TABLE I.

Subject	Lindhard % CO_2	Haldane-Priestley % CO_2
A	5.08	5.4
B	4.62	4.93
C	5.2	5.3
D	5.22	5.22

by a revolving electrically driven marker, so that they would breathe deeply at a rate of 6 times per minute. Haldane[2] has proved that voluntarily altering the rate of respiration does not affect the alveolar CO_2 percentage. However, the Lindhard samples were usually still slightly lower than the Haldane-Priestley samples as shown by Table I.

To obtain comparable results to the Haldane-Priestley method, it would probably be necessary to place the catheter further down the respiratory tract, as can be done with an anesthetized patient.

Summary. A mercury valve is described for obtaining samples of alveolar air over mercury at the end of expiration by a nasal catheter. The CO_2 values are lower than the Haldane-Priestley values, even when respiration is voluntarily slowed.

11629

Susceptibility of Spadefoot Toad and Tree Frog to Ouabain, Cymarin, and Coumingine Hydrochloride.

ALBERT P. BLAIR, CHESTER C. HARGREAVES AND K. K. CHEN.

From the Department of Zoology, Indiana University, Bloomington, and the Lilly Research Laboratories, Indianapolis.

Almost 85 years ago Vulpian[1] pointed out that the toad had a natural tolerance to digitalis. Subsequent investigators in confirmation with Vulpian's work reported that the toad was resistant to other cardiac drugs such as antiarin and strophanthin,[2, 3, 4] ouabain, cymarin, and coumingine hydrochloride.[5, 6] The African clawed

2 Haldane, J. S., *Am. J. Physiol.*, 1915, **38**, 20.

1 Vulpian, E. F. A., *Compt. rend. Soc. biol.*, 1856, **3**, 125.

2 Fornara, D., *J. de thérap.*, 1877, **4**, 882.

3 Heuser, O., *Arch. internat. de pharmacodyn. et de thérap.*, 1902, **10**, 483.

4 Epstein, D., *J. Pharm. and Exp. Therap.*, 1931, **43**, 697.

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

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

toad *(Xenopus laevis)*, however, behaves more like the frog than the true toad *(Bufo)*.[4, 7] In invertebrates Evans[8] found that antiarin and gitalin were inactive on the snail's heart *(Helix pomatia)* although strophanthin produced its typical effect. Straub[9] obtained negative results with antiarin on the sea-hare's heart *(Aplysia)*. The grass snake *(Tropidonotus natrix)* is very tolerant to strophanthin as compared with the frog.[10] Among warm blooded animals, the rat has been shown[11, 12] to have a high tolerance to strophanthin and digitalis.

The spadefoot toad, *Scaphiopus holbrookii holbrookii*, family *Scaphiopodidae*, has a pair of well-defined parotoid glands resembling in appearance those of the true toad. Phylogenetically, the spadefoot toad is generally considered more primitive than the true toad, family *Bufonidae*. The tree frog, *Hyla cinerea cinerea*, family *Hylidae*, on the other hand, is devoid of any structure suggestive of the parotoid glands, and in this respect it appears to be closer to the true frog, *Rana*.

The purpose of the present report is to show that, first, the venom from the spadefoot toad's glands contains no digitalis-like principles; and secondly, the spadefoot toad is less susceptible than the true frog to digitalis-like principles, such as ouabain, cymarin, and coumingine HCl, while the tree frog behaves more like the latter.

Our spadefoot toad was secured through Ross Allen's Reptile Institute, Silver Springs, Florida. The mean weight of 69 animals recorded at random was 13.62 ± 0.42 g. The parotoid glands are nearly circular in shape and much less conspicuous as compared with those of common toads such as *Bufo fowleri*. Histologically, they appear to have a morphology similar to the parotoid glands of *Bufo*, except that the acini are fewer in number and smaller in size, as illustrated in Fig. 1.

The secretion of the spadefoot toad can be expressed with a mosquito hemostat. The quantity is small and it is serous in color and consistency. It has a pungent odor not unlike that of fresh grass. It is acid to litmus and irritating to nasal mucous membranes causing incessant sneezing. In view of the fact that only little secretion

[7] Gunn, J. W. C., and Epstein, D., *Quart. J. Pharm. and Pharmacol.*, 1932, **5**, 180.

[8] Evans, C. L., *Z. f. Biol.*, 1912, **59**, 397.

[9] Straub, W., quoted by Evans.

[10] Clark, A. J., *J. Pharm. and Exp. Therap.*, 1912-1913, **4**, 399.

[11] Hatcher, R. A., *Am. J. Physiol.*, 1909, **23**, 303.

[12] Gunn, J. A., *J. Pharm. and Exp. Therap.*, 1912-1913, **4**, 225.

