SCIENTIFIC SECTION

THE POTENCY OF ELEVEN CRYSTALLINE CARDIAC PRINCIPLES FROM PLANTS.*,[1]

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In connection with our work on bufagins and bufotoxins isolated from the paratoid secretions of different species of toads (1), comparative studies were made with several crystalline substances of plant origin reported to have a digitalis-like action. In view of the fact that they have never been assayed under the same conditions, the presentation of our data may prove helpful to other workers who are interested in their relative activity. Furthermore, the differences in potency noted in this work may throw some light upon the significance of certain groups in their chemical structure and stereoisomerism, since progress in this field has been rapidly made (2), (3). The list includes convallatoxin, β-antiarin, ouabain, cymarin, scillaren A, uzarin, digoxin, digitoxin, gitoxin, erythrophlein sulphate and thevetin. With the exception of erythrophlein, which is an alkaloid of *Erythrophlœum guineense*, the other compounds are glucosides. The authors are indebted to Dr. W. A. Jacobs, the Rockefeller Institute for Medical Research, New York City, for a generous supply of cymarin, to Professor R. L. Stehle, McGill University, Montreal, for that of scillaren A, and to Dr. R. Tschesche, University of Göttingen, Germany, for that of uzarin and β-antiarin. Thevetin was isolated by ourselves from *Thevetia neriifolia* (4), while ouabain, erythrophlein sulphate and digitoxin were purchased from Merck and Company, Rahway, New Jersey, convallatoxin from Hoffmann-La Roche, Inc., Nutley, New Jersey, and digoxin and gitoxin from Burroughs Wellcome and Company, Tuckahoe, New York. The last two principles were first isolated by Smith from *Digitalis lanata* (5).

Of the eleven compounds, gitoxin is so insoluble in alcohol or water that its action and potency could not be accurately determined. The remaining substances are soluble in dilute alcohol. Ouabain and erythrophlein sulphate are the only members that are completely soluble in water. In our experiments, a stock solution of each to contain 0.1 per cent of the drug was prepared. Cymarin, scillaren A, digoxin and digitoxin required 47.5 per cent ethyl alcohol to effect solution (one part 95 per cent alcohol and one part saline), and convallatoxin and β-antiarin 28.5 per cent (three parts 95 per cent alcohol and seven parts water). With thevetin, 19 per cent alcohol was employed. Both ouabain and erythrophlein were dissolved in saline. Uzarin was made up into 1 or 2 per cent solution in 47.5 per cent alcohol. Appropriate dilutions were then made from the stock solution for the tests desired.

It may be pointed out that erythrophlein is the only alkaloid known to have a digitalis-like action (6), (7). The specimen (in the form of a sulphate) we obtained

* From the Lilly Research Laboratories, Indianapolis, Indiana.

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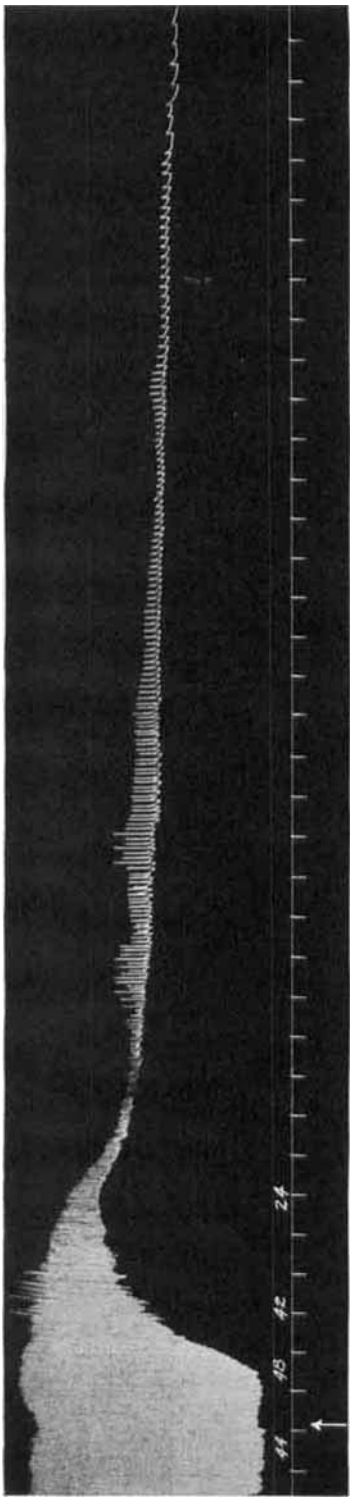


