

SUMMARY.

The centrifuge method for determining the percentage swelling of powdered drugs in liquids was modified so as to give more accurate results in case of drugs coarsely ground for percolation.

Swelling tests were carried out on eighteen drugs in a series of hydro-alcoholic liquids. All drugs except celery fruit swelled in alcohol, the average swelling in alcohol being 12 per cent in 10 minutes. Swelling usually increased with time, although the greatest swelling occurred in the first 10 minutes. Swelling usually increased with increasing concentration of water, except in the case of belladonna leaves and castanea. With belladonna leaves there was a shrinkage in intermediate concentrations of water, while castanea showed some irregularities.

The results tend to confirm the idea that each drug must be studied individually to determine the best menstruum and best method of extraction.

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THE BIOASSAY OF STROPHANTHUS PREPARATIONS.*¹

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The abundant literature dealing with the methods of assaying *Strophanthus* preparations (cited and reviewed in the original thesis) leaves one in considerable doubt concerning the answer to the following questions:

1. Do the several more widely accepted methods of assay furnish a reliable estimation of the clinical activity of *Strophanthus* preparations?
2. Do the various methods measure the same or different types of activity in the preparations?
3. If the various methods yield significantly different results, which method is the more reliable in insuring clinical efficiency?
4. If the various methods yield results which are not significantly different from one another, which method affords the greater precision and dependability in routinely standardizing *strophanthus* preparations?

In order to assist in answering the above questions, four tinctures were prepared by the U. S. P. X Method from as many different lots of *strophanthus*. The four specimens of the drug were selected from a number of recent importations. From the standpoint of physical characteristics, all appeared to be of better than average quality. These tinctures were carefully assayed periodically over a period of over a year by the methods indicated below.

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¹ Abstracted from a dissertation submitted by William H. Hunt, in May 1934, to the Graduate School of the University of Maryland in partial fulfilment of the requirements for the degree of Master of Science.

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TABLE I.—OUABAIN EQUIVALENT OF ONE CUBIC CENTIMETER OF TINCTURES OF STROPHANTHUS.*

Prep. No.	U. S. P. X Method. Mg.	P. X Method. Date of Assay.	Mortality Curve Method.		Cat Method. Standard Deviation.	Date of Assay.
			Mg.	Date of Assay.		
P. T. 206	5.00	5/2/33	7.203 ¹	4/3/33	4.246 ± 0.358	2/13/33
P. T. 210	7.10	5/2/33	8.399 ¹	4/3/33	5.217 ± 0.738	3/3/33
P. T. 222	8.33	5/1/33	15.120 ¹	4/3/33	6.801 ± 1.344	2/16/33
P. T. 231	10.00	5/4/33	10.799 ¹	4/3/33	8.026 ± 1.015	2/18/33
P. T. 206	5.00	7/28/33	5.538 ¹	8/11/33	3.935 ± 0.493	7/14/33
P. T. 210	6.66	7/28/33	10.780 ¹	8/11/33	5.857 ± 0.857	7/13/33
P. T. 222	8.00	7/30/33	15.370 ¹	8/11/33	6.735 ± 0.465	7/10/33
P. T. 231	10.00	7/30/33	15.090 ¹	8/11/33	7.117 ± 1.186	7/17/33
P. T. 206	5.33	12/21/33	5.410	1/11/34	3.385 ± 0.364	11/21/33
P. T. 210	6.66	12/21/33	6.018	1/11/34	5.704 ± 1.035	11/7/33
P. T. 222	8.88	12/21/33	8.569	1/11/34	5.896 ± 1.382	11/14/33
P. T. 231	10.00	12/21/33	8.463	1/11/34	6.849 ± 1.225	11/11/33
P. T. 206			4.907	8/9/34		
P. T. 210			5.830	8/9/34		
P. T. 222			8.200	8/9/34		
P. T. 231			8.885	8/9/34		

* These tinctures were prepared by the U. S. P. X procedure with the important exception that those showing a potency equivalent to more than approximately 4.0 mg. Standard Ouabain were obtained by the extraction of two or more times the prescribed amount of drug.

¹ Results obtained from unhealthy frogs having a high daily mortality in storage.

ASSAY METHODS EMPLOYED.