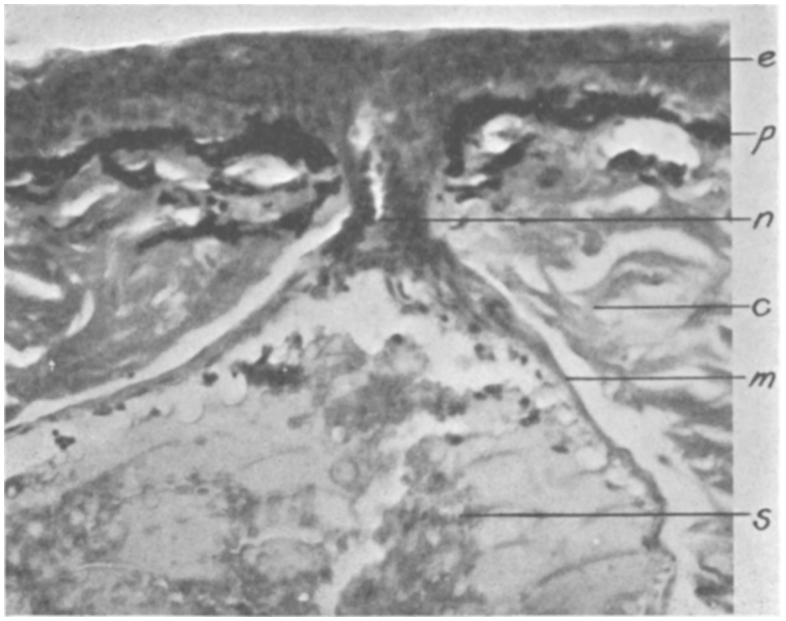

FIG. 1.
Section through a Portion and Duct of a Parotoid Gland.
e. Epidermis; *p.* melanophores; *n.* gland duct; *c.* cutis; *m.* muscular layer; and *s.* secretion.

could be collected by pressure, the glands were carefully excised, thoroughly dried, and pulverized. Exactly one-half gram of the dried glands was extracted under reflux with 30 cc of 95% ethyl alcohol on a water bath for approximately 2 hours. The extract was filtered off, and the process was repeated with a like volume of alcohol for 3 hours. The combined filtrate was evaporated to a small volume, and by addition of 1.4 cc of water, it was brought to a total of 5 cc. The extract gave a positive Liebermann-Burchard's reaction probably due to the presence of sterols such as cholesterol and ergosterol. When injected into the lymph sac or perfused through the inferior vena cava of frogs, no systolic effect could be demonstrated on the heart. This was substantiated in a cat by intravenous injection in which no rise of blood pressure, or bradycardia, or arrhythmia occurred. An alcoholic extract of the parotoid glands of *Bufo* (any common species), similarly prepared, showed a high degree of digitalis-like action. It is thus apparent that the parotoid secretion of the spadefoot toad, *Scaphiopus holbrookii holbrookii*, contains no principles like bufagins or bufotoxins. It, however, appears to have a substance or substances which increase the tone of

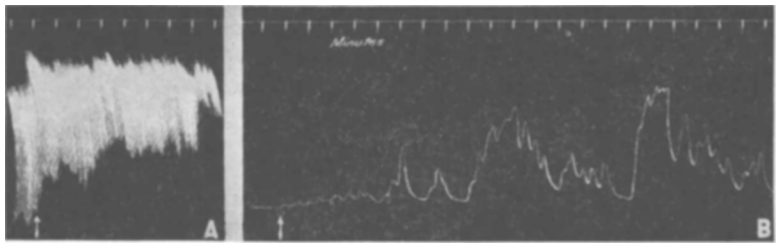

FIG. 2.
Action of the Alcoholic Extract of Parotoid Glands of the Spadefoot Toad.
A. Peristaltic movements of a strip of a rabbit's small intestines immersed in Tyrode's solution at 38°C. At arrow, 0.05 cc of the extract (1%) was applied. Note the increase in tone.
B. Myogram of a horn of a virgin guinea pig's uterus immersed in Tyrode's solution at 38°C. At arrow, 0.5 cc of the extract was added. It resulted in gradual development of contractions.

peristaltic movements of isolated rabbit's intestines and the isolated guinea pig's uterus, as exemplified in Fig. 2.

The tree frog, *Hyla cinerea cinerea,* employed in the present work was purchased from the Southern Biological Supply Company, Inc., New Orleans. The mean body weight of 79 specimens was 5.64 ± 0.103 g. Both the tree frog and the spadefoot toad were subjected to assays with ouabain, cymarin, and coumingine HCl, according to the U.S.P. one-hour frog method,[13] in order to evaluate their sensitivity to these drugs. The results are summarized in Table I and compared with the previously published figures[6] for the Leopard frog, *Rana pipiens,* and the nebulous toad, *Bufo valliceps.* It is obvious that the spadefoot toad is highly resistant to the glycosides, ouabain and cymarin, but slightly less susceptible than the frog to coumingine HCl, the alkaloid having a digitalis-like action. The tree frog is only slightly tolerant to cymarin but not to ouabain or coumingine HCl. No explanation is offered why one amphibian is naturally more sensitive than the other to cardiac poisons, or why the same animal is sensitive to one poison but resistant to others of the same pharmacological class. It is certain, however, that such a natural resistance does not depend upon the ability of the animal to produce digitalis-like substances in the sense of immunity development, for the spadefoot toad is free from these compounds in its venom. Similarly, the rat is very tolerant to digitalis and strophanthin[11, 12] without incorporating any chemical possessing a cardiac action. The secretion of digitalis-like substances in the venom of the nebulous toad *(Bufo valliceps)* must be considered, therefore, accidental, and not respon-

[13] *Pharmacopoeia of the United States,* XI Revision, Mack Printing Co., Easton, Pa., 1936, 136.