Fig. 1.—Action of Erythrophlein on Frog's Heart. Frog No. 567, female, weighing 61 Gm., was decerebrated and pithed. At arrow, the heart was perfused with erythrophlein sulphate, 1:50,000, *via* inferior vena cava.

was beautifully crystalline, and when heated in a capillary tube immersed in an oil bath it began to soften at 68° C., became a clear viscous mass at 88° C., and decomposed with evolution of gas at 140° C. The substance reduced Tollen's reagent but gave negative results with Legal, Liebermann-Burchard and Sakaguchi tests. Combustion analyses yielded the following figures:

C 59.12; H 8.24; N 3.12; S 2.42
C 59.15; H 8.31; N 3.09; S 2.53

It is possible that Harnack's empirical formula (8), $C_{28}H_{43}NO_7$ or $C_{28}H_{45}NO_7$, needs revision.

By perfusion into the inferior vena cava in frogs according to the method of Howell and Cooke (9), A-V dissociation and ventricular systolic standstill were demonstrated, as shown in Fig. 1. Convincing evidence of the digitalis-like action of erythrophlein sulphate on the mammalian heart such as that of the cat was recorded on the electrocardiogram by following the technique previously described (10), a 1:50,000 solution being injected intravenously at the rate of 1 cc. per minute. Figure 2 may be taken as an example to illustrate P-R prolongation, bradycardia, ectopic rhythm, secondary tachycardia, and finally ventricular fibrillation. In addition, the alkaloid caused nausea and vomiting in both pigeons and cats so that there is no doubt about its digitalis-like effect.

Regarding uzarin Gessner (11) observed systolic standstill of the amphibian ventricle either by perfusion or *in situ*. In our studies it was found that in frogs (*Rana pipiens*) a 1:5000 solution perfused into the inferior vena cava caused a moderate slowing of the heart rate and some decrease in amplitude of contractions, but no systolic stoppage. By injection into the lymph sac in frogs, however, large doses induced systolic standstill.

1. The Hatcher-Brody Method (12).—The solutions of the substances were so adjusted that when injected into the femoral vein at the rate of 1 cc. per minute they would kill a cat weighing less than 3 Kg. within about $1^1/_2$ hours. This time limit was arbitrary but seemed to be

