CONCLUSION.

1. The susceptibility of white rats to red squill powder follows the "standard curve."

2. By using a reference standard red squill powder and evaluating the potency of red squill preparations in terms of such a standard more precise results will be obtained than if attempts are made to determine potency in terms of dosage of body weight of white rats.

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STRYCHNINE VII. THE TOXICITY OF NUX VOMICA PREPARATIONS.*

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In other papers of this series the variation in physiological activity of strychnine alkaloids obtained from the same or different manufacturers during the last four years has been reported (1, 2). Simultaneous determinations of total alkaloidal content of the tincture, fluidextract and powdered extracts of nux vomica by the U.S.P.X method and the toxicity of these samples in form of tinctures were undertaken. In order to determine whether the same variations might be found in these galenicals as in the strychnine alkaloids studied, the same methods of bioassay were used.

The detailed results obtained in tests on nine tinctures and three fluidextracts of nux vomica commercially manufactured between 1929 and 1935 are given in Table I. For the taste tests the method previously used (3, 4, 5) was employed. In the determinations of toxicity, the $LD_{100\%}$ was determined on at least five, and in many instances up to twenty, mice, guinea pigs or rabbits. Since the susceptibility of these animals followed the "standard curve," the final conclusions are based on at least ten animals at the killing dose. The tinctures and fluidextracts were diluted with water to reduce the alcohol content below 5%: In some tests, a portion of the alcohol was evaporated off on a water-bath, but this did not affect the toxicity. Injections were made subcutaneously into mice and guinea pigs, and intravenously to rabbits. The mice weighed approximately 20 Gm., the guinea pigs 250 Gm. and the rabbits 2 Kg.

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TABLE I.—BIOASSAYS OF NUX VOMICA PREPARATIONS.

Product.	Total Alkaloids, Gm./l.	Taste Limen, Cc./l.	Toxicity. Mice Subcut., Cc./Kg.	G. Pigs Subcut., Cc./Kg.	Rabbits IV, Cc./Kg.
Tr. 1	2.65	0.9	1.25	4.0	0.3
2	2.64	1.0	1.0	5.0	0.275
3	2.61	0.9	1.5	4.0	0.275
4	2.57	0.9	1.2	4.0	0.225
5	2.56	0.6	1.3	3.0	0.275
6	2.55	0.9	1.6	3.0	0.25
7	2.55	1.0	1.1	7.0	0.275
8	2.53	0.8	1.2	3.0	0.225
9	2.51	0.9	1.5	5.0	0.275
FE. 1	24.90	0.06	0.19	0.8	0.0225
2	25.50	..	..	..	0.0325
3	25.60	..	..	..	0.035

Detailed toxicity results obtained in the study of three additional tinctures intraperitoneally injected into mice and rats are recorded in Tables II and III. As was observed in a few preliminary studies with the nine tinctures of Table I, toxicity to mice subcutaneously did not parallel toxicity to rats subcutaneously. In these tests a similar lack of agreement was found following intraperitoneal injections. Plotting the mouse toxicity findings, the $LD_{50\%}$ and the $LD_{100\%}$ were determined (Table IV).

TABLE II.—TOXICITY TR. NUX: MICE IP INJECTION.

	Dose Injected—Cc./Kg.													
Product.	0.25.	0.4.	0.5.	0.75.	1.0.	1.2.	1.5.	2.0.	2.5.	3.0.	4.0.	5.0.	7.5.	10.0.
Tr. 10	..	..	...	.	0/10	...	.	19/30	15/20	23/30	13/15	17/20	5/5	10/10
Tr. 11	0/20	..	2/20	.	3/10	...	1/5	7/10	7/10	8/10	14/15	18/20	5/5	5/5
Tr. 12	0/20	1/20	10/25	3/5	7/10	20/20	.	10/10	10/10	10/10	14/15	13/15	9/10	10/10

Numerator indicates number of animals dying; denominator number injected.

TABLE III.—TOXICITY TR. NUX VOMICA: RATS IP INJECTION.

	Dose Injected—Cc./Kg.							
Product.	1.5.	2.0.	2.5.	3.0.	5.0.	8.0.	9.0.	10.0.
Tr. 10	.	4/10	...	3/5	10/10	5/5	5/5	5/5
Tr. 11	0/5	2/10	5/15	6/10	9/10	5/5	5/5	5/5
Tr. 12	.	8/15	13/15	3/5	8/10	5/5	5/5	5/5

TABLE IV.—TOXICITY TR. NUX VOMICA: MICE IP INJECTION.

Product.	$10^{LD50\%}$, Cc./Kg.	$10^{LD100\%}$, Cc./Kg.
Tr. 10	1.80	7.5
Tr. 11	1.55	7.5
Tr. 12	0.62	1.2

Detailed comparisons of chemical assays by U. S. P. X and U. S. P. XI methods were made with taste tests and toxicity values following intraperitoneal injections into dogs. The number of animals used was limited but the results are considered indicative. When the doses, in terms of cc.'s per kilo are multiplied by the strychnine content shown in the U. S. P. XI assay, a hyperbolic relationship is observed:

The product of the dose administered in milligrams per kilo by the time between injection and death was approximately thirty minutes, or xy = 30. The results of the studies on dogs are given in Table V, and of taste tests in Table VI. Tests made with two of these tinctures upon a limited number of kittens were in agreement with the studies on dogs, in showing that tincture 12 produced a more rapid action than did tincture 11.

TABLE V.—TOXICITY TR. NUX VOMICA: DOGS IP INJECTION.

Interval in Minutes between Injection and Death.

Product.	Dose Injected: Cc./Kg. 0.25.	0.5.	1.0.	2.0.
Tr. 11	50	37	9	29
Tr. 12	45	23	19	9
Tr. 13	S	34	15	13:15

TABLE VI.—TASTE TESTS: TR. NUX VOMICA.

Product.	Strychnine Mg. Cc.	Concentration: Cc. /1. 0.1.	0.12.	0.15.	0.2.	1.0.	2.0.	Strychnine Equivalent in 5 Cc. Tasted, Gamma.
Tr. 11	2.09	0	1	2	.	.	.	1.25
Tr. 12	1.24	0	.	.	1	3	4	1.24
Tr. 13	1.20	.	0	1	2	.	.	0.90

Toxicity tests upon mice have shown variations in the lethal dose when samples have been obtained from the same manufacturer at different times, or from different manufacturers at the same time. Because of the lack of authentic history of these samples results are not included in this paper.

CONCLUSIONS.

1. Commercial nux vomica preparations have been found to exhibit significant differences in physiological activity.

2. Chemical assays for total alkaloid by the U. S. P. X process failed to agree with physiological potency. Somewhat better agreement was obtained between physiological activity and the U. S. P. XI strychine assay results. In a few samples the agreement was fairly close: In other samples marked discrepancies were obtained.

3. If adequate methods of chemical assay cannot be developed, the bioassay of nux vomica preparations may be necessary.

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