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Potency of Cymarin and Coumingine Hydrochloride as Influenced by Environmental Temperature.

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The influence of season and environmental temperature on the susceptibility of frogs to digitalis and its allied products has been repeatedly investigated. Moschkowitsch,[1] Edmunds and Hale,[2] and Gottlieb[3] reported that summer frogs were more resistant than winter frogs. Ziegenbein[4] believed that the season of the year made little difference in the susceptibility of frogs. Dixon[5] found less than 50% variation in frogs' sensitivity to digitalis throughout the year. Mansfeld and Horn,[6] using the isolated sinus of the frog's heart, stated that the months of June, July, and August did not give as reliable results as other months for strophanthin even though the temperature at which the tests were made was comparable. A majority of other workers observed an increase in sensitivity of frogs during the summer months. Thus, Focke[7,8] emphasized that summer frogs were more reactive to digitalis. Baker[9] concluded that ouabain increased in potency fourfold from 10° to 30°C, while digitalis showed only a difference of 25%. Weizsäcker[10] demonstrated that with the isolated frog's heart, the lower the temperature, the more time required for the completion of strophanthin action. Sollmann, Mendenhall, and Stingel[11] found that the toxicity of ouabain for frogs increased markedly with temperature, the increase per degree of temperature being much greater at the lower than at the higher temperatures. Smith and McClosky[12] proved that by intravenous injection the susceptibility of the frog's heart to digitalis increased with temperature in much the same manner as its susceptibility to ouabain. Gander[13] experimented with gitalin and gitaligenin on the isolated frog's heart, and came to the conclusion that their fixation and action were accelerated by elevation of temperature.

In this laboratory, a specially air-conditioned room was constructed. It was proposed that various drugs be studied at different room temperatures. In another communication,[14] results were presented to show that certain drugs at 40°C were several times as potent as at 20°C in mice. In the present investigation, cymarin and coumingine hydrochloride, both digitalis-like products, were assayed according to the U.S.P. XI[15] 1-hour frog method. The room was adjusted to 16°, 20°, 25°, 30°, 35°, and 40°C. Several concentrations of the solutions were employed in order to avoid an excessive volume of doses. Owing to the free air flow, evaporation took place at the surface of the water bath, so that the temperature of the bath, recorded by a thermometer, was lower than

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3 Gottlieb, R., *München. med. Wchnschr.*, 1914, **61**, 813.

4 Ziegenbein, H., *Arch. d. Pharm.*, 1902, **240**, 454.

5 Dixon, W. E., *Pharm. J.*, 1905, **75**, 155.

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11 Sollmann, T., Mendenhall, W. L., and Stingel, J. L., *J. Pharm. and Exp. Therap.*, 1915, **6**, 533.

12 Smith, M. I., and McClosky, W. T., *Pub. Health Rep.*, 1925, Supplement No. 52.

13 Gander, J., *Arch. f. exp. Path. u. Pharm.*, 1932, **164**, 324.

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15 *Pharmacopoeia of the United States of America*, 1936, 11th rev., 397.

the room temperature. The frogs (*Rana pipiens*) of the same batch were acclimatized at each temperature for 24 hours, except that at room temperature of 40°C they were injected without prolonged adaptation, since an overwhelming majority would have died at such temperature level if kept there for more than 3-4 hours. The entire work was carried out during the months of March, April, and May.

The results are summarized in Table I. The median systolic doses (SD_{50} ± standard error) were computed by the combined slope.[16] The general trend is unmistakable, namely, the higher the room or bath temperature, the more potent the drugs. With cymarin the data appear to fall into 3 groups: the frogs were least sensitive between 13° and 15.5°C, more sensitive between 20° and 29°C, and most sensitive at 33°C, bath temperature. No explanation is available as to why the frogs were more susceptible at 24.5° than at 20° and 29°C (bath temperature). With coumingine hydrochloride, the results ran a more uniform course, that is, without exception, the higher the temperature, the more potent the alkaloid as shown by the SD_{50}. Indeed, coumingine hydrochloride is approximately 5 times as potent at 33°C as at 13°C, bath temperature.

The frog is a poikilothermic animal, and its change in susceptibility to cymarin and

[16] Miller, L. C., Bliss, C. I., and Braun, H. A., *J. Am. Pharm. Assn.*, 1939, **28**, 644.

TABLE I.

Room temperature, °C	Temperature of bath, °C	Dose, μg/g	No. in systole / No. used	LD_{50} ± S.E. (by combined slope), μg/g
		Cymarin.		
		1.00	1/5	
		1.10	3/10	
16	13	1.25	1/5	1.374 ± 0.096
		1.40	1/5	
		1.60	4/5	
		1.80	3/3	
		1.00	2/10	
		1.10	4/10	
20	15.5	1.25	10/15	1.158 ± 0.061
		1.40	9/12	
		1.60	3/3	
		0.62	0/5	
		0.70	2/5	
25	20	0.80	3/5	0.816 ± 0.056
		0.90	3/5	
		1.00	3/5	
		1.10	4/4	
		0.56	0/5	
30	24.5	0.62	4/10	0.651 ± 0.043
		0.70	6/9	
		0.80	5/5	
		0.50	0/5	
		0.56	2/10	
		0.62	2/9	
35	29	0.70	5/10	0.788 ± 0.040
		0.80	4/10	
		0.90	5/10	
		1.00	3/4	
		1.10	4/4	
		0.45	2/5	
		0.50	5/10	
40	33	0.56	5/10	0.529 ± 0.032
		0.62	6/10	
		0.70	9/10	

TABLE I (Continued).

Room temperature °C	Temperature of bath, °C	Dose, μg/g	No. in systole / No. used	$LD_{50} \pm$ S.E. (by combined slope), μg/g
		Coumingine Hydrochloride.		
		3.00	3/10	
16	13	3.60	2/10	4.154 ± 0.263
		4.50	4/10	
		5.60	5/5	
		3.00	1/10	
		3.30	1/10	
20	15.5	3.60	4/11	3.868 ± 0.209
		4.00	9/13	
		4.50	3/3	
		1.80	1/10	
		2.00	7/11	
25	20	2.25	6/9	2.148 ± 0.103
		2.50	8/15	
		2.75	5/5	
		1.40	0/5	
		1.60	1/5	
30	24.5	1.80	5/10	1.961 ± 0.100
		2.00	5/10	
		2.25	7/10	
		2.50	3/4	
		1.25	1/10	
		1.40	2/10	
35	29	1.60	4/8	1.733 ± 0.095
		1.80	5/10	
		2.00	6/9	
		0.80	2/5	
40	33	0.90	2/5	0.810 ± 0.082
		1.00	5/5	
		1.10	5/5	

coumingine hydrochloride due to environmental temperature may be more apt to correspond to a chemical reaction than that of warm-blooded animals. The results of the present study, however, do not show a directly proportional relationship, for the points of temperatures versus doses (or logarithms) do not fall on a straight line.

Summary. The susceptibility of the frog's heart to cymarin and coumingine hydrochloride increases with the rise of environmental temperature. Coumingine hydrochloride is approximately 5 times as potent at 33°C as at 13°C (bath temperature), and cymarin is more than twice as active at 33°C as at 13°C.