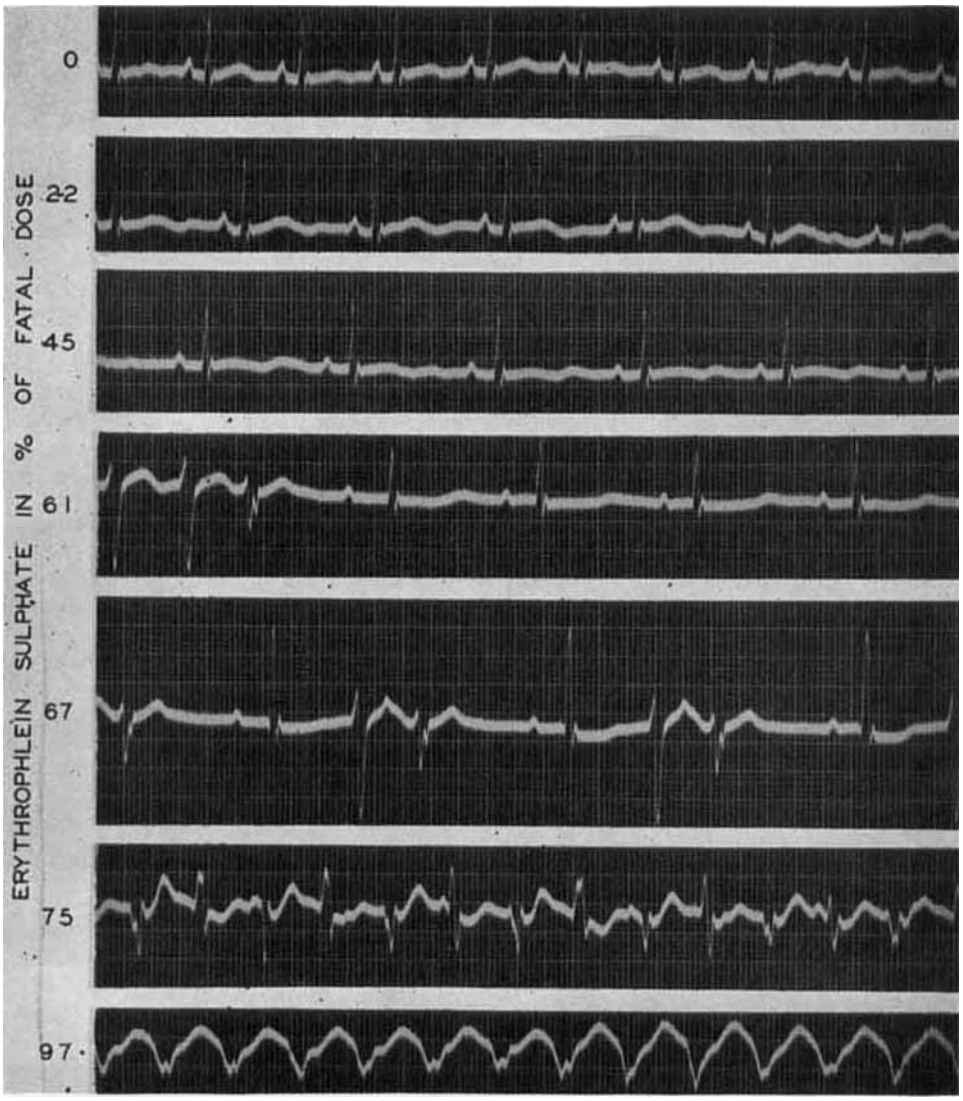


Fig. 2.—Electrocardiographic Changes Caused by Erythrophlein. Cat No. 1392, female, weighing 2.068 Kg., was anesthetized by ether. Erythrophlein sulphate in 1:50,000 solution was injected into the femoral vein at the rate of 1 cc. per minute. A total of 28 electrocardiograms was taken from lead II at different stages of this experiment. Only 7 selected tracings are shown in the figure. The abscissal time-lines make divisions equal to 0.02 and 0.10 second, and the ordinates those representing 0.0001 and 0.0005 volt.

suitable for both the easily eliminated compounds, such as ouabain, and the slowly acting ones, such as digitoxin. A stethoscope was used for the determination of the end-point. A group of 10 or more animals was employed in order to obtain an average of some statistical significance. The results are shown in Table I and summarized in Table IV. It should be noted that ouabain,

which for a long time has been known as the most potent of all cardiac glucosides, now ranks third according to the cat unit. The superiority of convallatoxin to ouabain in frogs has been noted by Karrer (13). Previously (1), it was shown that four bufagins were more powerful than ouabain. It may also be pointed out that in a series of 53 animals the cat unit of thevetin was found to be 0.92 mg. per Kg. as compared with 0.85 mg. in 16 cats, which has been reported before (14). The potency of uzarin is very low and variable. As indicated in Table I, the cat unit varied from 1.83 to 8.33 mg. per Kg., the average being 5.08 mg. There were 2 cats which did not die even with 14.5 and 13.2 mg. per Kg., respectively. They were not included in the table. Apparently certain animals can tolerate huge doses of uzarin without fatal outcome. The cat unit of digoxin in our series of experiments is equal to just one-half of that reported by White (15). Without knowing the strength of his solution and speed of his injection, it is difficult to account for the discrepancy.

2. The U. S. P. Frog Method (16).—The results by this well-known method are given in Tables II and IV. As a whole, they bear out the same order of activity of the different substances as that obtained by the cat method, except that thevetin in frogs proves to be more powerful than digitoxin and erythrophlein, while in cats the reverse is true. Quantitatively, the two methods do not yield the same ratio of activity among the different principles. For example, convallatoxin is fully 38 times as active as digitoxin in frogs, but is only 4.3 times as potent as the latter in cats. In some instances the difficulty of absorption in frog's lymph sac apparently accounts for the differences, but in a few cases, especially with erythrophlein, the material may be actually less poisonous to frogs than to cats.

3. The Cat Minimal Emetic Dose.—In previous reports (1), (17), mention was made of the minimal emetic doses of several substances. More complete and detailed data are presented in Table III. With convallatoxin, β-antiarin and ouabain, a 1:2000 solution was adopted, but with the remaining compounds a 1:1000 solution was employed. All injections were made intravenously in unanesthetized cats. Vomiting occurred in 10 to 15 minutes in the majority of instances, but with digitoxin, it was often delayed for 30 to 50 minutes, indicating its slowness of action. An examination of Table IV at once shows that the emetic effect does not follow the cardiac action, a phenomenon already observed by Eggleston and Hatcher (18). According to our data uzarin is the most powerful in causing vomiting. A dose equivalent to about 7 per cent of the average cat unit, rapidly injected, was sufficient to induce emesis in the majority of animals. Erythrophlein sulphate, on the other hand, required 81 per cent of the average cat unit as its minimal emetic dose. With the remaining substances, they varied from 24 to 75 per cent of their respective cat units.

