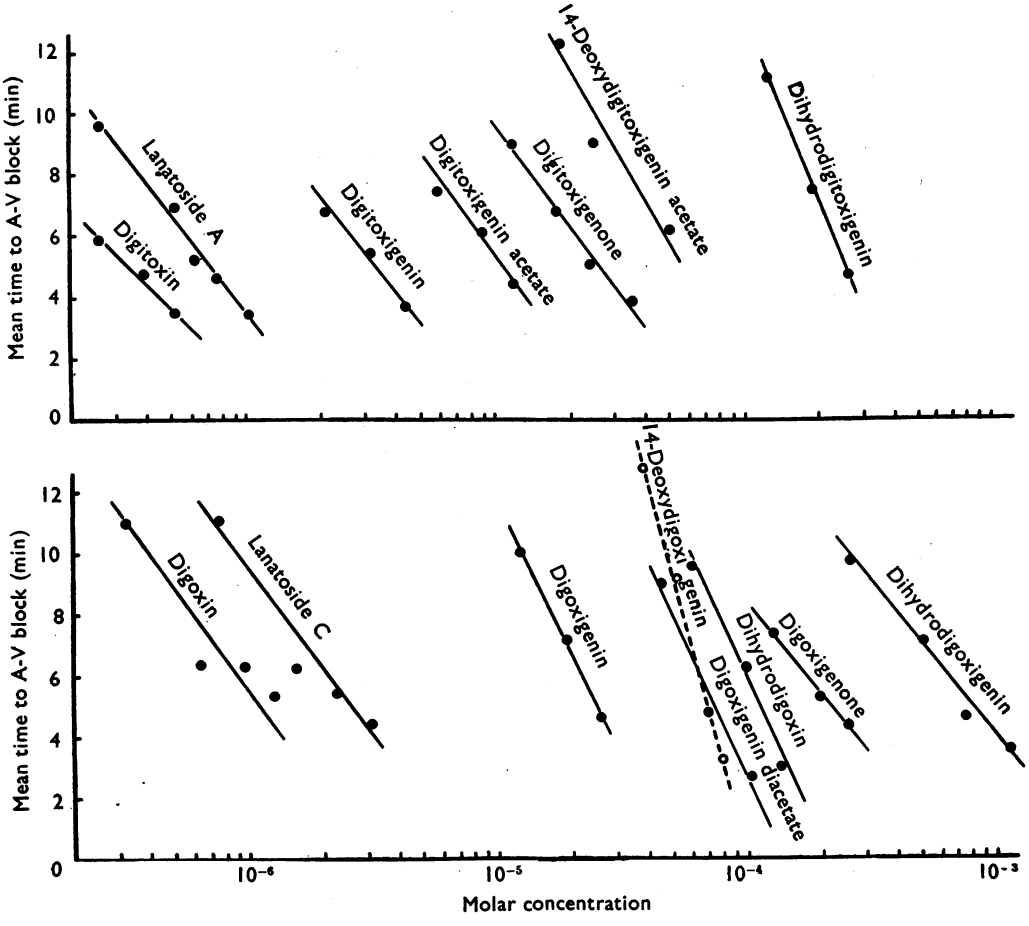

Fig. 1. Log dose-response lines for derivatives of digitoxigenin (upper figure) and digoxigenin (lower figure) on the isolated hearts of 48-hr chick embryos. Each point is the mean of 5 to 10 separate observations.

obtained. All the log dose-response regression lines were highly significant ($P<0.001$), but the slopes of the dose-response lines for dihydrodigitoxigenin, 14-deoxydigoxigenin, dihydrodigoxigenin and dihydrodigoxin were significantly different from those of the glycosides, aglycones, aglycone acetates and aglycone ketones, and this probably indicates a qualitatively different mechanism of action. Where the slope was significantly different from that of the parent compound, an approximate estimate of relative potency has been made by measuring the horizontal distance

between the log dose-response lines at the centre of the graph (see Fig. 1), that is, the relative doses which produced block in 7 sec. The molar activity ratios and their limits of error are shown in Table 4.

