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Relative Susceptibility of Warm Blooded Animals to Ouabain, Cymarin, and Coumingine Hydrochloride.

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In previous reports^{1, 2, 3} from this laboratory, it was shown that the nebulous toad and the spadefoot toad were more tolerant to ouabain, cymarin, and coumingine HCl than the Leopard frog and the tree frog. The work has now been extended to warm blooded animals with the same specimens of the digitalis-like substances, and the results may be presented as further examples of species difference of drug reaction.

Albino mice, albino rats, guinea pigs, New Zealand red rabbits, and cats were employed. The average weights were mice 16.3 g, rats 89.9 g, guinea pigs 334.1 g, rabbits 1.83 kg, and cats 2.38 kg. All drugs were administered subcutaneously, so that the data may be comparable to those already published⁴ on frogs and toads by injection into the ventral lymph sac. Solutions of each substance varying from 0.01 to 0.1% were used in most instances except in rats. On account of the low susceptibility of the latter, a 1% solution of ouabain and cymarin was required in order that the volume might not be excessive. Ethyl alcohol was added to ouabain and cymarin to effect a 1% solution—being 9.5 and 57% by volume, respectively. The alcohol was also present in the 0.1% solution of cymarin to the extent of 9.5% by volume. The remaining concentrations and dilutions were all prepared with water.

Following the subcutaneous injection of various doses of ouabain, cymarin, and coumingine HCl, a majority of the animals died within 24 hours, or fully recovered. A few rats succumbed on the second or third day. In any event, the injected animals were observed for one week, so that any delayed deaths, very few in the series, could be recorded. Control animals injected with an equivalent, or twice the amount of alcohol all survived. The protocols are summarized in Table I, and the median lethal doses ($LD_{50} \pm$ standard error) and the approximate ratios of susceptibility are computed and given in

¹ Chen, K. K., and Chen, A. L., *J. Pharm. and Exp. Therap.*, 1933, **47**, 295.

² Chen, K. K., Hargreaves, C. C., and Winchester, W. T., *J. Am. Pharm. A.*, 1938, **27**, 307.

³ Blair, A. P., Hargreaves, C. C., and Chen, K. K., *PROC. SOC. EXP. BIOL. AND MED.*, 1940, **45**, 209.

Table II. There can be little doubt from the results that cats, rabbits, and guinea pigs are much more susceptible than rats and mice, particularly with reference to ouabain and cymarin. The natural resistance of the rat to strophanthin and digitalis has been known for sometime in accordance with the works of Hatcher⁴ and Gunn.⁵ Our observations made with different substances are in full agreement with theirs. Since the mouse very frequently follows the rat in response to various drugs, it is not surprising that it, too, possesses natural tolerance to digitalis-like products.

It is interesting to note that coumagine HCl, an alkaloid having a digitalis-like action, confers a lower ratio of susceptibility on mice and rats, being 1:13 and 1:29, respectively, if the cat is taken as

TABLE I.
Acute Toxicity of Ouabain, Cymarin, and Coumagine HCl in 5 Species of Animals by Subcutaneous Injection.

Animal	Ouabain		Cymarin		Coumagine HCl	
	Dose: mg per kg	No. died No. inj.	Dose: mg per kg	No. died No. inj.	Dose: mg per kg	No. died No. inj.
Mouse	4.0	2/8	18.0	4/8	2.0	2/8
	6.2	3/8	22.5	4/8	4.0	2/8
	8.0	4/8	27.5	5/8	6.0	8/8
	12.0	7/8	33.0	6/8	8.0	8/8
	16.0	7/8				
	22.5	8/8				
Rat	27.5	1/5	40.0	0/5	6.0	0/5
	40.0	1/5	50.0	1/5	8.0	5/10
	62.0	1/5	62.0	5/5	10.0	9/10
	80.0	1/5	70.0	5/5	12.0	5/5
	120.0	5/5			14.0	5/5
	160.0	5/5				
Guinea pig	.140	0/5	.275	0/5	.140	0/4
	.180	0/5	.500	3/5	.180	2/5
	.225	3/5	1.000	5/5	.225	4/5
	.275	6/6	2.000	5/5	.275	4/5
					.330	5/5
					.400	5/5
Rabbit	.110	0/5	.225	0/5	.110	0/5
	.180	0/5	.330	2/5	.180	0/5
	.275	3/5	.500	5/5	.275	3/5
	.400	5/5	.600	5/5	.400	5/5
Cat	.100	1/5	.125	0/3	.180	0/5
	.125	4/5	.160	1/6	.225	0/6
	.140	8/11	.200	4/6	.275	2/5
	.160	7/8	.250	5/5	.330	5/5
	.200	5/6			.400	4/4

⁴ Hatcher, R. A., *Am. J. Physiol.*, 1909, **23**, 303.