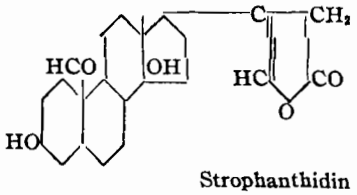
4. Persistence of Action.—By determining the fatal dose under ether anesthesia at various intervals after the initial injection in those cats which were used for the emesis test, information may be obtained regarding how long each substance stays in the body or is fixed in the heart; in other words, the persistence of action. Digitoxin was found to have the most prolonged effect, for 38 per cent of the cat unit persisted for more than 5 days. Digoxin, cymarin, convallatoxin and erythrophlein sulphate proved to be relatively persistent since one-half of the cat unit in each case remained in the circulation for 1 to 7 days. β-Antiarin, ouabain, thevetin and scillaren A were more rapidly eliminated. The least persistent drug was uzarin because 50 per cent of the cat unit often disappeared within an hour. The order of persistence of these substances is, therefore, digitoxin, digoxin, cymarin, convallatoxin, erythrophlein, β-antiarin, ouabain, thevetin, scillaren A and uzarin.

DISCUSSION.

Table IV clearly indicates that there is a disagreement of the results by three different methods of assay. It appears that digitoxin and erythrophlein are inherently more potent to feline than to amphibian hearts. The cat minimal emetic dose does not follow the frog's minimal systolic dose or cat unit. These data will be of importance from a therapeutic point of view, for none of the newer products should be applied to men unless their exact potency is known. The clinicians in this country appear to prefer the cat unit instead of the frog minimal systolic dose (19), (20).

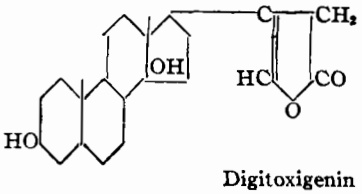
The results are also interesting from a chemical point of view. It is now well known that upon hydrolysis the cardiac glucosides yield aglucones of C_{23} derivatives and one or more molecules of a sugar or sugars (21), (22), (23), (24). Scillaridin A, the aglucone of scillaren A, however, conforms to the C_{24} formula (25). Furthermore, many of the bufagins which occur in toad poisons and which are closely related to aglucones, do not have the C_{23} pattern (17). The structural formulas of several aglucones have been proposed and in certain instances established with certainty. They are derivatives of cyclopentenophenanthrene with an unsaturated lactone side chain. A consideration of our results in light of the following equations should be, therefore, of some interest. These structural formulas were suggested by Jacobs and Elderfield (26), Kon (27), Tschesche and Bohle (28), Tschesche and Haupt (29), Stoll, Hofmann and Helfenstein (25), Smith (30) and Elderfield (31).

$C_{30}H_{44}O_9 + H_2O \longrightarrow C_7H_{14}O_4$
Cymarin — Cymarose



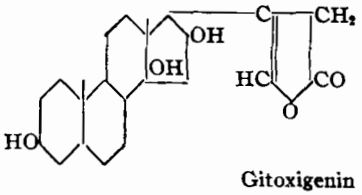
Strophanthidin

$C_{41}H_{64}O_{13} + 3H_2O \longrightarrow 3C_6H_{12}O_4$
Digitoxin — Digitoxose



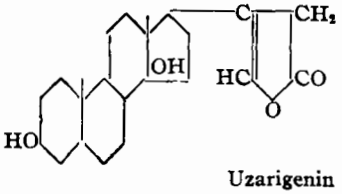
Digitoxigenin

$C_{41}H_{64}O_{14} + 3H_2O \longrightarrow 3C_6H_{12}O_4$
Gitoxin — Digitoxose



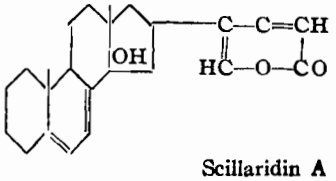
Gitoxigenin

$C_{36}H_{54}O_{14} \cdot 2H_2O \longrightarrow 2C_6H_{12}O_6 + H_2O$
Uzarin — Glucose