Cat papillary muscles. At no concentration did the synthetic acetyl and ketone derivatives produce as great an increase in the force of contraction of papillary

TABLE 4

THE POTENCIES OF DERIVATIVES OF DIGITOXIGENIN AND DIGOXIGENIN ON THE ISOLATED 48-HR EMBRYO CHICK HEART

	Regression response (sec) on log dose	Significance of difference from parallelism	Molar potency ratio	Fiducial limits $P=0{\cdot}05$
Compounds compared with digitoxigenin				
Lanatoside A	−632	$0{\cdot}8<P<0{\cdot}9$	4·51	4·08 – 5·00
Digitoxin	−473	$0{\cdot}2<P<0{\cdot}3$	9·43	8·26 –10·8
Digitoxigenin	−614		1	
Digitoxigenin acetate	−613	$P>0{\cdot}9$	0·305	0·266– 0·348
Digitoxigenone	−665	$0{\cdot}6<P<0{\cdot}7$	0·109	0·096– 0·125
14-Deoxydigitoxigenin acetate	−815	$0{\cdot}3<P<0{\cdot}4$	0·047	0·041– 0·056
β-Anhydrodigitoxigenin acetate	—	—	Inactive	—
Dihydrodigitoxigenin	−1,264	$P<0{\cdot}001$	0·01	—
Compounds compared with digoxigenin				
Lanatoside C	−667	$0{\cdot}1<P<0{\cdot}2$	12·8	11·1 –14·8
Digoxin	−600	$0{\cdot}05<P<0{\cdot}1$	31·8	27·8 –36·2
Digoxigenin	−885		1	
Digoxigenin diacetate	−1,268	$0{\cdot}05<P<0{\cdot}1$	0·313	0·277– 0·355
Digoxigenone	−595	$0{\cdot}1<P<0{\cdot}2$	0·143	0·116– 0·171
14-Deoxydigoxigenin	−1,820	$P<0{\cdot}001$	0·3	—
β-Anhydrodigoxigenin	—	—	0·017	—
Dihydrodigoxigenin	−640	$0{\cdot}02<P<0{\cdot}05$	0·042	—
Compounds compared with digoxin				
Dihydrodigoxin	−1,320	$P<0{\cdot}001$	0·01	—

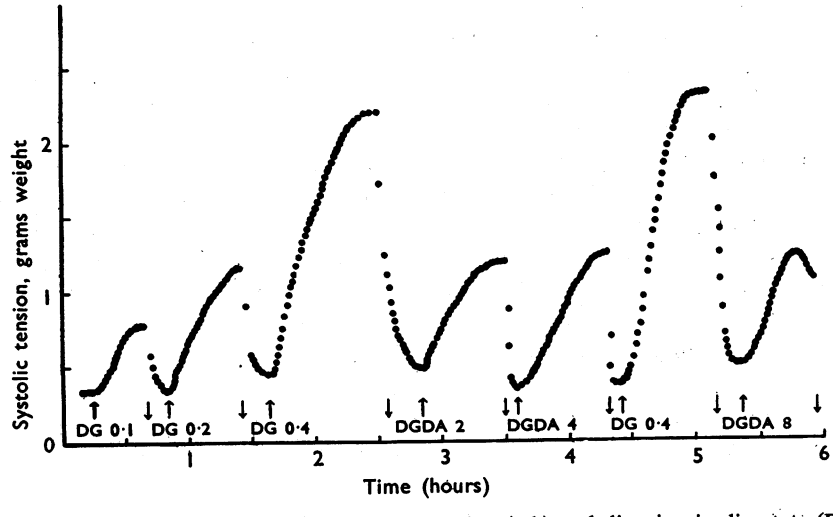

Fig. 2. The effects of digoxigenin (DG, 0.1, 0.2, 0.4 μg/ml.) and digoxigenin diacetate (DGDA, 2, 4, and 8 μg/ml.) on the force of contraction of a cat papillary muscle.

muscles as the parent aglycones. The result of an experiment comparing digoxigenin and digoxigenin diacetate is shown graphically in Fig. 2. Although the response to the high concentration of digoxigenin (0.4 μg/ml.) was reproducible, the increase in systolic tension produced by 8 μg/ml. of digoxigenin diacetate was not sustained, and still higher concentrations of digoxigenin diacetate produced only a decrease in the force of contraction. Similar results were obtained with the other aglycone acetates and ketones. Such a difference in properties makes meaningful estimates of potencies impossible on this preparation. However, comparisons of potency can be made with the lower dose levels of digitoxigenin and digoxigenin, where the synthetic derivatives produced equivalent responses. Table 5 lists the relative molar

