hydrochloric acid was added and the mixture allowed to stand over night. The precipitate which formed was filtered out. It was found to be a mixture of chrysophanic acid and potassium chloride. It was therefore boiled with water and the chrysophanic acid filtered out. Yield, 3.5 Gm. (95.8%), m. p. 193–194° (corr.). Naylor and Gardner (1) give m. p. 195.6–196.2°. Other investigators report slightly lower values.

SUMMARY.

A satisfactory method for the preparation of chrysophanic acid from chrysarobin has been developed.

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THE BIOASSAY OF SQUILL.*[1]

BY HARRY ROSEN.

Squill, a member of the heart stimulant or digitalis group of drugs is biologically assayed by the same methods as the other members of this so-called "Digitalis series."

Many test animals and methods of procedure have been proposed and employed for bioassay and standardization purposes. Consequently a wide divergence of opinion concerning the relative merits of the respective procedures is found.

Hale (1), (2), after working with the various methods of assay, concluded that they did not give proportional results and suggested that the one-hour frog procedure was probably the most suitable.

The American Drug Manufacturers' Association (3) undertook collaborative investigations of the various assay methods and concluded that the M. L. D. frog and M. L. D. guinea pig methods were more accurate than the one-hour frog or cat methods, and that the technique involved was much simpler.

Richaud (4), studying the various methods for the assay of cardiac tonics, concluded that the guinea pig method was unsuitable for the assay of these drugs.

Eckler (5) assayed a series of preparations by the cat, guinea pig and one-hour frog methods. He concluded that the cat method was complicated, time consuming, costly and gave results varying from 33 to 123 per cent.

Rowntree and Macht (6) concluded that the cat method was more reliable than the frog method. Van Leeuwen, den Besten and van Wijngaarden (7), (8), (9) reported that the cat method was more accurate and was independent of seasonal variations as compared with the frog methods.

Wible (10) concluded that the cat and the one-hour frog methods agree within the limits of biological error.

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* Scientific Section, A. PH. A., Washington meeting, 1934.

[1] From the laboratory of Marvin R. Thompson, Professor of Pharmacology, School of Pharmacy, University of Maryland. Compiled in part from a thesis submitted to the Faculty of the Graduate School of the University of Maryland in partial fulfilment of the requirements for the degree of Master of Science, June 1933.

Burn (11) stated that the results obtained by assaying Tr. of Squill, in terms of ouabain, by the cat method differed significantly from the results obtained by the frog method. The assay in terms of scillaren, gave the same results whether performed on the cat, frog or rabbit auricle.

Rowe (12) in an early report stated he preferred the M. L. D. frog to the cat method but believed he did not work with enough cats. In a later paper (13) he reported that the hour frog, M. L. D. frog and cat methods were inapplicable, while the guinea pig could be used but is too expensive.

Conflicting reports concerning the M. L. D. of squill for the various animals are also found in the literature. Eggleston (14) stated that he found for the cat M. L. D., 525, 545, 595 and 642 mg. per Kg., while Rowe (12) gave the value as 112 mg. per Kg. for one highly active sample of fluidextract. Pittenger, in his "Biologic Assays" (15), gives 575 mg. per Kg. as the M. L. D.

Vanderkleed (16) reported that season was of no direct significance in the guinea pig method of assay, while Haskell (17) disagreed with this statement since he had figures to show that during eight months, the original M. L. D. was at times doubled due to seasonal variation. Pittenger (15) stated the M. L. D. for guinea pigs is 0.0025 cc. Tr. or 0.00025 cc. Fluidextract of Squill.

Presentation of many other citations of the literature would only serve to illustrate further disagreements.

The U. S. P. X "one-hour frog," the "over night or mortality curve frog," "intravenous cat" and "subcutaneous guinea pig" methods have received the most impressive support.

It may thus be seen that many workers have subjected these and other methods to comparative study, and the evidence so obtained has resulted in conflicting opinions of such a magnitude as to cause some workers to roundly condemn certain of the methods and to praise others. It is worthy of note, however, that no one method has proved to be sufficiently superior to clearly establish universal preference for that method. Advantages and disadvantages are to be found in each, but the main reason for the preference of a given worker for a given method is usually to be found in his belief that the different methods yield significantly different results for a given squill preparation, and that the "preferred" method yields the results of greatest reliability and accuracy.