Uzarigenin

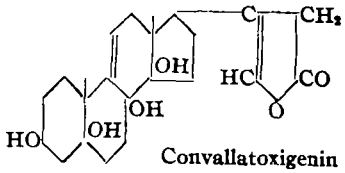
$C_{36}H_{52}O_{13} + H_2O \longrightarrow C_6H_{12}O_6 + C_6H_{12}O_5$
Scillaren A — Glucose — Rhamnose



Scillaridin A

$C_{29}H_{42}O_{10} + H_2O \longrightarrow C_6H_{12}O_5$
Convallatoxin Rhamnose

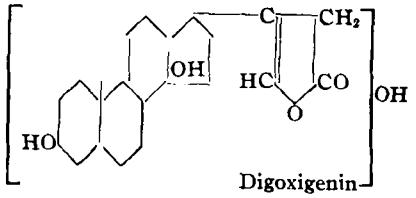
+



Convallatoxigenin

$C_{41}H_{64}O_{14} + 3H_2O \longrightarrow 3C_6H_{12}O_4$
Digoxin Digitoxose

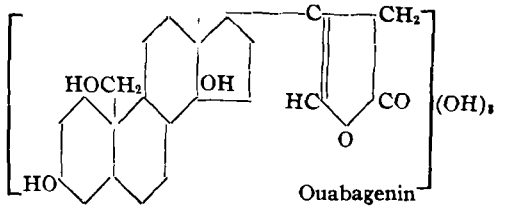
+



Digoxigenin

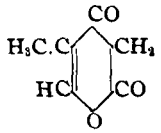
$C_{29}H_{44}O_{12} + H_2O \longrightarrow C_6H_{12}O_5$
Ouabain Rhamnose

+

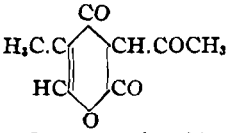


Ouabagenin

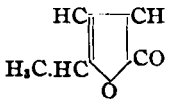
The most significant feature is that uzarin, which has an aglucone isomeric with digitoxigenin, has a very feeble cardiac action. The difference in steric arrangements probably accounts for the difference in their activity. Among the active members, the variation in potency is very great. For example, digoxin is 275 per cent, and digitoxin 412 per cent, less powerful than convallatoxin, gram for gram. It is possible that the two hydroxyl groups on C^5 and C^8 and the double bond between C^9 and C^{11} in the convallatoxigenin molecule are of significance in contributing to the high potency of convallatoxin. One is tempted to postulate that the presence of a carbinol group on C_{10} in the aglucone molecule of ouabain, and that of an aldehyde group on the same C-atom in the aglucone molecule of cymarin are also favorable to the high cardiac activity of the two substances. The sugar component alone has no action on the heart, but when conjugated with an aglucone it increases the potency (32). There is some suggestion from the results that the glucose-containing glucosides are rapidly eliminated while the digitoxose- or cymarose-containing members have a persistent action. The cyclopentenophenanthrene ring system apparently has no effect on the heart, for estrogenic hormones, bile acids and sterols have never been reported to stimulate the vagus or affect the myocardium like digitalis. The most important parts of the molecule are undoubtedly the double bond and the lactone ring, for hydrogenation and saponification both result in loss of activity (33), (34), (35), (36). It will be interesting to investigate pharmacologically the lactone side chains (α-pyrone and 2,3-dihydro-α-furone) of certain aglucones without the sterol ring system attached to them. The simple unsaturated lactones as listed below are devoid of any digitalis-like action, as concluded from our preliminary studies.



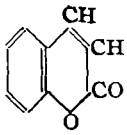
Triacetic lactone



Dehydracetic acid



α-Angelica lactone



Coumarin

The digitalis-like action of the alkaloid erythrophlein is of unusual interest. The negative Sakaguchi test indicates that the compound does not belong to the group of bufotoxins which also contains nitrogen. The failure of the sodium nitroprusside test makes it deviate from the constitution of common aglucones. As yet, there is no evidence that erythrophlein is a derivative of cyclopentenophenanthrene. The elucidation of its structure may thus lead to the discovery of another type of substances having a digitalis-like action but differing structurally from aglucones, bufagins and bufotoxins.

SUMMARY.

The potency of 11 crystalline digitalis-like substances—convallatoxin, β-antiarin, ouabain, cymarin, scillaren A, digoxin, digitoxin, erythrophlein sulphate, thevetin, uzarin and gitoxin—has been compared under the same conditions by the cat unit, the frog minimal systolic dose and the cat minimal emetic dose. Both convallatoxin and β-antiarin are more powerful than ouabain. The cat unit of thevetin should be revised to 0.92 ± 0.035 mg. per Kg. instead of 0.85 as previously reported.