TABLE 5

THE RELATIVE INOTROPIC POTENCY OF DERIVATIVES OF DIGITOXIGENIN AND DIGOXIGENIN ON THE CAT PAPILLARY MUSCLE

	Number of comparisons	Molar potency
Compounds compared with digitoxigenin		
Lanatoside A	1	0·78
Digitoxin	1	4·1
Digitoxigenin acetate	8	0·43
Digitoxigenone	9	0·19
Dihydrodigitoxigenin	4	0·007
Compounds compared with digoxigenin		
Lanatoside C	3	0·3
Digoxin	2	1·5
Digoxigenin diacetate	15	0·057
Digoxigenone	5	0·1
β-Anhydrodigoxigenin	3	$<0·002$
Compounds compared with digoxin		
Dihydrodigoxin	2	0·025

potencies calculated from the concentration of each synthetic derivative required to produce the same increase in force of contraction as digitoxigenin (0.1 μg/ml. or 2.7×10^{-7} M) or digoxigenin (0.2 μg/ml. or 5.1×10^{-7} M); these concentrations of digitoxigenin and digoxigenin produced equivalent submaximal responses. In the maximum concentration that could be tested (100 μg/ml.), β-anhydrodigoxigenin produced an immediate drop in resting tension, followed by a small increase in systolic tension; lower concentrations (down to 20 μg/ml.) changed only resting tension. In concentrations ranging from 60 to 100 μg/ml., 14-deoxydigoxigenin had marked negative inotropic activity, producing complete but reversible poisoning of the muscle in 5 to 15 min; lower concentrations were without effect.

Guinea-pig perfused heart. Three concentrations of each drug were tested. The "toxic" concentration was defined arbitrarily as that producing cardiac arrest after 20 to 50 min perfusion, and was determined first. Then the responses to one-half and one-quarter of this "toxic" concentration were observed. Table 6 shows the "toxic" concentrations of all the compounds, and the molar activity ratios calculated by dividing the "toxic" concentration of the reference compound by that of each of its derivatives.

TABLE 6

ACTIVITY OF DERIVATIVES OF DIGITOXIGENIN AND DIGOXIGENIN ON THE ISOLATED PERFUSED GUINEA-PIG HEART

	Inotropic activity. Mean % increase in amplitude of contraction	Toxic activity	
		Dose (mM) producing cardiac arrest in 20–50 min	Molar activity ratio
Compounds compared with digitoxigenin			
Lanatoside A	310	0·52	5·2
Digitoxin	300	0·52	5·2
Digitoxigenin	160	2·7	1
Digitoxigenin acetate	130	1·9	1·4
Digitoxigenone	80	5·4	0·48
Compounds compared with digoxigenin			
Lanatoside C	310	1·6	12·5
Digoxin	460	2·0	10
Digoxigenin	150	20	1
Digoxigenin diacetate	60	34	0·61
Digoxigenone	130	128	0·17
14-Deoxydigoxigenin	—	32	Actions qualitatively different from digoxigenin
β-Anhydrodigoxigenin	—	22	
Dihydrodigoxigenin	—	72	
Compounds compared with digoxin			
Dihydrodigoxin		72	0·03

Digitoxin, digoxin, the aglycones and their acetates and keto-derivatives produced an increase in amplitude of contraction at all three dose levels; heart rate changes were negligible before the onset of arrhythmias. The lanatosides A and C produced an increase in amplitude of contraction only at the two highest concentrations, and had no effect at one-quarter of the "toxic" concentration. With all the above drugs, half the "toxic" concentration sometimes produced arrhythmias, but did not stop the heart beating within 1 hr of administration, and one-quarter of the "toxic" concentration produced no arrhythmias. However, some of the synthetic derivatives had qualitatively different actions. 14-Deoxydigoxigenin and β-anhydrodigoxigenin decreased the amplitude of contraction, but did not affect rhythm. Dihydrodigoxigenin (7.2×10^{-5} M) produced a transient increase in the amplitude of beat followed by a decrease but no arrhythmias. Dihydrodigoxin, in 36 times the concentration of digoxin, had effects qualitatively similar to digoxin; it produced a marked increase in force of contraction and ventricular arrhythmias.

Records of heart contractions obtained with an isotonic lever provide relatively imprecise information that does not warrant detailed analysis. Over the range of concentrations of each drug tested, the inotropic responses (maximum percentage increase in amplitude of contraction before onset of arrhythmias) were variable and were not related to drug concentration. However, differences in the inotropic activities of the glycosides, aglycones and derivatives were sufficiently great to outweigh other variation. The inotropic responses to all 3 concentrations of each

drug were averaged, and these figures are included in Table 6, where it is apparent that the drugs which produced the greatest inotropic responses are those which are active at the lowest concentrations.