Because the four methods mentioned have been found to be possessive of merit in this laboratory, a comparative study involving these four methods was undertaken with a view toward establishing if possible, whether or not the respective methods actually do yield conflicting results, and if so, to ascertain the character of the discrepancies.

METHODS.

One-Hour Frog Method.—The procedure for this method was carried out as directed in the official U. S. P. X assay (18). The suitability of ouabain as a standard for substances, other than those of the strophanthus family is one of the doubtful points of the one-hour frog assay for squill. Burn (11) believes that it is not satisfactory and recommends a squill preparation as a standard. The standards used in this study were ouabain and scillaren A. It had been planned to also use scillaren B but this idea, as will be explained later, was abandoned.

Over Night Frog Method.—The Chapman and Morrell (19) modification of the Trevan (20) mortality curve method was used in the over night frog assays. Although the curve of Chapman and Morrell was prepared for digitalis and strophanthus assays, it was used here and the results compared to those of the other methods. Both ouabain and scillaren A were used here also as standards.

The Intravenous Cat Method.—The conventional intravenous method was used for this work with the exception that 30–45 minutes was strictly adhered to as a time limit in which the death of the animal was to occur. A further exception was that a dose of 6–10 cc. per Kg. was the range allowed for injection into the cat. Five cats, if giving consistent results, were used for each assay. If the results showed great variations, more cats were used, until consistent results were obtained.

All cat results were calculated in terms of ouabain and scillaren A. This necessitated the determination of the cat M. L. D. for ouabain and scillaren A, and the seasonal effect on the susceptibility of the cat to these standards. These studies afforded an opportune time to observe the stability of scillaren A solutions as preserved in this laboratory. This was not necessary for ouabain, since it had been determined before this work was begun, that ouabain solutions as preserved in this laboratory are very stable.

Subcutaneous Guinea Pig Method.—This method was used as developed by Reed and Vanderkleed (21). Six hours was the time limit originally planned for this method, but could not be adhered to, as will be explained later. Results were calculated in terms of ouabain and scillaren A.

EXPERIMENTAL.

TABLE I.—SHOWING M. L. D. OF OUABAIN, SCILLAREN A AND SCILLAREN B, SEASONAL VARIATIONS OF CAT SUSCEPTIBILITY TO OUABAIN AND SCILLAREN A, AND STABILITY OF THE SCILLAREN A SOLUTIONS.

Solution.	Date of Test.	Number of Cats.	M. L. D. in Mg.	Standard Deviation.
Ouabain sol. of 6–22–32	6–(23–30)–32	20	0.1111	±0.0162
Ouabain sol. of 6–22–32	10–14–32	6	0.1059	±0.0192
Ouabain sol. of 6–22–32	12–2–32	5	0.1139	±0.0168
Ouabain sol. of 6–22–32	3–13–33	5	0.1052	±0.0125
Scillaren A sol. of 7–5–32	7–(5–14)–32	20	0.2185	±0.0314
Scillaren A sol. of 9–26–32	9–26–32	5	0.2104	±0.0256
Scillaren A sol. of 9–26–32	11–29–32	5	0.2161	±0.0435
Scillaren A sol. of 9–26–32	3–14–33	5	0.1749	±0.0344
Scillaren A sol. of 9–26–32	4–24–33	5	0.2300	±0.0331
Scillaren A sol. of 2–23–33	2–23–33	5	0.2108	±0.0158
Scillaren A sol. of 5–2–33	5–2–33	5	0.2043	±0.0276
Scillaren B sol. of 7–5–32	7–(14–19)–32	12	....	.....
Scillaren B sol. of 7–5–32	8–4–32	6	....	.....

DISCUSSION.

Table I shows the M. L. D. of ouabain per Kg. of cat to be 0.1111 mg. and that of scillaren A to be 0.2185 per Kg. The values for scillaren B with 12 cats varied from 0.05987 to 0.1511 mg. per Kg. Repeated with six cats, the figures ranged from 0.08277 to 0.1865 mg. per Kg. Due to this great variation in results of the individual cats, no conclusions as to the M. L. D. could be made. For this reason, all further work planned with scillaren B as a standard was abandoned.

Cats show no seasonal variation in susceptibility to either ouabain or scillaren A.

Scillaren A solutions as preserved in this laboratory are stable for about three months. It is proved that the change in M. L. D. after this time is due to decomposition and not seasonal variation, by the fact that a while previous to this test, a new solution gave results, which were the same as the original M. L. D.