The emetic action does not run parallelly with the cardiac action. Uzarin having the least cardiac effect is very highly efficient in causing vomiting.

The order of persistence of action from high to low is digitoxin, digoxin, cymarin, convallatoxin, erythrophlein, β-antiarin, ouabain, thevetin, scillaren A and uzarin.

Gitoxin is so insoluble in alcohol and water that its cardiac action cannot be accurately determined.

The significance of certain chemical structures with reference to their pharmacological activity has been discussed.

TABLE I.—CAT UNITS OF 10 CARDIAC PRINCIPLES OF PLANT ORIGIN.

Drug.	Solution.	Cat No.	Sex.	Weight, Kg.	Fatal Dose, Mg. per Kg.	Drug.	Solution.	Cat No.	Sex.	Weight, Kg.	Fatal Dose, Mg. per Kg.
Convallatoxin	1:400,000	1438	M	2.477	0.06	Digitoxin	1:100,000	491	M	1.459	0.37
		1439	F	1.848	0.06			492	M	1.944	0.32
		1440	F	1.889	0.08			494	F	1.590	0.34
		1441	F	2.537	0.08			495	M	1.452	0.41
		1442	M	2.798	0.08			496	M	1.608	0.28
		1445	F	2.489	0.06			501	F	1.647	0.34
		1449	F	2.261	0.09			502	F	1.800	0.34
		1450	F	2.406	0.10			503	M	1.512	0.27
		1451	F	1.707	0.07			504	F	1.972	0.31
		1452	F	1.823	0.08			505	M	1.733	0.31

TABLE I.—CAT UNITS OF 10 CARDIAC PRINCIPLES OF PLANT ORIGIN.—(*Continued from page 585.*)

Drug.	Solution.	Cat No.	Sex.	Weight, Kg.	Fatal Dose, Mg. per Kg.
β-Antiarin	1:200,000 and 1:100,000	1444	F	2.381	0.13
		1446	F	1.632	0.11
		1447	F	1.915	0.12
		1448	F	2.737	0.12
		1453	F	2.543	0.07
		1454	M	2.879	0.10
		1455	F	2.227	0.09
		1456	F	2.634	0.12
		1457	F	2.120	0.09
		1458	M	2.076	0.09
Ouabain	1,100:000	30	F	2.290	0.14
		31	F	1.830	0.15
		32	F	2.264	0.09
		33	M	1.846	0.10
		34	F	2.815	0.11
		35	F	2.417	0.14
		36	F	2.171	0.14
		37	F	2.703	0.10
		40	M	2.353	0.13
		41	M	2.660	0.11
		42	M	2.967	0.12
		43	M	1.846	0.14
		44	F	2.828	0.12
		45	F	1.750	0.12
		98	M	2.157	0.11
		99	M	2.263	0.12
		100	M	2.135	0.12
		101	M	2.155	0.13
		102	M	1.928	0.11
		129	M	2.114	0.11
		130	F	2.286	0.11
		131	F	2.215	0.11
		558	M	1.492	0.15
		559	F	1.411	0.13
		560	F	1.713	0.12
		561	M	2.409	0.11
		562	F	1.880	0.17
		563	M	2.011	0.12
		564	F	1.958	0.10
		565	M	1.826	0.13
		566	M	1.553	0.13
		567	M	1.790	0.10
		699	F	2.330	0.10
		698	M	2.273	0.09
		700	M	2.280	0.13
		701	F	2.261	0.12
		702	F	1.990	0.12
		1144	F	1.793	0.15
		1145	F	1.830	0.14
		1146	M	2.151	0.15
		506	F	1.891	0.29
		538	F	1.519	0.36
Erythrophlein Sulphate	1:50,000	1169	F	1.650	0.48
		1170	M	2.064	0.42
		1171	M	2.740	0.34
		1172	F	2.310	0.55
		1173	F	2.245	0.34
		1174	M	2.210	0.30
		1175	F	2.758	0.36
		1176	F	2.470	0.38
		1177	M	3.060	0.28
		1178	M	2.651	0.32
		1179	F	2.052	0.30
Thevetin	1:20,000	1070	M	2.124	0.85
		1071	M	1.800	0.69
		1072	M	1.880	0.67
		1073	F	1.785	0.70
		1074	F	2.274	0.83
		1079	F	2.186	0.79
		1080	F	1.775	0.79
		1081	M	1.992	0.81
		1082	F	1.762	1.36
		1083	M	1.808	0.77
		1084	F	2.140	1.36
		1085	F	2.060	0.67
		1086	F	2.850	1.24
		1089	M	1.536	0.62
		1090	M	3.300	0.59
		1091	M	1.720	0.80
		1291	M	2.436	0.93
		1290	F	2.314	1.04
		1229	F	2.370	0.63
		1228	F	2.855	0.60
		1259	F	1.690	1.14
		1257	F	2.184	0.84
		1256	F	2.292	0.61
		1260	M	1.951	0.80
		1258	F	1.533	0.72
		1253	F	2.209	0.63
		1254	M	2.480	0.65
		1255	F	2.111	1.14
		1244	F	2.422	1.75
		1242	F	2.837	0.68
		1240	F	2.495	1.00
		1241	M	1.910	0.78
		1238	M	2.070	0.94
		1236	F	1.924	0.77
		1237	F	2.470	1.20
		1269	F	2.157	1.16
		1264	F	2.607	1.72