⁵ Gunn, J. A., *J. Pharm. and Exp. Therap.*, 1912-13, **4**, 225.

TABLE II.
Ratio of Susceptibility.

Animal	Drug	LD ₅₀ ± S.E. mg per kg	Approximate ratio of susceptibility when the cat is unity
Cat	Ouabain	.1119 ± .0158	1
	Cymarin	.1853 ± .0102	1
	Coumingine HCl	.2790 ± .0126	1
Rabbit	Ouabain	.2648 ± .0265	1:2
	Cymarin	.3416 ± .0290	1:2
	Coumingine HCl	.2648 ± .0265	1:1
Guinea Pig	Ouabain	.2205 ± .0110	1:2
	Cymarin	.4760 ± .0571	1:3
	Coumingine HCl	.2003 ± .0160	1:1
Mouse	Ouabain	6.977 ± .986	1:62
	Cymarin	19.78 ± 3.56	1:107
	Coumingine HCl	3.560 ± .498	1:13
Rat	Ouabain	75.08 ± 12.76	1:671
	Cymarin	52.90 ± 2.64	1:285
	Coumingine HCl	8.118 ± .395	1:29

unity. This appears to agree with the response of the spadefoot toad which is much more resistant to ouabain and cymarin than to coumingine.³ Ouabain and cymarin are monosides of the aglycones, ouabagenin and strophanthidin, respectively. Both of these aglycones are derivatives of cyclopentanophenanthrene with a side-chain of unsaturated lactone.^{6, 7, 8} Coumingine, on the other hand, has apparently a different configuration.⁹ The deviation in chemical structure of coumingine from that of ouabain and cymarin may account for the relatively lower resistance of mice and rats to its toxic action.

It has been suspected sometimes that toads are resistant to digitalis-like substances, because they immunize themselves with bufagins and bufotoxins of their own venom, both of which belong to the digitalis class. Such a possibility may be excluded now for several reasons: first, bufagins and bufotoxins are not present in toads' circulation;¹⁰ secondly, the same substances, having small molecular

⁶ Fieser, L. F., and Newman, M. S., *J. Biol. Chem.*, 1936, **114**, 705.

⁷ Jacobs, W. A., and Elderfield, R. C., *Science*, 1934, **80**, 533.

⁸ Paist, W. D., Blout, E. R., Uhle, F. C., and Elderfield, R. C., *J. Org. Chem.*, 1941, **6**, 273.

⁹ Ruzicka, L., Dalma, G., and Scott, W. E., *Helv. Chim. Acta*, 1941, **24**, 63.

¹⁰ Chen, K. K., and Chen, A. L., *Arch. internat. de pharmacodyn. et de therap.*, 1934, **47**, 297.

weights, are unlikely to induce an immunological process like proteins; and thirdly, rats and mice, devoid of any digitalis-like principles in their body secretions, are comparable to toads in their tolerance to cymarin, ouabain, and coumignine HCl.

In the assay of digitalis-like principles, cats and frogs are most frequently used. When the results in cats are compared with those of frogs published in a previous communication,³ cats appear to be more susceptible. However, part of the difference may be due to temperature variations, for the frogs were studied in a water bath maintained at 20°C, while the cats were investigated at a room temperature of 24.5°C.

Summary. 1. The acute toxicity of ouabain, cymarin, and coumignine hydrochloride has been compared by subcutaneous injection in cats, rabbits, guinea pigs, rats, and mice. 2. Both mice and rats are much more tolerant to ouabain and cymarin than cats in the ratios of 62:1 to 671:1. Regarding the alkaloid, coumignine HCl, which also has a digitalis-like action, their resistance is not so marked, being in the ratios of 13:1 to 29:1. 3. Guinea pigs and rabbits, like cats, are highly susceptible to ouabain, cymarin, and coumignine HCl.

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Preparation of Living Nuclei from Hen Erythrocytes.

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The early attempts to separate the nuclei from avian erythrocytes resulted in obtaining an amorphous mass of nuclear material. Warburg¹ was the first to isolate the separate nuclei. He used a freezing-melting technic. The same method was used by Miyake.² The disadvantages of this method are: partial agglutination and damage of nuclei, incomplete hemolysis and the necessity of further separation.

The method to be described leads to a stable suspension of free

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Paper No. 1959 Scientific Journal Series, Minnesota Agricultural Experiment Station.

¹ Warburg, O., *Z. physiol. Chemie*, 1910, **70**, 413.

² Miyake, M., *Keijo J. Med.*, 1933, **4**, 247.