As stated before, other workers in this laboratory proved that ouabain solutions as preserved are very stable.

Table II shows that the one-hour frog, over night frog and cat methods give results in good agreement within the limits of experimental error. Much of the disagreement shown by other workers is most likely due to the fact that they obtained results in cat units and not in terms of a standard. Cat units vary in different laboratories due to differences in type and depth of anesthesia, modifications in technique, environment and diet of the cats. For this reason, cat units established by any one laboratory cannot be used universally, while a standard of comparison can. Results should be expressed in terms of a standard substance, regardless of what assay method is used.

The guinea pig method shows agreement with the other methods within the limits of experimental error in about 60 to 70% of the assays. Variation in guinea pig susceptibility is found in this laboratory to occur only when the source of supply or diet of the animals is changed. So long as they are obtained from one source and kept on one diet, they show no variations. The reason for the disagreement of some of the guinea pig results is thought to be due to the great variation in susceptibility of the individual animals and not due to so-called seasonal variation.

The six-hour limit for the occurrence of death in the guinea pigs was altered to an over night time limit. Absorption was very variable in the individual animals, and it was thought that the longer period of observation would overcome this difficulty.

From the standpoint of accuracy and routine dependability in assaying squill preparations, and also taking the matter of time and expense into consideration, the results of this investigation have convinced the author as well as the other workers in this laboratory that the over night frog method is definitely superior to any of the other methods employed.

For those workers having an adequate supply of suitable cats the cat method is thoroughly reliable, though somewhat less accurate than the over night frog method, provided that the "cat unit" idea is abandoned and samples are assayed in terms of ouabain or scillaren A as in the frog methods.

The one-hour frog method requires much experience in order to eliminate the sources of error. The assayist lacking thorough experience cannot hope to obtain consistently accurate results. For this reason, either the over night frog or cat methods are distinctly preferable.

The time and expense necessary before results can be obtained by the guinea pig method is not justified by the results. The other methods require no more time, in fact two of them require much less time before results are known and all of them are more accurate.

CONCLUSIONS.

(1) The one-hour frog, over night frog and intravenous cat methods of assay for squill give results in agreement within the limits of experimental error provided the results are expressed in terms of an appropriate standard. The guinea pig method gives the same results in 60–70% of the assays.

(2) Ouabain is a satisfactory standard for squill since scillaren A, a squill body, showed no advantages.

(3) The over night frog, intravenous cat and one-hour frog methods are most suitable in the order indicated. The guinea pig method is not as reliable.

TABLE II.—A COMPOSITE TABLE OF ALL ASSAY RESULTS EXPRESSED IN TERMS OF MILLIGRAMS OF OUABAIN AND SCILLAREN A TO WHICH EACH CC. OF PREPARATION IS EQUIVALENT.

Results—Summer, 1932.

Preparation.	Cat.		One-Hour Frog.		Over Night Frog.		Guinea Pig.	
	Ou[1].	A[2].	Ou[1].	A[2].	Ou[1].	A[2].	Ou[1].	A[2].
F. E. Squill P. T. No. 180	0.8881 ±0.1000	1.7467 ±0.1964					1.2000	2.8000
F. E. Squill P. T. No. 82	1.1236 ±0.1345	2.2097 ±0.2646					1.000	2.3333
F. E. Squill P. T. No. 233	0.3656 ±0.0389	0.7191 ±0.0765						
Tr. Squill H. R. No. 1	0.0745 ±0.0050	0.1466 ±0.0099						
Tr. Squill H. R. No. 2	0.0652 ±0.0039	0.1283 ±0.0076						
F. E. Squill P. T. No. 283								
F. E. Squill P. T. No. 198	0.6386 ±0.0704	1.2559 ±0.1385	0.6249				1.1579	2.7019

Results—Winter, 1932–1933.

