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Comparison of Digoxin, Digilanids *A*, *B* and *C*, and Deacetyldigilanids *A* and *B**†

By K. K. Chen, Chester C. Hargreaves and E. Brown Robbins

After *Digitalis lanata* was shown to have more physiologic units quantitatively than *Digitalis purpurea* (1), chemical investigations were carried out in a few laboratories. The most pertinent question to settle is whether or not the active constituents of one species are identical with those of the other. Smith (2, 3) succeeded in isolating digoxin and gitoxin, and studied the constitution of the aglycone of digoxin, digoxigenin (4). Mannich and his associates (5) announced the separation of lanata-glycosides I, II, III and IV, and later made several revisions (6, 7, 8) in the light of other workers' findings. Much systematic information has been furnished by Stoll, Kreis and Hofmann (9, 10, 11, 12, 13, 14, 15, 16). According to their results, the leaves of *Digitalis lanata* contain three polysides, digilanids *A*, *B* and *C*. The latter under suitable conditions of hydrolysis give rise to glycosides of smaller molecular sizes as follows:

Digilanid *A* → Deacetyldigilanid *A* + CH_3COOH
↓
Digitoxin + $C_6H_{12}O_6$ (glucose)
↓
Digitoxigenin + 3 $C_6H_{12}O_4$ (digitoxose)

Digilanid *B* → Deacetyldigilanid *B* + CH_3COOH
↓
Gitoxin + $C_6H_{12}O_6$
↓
Gitoxigenin + 3 $C_6H_{12}O_4$

Digilanid *C* → Deacetyldigilanid *C* + CH_3COOH
↓
Digoxin + $C_6H_{12}O_6$
↓
Digoxigenin + 3 $C_6H_{12}O_4$

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Interestingly, Stoll and Kreis (17) have shown that deacetyldigilanids *A* and *B* also occur in the leaves of *Digitalis purpurea*, although, heretofore, digitoxin and gitoxin have been long considered as the main crystalline principles of this well-known plant (18). The simpler glycosides of digilanid *C*, namely, deacetyldigilanid *C* and digoxin, are apparently absent in the leaves of *D. purpurea*.

The potencies of the above compounds have been repeatedly determined. The results as published by different workers are compiled in Table I. The discrepancies are undoubtedly due to many factors such as the strain of animals, the atmospheric temperature and variations in experimental procedures. The anesthetic in cats exerts a great influence on the outcome of tests, as is well illustrated by Kaplan and Visscher (23). Digoxin has been assayed on guinea pigs, rabbits, pigeons and dogs (23, 26, 28, 29). Quantitative evaluation of digoxin and digilanid *C* can also be made on the embryonic chick heart (30). Moe and Visscher (22), by means of the heart-lung preparation, presented evidence that digilanid *C* was especially effective in increasing the cardiac efficiency in relatively small doses, as compared with digilanids *A* and *B*. This has been questioned by Cattell and Gold (31).

The present study deals with the evaluation of digoxin, digilanids *A*, *B* and *C*, and deacetyldigilanids *A* and *B*, in cats and frogs under as nearly comparable conditions as possible. The results so obtained furnish secure grounds for correct assignment of the order of activity. Besides, they reliably demonstrate the significance of the

TABLE I.—PUBLISHED DATA ON FROGS AND CATS

Compound	Cat Lethal Dose		Frog Lethal Dose	
	Mg./Kg.	Author	Mg./Gm.	Author
Digilanid *A*	0.380	Rothlin (19, 20, 21)	0.00145	Rothlin (20)
	0.45	Moe and Visscher (22)		
	0.32	Kaplan and Visscher (23)		
	0.368	Rothlin (12)		
Digilanid *B*	0.403	Rothlin (19, 20, 21)	0.00185	Rothlin (20)
	0.65	Moe and Visscher (22)		
	0.58	Kaplan and Visscher (23)		
	0.40	Kaplan and Visscher (23)		
	0.346	Rothlin (12)		
Digilanid *C*	0.280	Rothlin (19, 20, 21)	0.00156	Rothlin (20)
	0.390	Moe and Visscher (22)	0.0016	Kwit, Gold and Cattell (24)
	0.34	Kaplan and Visscher (23)		
	0.23	Kaplan and Visscher (23)		
	0.255	Rothlin (12)		
	0.29	Kwit, Gold and Cattell (24)		
	0.261	DeGraff and Lehman (25)		
Deacetyldigilanid *A*	0.337	Rothlin (12)	0.00145	Rothlin (15, 16)
	0.368	Rothlin (15, 16)		
Deacetyldigilanid *B*	0.228	Rothlin (12)	0.00318	Rothlin (15, 16)
	0.369	Rothlin (15, 16)		
Deacetyldigilanid *C*	0.228	Rothlin (12)		
Digoxin	0.442	White (26)	1 mg. ≐ 0.28 mg. of ouabain	Trevan (2)
	0.22[a]	Chen, Chen and Anderson (27)	0.0025	Chen, Chen and Anderson (27)
	0.424	Walker (28)	0.00154	Rothlin (15, 16)
	0.335	DeGraff and Lehman (25)		
	0.280	Rothlin (21)		