Cymarin 1:100,000	1147	M	2.360	0.12	Uzarin 1:3000	1266	M	2.739	1.34
	1148	M	2.333	0.11		1265	F	2.457	1.17
	1149	F	1.646	0.12		1263	F	2.185	0.99
	1150	F	1.962	0.12		1262	M	2.697	1.03
	1151	F	1.964	0.11		1261	F	2.423	1.47
	1152	F	1.990	0.11		1271	F	2.177	0.88
	1153	F	2.034	0.12		1270	F	2.260	1.19
	1154	F	1.822	0.13		1280	F	2.006	0.98
						1278	F	2.128	1.06
Scillaren A 1:100,000	594	F	1.762	0.19		1224	F	3.215	0.83
	595	F	2.608	0.09		1223	M	3.025	0.56
	596	M	1.770	0.16		1166	F	2.290	0.88
	597	F	1.765	0.16		1165	M	1.818	0.99
	598	M	1.827	0.21		1168	F	2.283	0.81
	602	F	2.073	0.15		1167	M	2.116	0.91
	603	M	1.612	0.15		1109	F	2.196	0.83
	604	F	1.749	0.14		1110	F	1.955	0.87
	605	F	2.155	0.11		1111	F	2.260	0.76
	606	F	1.700	0.15		1106	M	2.145	0.82
						1107	F	2.773	1.15
						1108	F	2.833	0.74
Digoxin 1:100,000	1198	M	2.265	0.28					
	1199	F	1.955	0.28		1402	F	1.889	3.07
	1200	M	2.290	0.17		1403	F	1.960	6.72
	1201	M	2.528	0.26		1404	F	1.764	2.91
	1202	M	1.882	0.25		1405	M	1.600	1.83
	1203	F	2.075	0.23		1406	F	1.565	5.98
	1204	F	1.910	0.21		1407	F	2.543	3.85
	1205	F	1.830	0.24		1410	F	2.537	7.96
	1206	M	2.538	0.17		1418	F	2.100	3.62
	1207	M	1.830	0.22		1420	F	2.023	6.52
						1421	F	2.717	8.33

TABLE II.—FROG MINIMAL SYSTOLIC DOSES OF 10 CARDIAC PRINCIPLES OF PLANT ORIGIN.

Drug.	Solution.	Dose, Mg. per Kg.	No. in Systolic Standstill/ No. of Frogs Used.
Convallatoxin	1:40,000	0.00014	0/4
		0.00018	2/8
		0.00021	3/4
		0.00025	3/4
β-Antiarin	1:20,000	0.00032	1/4
		0.00035	1/4
		0.00039	10/16
		0.00043	4/4
Ouabain	1:20,000	0.00040	0/4
		0.00045	0/4
		0.00050	3/4
		0.00055	4/4
Cymarin	1:20,000	0.00050	0/4
		0.00055	0/4
		0.00060	3/4
		0.00065	3/4
		0.00070	4/4
Scillaren A	1:20,000	0.00065	0/7
		0.00070	7/12
		0.00075	4/7

TABLE II.—(*Continued from page 587.*)

Drug.	Solution.	Dose, Mg. per Kg.	No. in Systolic Standstill/ No. of Frogs Used.
Digoxin	1:5000	0.00225	1/4
		0.00250	3/4
		0.00300	7/8
Digitoxin	1:2000	0.00650	0/4
		0.00700	3/12
		0.00750	4/8
		0.00800	6/8
		0.00850	4/4
Erythrophlein Sulphate	1:1000	0.01000	1/4
		0.01100	5/8
		0.01200	3/4
		0.01300	7/8
Thevetin	1:2000	0.00400	0/12
		0.00450	18/24
		0.00500	7/8
Uzarin	1:50	1.00000	1/4
		1.10000	0/1
		1.20000	0/1
		1.30000	0/1
		1.40000	0/3
		1.50000	2/3

TABLE III.—MINIMAL EMETIC DOSES OF 10 CARDIAC PRINCIPLES OF PLANT ORIGIN IN CATS.

