Since the method of the Association of Official Agricultural Chemists gives the same results as that of the U. S. P., and is easier to use, the analyses by the new method were checked against it. Over one hundred and fifty analyses were run by this method. Six different preparations of silver proteinates, which represent three of the four different types, were used. No work was done on the electric type of solution.

TABLE I.

Method Used, Time for Digestion.	A. O. A. C. 45–90 Min., % Ag.	New Method 5–20 Min., % Ag.
Strong Silver Protein (Merck)	8.19	8.21
Mild Silver Protein (Merck)	19.54	19.59
Argyrol	20.95	20.95
Silloid	17.67	17.67
Collargol	78.22	78.42

A ten per cent Argyrol ointment (Norwich Pharmacal Co.) was analyzed by this method. For complete oxidation it required from one to two hours and the use of 50 cc. of H_2SO_4 and 50 cc. of fuming HNO_3. The results indicated the presence of 2.15% silver, $\pm 0.02\%$. Samples of the same ointment were treated by the A. O. A. C. method. After heating for two days and repeatedly adding HNO_3 and H_2SO_4, the samples were discarded.

SUMMARY.

1. An accurate method, considerably less involved than that of the U. S. P., has been developed for the assay of silver proteinates.

2. It has the following advantages over that of the U. S. P. and other methods reported in the literature:

(*a*) It gives the same or slightly higher percentage of silver.

(*b*) The oxidation is complete in from one-tenth to one-third of the time.

(*c*) The complete analysis is made in one container, a 250-cc. Erlenmeyer flask.

3. It can be used for the analysis of oil solutions and of ointments of silver proteinates.

REFERENCES.

(1) Marschner, J., *Apoth. Ztg.*, 27, 887 (1912).
(2) Tabern, D. L., and Shelberg, E. F., *Ind. Eng. Chem., Anal. Ed.*, 4, 401–403 (1932).
(3) Eaton, E. O., *J. Assoc. Official Agr. Chem.*, 8, 551–552 (1925).
(4) Antoine, Georges, *J. pharm. chim.*, 21, 457–465 (1935).
(5) Inabinette, I. H., *Am. J. Pharm.*, 103, 42 (1931).

COMPARATIVE SENSITIVITY OF THE TOAD AND THE FROG TO CYMARIN AND COUMINGINE.*

BY K. K. CHEN, CHSETER C. HARGREAVES AND WILLIAM T. WINCHESTER.[1]

In a previous report (1), it was concluded that the nebulous toad, *Bufo valliceps*, was many times more tolerant to *G*-strophanthin and scillaren B than the common frog, *Rana pipiens*. It was also noted that the tolerance of the toad is confined to the cardiac muscles. Of a large number of crystalline cardiac principles studied

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[1] From the Lilly Research Laboratories, Indianapolis, Indiana.

in this laboratory (2), (3), there were two, coumingine hydrochloride and cymarin, that were sufficiently soluble in water to be suitable for administration to toads. The purpose of this communication is therefore to present additional data demonstrating the natural tolerance of the toad to cardiac drugs, irrespective of their origin, as compared with the frog. The same species of animals were employed as before, that is, *Bufo valliceps* to represent the toad, and *Rana pipiens*, the frog. The specimen of cymarin was courteously supplied by Dr. Walter A. Jacobs, the Rockefeller Institute for Medical Research, New York City, and that of coumingine hydrochloride by Dr. Gustavo Dalma, Provincial Chemical Laboratory, Fiume, Italy. To determine the minimal systolic dose in toads, a 1 per cent solution of coumingine hydrochloride in saline, and a 2 per cent solution of cymarin with 57 per cent ethyl alcohol by volume, were prepared. For frogs, however, a 1:20,000 concentration of cymarin and 1:2000 concentration of coumingine hydrochloride, were adequate for injection purposes. The procedure of testing conforms to the U. S. P. one-hour method (4), the temperature of the bath being kept at 20° C.

TABLE I.—DETERMINATION OF MINIMAL SYSTOLIC DOSES OF OUABAIN-MERCK, COUMINGINE HYDROCHLORIDE AND CYMARIN.