DISCUSSION

To evaluate the effects of small changes in the structure of the cardiac glycosides and aglycones on their pharmacological activities it must be assumed that the drugs act as such, and that metabolic processes in the whole animal or isolated tissues do not produce compounds with either increased or decreased activity. Work by Wright and his co-workers (Ashley, Brown, Okita & Wright, 1958), Megges & Repke (1961) and Repke (1959) has shown that metabolic alteration to cardiac glycosides does occur after administration to animals, particularly at the carbon-3 substituents. Our results, which are summarized in Table 7, show a marked corre-

TABLE 7
MOLAR POTENCIES RELATIVE TO THE PARENT AGLYCONE

	Toxicity			Positive inotropic activity	
Compound	Cat	Guinea-pig	Chick embryo heart	Papillary muscle	Langendorff heart
Lanatoside A	1·4	6·6	4·5	0·8	3·6
Digitoxin	0·7	4·4	9·4	4·1	5·1
Digitoxigenin	1	1	1	1	1
Digitoxigen acetate	0·5	0·5	0·3	0·4	1·4
Digitoxigenone	0·3	0·4	0·1	0·2	0·5
20 : 22-Dihydrodigitoxigenin	<0·05	<0·1	0·02	0·007	None
14-Deoxydigitoxigenin acetate	—	—	0·05	—	—
Digoxigenin (digitoxigenin=1)	0·9	0·4	0·1	0·5	0·1
Lanatoside C	1·4	23	13	0·3	8·9
Digoxin	1·9	23	32	1·5	10
Digoxigenin	1	1	1	1	1
Digoxigen diacetate	0·2	0·4	0·3	0·06	0·6
Digoxigenone	0·07	<0·5	0·1	0·1	0·2
20 : 22-Dihydrodigoxigenin	<0·08	<0·5	0·04	—	None
14-Deoxydigoxigenin	<0·04	<0·2	0·3	None	None
β-Anhydrodigoxigenin	<0·02	<0·2	0·02	None	None

lation between the activities of all the derivatives of digitoxigenin and digoxigenin on five different preparations. If any drug became active only after metabolic alteration, it would be expected to show relatively greater activity in the intact cat or guinea-pig than on isolated tissues. That the order of activities remains approximately the same with all methods of testing suggests that these compounds act without prior alteration or are altered at the site of action.

The influence of the sugar residue on activity. Chen and his colleagues (Chen, Henderson & Anderson, 1951 ; Chen & Henderson, 1954) investigated the influence of sugars attached at carbon-3 on the toxicity of cardiac glycosides in the cat. From the differences in toxicity between the naturally occurring glycosides and their aglycones, they concluded that the presence of a sugar usually increased the potency. Chen's comparisons were made on a weight basis, but recalculation with allowance for molecular weight only exaggerates the existing differences in potency. With a few exceptions, our measurements of toxic and positive inotropic potency support

Chen's conclusions, which were based on toxicity alone. The way in which the sugars enhance activity is not yet clear. Repke (1961) has suggested that sugars increase the binding to heart muscle, and protect the 3-OH from epimerization or conjugation, reactions in the metabolic process leading to a reduction of pharmacological activity. However, on the embryo chick heart, where the likelihood of extensive metabolic alteration is small, the discrepancy in potency between the aglycones and the glycosides is greater than on any other preparation (Table 7; DeGraff, Paff & Lehman, 1941).

The influence of oxidation and esterification of the hydroxyl at carbon-3. Since all the glycoside sugars are linked to the steroid nucleus by the C-3 hydroxyl, it is obvious that a free C-3 hydroxyl is not a prerequisite for activity. Chen (1945) found that various C-3 esters of strophanthidin were more toxic in the cat than the original aglycone; also hellibrigenin acetate was more toxic than hellibrigenin (Chen, Henderson & Anderson, 1950). On the other hand, the acetylation of scillirosidin was accompanied by a loss of activity (Chen, Henderson & Anderson, 1951). We found that the acetylation of digitoxigenin and digoxigenin reduced their activities to about one-half on every method except the guinea-pig Langendorff heart, where digitoxigenin acetate was slightly more toxic than digitoxigenin. It is somewhat surprising that, while the acetylation of the free C-3 hydroxyl groups of digitoxigenin and digoxigenin will lead to a reduction in activity, glycoside formation will increase activity. One explanation is that addition of a relatively polar sugar residue has increased water solubility, and this may be a factor which enhances access to the receptor sites.

Oxidation of the C-3 hydroxyl resulted in a reduction of activity in all test preparations to about a quarter of that of the parent aglycones. The effect of oxidation of the C-3 hydroxyl upon cardiac toxicity has been described by other workers; for example, toxicity in the cat is reduced, but not abolished, in 5-anhydroperiplogenone (Chen, 1945) and in scillarenone (Stoll, Renz & Brack, 1951).