1. The U. S. P. X One-hour Frog Method (1).
2. The Over-night Lethal Frog Method (2).
3. The Cat Method.
4. The Colorimetric Method of Knudson and Dresbach (3).

In the application of the above methods, the potency of the tinctures was ascertained, in all instances, in terms of U. S. P. Standard Ouabain. Even in the case of the cat method, the conventional practice of ascertaining and expressing the potency in terms of "cat units" was abandoned because of the well-established fact that type and depth of anesthesia, perfusion rate, etc., determine to a great extent the size of the so-called "cat unit." In the present studies the cat assays consisted of using a minimum of five cats for each preparation and five cats for the standard ouabain at each assay period, under identical technique and conditions, ascertaining and expressing the potency of each preparation directly in terms of ouabain, as in the other methods. Light ether anesthesia was used, and the preparation, diluted with saline so as to require approximately 10 cc. per Kg. of cat, were perfused into the femoral vein at a rate such that approximately two-thirds to three-fourths of the M. L. D. was given during the first fifteen to twenty minutes, the remainder being given more slowly so as to cause death in thirty to forty minutes. Pregnant or lactating cats were not used. Males or females, weighing from two to three Kg. were selected for the study, being further chosen so that the total weight of the five cats for each preparation was approximately equal (within 1 Kg.). Concerning sex, two males and three females, or vice versa, were used for each preparation each time it was subjected to assay.

At each experimental period extreme care was exercised to avoid any variation from previously employed technique, so as not to introduce any error from this standpoint.

From Table I it may be readily observed that the official U. S. P. X "One-hour" Frog Method (1), the Over-night Lethal Frog Method (2) and the Cat Method agree within the range of experimental error in showing the relative potency of the tinctures. There was no evidence of deterioration in any of the tinctures over the period of one year.

The results obtained by the Over-night Lethal Frog Method (2), for the months of April and August 1933, show wide variations from the other methods of assay, and also from the assays performed in January and August 1934. However, at the time of the assays in April and August 1933, it was noted that the frogs, which were obtained from the same source throughout this work, were very unhealthy and showed a high mortality rate in storage. It is quite obvious that the abnormally high values obtained from these frogs were clearly due to the unhealthy condition of this lot of frogs. Subsequent tests upon other lots of frogs showed this to be true.

The Knudson and Dresbach Colorimetric Method (3) of assay was conducted in accordance with their directions. The assays by this method were compared with the prescribed solution of potassium bichromate as the standard. It was found after ten trials that it was impossible to satisfactorily duplicate a previous result. For this reason, all of the solutions which were utilized in the assay were remade and carefully checked. Again it was not possible to obtain consistently reproducible results. At this point the standard solution of potassium bichromate was replaced by a U. S. P. Standard Ouabain solution. After five trials upon each of the preparations involved in this study, the inability to secure satisfactorily consistent results caused the abandonment of this method of assay as a reliable standardization procedure.

Disregarding the Over-night Lethal frog results obtained on April 3, 1933, and August 11, 1933 (these results being included merely to show the error that may be introduced through the use of unhealthy frogs), Table I shows closer agreement between the Over-night Lethal frog and the cat methods than between the One-hour frog and cat methods. Although the One-hour frog results in the Table are remarkably consistent, experience gained through assaying more than 500 preparations of strophanthus and other allies of the digitalis series in this laboratory by the cat and various modifications of the frog method, has caused the authors to regard the Over-night Lethal Frog Method as being more reliable and accurate than the one-hour frog method. Considerable difficulty is frequently encountered in interpreting the decidedly obscure end-point in the one-hour method, even though from 30 to 100 frogs are used per test, whereas the Over-night Lethal frog method is obviously entirely free from such difficulties.

The cat method yielded results which, in some instances, disagreed somewhat with certain of the frog values, but the variations are of such magnitude that a claim that the differences were significant would undoubtedly be subject to challenge by experienced workers. The cat method, using a minimum of five cats per test, while possessing certain advantages in some laboratories, can hardly be depended upon to yield a thoroughly accurate "statistical result" comparable to the Over-night Lethal frog method simply because a larger number of animals would

have to be used. To insist upon the use of appreciably more than five cats per test places the method outside the realms of routine practicability.