	Rana pipiens.		*Bufo valliceps.*		
Drug.	Dose, Mg. per Gm.	No. of Hearts in Systole/No. of Frogs Used.	Dose, Mg. per Gm.	No. of Hearts in Systole/No. of Toads Used.	Ratio of *R. pipiens* to *B. valliceps.*
Ouabain-Merck	0.00050	0/4	0.03000	0/4	
	0.00055	0/4	0.04000	1/4	
	0.00060	1/4	0.04500	0/4	1:77
	0.00065	4/4	*0.05000*	3/4	
			0.05500	3/4	
Cymarin	0.00035	0/4	0.05500	0/4	
	0.00040	0/4	0.07500	0/4	
	0.00045	0/4	0.15000	0/4	
	0.00050	0/4	0.30000	0/1	
	0.00055	0/4	0.40000	0/1	1:167
	0.00060	3/4	0.60000	0/2	
	0.00065	3/4	0.80000	0/4	
	0.00070	4/4	1.00000	0/4	
	0.00075	4/4	*1.10000*	4/4	
			1.20000	4/4	
Coumingine Hydrochloride	0.00200	0/4	0.10000	0/1	
	0.00250	2/8	0.12500	0/1	
	0.00300	6/8	0.15000	3/7	
	0.00300	4/4	*0.17500*	4/7	1:58
	0.00750	4/4	0.20000	5/5	
	0.01000	4/4	0.22000	4/4	

The results are summarized in Table I. It may be noted that the minimal systolic dose of cymarin in frogs is 0.0006 mg. per Gm., and that in toads 1.1 mg. per Gm. The ratio of sensitivity between frogs and toads is therefore 1:167. Similarly, the minimal systolic dose of coumingine hydrochloride in frogs is 0.003 mg. per Gm., and that in toads 0.175 mg. per Gm. The ratio of sensitivity between frogs and toads in this case is 1:58. It may be pointed out here that the figures for the frogs in Table I are larger than those reported previously (2), (3). This is due to the fact that different batches of frogs respond differently to ouabain, that is, the minimal systolic dose may vary from time to time. In order to make

accurate comparisons of various cardiac principles in frogs, it has been our custom to recalculate the dose by proportion as if every batch of frogs has the same minimal systolic dose to ouabain, namely, 0.0005 mg. per Gm. In the present work no such correction was made because the main point of interest was to find the difference of sensitivity between frogs and toads.

In the previous paper (1), it was shown that the toad was 150 times more tolerant to *G*-strophanthin than the frog. The ratio was redetermined with a new lot of ouabain-Merck (*G*-strophanthin), and it was found, as shown in Table I, to be 1:77, practically one-half of the former figure. It is difficult to account for the discrepancy of results at present, except to mention that the former tests were carried out in the fall, and the present ones in the spring although both were made at 20° C.

SUMMARY.

The nebulous toad, *Bufo valliceps*, has a natural tolerance to cardiac glycosides as well as alkaloids. When compared with the common frog, *Rana pipiens*, it is 167 times less sensitive to cymarin, and 58 times less sensitive to coumingine hydrochloride. In the present investigation it is also found that the ratio of susceptibility to ouabain between the same species of the frog and the toad is 1:77 as contrasted with that of 1:150 reported previously.

REFERENCES.

(1) Chen, K. K., and Chen, A. L., *J. Pharmacol.*, 47, 295 (1933).
(2) Chen, K. K., Chen, A. L., and Anderson, R. C., JOUR. A. PH. A., 25, 579 (1936).
(3) Chen, K. K., Hargreaves, C. C., and Winchester, W. T., *Ibid.*, 27, 9 (1938).
(4) Pharmacopœia of the United States, XI Revision, Mack Printing Co., Easton, Pa., 397 (1936).

DIGITALIS ASSAY BY THE CAT METHOD UNDER "SODIUM AMYTAL" ANESTHESIA.*

BY WILLIAM E. FRY AND EDWARD E. SWANSON.[1]

In the assay of digitalis by the Hatcher-Brody Cat Method (1), the question of anesthesia has been a problem of much discussion. Rowe (2) and Chopra and Chowhan (3) suggested the substitution of chlorobutanol in place of ether, assuming that this would not affect the size of the cat unit. Epstein (4) found that paraldehyde lowered the resistance of the cat to digitalis. With ether, chlorobutanol, urethane, chloralose and paraldehyde, David and Rajaminchan (5) observed that the choice of anesthetic was of much importance in the results of the cat unit. Haskell (6) noted a larger cat unit with dial-urethane anesthesia. With urethane, Edmunds, Moyer and Shaw (7) reported more of an increase in the cat unit for the U. S. P. Standard Powder than with ether, and concluded that the effect of anesthetics has a marked influence upon the amount of digitalis in the cat unit. Bauer and Fromherz (8) in the assay of ouabain obtained a larger cat unit with allylisopropyl barbituric acid than with ether.

EXPERIMENTAL.

In the present work, experiments were carried out with two short-acting barbituric acid derivatives as anesthetics. The compounds used were "Sodium Amytal" (Sodium Iso-amyl

* Scientific Section, A. PH. A., New York meeting, 1937.

[1] Eli Lilly & Co., Research Laboratories.