[a] ±0.008.

acetyl and glucose radicals upon the potency. With the exception of digoxin, which was courteously supplied by Burroughs Wellcome and Company, Tuckahoe, New York, all the glycosides were provided by Dr. Arthur Stoll of Basel, Switzerland.

EXPERIMENTAL

To effect 0.1% stock solutions, each compound was dissolved in 47.5% ethanol (by volume), except deacetyldigilanid *B* which required 57% ethanol. Frog assays were made according to the U. S. P. (32) with dilutions of 1:4000 and 1:2000; and cat assays, according to procedures previously published (27,

TABLE II.—ASSAY IN CATS

Compound	Sex of Cat	Body Weight, Kg.	Heart Weight, Gm.	Fatal Dose by Body Weight, μg./Kg.	Fatal Dose by Heart Weight, μg./Gm.	Mean Lethal Dose by Body Weight, μg./Kg.	Mean Lethal Dose by Heart Weight, μg./Gm.
Digilanid *A*	*M*	2.702	8.4	288.7	92.9	361.0 ± 17.25	97.2 ± 4.99
	F	2.037	8.9	437.9	100.2		
	M	2.073	7.3	405.2	115.1		
	F	1.741	5.9	399.5	117.9		
	F	2.733	10.5	337.0	87.7		
	M	2.594	11.1	327.7	76.6		
	F	2.531	10.4	370.6	90.2		
	F	2.293	7.6	344.5	103.9		
Digilanid *B*	*F*	2.358	9.9	448.7	106.9	387.8 ± 27.68	98.6 ± 6.65
	F	2.196	9.3	401.5	94.8		
	M	2.265	8.1	376.2	105.2		
	F	2.663	10.2	250.1	65.3		
	F	2.110	8.6	484.4	118.8		
	F	2.343	9.0	358.5	93.3		
	F	2.343	8.5	430.2	118.6		
	M	2.442	10.1	403.8	97.6		

(Table II continued on p. 238)

Compound	Sex of Cat	Body Weight, Kg.	Heart Weight, Gm.	Fatal Dose by Body Weight, μg./Kg.	Fatal Dose by Heart Weight, μg./Gm.	Mean Lethal Dose by Body Weight, μg./Kg.	Mean Lethal Dose by Heart Weight, μg./Gm.
Digilanid *C*	*F*	2.597	10.8	219.1	52.7	232.6 ± 18.10	61.6 ± 3.50
	M	2.593	9.4	207.1	57.1		
	F	2.708	11.0	272.2	67.0		
	F	2.776	8.6	180.1	58.1		
	F	1.918	8.4	260.7	59.5		
	F	2.150	9.5	294.7	66.7		
	F	2.235	7.8	296.6	85.0		
	F	2.005	6.5	169.1	52.2		
Digoxin	*M*	2.626	9.6	181.2	49.6	235.0 ± 13.96	58.8 ± 3.61
	F	1.843	7.3	309.3	78.1		
	F	1.973	8.7	204.8	46.4		
	M	2.440	9.8	209.8	52.2		
	F	2.723	11.7	266.8	62.1		
	F	2.200	9.1	253.6	61.3		
	F	2.725	10.8	230.5	58.1		
	M	2.238	8.0	247.5	69.2		
Deacetyldigilanid *A*	*F*	1.779	8.3	530.6	113.7	469.2 ± 13.24	123.9 ± 3.49
	M	2.349	8.4	485.3	135.7		
	F	2.612	9.1	419.9	120.5		
	M	1.989	7.4	467.6	125.7		
	M	2.346	7.6	447.6	138.2		
	M	2.303	8.9	419.5	108.5		
	F	2.443	10.3	502.7	119.2		
	F	2.707	9.8	425.6	117.6		
	F	2.332	9.4	488.9	121.3		
	F	2.308	8.4	521.7	143.3		
Deacetyldigilanid *B*	*M*	2.522	9.8	420.3	108.2	548.4 ± 21.46	140.1 ± 6.19
	F	1.868	7.8	636.0	152.3		
	F	2.074	8.0	617.2	160.0		
	F	2.682	8.9	498.1	150.1		
	F	2.049	8.3	581.7	143.6		
	M	2.254	8.4	587.4	157.6		
	F	2.160	8.8	575.0	141.1		
	F	1.968	8.1	558.9	135.8		
	F	1.586	5.7	552.3	153.7		
	M	2.968	13.3	493.3	110.1		