Drug.	Dose, Mg. per Kg.	No. of Cats Vomited/ No. of Cats Used.
Convallatoxin	0.050	0/2
	0.060	2/3
	0.070	2/3
	0.080	2/2
β-Antiarin	0.030	0/3
	0.040	2/2
	0.050	2/2
Ouabain	0.050	0/2
	0.060	2/3
	0.070	2/2
Cymarin	0.070	0/3
	0.080	3/3
	0.090	2/2
Scillaren A	0.080	1/3
	0.090	1/3
	0.100	2/2
Digoxin	0.060	0/2
	0.070	2/2
	0.080	2/3
Digitoxin	0.100	0/2
	0.125	1/3
	0.150	2/2
Erythrophlein Sulphate	0.200	0/2
	0.250	1/3
	0.275	1/3
	0.300	2/2

	0.200	0/2
	0.225	2/3
Thevetin	0.250	2/2
	0.275	2/2
	0.300	2/2
	0.300	0/2
	0.325	0/2
Uzarin	*0.350*	2/3
	0.400	1/1
	0.500	1/1

TABLE IV.—SUMMARY OF ALL RESULTS.

Drug.	Cat Unit (Mean ± Probable Error), Mg. per Kg.	Frog Minimal Systolic Dose, Mg. per Kg.	Minimal Emetic Dose in Cats, Mg. per Kg.
Convallatoxin	0.08 ± 0.002	0.00021	0.060
β-Antiarin	0.10 ± 0.004	0.00039	0.040
Ouabain	0.12 ± 0.002	0.00050	0.060
Cymarin	0.13 ± 0.003	0.00060	0.080
Scillaren A	0.15 ± 0.007	0.00070	0.100
Digoxin	0.22 ± 0.008	0.00250	0.075
Digitoxin	0.33 ± 0.008	0.00800	0.150
Erythrophlein Sulphate	0.37 ± 0.017	0.01100	0.300
Thevetin	0.92 ± 0.035	0.00450	0.225
Uzarin	5.08 ± 0.437	1.50000	0.350
Gitoxin*	Unable to determine	Unable to determine	Unable to determine

* Insoluble.

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STRYCHNINE VI. VARIATION IN PHYSIOLOGICAL ACTION OF C.P. STRYCHNINE.*

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For many years, men using strychnine alkaloid for the control of noxious rodents and predatory animals have noticed variation in the results obtained. The earlier explanations were that variations in field and animal conditions or in the methods of placing the poison baits were responsible. It was incredible that a substance as chemically stable as strychnine would not be uniform in its toxic properties. Wide-spread complaints, traceable to the same lot of poison, have become so prevalent within the past few years, however, that we have been forced to recognize the probability that something was wrong with those lots.

Since we had, for several years, been running tests for the "free base," and the crystal size of the incoming lots of alkaloid, we made an attempt to associate the per cent of free base or the physical size of the particles with the reported troubles. There was no correlation—in fact, the alkaloid carrying the lowest free base very often proved the most efficient toxic agent, and the crystal size was entirely too variable a factor for any conclusions to be drawn. The most toxic alkaloids and the least toxic were often practically the same size—either large or small.

During the past few years, the Denver laboratory has tested biologically 27 lots of the alkaloid, originating from five wholesale distributors. Our system of tests has been by means of stomach tube to white rats. A test suspension of the alkaloid in the concentration of 1 mg. of strychnine to each cc. of suspension was made with the assistance of 1 per cent acacia. The animals to be used were weighed and divided into comparable series. The doses were computed for each animal, and the dose for the first rat measured out in a hypodermic syringe. The animal was then placed on a holding board and a wooden gag, having a $^3/_{16}$" hole in it, was placed in its mouth. A No. 8 soft rubber catheter was then passed through the hole in the gag into the animal's stomach. The syringe was shaken to insure

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