Preparation.	Cat.		One-Hour Frog.		Over Night Frog.		Guinea Pig.	
	Ou[1].	A[2].	Ou[1].	A[2].	Ou[1].	A[2].	Ou[1].	A[2].
F. E. Squill P. T. No. 180	0.9039 ±0.0674	1.7777 ±0.1326	0.8000	1.4000				
F. E. Squill P. T. No. 82								
F. E. Squill P. T. No. 233								
Tr. Squill H. R. No. 1	0.0602 ±0.0030	0.1185 ±0.0059	0.0666	0.1166	0.0500	0.1252	0.0499	0.1166
Tr. Squill H. R. No. 2								
F. E. Squill P. T. No. 283	1.2318 ±0.0179	2.4226 ±0.0179	1.6000	2.8000	1.1966	3.0000	2.1423	4.9887
F. E. Squill P. T. No. 198	0.7494 ±0.0581	1.4738 ±0.1143	0.6666	1.6666			0.8571	2.000

Results—Spring, 1934.

Preparation.	Cat.		One-Hour Frog.		Over Night Frog.		Guinea Pig.	
	Ou[1].	A[2].	Ou[1].	A[2].	Ou[1].	A[2].	Ou[1].	A[2].
F. E. Squill P. T. No. 180								
F. E. Squill P. T. No. 82	0.5666 ±0.0889	1.7143 ±0.1748	0.5833		0.4062			
F. E. Squill P. T. No. 233								
Tr. Squill H. R. No. 1	0.0422 ±0.0056	0.0830 ±0.0109	0.0350		0.0206			
Tr. Squill H. R. No. 2	0.0211 ±0.0033	0.0415 ±0.0065	0.0175		0.0160			
F. E. Squill P. T. No. 283	1.1665 ±0.1111	2.2942 ±0.2185	1.000		0.7777			
F. E. Squill P. T. No. 198								

Ou[1]. = Ouabain. A[2]. = Scillaren A.

Results—Autumn, 1932.

Cat.		One-Hour Frog.		Over Night Frog.		Guinea Pig.	
Ou[1].	A[2].	Ou[1].	A[2].	Ou[1].	A[2].	Ou[1].	A[2].
0.9121 ±0.0454	1.7939 ±0.0894	0.5833	0.1000			1.000	2.3333
0.7854 ±0.0865	1.5446 ±0.1702	0.7777	1.3333			1.2000	2.8000
0.3615 ±0.0513	0.6910 ±0.1009	0.5000	0.6667			0.6000	1.4000
0.0563 ±0.0057	0.1108 ±0.0112	0.0750	0.1000			0.0600	0.1400
0.0586 ±0.0092	0.1153 ±0.0180	0.0750	0.1000			0.0600	0.1400
0.7125 ±0.0928	1.4012 ±0.1824	0.6000	1.4000			0.8571	1.9999

Results—Spring, 1933.

Cat.		One-Hour Frog.		Over Night Frog.		Guinea Pig.	
Ou[1].	A[2].	Ou[1].	A[2].	Ou[1].	A[2].	Ou[1].	A[2].
0.7373 ±1.004	1.4499 ±0.1975	0.7143	1.7142	0.6725	1.7800	1.0000	2.3333
0.3399 ±0.0355	0.6684 ±0.0699	0.3572	0.8572	0.2988	0.8188	0.3333	0.7777
0.0528 ±0.0152	0.1038 ±0.0298	0.0417	1.000	0.0[illegible]36	0.0921	0.0375	0.0875

(4) Official squill preparations retain over half of their activity for a period of one year.

(5) The simple determination of the M. L. D. of a squill preparation by the well-known cat method does not yield a reliable indication of potency. The M. L. D. of a standard substance, such as ouabain or scillaren A, must be determined by the identical technique used for the sample, and the results expressed in terms of the standard instead of "cat units."

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FOOT-NOTE: The Sandoz Chemical Works very kindly supplied the scillaren A and B used in this work.

SALIVA TESTS. II. HEROIN.

BY JAMES C. MUNCH.*

Success in using the mouse test for the detection of morphine (1) suggested the possibility of employing it for the detection of heroin in the saliva of horses and in pharmaceutical products (2). A standardized technique was developed for this saliva test (1).

A series of normal mice, weighing approximately 20 Gm., were injected subcutaneously with heroin, and various symptoms observed over a period of half an hour. In general, the *S*-tail curve resembles that produced by morphine. Literature reports (2) indicate that morphine and heroin are equally potent, threshold doses being stated to be 10 gamma per 20-Gm. mouse, or 0.5 mg. per Kg. We found the threshold dose of heroin to be much smaller (Table I). In addition to the tail curve, mice injected with heroin showed a series of symptoms differing from those following the administration of morphine. The mice tended to become much more restless, and hyper-irritable. A common heroin symptom was the

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