The hydroxyl group at carbon-14 and the cis *C-D ring junction.* All the naturally occurring cardiac glycosides except adynerin contain a 14-β hydroxyl group and a *cis* C-D ring junction. Adynerin, with an 8:14 unsaturated bond (Tschesche & Snatzke, 1955), and the synthetic 8:14 and 14:15 anhydrodigitoxigenins (Cardwell & Smith, 1954) are not toxic in cats (Chen, 1945). Resibufogenin and artebufogenin, isolated from toads, do not possess a 14 hydroxyl group, and have a *trans* C-D ring junction (Meyer, 1952; Meyer & Reichstein, 1953); these aglycones are not toxic in cats in doses of 6 mg and 8 mg/kg respectively (Chen & Henderson, 1954).

The β-anhydro- and 14-deoxydigoxigenins which we prepared lack the 14-OH and *cis* C-D ring arrangement, and had no demonstrable toxicity and only negative inotropic activity on isolated heart muscle. Both toxicity and positive inotropic activity, therefore, are dependent on this part of the molecule. Examination of a scale molecular model of digoxigenin shows that the 14-β hydroxyl and 17-β unsaturated lactone ring are exposed groups. In models of the 14:15-anhydro and 14-deoxy compounds, the loss of a *cis* C-D ring configuration markedly alters the spatial position of the unsaturated lactone ring relative to the steroid nucleus. It may be that attachment of cardiac glycosides to receptor sites requires exposed structural

groups in both the 14- and 17- positions. So far a cardiac aglycone with a *cis* C-D ring junction but without a 14-β hydroxyl group has not been synthesized; hydrogenation of the 14:15 anhydrogenins produces a *trans* C-D ring junction.

The unsaturated lactone ring. For some time, the β-orientated, unsaturated lactone ring was considered to be an essential feature of the cardiac glycosides, because alterations in this part of the molecule led to a marked reduction in pharmacological activity. Chen & Elderfield (1940) found that dihydrostrophanthidin was not toxic in the cat in a dose level of 65 mg/kg; and the 17-α compounds, allostrophanthidin and allocymarin, have indeterminably low toxicities (Jacobs, 1930; Chen & Elderfield, 1940). Not only toxic, but positive inotropic, activity appeared to be reduced by hydrogenation of the lactone ring; Wollenberger (1954) found that hexahydroscillaren A was inactive in the dog heart-lung preparation. However, Vick *et al.* (1957) found that hydrogenation of the lactone rings of ouabain, digoxin and digitoxin reduced toxicity in the dog heart-lung preparation more than positive inotropic activity. Taeschler & Cerletti (1961) drew the same conclusions from experiments with dihydrodigoxin on the isolated guinea-pig auricle. We have found dihydrodigitoxigenin and dihydrodigoxigenin to be virtually inactive, and both toxic and positive inotropic activity to be vastly reduced in dihydrodigoxin.

Hydrogenation of the lactone ring produces another centre of asymmetry (carbon-20), and it may be assumed that previous workers used mixtures of the 20-α and 20-β isomers. Though these isomers have now been separated chemically (Brown & Wright, 1961) they have not been tested separately for pharmacological activity. It is possible that different proportions of 20-α and 20-β isomers in the material tested may contribute to the conflicting results described above.

Conclusions. Our results confirm structure-activity relationships which have previously been based almost entirely on cat lethal dose figures. Toxicity and positive inotropic activity are properties which are closely related, and it has not been possible to reduce toxicity without a corresponding reduction in inotropic potency by alteration of the molecular structure of the aglycone. It might appear from these conclusions that we believe that measurement of toxicity alone would give sufficient information about the pharmacological activity of a new cardiac glycoside. Other workers have pointed out the inadequacy of toxicity measurements in general for assessing the activity of cardiac glycosides (Tanz & Kerby, 1961; Vick *et al.*, 1957) on fairly obvious grounds which have been discussed in this paper. However, we wish to emphasize the importance of estimating not only the drug concentrations that are required to produce toxic and positive inotropic effects, and the ratio between them, but also the maximum intensity of effect that can be achieved. This concept is meaningless for toxicity measurements, but is important when considering positive inotropic activity. A similar situation is well known in other fields of pharmacology, for example, the analgesics. Relative toxicities of morphine and codeine are easily obtained, and a figure can be produced even for relative analgesic potency, provided the experimental conditions are limited to those in which equivalent responses can be produced. But when maximum intensity of analgesic response is considered, the therapeutically important difference between morphine and codeine is manifest. We see now that cardiac glycosides and aglycones may present a

similar picture. This has not been mentioned by other workers probably because it has been obscured by the difficulties attached to quantitative measurements of positive inotropic activity. It is important to emphasize this difference in properties, because, by devising situations in which conventional comparisons of potency can be made, the therapeutically more important concept of maximum intensity of effect may be completely overlooked.

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