33). It was found that for intravenous injection in cats at the rate of 1 cc. per minute, a solution of 1:100,000 was best suited for digoxin and digilanid *C;* one of 1:50,000, for digilanids *A* and *B;* and one of 1:25,000, for deacetyldigilanids *A* and *B*. Upon the death of each animal, the heart was dissected out and weighed, so that the individual dose could be calculated on the basis of heart weight, in addition to body weight.

RESULTS

The results in cats are shown in Table II. There is a high correlation between heart weight and body weight. It should be noted that digilanids *A* and *B* are less potent in cats than digilanid *C*. This is in agreement with our predecessors' reports (see Table I). Digoxin and digilanid *C* have practically the same activity. In cats, the larger molecule of the latter apparently does not diminish the potency as compared with the former, namely, digoxin. Previously, it was demonstrated that periplocin was actually stronger than periplocymarin while K-strophanthoside proved weaker than K-strophanthin-β (34). Thus, the effect of the extra sugar molecule on the activity of simpler glycosides is unpredictable. Aglycones are, however, less potent than their parent glycosides (35)—indicating the importance of the sugar radicals in simpler molecules.

According to our data, in cats (Table II) deacetyl-

TABLE III.—ASSAY IN FROGS BY THE 1-HR. METHOD

Compound	Dose, μg./Gm.	No. of Frogs in Systole/ No. of Frogs Used	SD_{50} ± S.E., μg./Gm.
Digilanid *A*	1.3	2/10	1.61 ± 0.13
	1.6	5/10	
	2.0	4/5	
	3.0	5/5	
Digilanid *B*	5.0	0/5	5.94 ± 0.60
	6.0	3/5	
	7.0	4/5	
	9.0	5/10	
Digilanid *C*	2.75	0/5	6.24 ± 0.44
	3.5	1/5	
	6.0	4/10	
	7.0	8/15	
	8.0	12/15	
	9.0	5/5	
Digoxin	3.0	0/5	4.22 ± 0.32
	4.0	6/10	
	5.0	6/10	
	6.0	5/5	
Deacetyl-digilanid *A*	1.6	2/10	2.40 ± 0.17
	2.0	3/10	
	2.5	6/10	
	3.5	7/10	
	4.0	5/5	
Deacetyl-digilanid *B*	5.0	1/5	5.58 ± 0.53
	5.5	2/5	
	6.0	4/5	
	7.0	4/5	

digilanids *A* and *B* are less potent than digilanids *A* and *B*, suggesting the favorable influence of the acetyl group on the cardiac action. The acetyl group in both compounds is presumably also attached to one of the digitoxose molecules as in the case of digilanid *C* (14). The differences observed in this investigation are far more decisive than those recorded by Rothlin (12, 15, 16). It must be pointed out that among the synthetic glycosides of strophanthidin the deacetyl members are much more potent than the acetyl derivatives (36).

The results in frogs as summarized in Table III are extraordinary in that they do not correspond to those in cats. In fact they reverse the order of activity in several instances. Our confidence in the data is enhanced by the fact that frogs from the same lot were employed for a repetition of the assay by the same person with similar results. Digilanid *A* is far more potent than digilanids *B* and *C*. Digoxin is significantly more active than digilanid *C*. While deacetyldigilanid *A* is less potent than digilanid *A*, deacetyldigilanid *B* and digilanid *B* have closely similar median systolic doses. The rating of activity of the six glycosides in frogs can be expressed as follows: digilanid *A*, 100; deacetyldigilanid *A*, 67.2; digoxin, 38.2; deacetyldigilanid *B*, 28.9; digilanid *B*, 27.1; and digilanid *C*, 25.8.

SUMMARY

Six glycosides of *Digitalis lanata*, digoxin, digilanids *A*, *B* and *C* and deacetyldigilanids *A* and *B*, have been assayed in cats and frogs. In cats, the order of activity from high to low is: digoxin and digilanid *C*, digilanids *A* and *B*, deacetyldigilanid *A*, deacetyldigilanid *B*. The results in frogs do not follow those in cats. The order of potency from high to low is: digilanid *A*, deacetyldigilanid *A*, digoxin, deacetyldigilanid *B*, digilanids *B* and *C*. The differences of the last three compounds are apparently not significant.

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