TABLE I.
Action of Ouabain, Cymarin, and Coumingine HCl in the Spadefoot Toad and the Tree Frog.

Animal	Drug	Solution	Dose, γ per g	No. in systole / No. used	Median systolic dose ± standard error γ per g	Approximate ratio of susceptibility when the frog is unity
Leopard Frog,	Ouabain	1:20,000	*	*	0.6096 ± 0.0096	1
Rana pipiens	Cymarin	1:20,000	*	*	0.5999 ± 0.0159	1
	Coumingine HCl	1:2000	*	*	2.733 ± 0.111	1
Nebulous Toad,	Ouabain	1:250	*	*	48.65 ± 2.86	1:80
Bufo valliceps	Cymarin	1:50, alcohol 57% by volume	*	*	1050†	1:1750
	Coumingine HCl	1:100	*	*	159.4 ± 9.1	1:58
Spadefoot Toad,	Ouabain	1:200	10	0/5	45.94 ± 1.28	1:75
Scaphiopus holbrookii holbrookii			**30**	**0/5**		
			40	1/10		
			45	4/10		
			50	8/10		
	Cymarin	1:200, alcohol 15% by volume	10	0/2	41.57 ± 2.30	1:69
			30	**0/2**		
			35	**3/10**		
			40	4/10		
			45	7/10		
			50	7/10		
	Coumingine HCl	1:500	4.00	1/5	4.317 ± 0.228	1:2
			4.50	4/5		
			5.00	4/5		
			8.00	2/2		
Tree Frog	Ouabain	1:50,000	0.75	0/5	0.850 ± 0.022	1:1
Hyla cinerea cinerea			0.80	1/5		
			0.90	4/5		
			1.00	5/5		
	Cymarin	1:10,000	3.00	0/5	3.943 ± 0.180	1:7
			3.50	1/5		
			4.00	3/5		
			4.50	4/5		
	Coumingine HCl	1:10,000	2.50	0/5	3.485 ± 0.336	1:1
			3.00	1/5		
			3.50	4/5		
			4.00	4/5		
			6.00	4/5		

*See previous report for protocols.[6]

†This figure was taken from the curve at the level of probit 5.

sible for its tolerance to ouabain, cymarin, and coumingine HCl.

Summary. 1. The parotoid secretion of the spadefoot toad *(Scaphiopus holbrookii holbrookii)* has no digitalis-like effect, but it stimulates the isolated rabbit's intestines and the isolated guinea pig's uterus. 2. The spadefoot toad, like the nebulous toad, *Bufo valliceps,* is definitely more resistant to ouabain and cymarin, and only slightly more resistant to coumingine HCl, as compared with the Leopard frog, *Rana pipiens.* 3. The tree frog, *Hyla cinerea cinerea,* is less susceptible to cymarin than the frog, but almost equally susceptible to ouabain and coumingine HCl.

11630

Toxicity of Saline Extracts of Rabbit Uterus After Estrogen Administration.*

BORIS KRICHESKY AND C. D. HONIG.

From the Department of Zoology, University of California at Los Angeles.

In a previous communication it was demonstrated that saline extracts of full term or immediately post-partum rabbit uteri contain a toxic substance lethal to other rabbits when injected intravenously in doses of 6 cc or less.[1] It was also reported that this toxic substance possesses some physiological properties similar to those of histamine.[2]

Among the consistent characteristics of a full term or immediately post-partum rabbit uterus containing the toxic substance, there are: (1) increased color, of vascular origin;[3] (2) increased volume of the entire uterus;[4] and (3) increased amounts of intercellular fluid, or edema.[5] Similar uterine changes after administration of estrogens to non-pregnant animals have been adequately demonstrated by several investigators. Moreover, it has been demonstrated, at least in other species, that estrogens are present in both blood and urine in increasing amounts as pregnancy progresses.[6] The presence of high concentrations of estrogens in the final stages of pregnancy, and the

* Aided by a research grant from the University of California. The stilbesterol was generously supplied by the Eli Lilly and Company, Indianapolis.

1 Krichesky, B., and Pollock, W., *Science,* 1940, **91**, 410.

2 Krichesky, B., and Pollock, W., *Am. J. Physiol.,* 1940, in press.

3 Markee, J. E., and French, H. M., *Anat. Rec.,* 1934, **58**, Suppl., p. 79.

4 Markee, J. E., and Andersen, E., *Anat. Rec.,* 1934, **58**, Suppl., p. 78.

5 Krichesky, B., unpublished.

6 Frank, R. T., *Glandular Physiology and Therapy,* Chap. 16, Amer. Med. Assn., Chicago, 1935.