The authors are in complete agreement with Trevan, Chapman, Morrell, Gaddum and others, that the Over-night Lethal Frog Method is at present the nearest approach to an ideal method for the control of strophanthus preparations intended for distribution for therapeutic use. It is further believed that results obtained by this method disagree with those of other popular methods to such a degree, if any, as to be of no practical importance, since the relative values obtained in the series of tinctures were reflected similarly by all three bioassay methods.

CONCLUSIONS.

1. The application of the U. S. P. X frog method, the cat method and the Chapman and Morrell modification of the lethal frog method to four tinctures of strophanthus at different seasons of the year yielded evidence which indicates that the respective methods provide the same type of quantitative information concerning tinctures of strophanthus.

2. The inconsistencies in results obtained by the same or different methods are believed, because of experience gained in assaying a great many other preparations of Digitalis, Strophanthus and other allies, to be due to inherent characteristics of the respective methods.

3. The U. S. P. X one-hour method, owing partially to the fact that absorption is rather erratic in different frogs during a one-hour period, is the least reliable of the two frog methods. The end-point is not distinct and consistent, and the interpretation of results is of necessity a matter of opinion or judgment. It is not at all unusual to observe a decrease in the percentage of cardiac arrests with significant increases in dosage.

4. The Chapman and Morrell modification of the lethal frog method is beset with no objections concerning the sharpness and clarity of the end-point, and yields accurate and reproducible results under conditions which impose no serious difficulties from the standpoint of routine practicability. Consequently this method is definitely favored as the method of choice. Its adoption in principle as the official method is recommended.

5. The cat method, using five cats per test, is less accurate than the Over-night lethal frog method but definitely serviceable where cats are available. In the application of this method, each worker should "standardize" his own technique and determine the M. L. D. of the standard ouabain by his technique so as to make possible a direct expression of potency of the tincture in terms of the standard ouabain, rather than to simply determine the M. L. D. of the tincture and to express the potency in terms of cc. per Kg. or so-called "cat units." The so-called "cat unit" is essentially meaningless unless the type and depth of anesthesia, injection rate and other details of technique are carefully and rigidly specified. The use of a reference standard, such as ouabain (for strophanthus preparations) places the method on a rational basis. Owing to the fact that cats are not subject to more than slight variations in susceptibility, it is not necessary to repeat the standard ouabain determination for each assay, but only three or four times per year to avoid errors which might gradually creep in due to slight changes in technique in applying the method. Cats weighing between two and three Kg. yield the most consistent results, and

the weights of the cats used for the assay should be as nearly as possible the same for both the preparation being assayed and the reference standard.

6. None of the methods provided evidence of loss of potency in the tinctures during a period of approximately a year.

7. The U. S. P. X process of extraction of the drug fails to produce tinctures meeting the official potency requirements. To achieve the required potency, a quantity of drug in excess of that specified by the U. S. P. X must be used.

8. It has not been possible to obtain specimens of strophanthus which would meet U. S. P. X potency requirements no matter how completely the drug was extracted. The fault therefore lies, not in the specified procedure of extraction, but in the fact that the required amount of potency is not contained in available lots of the drug. It is concluded, therefore, that the official potency requirements for the drug and the tincture should be reduced to 50 or 60 per cent of U. S. P. X requirements.

REFERENCES.

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RED SQUILL IV. BIOASSAY METHODS.*¹

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In connection with our studies on red squill powders and preparations great variations in potency have been observed. The methods of chemical assay have not furnished adequate indications of physiological potency, so bioassays are required. After studying the pharmacodynamic action on various animals, a standard technique was developed using the white rat (1, 2, 3). In this series of toxicity determinations every effort was made to conform rigidly to the following specifications: Normal male rats, not previously used for any other test and weighing between 100 and 200 Gm., were starved for eighteen hours, after having been fed on a stock diet for at least one week. The squill powder tested was weighed and mixed with the stock diet, then the quantity for each rat was weighed and placed in an individual dish, which was offered to the animal. The bait was consumed within fifteen minutes; squill symptoms developed before death, which occurred within five days, approximately.

The detailed results with five lots of commercial red squill powder furnished by John L. Hopkins, International Sales Corporation, S. B. Penick and K-R-O Company are recorded in Table I. Equal weights of each of these five powders were thoroughly mixed, in the preparation of "BSS-1." The results obtained with the five squill powders represent assays made in October and November 1935; the tests on BSS-1 were made in March and April 1936.

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